Growth and neurodevelopmental outcomes of children with *in utero* growth restriction due to placental insufficiency and modification by maternal HIV status

by

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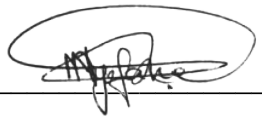
DECLARATION

"I declare that the thesis, which I hereby submit for the degree Doctor in Philosophy in Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university".

Signature:  Date: 17 July 2024

DEDICATION

In memory of my father, Leabitsoa, who passed on during this work, and my mother, Makhethang, who never saw this adventure coming. You are missed terribly; I wish heaven had visiting hours. I love you always.

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EXECUTIVE SUMMARY

Background: South Africa is burdened with a high prevalence of HIV infection in pregnant women (30.0%). Nevertheless, access to antiretroviral therapy (ART) has greatly increased, leading to an expanding population of HIV-exposed-uninfected children (CHEU). Adverse birth outcomes, including intrauterine growth restriction (IUGR), have been documented in women living with HIV, even when on ART. Child HIV exposure, anaemia and IUGR due to placental insufficiency carry significant risks to early child growth and neurodevelopment. This study determined and compared growth and neurodevelopmental outcomes, micronutrient intakes, and anaemia in CHEU compared to a control group of HIV-unexposed-uninfected children (CHUU) stratified by history of placental insufficiency.

Methods: An abnormal umbilical artery resistance index (UmA-RI) on pregnancy Doppler ultrasound was used to detect placental insufficiency as a proxy for IUGR. The cross-sectional study investigated 271 mother-child pairs at 18-months postnatal, grouped into four subgroups: CHUU with normal UmA-RI (CHUU/N-RI; control group), CHEU with normal UmA-RI (CHEU/N-RI; single exposure), CHUU with abnormal UmA-RI (CHUU/AbN-RI; single exposure) and CHEU with abnormal UmA-RI (CHEU/AbN-RI; double exposure). Pregnancy and birth information was available. World Health Organisation standard procedures were followed to collect anthropometric data and compute z-scores. International Guide for Monitoring Child Development (GMCD) was used for developmental screening, and Bayley Scale of Infant and Toddler Development III (Bayley-III) was used to test for cognitive, language and motor development. Premature births were corrected for gestational age. Previously used questionnaires and quantified 24-hour dietary recall were used to collect sociodemographic variables and dietary intake. FoodFinder™ 3.0 was used for meal analysis, quantifying dietary intake of iron, zinc, and iodine. Both maternal and children's haemoglobin concentrations were tested using HemoCue® Hb 201^{+}. Comparisons were performed using

independent t-test and Mann-Whitney U test. Spearman's correlation and regression models were used to determine associations.

Results: Lower length-for-age z-scores (LAZ) were observed in CHEU than CHUU (-0.71±1.23 vs -0.05±1.32; p=0.004), and children who had abnormal UmA-RI than normal counterparts (-0.68±1.53 vs -0.14±1.29; p<0.001). CHEU/AbN-RI had the lowest LAZ compared to CHUU/N-RI (-1.3±1.3 vs -0.03±1.30; p<0.001). The prevalence of stunting (LAZ <-2) was higher in CHEU/AbN-RI (40.0%) and CHEU/N-RI (16.0%) than in CHUU/N-RI (4.8%); p<0.001 and p=0.016, respectively. GMCD screening indicated a concern for delay in gross motor development among 21.4% of CHEU/AbN-RI. Bayley-III test demonstrated lower mean cognitive scores in CHEU/AbN-RI compared to CHUU/N-RI: 93.9±12.9 vs 100.0±10.6; p=0.045, with 21.4% of CHEU/AbN-RI having mild delay in cognitive development. Further, zinc intake and weight-for-age z-scores were positively associated with language (r=0.10; p=0.042) and motor (r=0.10; p=0.028) development, respectively. Above one-third of children were mildly anaemic: CHUU/N-RI: 44.4%, CHEU/N-RI: 44.7%, CHUU/AbN-RI: 40.0% and CHEU/AbN-RI: 33.3%. In the CHEU group, maternal haemoglobin concentrations were associated with child haemoglobin concentrations: β=0.19, 95% confidence interval (CI) (0.02,0.36); p=0.028. There was no evidence to suggest an association between maternal or child haemoglobin concentration and child neurodevelopment. On further analysis cognitive development was positively associated with LAZ: β=3.34, 95%CI (1.13,5.54), P=0.004 in the CHEU group.

Conclusion: Maternal HIV exposure and placental insufficiency are risk factors for stunting and cognitive deficits, both independently and compounded. CHEU and children who had IUGR are a high-risk population in need of identification and appropriate interventions within child health and nutrition-sensitive programmes. Childhood anaemia remains a paramount public health concern.

Keywords: HIV-exposed children; placental insufficiency; intrauterine growth restriction; growth; neurodevelopment; micronutrient intake; anaemia

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ABBREVIATIONS AND ACRONYMS

AEDF	Absent end diastolic flow
AGA	Appropriate for gestational age
AI	Adequate intake
ART	Antiretroviral therapy
BANC	Basic antenatal care
BAZ	BMI-for-age z-score
BMI	Body mass index
BSID-II	Bayley Scale for Infant Development second edition
BSITD-III	Bayley Scale for Infant and Toddler Development, third edition
CE	Conformité Européenne
CHC	Community Health Centre
CHEU	HIV-exposed-uninfected children
CHEU/AbN-RI	CHEU with abnormal UmA-RI
CHEU/N-RI	CHEU with normal UmA-RI
CHUU	HIV-unexposed-uninfected children
CHUU/AbN-RI	CHUU with abnormal UmA-RI
CHUU/N-RI	CHUU with normal UmA-RI
COVID-19	Coronavirus disease of 2019
CSIR	Centre for Scientific and Industrial Research
CTB	Cytotrophoblast
DRI	Dietary reference intake
EAR	Estimated Average Requirement
EBF	Exclusive breastfeeding
FFM	Fat-free mass
FFMZ	Fat-free mass z-score
FGR	Foetal growth restriction
FVWs	Flow velocity waveforms
GA	Gestational age
GEE	Generalized estimating equations

GMCD	International Guide for Monitoring Child Development
HAART	Highly-active ART
HAZ	Height-for-age z-score
HC	Head circumference
HCZ	Head circumference-for-age z-score
HINE	Hammersmith Infant Neurological Examination
HIV	Human immunodeficiency virus
INTERGROWTH	International Foetal and Newborn Growth Consortium
IUGR	Intrauterine growth restriction
IYCF	Infant and Young Child Feeding
LAZ	Length-for-age z-score
LBW	Low birth weight
LFA	Length-for-age
LMICs	Low- and middle-income countries
MTCT	Mother-to-child HIV transmission
MUAC	Mid-upper arm circumference
MUACZ	Mid-upper arm circumference z-score
PCR	Polymerase chain reaction
PLHIV	People living with HIV
PMTCT	Prevention of mother-to-child HIV transmission
REDCap	Research Electronic Data Capture
REDF	Reversed end diastolic flow
RI	Resistance index
RNA	Ribonucleic acid
RTHB	Road to Health Book
SAMRC	South African Medical Research Council
SDG	Sustainable development goal
SGA	Small for gestational age
SSA	Sub-Saharan Africa
STB	Syncytiotrophoblast

TSF	Triceps skinfold
TSFZ	Triceps skinfold- for-age z-score
UmA-RI	Umbilical artery resistance index
VLBW	Very low birth weight
WAZ	Weight-for-age z-score
WFA	Weight-for-age
WFL	Weight-for-length
WHO	World Health Organisation
WHO MGRS	World Health Organization Multicentre Growth Reference Study
WLWH	Women living with HIV
WLZ	Weight-for-length z-score

GLOSSARY

Abnormal UmA-RI	Umbilical artery resistance index value ≥75th percentile and is regarded as high risk for placental insufficiency, with previously proven increased risk of stillbirth, here used as a marker for intrauterine growth restriction.
Anaemia	A blood condition diagnosed with haemoglobin levels <11.0g/dL for children under 60 months and <12.0g/dL for non-pregnant women.
HIV-exposed-uninfected	Child born to a woman living with HIV and not HIV-infected him/herself.
HIV-unexposed-uninfected	Child born to an HIV-uninfected women and not HIV-infected him/herself.
Intrauterine growth restriction	Failure of a foetus to reach the genetically determined growth potential.
Neurodevelopment	Child development of the neurological pathways of the brain, which sway functioning or performance, including social skills, attention or focus skills, ability to read and intellectual functioning.
Normal UmA-RI	Umbilical artery resistance index value < 75th percentile and is regarded as low risk for compromised placental function.
Placental insufficiency	A condition related to the failure of placental vascular remodelling, followed by deterioration of placental functioning and subsequently acidosis and foetal hypoxemia. It results in reduced delivery of oxygen and nutrients to the foetus.
Umbilical artery resistance index	Measurement produced by Doppler ultrasound reflecting changes in the blood circulation of the umbilical artery; that is circulation between the placental and the foetus.

CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT

1.1 CONTEXTUALIZATION

Placental insufficiency is one immediate cause of intrauterine growth restriction (IUGR), which can lead to stillbirth (Figure 1.1) (Audette and Kingdom, 2018; Wardinger and Ambati, 2021). Intrauterine growth restriction is the second most important cause of perinatal death following prematurity and affects 3 to 9% of all pregnancies (Audette and Kingdom, 2018). The IUGR is a clinical term describing a pathological inhibition of foetal growth, preventing the foetus (unborn baby) from growing according to its genetic growth potential, therefore being smaller than it should be (Burton and Jauniaux, 2018). Other risk factors for IUGR include maternal HIV infection, undernutrition (mid-upper arm circumference (MUAC) below 23 cm or body mass index (BMI) below 18.5kg/m^2) and short stature (below 145cm), overweight, obesity, and iron or other micronutrient deficiencies during early pregnancy (Black *et al.*, 2013; Flores-Guillén *et al.*, 2020). Maternal nutritional deprivation during pregnancy leads to foetal starvation and, ultimately, IUGR. IUGR is linked in its most severe form to low birth weight (LBW) (birth weight less than 2500g) and born small for gestational age (SGA) (birth weight less than the 10th percentile for gestational age (GA) and sex) (Flores-Guillén *et al.*, 2020; Longo *et al.*, 2014; Ndirangu *et al.*, 2012; Saleem *et al.*, 2011; Vayssière *et al.*, 2015). Appropriate for gestational age (AGA) babies with growth restriction and features of malnutrition at the time of birth are also regarded as growth-restricted *in utero* (Sharma *et al.*, 2016). Although SGA is used as a proxy for IUGR (Kesavan and Devaskar, 2019; Sharma *et al.*, 2016), this remains problematic because many SGA babies are not growth-restricted but constitutionally small yet healthy (Hiersch and Melamed, 2018; Karlsen *et al.*, 2016), while additionally there are growth-restricted AGA babies whose birth weight is above the 10th percentile for GA and sex (Hiersch and Melamed, 2018; Iliodromiti *et al.*, 2017).

Globally, IUGR is present in about 30% of cases of preventable stillbirths (Audette and Kingdom, 2018; Flenady *et al.*, 2017). Stillbirths remain an important health issue (Reinebrant *et al.*, 2018), with approximately 2 million third-trimester stillbirths every year, globally (United Nations Inter-agency Group for Child Mortality Estimation (UN IGME), 2020). The World Health Assembly's Every Newborn Action Plan sets the target to reduce stillbirths to ≤12 per 1000 births in every country by 2030 (WHO, 2014). Low- and middle-income countries (LMICs) are the most burdened with a high prevalence (98%) of stillbirths and perinatal deaths (Lawn *et al.*, 2016). About 75% of cases are reported in sub-Saharan Africa

(SSA) and South Asia, which is 10-fold higher than in developed countries, with a stillbirth rate of 26 per 1000 being reported in SSA (Anu *et al.*, 2019). Stillbirth secondary to IUGR can be attributed to unrecognised placental insufficiency (Lavin *et al.*, 2020; Lawn *et al.*, 2016; Mufenda *et al.*, 2015; Zohdi *et al.*, 2012). In view of the above, screening for placental insufficiency has become critical to identifying *in utero* growth-restricted foetuses as they are at higher risk of prematurity, perinatal death, poor neurodevelopmental outcomes, and health risk of reprogramming the foetus for long-term diseases like metabolic syndrome (Audette and Kingdom, 2018).

The Umbiflow™ Doppler device is a precise and low-cost continuous Doppler ultrasound device that can be used as a screening tool to detect placental insufficiency in low-risk pregnancies (without medical complications) (Theron *et al.*, 2005). It measures the blood velocity within the umbilical artery, enabling the calculation of the umbilical artery resistance index (UmA-RI) (Mufenda *et al.*, 2015). Placental insufficiency is detected by an abnormally high UmA-RI, as this is linked to vascular resistance and poorer blood flow from the placenta to the foetus, with insufficient supply of nutrients and oxygen to the foetus and subsequently foetal starvation and IUGR.

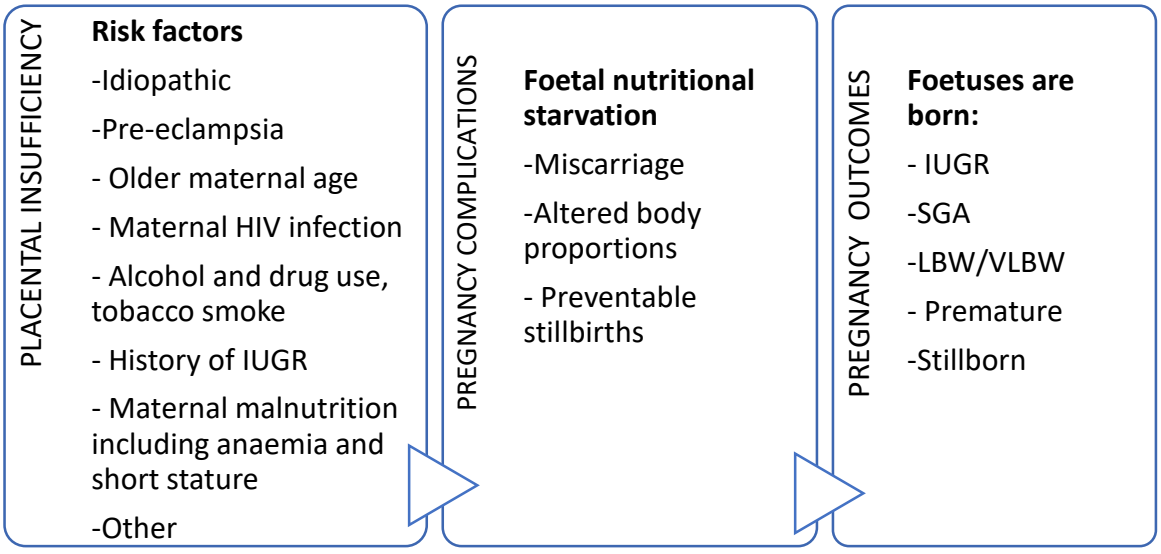

Figure 1.1: The maternal risk factors for placental insufficiency and the consequences of IUGR in the absence of the screening intervention for placental insufficiency

Abbreviations: AGA: appropriate for gestational age; LBW: low birth weight; IUGR: intrauterine growth restriction; SGA: small for gestational age; VLBW: very low birth weight

1.2 BACKGROUND

In South Africa, about 31.6% of women of reproductive age are living with human immunodeficiency virus (HIV) (Clouse *et al.*, 2020). The World Health Organisation (WHO) recommends the use of antiretroviral therapy (ART) for all pregnant and breastfeeding women for the prevention of mother-to-child HIV transmission (PMTCT) (WHO, 2013; WHO, 2016). Access to ART has increased over the years in South Africa, with an estimated >95% ART coverage during pregnancy and delivery (Clouse *et al.*, 2020); this has led to a significant decline in vertical transmission of HIV among children born to women living with HIV (WLWH).

Maternal HIV infection has been associated with an increased risk of IUGR (Flores-Guillén *et al.*, 2020; Ndirangu *et al.*, 2012) and LBW (Xiao *et al.*, 2015). It has been suggested that maternal HIV infection may be associated to IUGR, irrespective of ART treatment (Canlorbe *et al.*, 2015). Moreover, increased prevalence of unfavourable birth outcomes such as IUGR, prematurity, LBW and stillbirths, have been reported in WLWH, compared to their HIV-uninfected counterparts (Conroy *et al.*, 2017; Papp *et al.*, 2014; Zash *et al.*, 2017). Studies have indicated that WLWH may have a higher risk of placental insufficiency, as infections, including HIV, have the potential to disrupt placental development and function, leading to adverse birth outcomes (Conroy *et al.*, 2017; Weckman *et al.*, 2019). Kalk *et al.* (2017) have pinpointed HIV as a risk factor for maternal vascular malperfusion among South African WLWH. Children with a medical history of IUGR (in this study to be referred to as children with a history of an abnormal UmA-RI during their foetal phase) are a high-risk group with short- and long-term complications in terms of morbidity and mortality, differentiating them from the normal population. Further, maternal HIV exposure may potentially aggravate the *in utero* and postnatal growth and development deficits in children with abnormal UmA-RI.

Around one quarter (27.4%) of South African children under the age of five years were reported to be stunted (length-for-age z-score (LAZ) below -2 on WHO Growth Standards) in 2016 (National Department of Health (NDoH) *et al.*, 2018). HIV infection in children is a well-known risk factor for stunting. Nonetheless, the number of HIV-infected children is decreasing, resulting in an expanding population of HIV-exposed-uninfected children (CHEU) globally due to the success of PMTCT programmes (Chandna *et al.*, 2020; Neary *et al.*, 2021). Even though Ramokolo *et al.* (2013) reported that the CHEU present similar growth patterns with HIV-unexposed-uninfected children (CHUU), CHEU are classified as a high-risk group due to *in utero* and postnatal ART exposure, as well as exposure to pathogens from contacts with

immunocompromised family members (Slogrove *et al.*, 2009). Delicio *et al.* (2018) reported a high prevalence of anaemia (25.7%) and liver changes (36%) attributed to ART in new-borns of WLWH. Anaemia in itself is associated with stunting in 6 to 23-month-old children (Malako *et al.*, 2019). Further, CHEU are at greater risk of impaired growth and neurodevelopmental outcomes than CHUU (Evans, 2016; Wedderburn *et al.*, 2019a). Jumare *et al.* (2019) reported stunting and wasting (weight-for-length z-score (WLZ) below -2 on WHO Growth Standards) among CHEU during the first 18 months as compared to their CHUU counterparts. Stunting indicates chronic undernutrition and is associated with suboptimal neurodevelopment (Black *et al.*, 2015). Neary *et al.* (2021) reported poorer growth outcomes (stunting, underweight and microcephaly) in CHEU than in CHUU in the context of high maternal ART coverage. Underweight is defined as a weight-for-age z-score (WAZ) below -2, and microcephaly is the head circumference-for-age z-score (HCZ) below -2, according to WHO Growth Standards (De Onis et al., 2006).

Black *et al.* (2013) stated that IUGR is associated with postnatal child wasting and stunting. Flores-Guillén *et al.* (2020) reported a 21% prevalence of stunting associated with IUGR among adolescents from Mexico. Children with abnormal UmA-RI are not only prone to poor physical growth but are also at increased risk of overweight and obesity during the adolescent years, as well as intellectual and physical developmental modifications (Flores-Guillén *et al.*, 2020). IUGR is also linked with long-term metabolic syndrome and neurodevelopmental abnormalities, such as cognitive deficits, leading to reduced school performance, lower economic productivity, and mental illness throughout life (Baschat, 2011; Kesavan and Devaskar, 2019; Shonkoff and Garner, 2012; Vayssière *et al.*, 2015). In addition, IUGR makes term and preterm infants more vulnerable to neurodevelopment impairment, as noted by lower neurodevelopmental scores at two years (Belfort *et al.*, 2011; Vayssière *et al.*, 2015).

Child neurodevelopment is a global health priority. Estimates are that over one-third of children under the age of five years are at risk of not attaining their full developmental potential, particularly in LMICs (John *et al.*, 2017). Child development, including motor and cognitive development, is highly influenced by pre- and post-natal circumstances and maternal and infant exposure to environmental and biological risks (Belfort *et al.*, 2011; Hamadani *et al.*, 2012). The first 1000 days of life (starting at conception to the age of two years) are a crucial developmental window, with heightened susceptibility to poor growth, including stunting (Rieger and Trommlerová, 2016), a common predictor of inadequate development (Black *et*

al., 2013; Smuts *et al.*, 2019). Three micronutrients, namely iron, zinc and iodine, have been positively associated with child neurodevelopment (John *et al.*, 2017).

Globally, 30% of women of childbearing age are anaemic (haemoglobin level below 10g/dL (Takuva *et al.*, 2013)), and the prevalence of iron deficiency anaemia during pregnancy has been estimated to be 38% (Juul *et al.*, 2019). The world is not on track to reach the target of a reduction in the prevalence of anaemia in women of childbearing age by 2030 (SDG Indicator 2.2.3) (WHO, 2021). In South Africa, the prevalence of anaemia in women has previously been reported as 28.1%, with iron deficiency being the most common cause (Tunkyi and Moodley, 2018). The nutrient deficiencies are compounded in HIV settings, with WLWH adversely affected, especially in terms of iron deficiency (Takuva *et al.*, 2013). A higher prevalence of anaemia in WLWH than in HIV-uninfected women was reported by Tunkyi and Moodley (2018). Pregnancy itself increases nutritional needs (Montgomery, 2003); likewise, HIV infection, thereby compounding increased nutrient requirements in pregnant WLWH, which is essential for their own health outcomes and that of their foetuses. Maternal anaemia is a risk factor for IUGR (Figure 1.1). There is a known association between iron deficiency anaemia and impaired neurodevelopment in children, including suboptimal motor development, even in iron deficiency without anaemia (Pala *et al.*, 2010). Delays in motor, cognitive, social-emotional, and neurophysiologic development have been attributed to iron deficiency anaemia in 6- to 24-month-old children (Engle *et al.*, 2007; Hamadani *et al.*, 2012; Juul *et al.*, 2019; Lozoff, 2007). Iron is contained in numerous proteins in the body, including haem proteins such as haemoglobin, known to be the most bountiful iron-containing protein (Miller, 2013). The production, structure, and functionality of haemoglobin depend on iron (Miller, 2013; Perutz, 1982). Therefore, growth and development of the foetal and children's brains necessitate iron for haemoglobin; thus, insufficient production of haemoglobin, owing to iron deficiency, is a risk factor for deficits in cognition (Juul *et al.*, 2019; Prado and Dewey, 2014). Further, zinc deficiency has been linked with poor cognitive and mental health function (Black, 2011).

Additionally, a positive correlation exists between breastfeeding and a child's growth and development. Jumare *et al.* (2019) pointed out that exclusive breastfeeding (EBF) stimulates growth among CHEU and CHUU, while Eidelman (2013) found that breastfeeding results in enhanced cognitive development. It is believed that optimal breastfeeding can also stimulate growth and development in children with abnormal UmA-RI. In an effort to ensure optimal

feeding practices and enhance favourable growth and developmental outcomes, the 2016 WHO guideline updates on HIV and infant feeding recommend EBF for the first six months and continued breastfeeding for two years or longer with complementary feeds for both WLWH and HIV-uninfected mothers, within the context of the provision of ART for WLWH (WHO and UNICEF, 2016). The WHO and UNICEF (2021) strongly recommend EBF until the age of six months. Exclusive breastfeeding is defined as breast milk feeding without other food or liquids, not even water, except oral rehydration solution, vitamins, minerals, or medicines (WHO and UNICEF, 2021).

Adequate child nutrition is central for catch-up growth and brain development (Prado and Dewey, 2014). It is of great importance to better understand the outcomes of postnatal growth and development of children with abnormal UmA-RI and modifying factors within the settings of high HIV prevalence in LMICs.

The present study is centred on assessing and comparing the growth and developmental outcomes and anaemia among 18-month-old children with maternal HIV exposure and placental insufficiency/abnormal UmA-RI (IUGR) (Figure 1.2). Since there are important confounders, such as socio-demographic characteristics, maternal health and nutrition, education, food security and alcohol and tobacco use, that are known to influence child growth and neurodevelopmental outcomes, the comparison with CHUU will be of great importance in bettering our understanding of the impact of HIV-exposure and placental insufficiency on child outcomes (Richter *et al.*, 2017; Springer *et al.*, 2018). Lastly, due to the impact of the COVID-19 pandemic, which included increases in existing family food insecurity of 32.3% and new household food insecurity of 35.5% (Diao and Mahrt, 2020), the present study considered COVID-19 as a crucial potential influencer of maternal and child health and nutritional status.

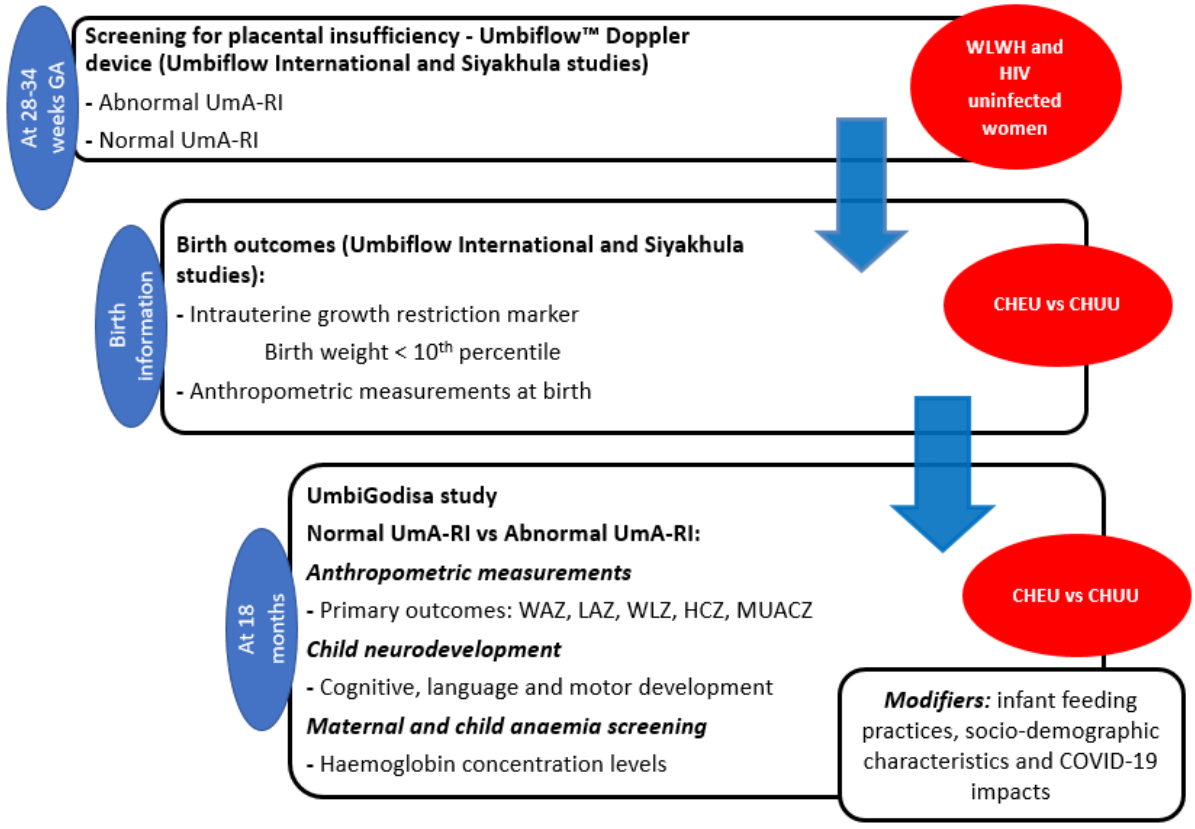

Figure 1.2: Conceptual framework of the study

Abbreviations: CHEU: HIV-exposed-uninfected children; CHUU: HIV-unexposed-uninfected children; GA: gestational age; UmA-RI: umbilical artery resistance index; WAZ: weight-for-age z score; LAZ: length-for-age z score; WLZ: weight-for-length z score; WLWH: women living with HIV; HCZ: head-circumference-for-age z score; MUACZ: mid-upper arm circumference-for-age z score; COVID: coronavirus disease.

1.3 THE PROBLEM STATEMENT

Since IUGR is a crucial risk factor for child undernutrition and neurodevelopmental delays, children who have IUGR are known to be a high risk population, possibly throughout their life course (Baschat, 2011; Kesavan and Devaskar, 2019; Sania *et al.*, 2015). Similarly, exposure to maternal HIV infection has potentially negative consequences on child growth and development. Malnutrition is more prevalent in CHEU and is known to negatively affect neurodevelopment and increase the risk of morbidity and mortality. Even though the cause of poor growth in CHEU remains uncertain and is likely multifactorial, implicated factors include lower economic backgrounds, antiretroviral (ARV) drug exposure, differences in infant feeding and increased postnatal exposure to infectious pathogens. Despite the expanding population of CHEU, the growth differences between CHEU and CHUU at the population level

are under-investigated. Additionally, the influence of HIV exposure on their growth and neurodevelopment is not yet fully defined (Evans, 2016; Omoni *et al.*, 2017).

Given the above, HIV exposure and placental insufficiency both carry major risks to early and late child growth and development, possibly compounding each other. However, the postnatal growth and neurodevelopment in children born to otherwise low-risk pregnancies with documented abnormal UmA-RI measurements, indicating placental insufficiency leading to IUGR, have not been intensively investigated, particularly in high HIV burden settings. There is still a lack of evidence on the nutritional management of children with a history of placental insufficiency to optimise postnatal catch-up growth, including the impacts of feeding practices on the growth of children with a history of placental insufficiency in the context of HIV exposure. Additionally, the influence of maternal HIV infection on placental insufficiency and the postnatal consequences thereof are not fully understood; therefore, there is a need to investigate this further.

Moreover, starvation during early life is known to affect brain development negatively (John *et al.*, 2017) and may even lead to permanent impairment. It is well known that iron, zinc, and iodine are vital nutrients for neurological development. Their low intakes and associated conditions such as anaemia have been documented to be associated with poor child development; however, such documentation is scarce in IUGR and HIV-exposed settings. The influence of HIV exposure on the intake of the mentioned micronutrients, either due to financial constraints or suboptimal feeding practices, and the subsequent developmental outcomes of children with abnormal UmA-RI have not been adequately explored. Lastly, the availability of UmA-RI measurement from the portable, low-cost Umbiflow^{TM} Doppler, which can be used at the primary healthcare level in low-risk pregnancies and the present data on the follow-up in this population are novel to the study.

1.4 AIM AND OBJECTIVES

This study, named UmbiGodisa (Umbi referring to the umbilical cord and Godisa being an African word meaning 'bring me up, let me grow and develop'), was a follow-up of a sub-set of infants at 18 months, recruited from the South African arm of the Umbiflow International study. Also, it includes children from a longitudinal study, the UmbiBaby study, who were also recruited from the South African arm of the Umbiflow International study. The UmbiGodisa study also included participants from the Siyakhula study (refer to section 3.2) as they complemented the study group and enabled the inclusion of further WLWH who had an abnormal UmA-RI in pregnancy (Figure 1.2). The thesis is meant for a PhD by publication.

1.4.1 Overall aim of the doctoral study

To study how *in utero* growth restriction, resulting from placental insufficiency, in addition to *in utero* and early postnatal environments altered by maternal HIV infection and anaemia, influence the child's growth and neurodevelopment, as well as anaemia, at age 18 months.

1.4.2 Hypotheses

A. CHEU with abnormal UmA-RI have suboptimal growth outcomes at age 18 months.
    - Rationale: Intrauterine stresses have been linked to disturbed health, growth and development through foetal programming (Thompson and Al-Hasan, 2012); the programmed modifications of growth factors, hormones and enzyme concentrations result from epigenetic alteration of gene expression (Nüsken *et al.*, 2011). Also, exposure to maternal HIV infection may disrupt a child's growth, especially length growth (Prendergast *et al.*, 2019; Wedderburn *et al.*, 2019a).

B. CHEU with abnormal UmA-RI have neurodevelopmental delays in at least one of the developmental domains at age 18 months.
    - Rationale: Literature shows that children with a history of placental insufficiency indicating IUGR have macrostructural alterations, including reductions in cerebral cortical grey matter and sulcation (atypical brain development), associated with microstructural and metabolic changes that increase the risk of neurodevelopmental deficits (Padilla *et al.*, 2010). This finding might be due to direct exposure to HIV virions, ART toxicity, immune activation and indirectly through low socioeconomic status, maternal psychosocial factors, including maternal depression

resulting in lack of psychosocial stimulation/parent and child interaction and maternal education (Mofenson, 2015; Wedderburn *et al.*, 2019b).

C. Suboptimal infant feeding practices, including low dietary iron, zinc and iodine intake at age 18 months, are associated with delays in at least one functional domain of development, particularly in CHEU with a history of an abnormal UmA-RI.
    - Rationale: During the period of rapid infantile growth, body and brain growth require a high energy supply and metabolism, and the cellular energy metabolism is dependent on oxygen supply, which is reliant on adequate haemoglobin and iron stores (Soliman *et al.*, 2014). Zinc deficiency alters the production of neurotransmitters, cellular replication and bone metabolism (Salgueiro *et al.*, 2002), and insufficient thyroid hormone, linked to iodine deficiency, impairs myelination, cell migration, differentiation and bone maturation (Zimmermann, 2011).

D. The maternal HIV infection, anaemia and placental insufficiency are associated with child anaemia and delayed neurodevelopment at age 18 months.
    - Rationale: High anaemia prevalence in HIV settings is due to nutritional deficiencies compounded by vulnerability to infections, compromised immunity, and the haematological consequences of chronic and systemic inflammation (Uneke *et al.*, 2007). Maternal HIV infection and placental insufficiency predispose children to anaemia.

1.4.3 Specific Aim

To assess and compare, at the age 18 months, the growth and neurodevelopmental outcomes of children who had IUGR due to placental insufficiency, as measured by an abnormal UmA-RI on Umbiflow™ Doppler screening during pregnancy and as modified by maternal HIV infection, together with the impact of anaemia, in the Tshwane District in the Gauteng Province of South Africa.

1.4.4 Objectives

The following objectives were investigated in children who had normal or abnormal UmA-RI, between CHEU and CHUU:

A. To determine the socio-demographic, household food security and lifestyle factors, and health and nutrition of mother-child pairs.
B. To assess and compare the anthropometric indices and z-scores at the age 18 months.

C. To assess and compare neurodevelopment at age the 18 months, including cognitive, motor and language development.
D. To compare haemoglobin concentrations, determine prevalence and the impact of maternal anaemia on child neurodevelopment and anaemia.
E. To determine and compare the feeding practices over 18 months and the dietary intake of iron, zinc, and iodine at age 18 months and relate to neurodevelopmental outcomes.

1.5 THE STRUCTURE OF THE THESIS

The present thesis is prepared in articles. The thesis comprises seven chapters: Chapter 1 covers the introduction, problem statement, objectives and impact of the study; Chapter 2 entails the literature review; and a detailed description of the methodology is presented in Chapter 3. Chapters 4, 5 and 6 are manuscripts, and the titles are listed below. The manuscripts have been prepared and presented according to guidelines to authors for publication in peer-reviewed journals accredited by the Department of Higher Education and Training (DHET). Chapter 7 summarises the key findings, study limitations and conclusions. Annexes have been attached at the end of this thesis.

Research paper publications:

A. Chapter 4: Article 1: Early childhood growth parameters in South African children with exposure to maternal HIV infection and placental insufficiency. This manuscript was published in an international journal called Viruses.
B. Chapter 5: Article 2: Growth, neurodevelopmental outcomes and micronutrient intake in 18-month-old South African children with maternal HIV exposure and placental insufficiency: the UmbiGodisa cross-sectional study. This manuscript was submitted for publication in an International Journal of Paediatrics.
C. Chapter 6; Article 3: The impact of maternal HIV infection and anaemia together with placental insufficiency on neurodevelopment and anaemia in South African children. This manuscript was submitted for publication in an international journal called Anaemia.

1.6 IMPACT OF THE STUDY

Assessing growth and screening for developmental delays during the first 1000 days of life, a critical period of child development is important for early detection and intervention. This study provided high-quality primary data about the early postnatal growth and neurodevelopment of CHEU with abnormal UmA-RI, which provided substantial evidence for future nutrition management of these high-risk children. The generation of such valuable information in the context of maternal HIV exposure possibly filled the existing gap. It enabled

comparisons between CHEU with histories of normal or abnormal UmA-RI measurements and their CHUU counterparts. The study findings are essential for policymakers to provide interventions and support future surveillance.

This study contributes to improving nutrition knowledge involving the impact of nutrient intake and feeding practices on growth, which can then be translated into the clinical domain towards improved nutrition and child health care outcomes. South Africa is among the countries committed to achieving the United Nations Sustainable Development Goals (SDGs). Therefore, the present study also contributed towards achieving the United Nations SDGs, precisely goal number 3, "ensuring healthy lives and promoting well-being for all ages", and 4, underscoring "unbiased quality education and promoting lifelong learning opportunities for all" in South Africa (Hawkes and Popkin, 2015). Assessing early child developmental milestones forms an SDG element that safeguards preparedness for early education (United Nations, 2020).

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CHAPTER 2: LITERATURE REVIEW

2.1 PLACENTAL DEVELOPMENT AND FUNCTION

The placenta is described as a transitory, highly specialised supporting organ of pregnancy linking the mother and the embryo (Hemberger *et al.*, 2020). The placental development begins at embryonic days 5 to 6. The formation of the placenta involves localised growth of chorionic villi comprising of a mesenchymal core, each with foetal blood vessels and layers of cytotrophoblast (CTB) and syncytiotrophoblast (STB) cells (outer layer) that are directly bathed in the maternal blood (Hemberger *et al.*, 2020) (Figure 2.1). The mentioned basic structure of the complete placenta is usually formed around the fourth week of pregnancy (Knöfler *et al.*, 2019b). However, the establishment of blood supply from the maternal uterus to the placenta occurs only at weeks 10 to 12 of the pregnancy (Burton *et al.*, 2002; Hemberger *et al.*, 2020). Maternal blood forms uteroplacental circulation as it moves in and out of the networks within the STB (Gude *et al.*, 2004).

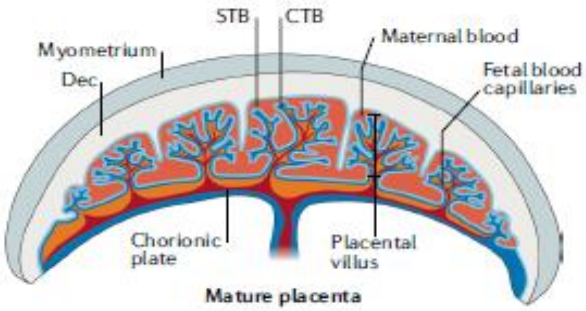

Figure 2.1: Basic structure of mature human placenta at embryonic day 35 or fourth week of pregnancy

Abbreviations: CTB: cytotrophoblast; Dec: decidua; STB: syncytiotrophoblast.

Sourced from Hemberger *et al.* (2020).

The placental function can be classified as 'transport, protection, and endocrine' because it serves as an agent of human symbiosis and foetal renal, gastrointestinal, respiratory, and immune systems throughout foetal development (Guttmacher *et al.*, 2014). The placenta supports the normal growth and development of the foetus and forms the interface between the foetus and its mother (Burton *et al.*, 2016; Gude *et al.*, 2004). It provides nourishment to the foetus by delivering oxygen, water, amino acids (about 20 amino acids for foetal protein synthesis), lipids, carbohydrates (mainly glucose), minerals, vitamins, and other protective non-nutritive chemicals such as phytochemicals, while eliminating carbon dioxide and waste products, and additionally, providing protection against maternal infections and diseases, as

well as some xenobiotic molecules that could be circulating in the maternal blood (Gude *et al.*, 2004). The placenta has a highly permeable membrane that permits exchange between the mother and the foetus through diffusion and passive transport. Further, the placenta secretes hormones into the blood circulation of the mother and foetus, regulating the maternal physiology to stimulate the maintenance of pregnancy and inducing foetal growth and birthing (Gude *et al.*, 2004; Knöfler *et al.*, 2019a). The mentioned placental functions infer that the placenta directly influences the intrauterine development of the foetus; thus, the placental growth and function needs to be monitored to ensure that the exchange of oxygen, nutrients and waste products between the maternal and foetal circulatory systems functions efficiently. The reduced ability of the placenta to efficiently perform the above may be referred to as a poor placental function or placental insufficiency (Wardinger and Ambati, 2021). Placental insufficiency is a well-documented placental phenotype that leads to pregnancy complications.

2.2 THE AETIOLOGY AND PATHOPHYSIOLOGY OF THE PLACENTAL INSUFFICIENCY

According to Wardinger and Ambati (2021), placental insufficiency is defined as a condition related to the breakdown of placental vascular remodelling, followed by deterioration of placental functioning and, subsequently, acidosis and foetal hypoxemia. This process leads to reduced placental transfer of oxygen and nutrients to the foetus (Gagnon, 2003). The foetal growth and development depend on uteroplacental blood supply and placental villous development to deliver nutrients (Burton and Jauniaux, 2018). Foetal hypoxemia leads to lowered metabolic demands by the growing foetus, leading to reduced foetal growth, clinically known as IUGR or foetal growth restriction (FGR) (Gagnon, 2003; Wardinger and Ambati, 2021). Burton and Jauniaux (2018) and Brosens *et al.* (2011) stressed that the primary cause of placental-derived FGR is the insufficient remodelling of the uterine spiral arteries that supply the placenta in early pregnancy, resulting in malperfusion of the placenta or uteroplacental dysfunction. Furthermore, there are obstetric disorders that are associated with placental insufficiency, IUGR and pre-eclampsia, which equally predispose to preterm labour, a globally known prominent cause of perinatal morbidity and mortality (Wardinger and Ambati, 2021). Intrauterine growth restriction and pre-eclampsia originate from the invasion of trophoblasts into the placenta which displays features of decidua during early pregnancy (Mandò *et al.*, 2014). Histopathologic examination of placental insufficiency shows the presence of placental infarcts, chorionic villi fibrosis, fibrin deposits, uteroplacental thrombosis and a reduced

number and surface area of the villous capillary tree (Mazarico *et al.*, 2020; Wardinger and Ambati, 2021).

Wardinger and Ambati (2021) pointed out that the aetiology of placental insufficiency is presently poorly understood and requires further investigation. Nonetheless, maternal known lifestyle behaviours, including alcohol consumption, cigarette smoke and use of drugs, pre-eclampsia or other maternal hypertensive disorders, old maternal age, nulliparity, and previous IUGR are maternal risk factors associated with placental insufficiency (Gagnon, 2003; Pintican *et al.*, 2019; Wardinger and Ambati, 2021). In their systematic review that explored the effects of maternal smoking on placental vascularisation, Pintican *et al.* (2019) documented that Doppler findings revealed abnormal RI of the umbilical, uterine and foetal middle cerebral arteries and reduced blood flow velocity waveforms. A different study indicated an association between maternal malnutrition, including anaemia (contributing up to 40% of cases globally), and the development of IUGR neonates (Audette and Kingdom, 2018). Genetic syndromes or chromosomal aneuploidies are foetal factors that also lead to IUGR (Audette and Kingdom, 2018). Figure 2.2 showcases the four causes that can lead to IUGR.

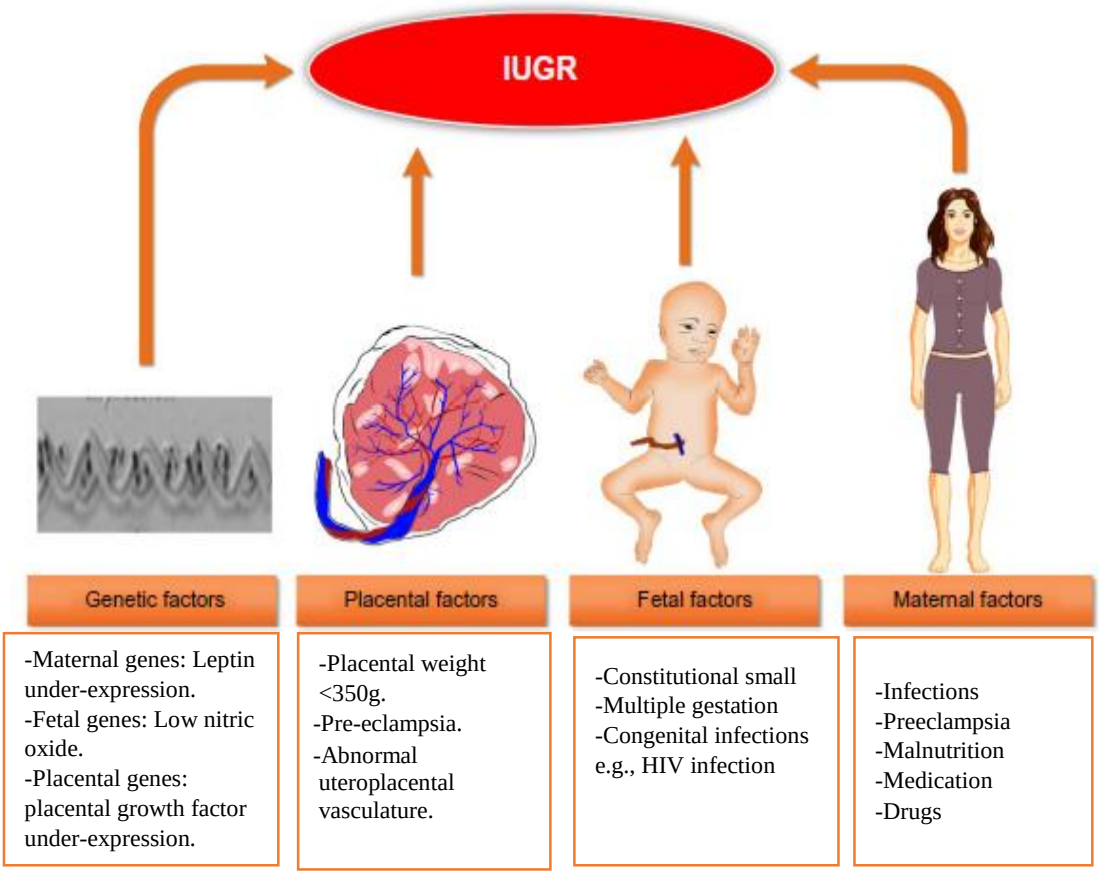

Figure 2.2: Factors that are linked to IUGR.

Adapted from Sharma *et al.* (2016)

Placental insufficiency causes a decreased blood flow in the umbilical artery, probably due to high umbilical-placental vascular resistance, such as detected by an abnormal UmA-RI on Doppler ultrasound (Gagnon, 2003; Wardinger and Ambati, 2021). Wardinger and Ambati (2021) stated that the foetus with suspected IUGR can benefit from regular Doppler monitoring, as Doppler screening allows detection of placental insufficiency through notching in the uterine arteries or high resistance in the umbilical artery as well as end-stage placental disease presented as absent or reversed end-diastolic flow (AEDF or REDF). Placental insufficiency can be categorised into maternal (indicated by RI of the uterine artery), foetal (indicated by UmA-RI) or maternal and foetal placental insufficiency (Lopez *et al.*, 2015). The present study focuses on foetal placental insufficiency. In South Africa, several studies have been carried out to measure placental insufficiency using the Umbiflow™ Doppler ultrasound screening tool, which measures the UmA-RI and indicates placental function and risk of IUGR. The use of this device is still in the implementation phase in healthcare centres.

2.3 THE DETECTION, DIAGNOSIS AND DOWNSTREAM FOETAL CONSEQUENCES OF PLACENTAL INSUFFICIENCY

Normal placental development and function are critical for a successful pregnancy (Forbes and Westwood, 2010). Doppler ultrasound serves as a proxy measure of the functioning of the placenta, through the blood flow velocity, and therefore, by proxy, the capability to transport adequate oxygen and nutrients to the foetus. The Umbiflow™ is a low-cost hand-held continuous wave Doppler device that can detect compromised placental blood flow. Umbiflow™ is used with a standard personal computer and ultrasound transducer, analysing the flow velocity waveforms (FVWs) of the umbilical artery (measuring resistance in the umbilical artery); this device was developed by the South African Medical Research Council (SAMRC) and the Council for Scientific and Industrial Research (CSIR) (Hugo *et al.*, 2007; Theron *et al.*, 2005). Mueller *et al.* (2021) stated that the Umbiflow™ Doppler technology is presently on Conformité Européenne (CE) tests for accessibility in markets.

Research on the use of Doppler technology has been carried out in various studies in South Africa and in five LMICs (South Africa, Ghana, India, Kenya, and Rwanda) in a WHO-sponsored study, the Umbiflow International Study. The present study recruited mother-child pairs from the South African arm of the Umbiflow International Study. The device has been proven to be accurate, cost-effective and user-friendly, with the FVWs displayed on the screen of the computer and the calculated RI from the waveform plotted on the suitable percentile graph against the approximated GA (Hugo *et al.*, 2007; Theron *et al.*, 2005). The recent South

African study that evaluated the ability of continuous wave Doppler ultrasound of the umbilical artery to detect IUGR in low-risk pregnancies reported that increased RI can effectively detect IUGR (Feucht *et al.*, 2021). The Umbiflow™ calculates three indices of systolic and diastolic flow. These are the RI (calculated as systolic-diastolic/systolic (S-D/S)), pulsatility index (PI) (S-D)/mean velocity Vm), and systolic: diastolic ratio (SDR) (S/D) (Mufenda *et al.*, 2015). There are reference values according to GA in weeks for the measurement of umbilical artery RI, PI and SDR published by Pattinson and co-workers in the late 1980s, and recently, the international reference values were established in a longitudinal prospective cohort study of the INTERGROWTH-21st Project (Drukker *et al.*, 2020; Pattinson *et al.*, 1989). An RI less than the 75th percentile is considered low risk for compromised placenta function, while an RI ≥ 75th percentile is regarded as high risk for placental insufficiency (Theron *et al.*, 2005).

Previously, SGA infants were reported from pregnancies with an RI between the 75th and 95th percentiles, as well as over the 95th percentile (Hugo *et al.*, 2007). The increased RI (also known as an abnormal Doppler) has been associated with placental insufficiency (Theron *et al.*, 2002), as well as a higher percentage of caesarean sections compared to pregnancies with a normal Doppler (Hlongwane *et al.*, 2021; Mufenda *et al.*, 2015). For past decades, placental insufficiency has been regarded as the primary cause of IUGR (Mufenda *et al.*, 2015; Pardi *et al.*, 2002). The foetuses with IUGR are at risk of stillbirth, and hence, IUGR has been regarded as the leading cause of perinatal mortality and morbidity (Parra-Saavedra *et al.*, 2013). IUGR contributes up to 30% of cases of stillbirth and has been documented as a risk factor for preventable stillbirth (Audette and Kingdom, 2018). Evidence has shown that about one-quarter of stillbirths are either SGA, IUGR, or both (Hlongwane *et al.*, 2021; Lavin *et al.*, 2020; Lavin *et al.*, 2016). Lavin *et al.* (2016) and Nkosi *et al.* (2019) stated that most perinatal deaths in South Africa are unexplained stillbirths, and these are mainly (about two-thirds) macerated stillbirths.

The WHO defines stillbirth as a "baby born with no signs of life, weighing more than 1000 g or at more than 28 completed weeks of gestation" (WHO, 2014). Stillbirths can be categorised into macerated stillbirths due to antepartum death (Gold *et al.*, 2014) and fresh or intrapartum stillbirths, referring to death following the onset of labour (Lawn *et al.*, 2016). Other risk factors associated with stillbirths include congenital abnormalities, non-communicable diseases including diabetes and hypertensive disorders, older maternal age (>35 years), maternal infections such as syphilis, placental or foetal infections (Lawn *et al.*, 2016; Madhi *et al.*, 2019), lack of antenatal care, and maternal malnutrition (Anu *et al.*, 2019). The weakened

psychological health and well-being of women are also linked with stillbirths (Madhi *et al.*, 2019).

In the clinical management of pregnancies with suspected IUGR, the use of umbilical artery Doppler has great value and potential to reduce perinatal deaths by decreasing the prevalence of stillbirths in South Africa (Nkosi *et al.*, 2019). Research has shown that this technology can potentially reduce the perinatal mortality rate by at least 38% in the management of low-risk pregnancies (Alfirevic *et al.*, 2017; Hlongwane *et al.*, 2021). In 2019, the global (about 195 countries) rate of stillbirth was estimated at 13.9 stillbirths per 1000 total births, with SSA estimated at 21.7 stillbirths per 1000 total births (Hug *et al.*, 2021). Hug and colleagues further stated that the global annual reduction rate of stillbirth was estimated at 2.3% from 2000 to 2019. Saleem et al. (2018) pointed out that even though there are some declines in stillbirth rates, there is a low possibility of reaching the United Nations' Every New-born Action Plan goal of 12 stillbirths per 1000 births by 2030 if adequate attention is not given to managing the causes and risk factors of stillbirth. Incorporating the detection of placental insufficiency can ensure even more successful pregnancies and decrease the stillbirth rates.

2.3.1 Defining intrauterine growth restriction (IUGR)

Burton and Jauniaux (2018) defined IUGR as the pathological inhibition of intrauterine foetal growth and failure to reach its genetically determined growth potential. The pathological IUGR cannot simply be distinguished from SGA (described in Chapter 1) (Albu *et al.*, 2014). In some studies, LBW and/or SGA are regularly used as proxies for IUGR (Dos Reis *et al.*, 2015; Sania *et al.*, 2015; Schlaudecker *et al.*, 2017). The IUGR can be classified as 1) asymmetrical IUGR, referring to some foetal biometric measurements being significantly lower than others and below the 10th percentile. The commonly affected parameter is abdominal circumference; 2) symmetrical IUGR, involving all foetal biometric measurements, often falls below the 10th percentile for the GA, which is less than what would be expected. Reduced parameters include length and weight, and 3) mixed IUGR: babies having clinical features of both symmetrical and asymmetrical IUGR (Sharma *et al.*, 2016). The IUGR babies can be detected through an anthropometry index immediately after delivery (Chauhan and Magann, 2006; Lubchenco *et al.*, 1966). The symmetrical IUGR is characterised by weight, length and head circumference (HC) below the 10^{th} centile, while for asymmetrical IUGR, weight is below the 10^{th} centile and the length and HC are as per GA (Sharma *et al.*, 2016).

2.4 EPIDEMIOLOGY OF PLACENTAL INSUFFICIENCY AND IUGR GLOBALLY AND IN SOUTH AFRICA

Normal intrauterine growth requires a well-developed structure of the placenta, the umbilical vessels and the entire cord, with sufficient placental perfusion (da Rocha, 2020; Krzyżanowski *et al.*, 2019; Su, 2015). According to Pattinson *et al.* (2019), major causes of perinatal deaths are tied to placental insufficiency and HIV infection. Placental insufficiency can affect 10 to 15% of pregnancies (Wardinger and Ambati, 2021). Placental insufficiency accounts for about 60% of cases of IUGR in normally-formed foetuses (Gagnon, 2003). In developing countries, observations showed that 24% of new-borns, that is about 30 million, experience IUGR annually (Saleem *et al.*, 2011). A recent Ethiopian study by Tesfa *et al.* (2020) stated that IUGR can affect about 10 to 15% of all pregnant women and is seen in 23.8% of neonates, accounting for almost 30 million neonates being affected worldwide every year in the general population. The Nigerian study reported 26.0% and 50.0% of children who were born with a history of IUGR and LBW, respectively, in the general population of low-risk nulliparous women (Adefisan *et al.*, 2020). In Ethiopia, the prevalence of IUGR and SGA (IUGR was not used as a proxy of SGA) was 23.5% and 19.7%, respectively (Tesfa *et al.*, 2020). The evaluation of the utilisation of uterine artery Doppler indices in the second trimester for prediction of adverse pregnancy outcomes presented 60.8% of low-risk pregnancies with an abnormal RI (above 0.58) at 22 to 26 gestational weeks (Adefisan *et al.*, 2020). Adefisan and co-workers used uterine artery Doppler while most countries use the umbilical artery Doppler; hence, this may be the reason for the high percentage of abnormal RI.

A recent American study that assessed the progression rate of severe early-onset IUGR in singleton pregnancies at 28 weeks gestation reported 15.8% of pregnancies with an abnormal UmA-RI; however, this study defined abnormal UmA-RI as above the 95th percentile for GA (Martins *et al.*, 2021). In India, the percentage of abnormal UmA-RI was 19% in singleton pregnancies at 28 to 30 weeks gestation (Rajpoot *et al.*, 2020). The Umbiflow International Study reported 6.9% overall (495 of 7151 screened women) with an abnormal UmA-RI, of which 5.9% was observed in South Africa, 9.9% in Ghana, 8.3% in Rwanda, 5.6% in India and 4.6% in Kenya (Figure 2.3) (Vannevel *et al.*, 2022). The same study reported AEDF in 0.2% overall (14 of 7151, of which 10 (0.7%) of these women were observed in South Africa) (Vannevel *et al.*, 2022). Furthermore, still in South Africa, Hlongwane *et al.* (2021) reported a high prevalence of abnormal UmA-RI (13.0%) and AEDF (1.2%) among low-risk pregnancies across eight provinces; this prevalence is around ten times higher than the previous reports

from developed countries. Hlongwane and co-workers also recorded a higher prevalence of about 32.1% in SGA infants who had an abnormal UmA-RI compared to 23.1% of SGA infants with a normal UmA-RI. Another South African study by Nkosi *et al.* (2019) reported 11.7% of pregnancies with an abnormal UmA-RI in a single study site in Pretoria.

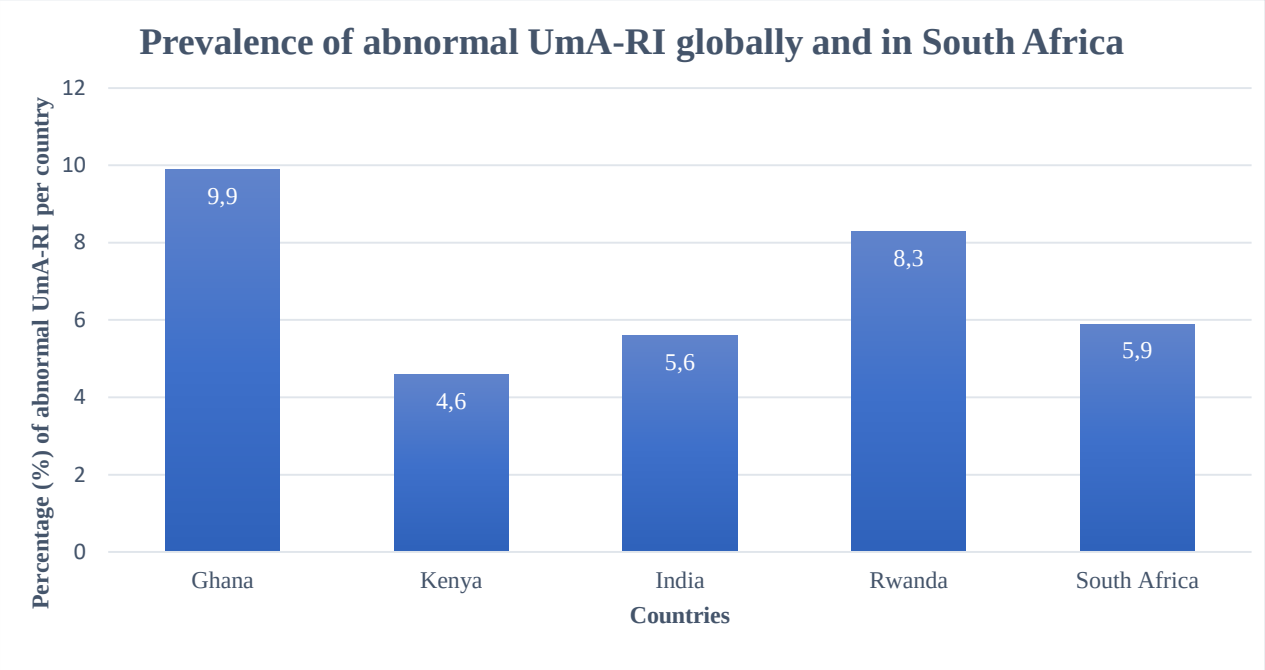

Figure 2.3: The prevalence of abnormal resistance index of the umbilical artery (UmA-RI) in five low- and middle-income countries in the Umbiflow International study.

Adapted from Vannevel *et al.* (2022)

2.5 MATERNAL HIV INFECTION AND CHILD HIV EXPOSURE

The eastern and southern African region is deeply affected by HIV, particularly adolescent girls and young women. In 2022, women accounted for 61.0% of all 20.8 million people living with HIV (PLHIV) in the region (UNAIDS, 2023). Women are at increased risk of HIV due to poverty and gender inequalities, which deny them economic opportunities and control over their sexual relations (UNAIDS, 2023). Around 1.4 million WLWH are estimated to become pregnant globally each year (Chandna *et al.*, 2020). South Africa is one of the southern African countries that is highly burdened with HIV infection, with women being more affected than men. In 2017, the South African National HIV Survey reported an HIV prevalence of 33.3% in women aged between 25 and 49 years and 26.3% in women aged 15 to 49 years (Simbayi *et al.*, 2019). In the same survey, black African women (16.6%) were found to be more affected than Coloured (5.3%), White (1.1%) or Indians (0.8%) per overall prevalence. Furthermore, South African statistics reported that more than 1 in 5 women of childbearing age live with

HIV infection (2019 mid-year population estimates) (South African Statistics, 2019). According to South Africa's National Strategic Plan for HIV, TB and STIs 2017-2022, HIV infection in pregnant women was estimated at 30%, with a 1.8% rate (as of 2014) of mother-to-child transmission at six weeks in South Africa (South African National AIDS Council, 2018). The WHO 2016 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection urges the provision of ART to all PLHIV (WHO, 2016). The UNAIDS global AIDS update of 2023 reported that in 2022, 82.0% of pregnant and breastfeeding WLWH were receiving ART globally, with high coverage in eastern and southern African countries (93.0%) (UNAIDS, 2023). Approximately 65.5% of South African WLWH were on ART in 2017, and the HIV viral load suppression (viral load below 1000 copies/mL of HIV ribonucleic acid (RNA), indicating successful ART) had been attained in 69.0% of women aged 25 to 44 years and in 75.0% of those aged 45 to 49 years (Simbayi *et al.*, 2019). Consequentially, a high prevalence of HIV serodiscordance among 415 mother-child pairs was documented, in which 90.2% of WLWH had children under 24 months with negative polymerase chain reaction (PCR) status (Simbayi *et al.*, 2019).

Globally, about 3.4 million HIV infections in children were prevented during pregnancy, birth and breastfeeding, with programmes for preventing HIV vertical transmission since 2000 (UNAIDS, 2023). Malawi and Botswana have remarkably succeeded in reducing HIV vertical transmission rates by 74.0% and 83.0%, respectively (UNAIDS, 2023). In South Africa, the PMTCT programme was reported to have reduced new HIV infections in children born to WLWH, evidenced by the low HIV prevalence in the children (Chandna *et al.*, 2020; Rossouw *et al.*, 2016; Simbayi *et al.*, 2019). Nonetheless, over 1.2 million new-borns are exposed to maternal HIV infection every year worldwide (UNAIDS, 2023). Globally, the population of CHEU is increasing. The global statistics for the population of CHEU was estimated at 14.8 million in 2018 and 15.4 million in 2020, an increase of 0.6 million (Slogrove, 2021; Slogrove *et al.*, 2020). About 90% (13.2 million) of these CHEU lived in SSA, and about 25% in Southern African countries (Slogrove, 2021; Slogrove *et al.*, 2020). Slogrove *et al.* (2020) reported that South Africa had the largest percentage of 23.8% (3.5 million) of CHEU compared to other SSA countries (21 countries) with a high HIV burden (Figure 2.4). In Southern Africa, South Africa was ranked number 3 among countries with the percentage of CHEU exceeding 15% of the general child population (Figure 2.5) (Slogrove *et al.*, 2020).

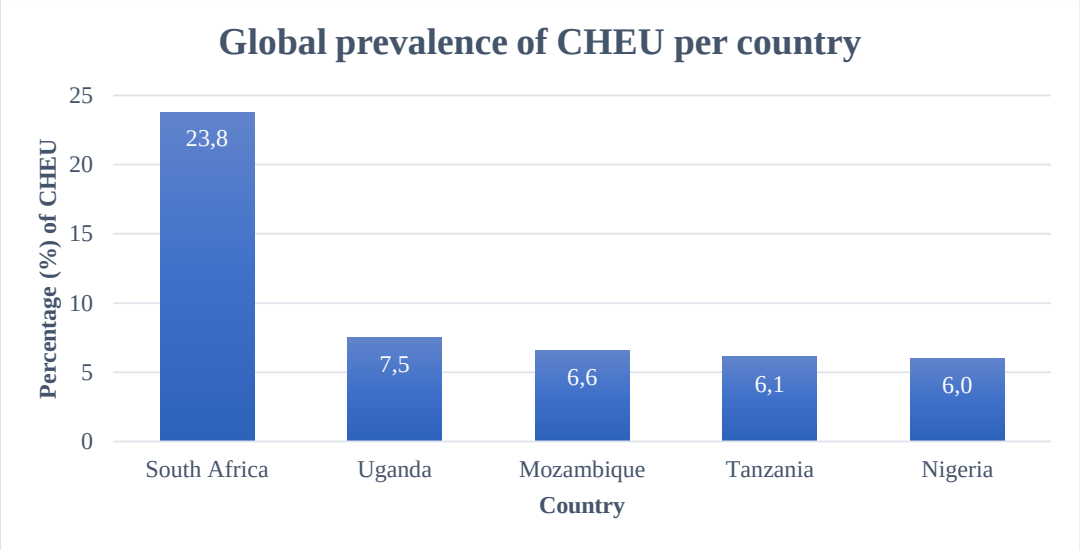

Figure 2.4: The 2018 global prevalence of CHEU per African country

Figure 2.4 above shows the five countries contributing to 50% of the 14.8 million CHEU population globally for 2018.

Adapted from Slogrove *et al.* (2020).

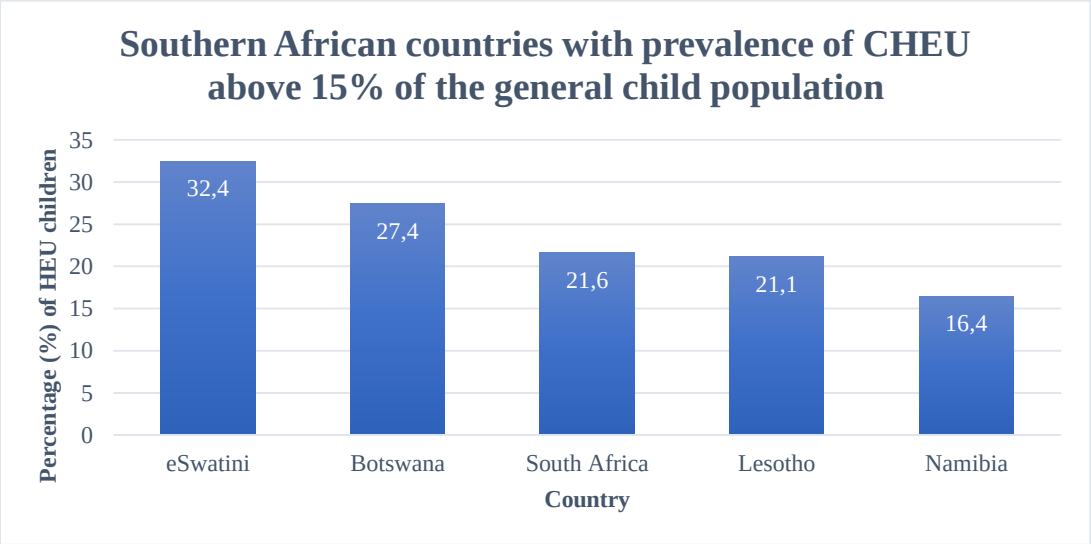

Figure 2.5: The five Southern African countries with a prevalence of CHEU beyond 15% of the entire child population (aged 0-14 years) for the year 2018

Adapted from Slogrove *et al.* (2020).

2.5.1 Maternal HIV exposure and pregnancy or birth outcomes

Maternal HIV infection during pregnancy negatively affects pregnancy outcomes, the development of the foetus and subsequently, the health of the child (Altfeld and Bunders, 2016). Children born to WLWH experience adverse exposures, which include pre-and post-

natal HIV and ART exposure; these are well-known and unique exposures to CHEU, as well as other common exposures that can potentially affect child growth and developmental outcomes undesirably (Figure 2.6) (Slogrove, 2021). Slogrove (2021) and Malaba *et al.* (2021) stressed that even in the era of ART, WLWH are still experiencing a doubled risk of adverse birth outcomes such as IUGR or SGA, preterm and stillbirth or pregnancy loss. Higher prevalence of preterm births (18.6% vs 8.0%) and neonatal deaths (3.6% vs 1.1%) have been reported in WLWH compared to their HIV-uninfected counterparts, respectively (Canlorbe *et al.*, 2015). In addition, neonatal anaemia, LBW, SGA and liver test abnormalities were reported to be associated with ART in CHEU (Delicio *et al.*, 2018). The consequences of maternal HIV infection associated with immune activation and ART, which lead to adverse pregnancy outcomes and the health of the CHEU, are summarised in Figure 2.7, adapted from Altfeld and Bunders (2016). The adverse birth outcomes may be consequential from the interplay between maternal HIV infection and placental insufficiency.

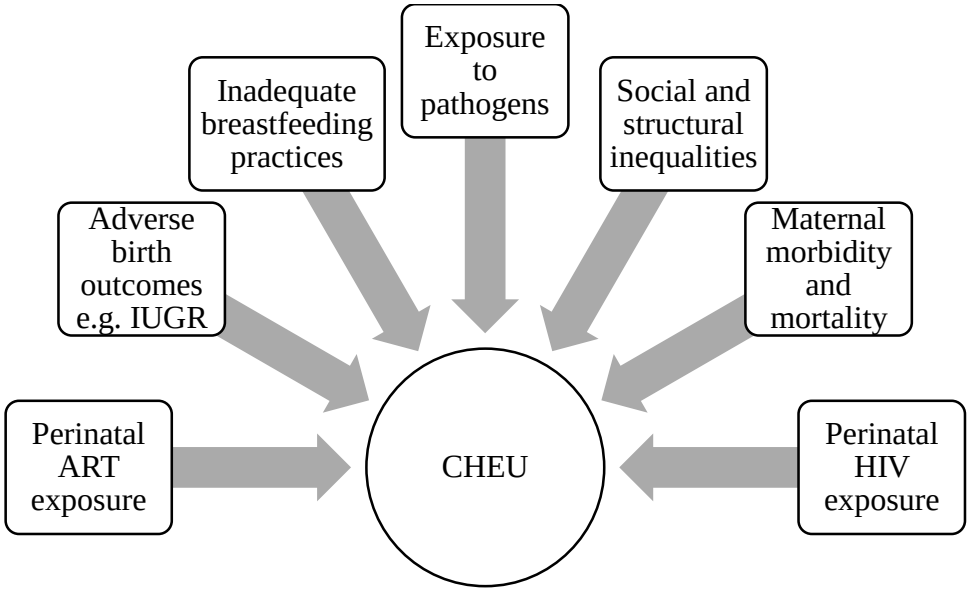

Figure 2.6: The unique and common risks for inadequate health, growth, and development in CHEU

Abbreviations: ART: antiretroviral therapy; CHEU: HIV-exposed-uninfected children; IUGR: intrauterine growth restriction;

Adapted from Slogrove (2021)

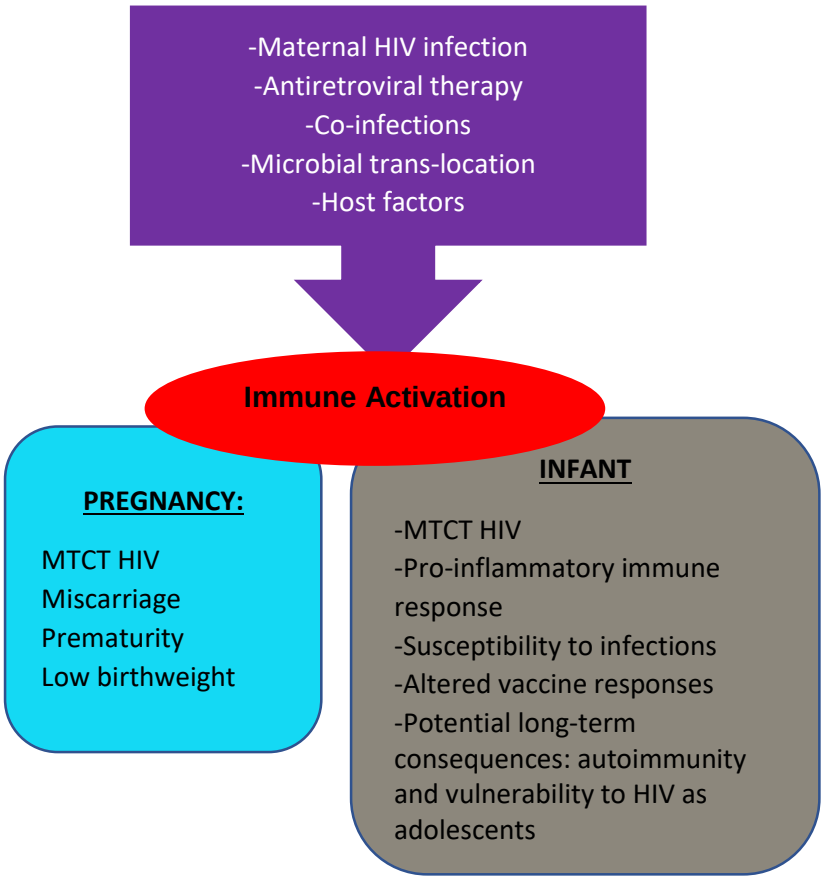

Figure 2.7: The consequences of maternal HIV infection-related-immune activation on pregnancy outcome and CHEU

Abbreviations: MTCT: mother-to-child transmission.

Adapted from Altfeld and Bunders (2016)

2.5.2 Maternal HIV infection and placental insufficiency

Maternal HIV infection stimulates inflammation and alters the maternal and foetal equilibrium (Altfeld and Bunders, 2016). It has been related to inflammation and dysregulation of placental vasculogenesis (the new formation of blood vessels) and angiogenesis (the branching and remodelling of existing vasculature), which ultimately alters placental function and results in poor birth outcomes such as IUGR (Conroy *et al.*, 2017; Weckman *et al.*, 2019; Yampolsky *et al.*, 2021). A Canadian study that explored the effects of HIV on placental morphology (alterations in placental shape, thickness, and/or weight) observed lower placenta weight (less than 350 grams) and reduced placental area in the WLWH compared to the HIV-uninfected counterparts (Sharma *et al.*, 2016; Yampolsky *et al.*, 2021). Yampolsky *et al.* (2021) concluded that maternal HIV infection and ART exposure modified placental morphology, resulting in higher rates of abnormal (velamentous or marginal) cord insertion (35% in WLWH vs 13% in

HIV uninfected mothers). Abnormal (velamentous) cord insertion has been associated with IUGR encompassing LBW and SGA (Räisänen *et al.*, 2012). Abnormal RIs on umbilical artery Doppler examination have been reported to be common among South African WLWH (33.2%) (Hlongwane *et al.*, 2021). A study that described Doppler findings in a cohort of HIV-infected pregnancies in Spain reported 66.7% of Doppler abnormalities well-matched with maternal and foetal placental insufficiency (Lopez *et al.*, 2015). In the same study, the foetal placental insufficiency was reported to be 50.0%. Another study from France reported a higher incidence of abnormal uterine artery Doppler waveforms of 16.8% among WLWH than their HIV uninfected counterparts (12.5%) (Canlorbe *et al.*, 2015). Nonetheless, an observation in France showed that pregnant WLWH experienced the same rate of vasculoplacental problems as women who were HIV-uninfected (Canlorbe *et al.*, 2015).

2.5.3 Prevalence of intrauterine growth restriction in HIV-exposed settings

Maternal HIV infection is reported to contribute to 5 to 10% of IUGR (Iqbal *et al.*, 2010; Schlaudecker *et al.*, 2017). Studies have reported HIV infection as one of the foetal risk factors for IUGR or SGA (Albu *et al.*, 2014; Cambrea *et al.*, 2013). Yampolsky *et al.* (2021) observed that small placental weight and area in WLWH was associated with SGA births, while none of the HIV-uninfected women had SGA babies. In India, IUGR was found to be more common in WLWH (9.9%) than in the HIV-uninfected counterparts (5.0%) (Dadhwal *et al.*, 2017). In their study that assessed the percentage of CHEU born with a history of IUGR in Romania, Cambrea *et al.* (2013) reported 22.8% of IUGR in CHEU, 11.4% of children with symmetrical IUGR and about 58.5% of SGA children. Lopez and co-workers (2015) also observed a high percentage (23.4%) of IUGR among WLWH in Spain. Conversely, a low rate of IUGR (4.4%) was documented in Brazil among WLWH between 2000 and 2015 (Delicio *et al.*, 2018). Canlorbe *et al.* (2015) reported similar percentages of IUGR (10.4% vs 7.7%) between WLWH and HIV-uninfected women, respectively, in France. In South Africa, two studies have shown that pregnant WLWH are at greater risk of IUGR than their HIV-uninfected counterparts (4.7% vs 0.0% and 1.0% vs 0.0%, respectively) (Allanson *et al.*, 2018; Bodkin *et al.*, 2006). Another South African study reported a higher risk of SGA (using birthweight-for-gestational-age, which were not necessarily IUGR) in WLWH than HIV-uninfected women (18.1% vs 15.1%; of which 16.6% the SGA children were reported to be severely growth restricted) (Ndirangu *et al.*, 2012). Furthermore, Ndirangu *et al.* (2012) added that there were higher odds for severe growth restriction in children born to WLWH than children born to HIV-uninfected women. The prevalence of IUGR in the studies mentioned above and countries is summarised in Figure

2.8. The above studies defined IUGR as a birthweight below the 10th percentile for GA. All mothers were receiving ART.

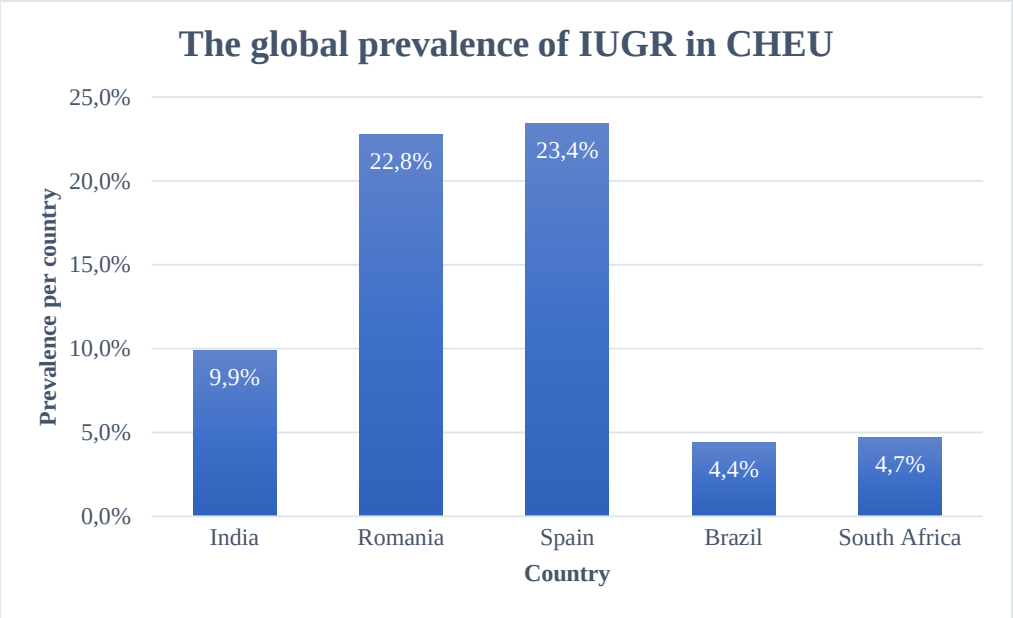

Figure 2.8: The global prevalence of intrauterine growth restriction in HIV-exposed and -uninfected children

2.6 POSTNATAL GROWTH AND LATER LIFE OUTCOMES OF CHILDREN WITH A HISTORY OF INTRAUTERINE GROWTH RESTRICTION

It is well documented that IUGR is associated with higher morbidity and mortality rates during perinatal and neonatal periods. Mortality rate and cases of perinatal complications have been reported to be 8.0% and 24.4%, respectively, in children with a history of IUGR (von Beckerath *et al.*, 2013).The IUGR is further associated with postnatal short- and long-term risks detrimental to a child's growth and health. Children with a history of IUGR are at higher risk of long-term health-related consequences such as the increased risk of developing obesity, diabetes mellitus type II, hypertension, and metabolic syndrome, because of changes in the foetal nutritional environment (Figure 2.9) (Salam *et al.*, 2014). Literature has shown that Americans children with a history of IUGR experience catch-up growth at a higher rate than children without a history of IUGR in the first 12 months (BMI: 3.58kg/m^2 vs 2.36 kg/m^2) (Crume *et al.*, 2014). Crume *et al.* (2014) added that at the adolescence stage, these children have higher waist circumference, higher insulin, and lower adiponectin levels. In addition, Beltrand *et al.* (2009) reported that catch-up growth in children with a history of IUGR promotes restoration of fat stores at 12 months of age. It has been documented that children born with a history of IUGR are likely to be stunted and at risk of developmental origins of

health and disease (Figure 2.9) (Sharma *et al.*, 2016). Salam *et al.* (2014) stated that IUGR leads to wasting and stunting, and estimations are that a fifth of stunted children have a history of IUGR in developing countries. A high percentage (21.2%) of delayed growth in children with a history of IUGR at age 12 months was reported in Austria by von Beckerath *et al.* (2013). In their study that focused on the effects of LBW and SGA (the assumption was that SGA was due to the pathological process of IUGR) on postnatal growth and nutritional status at 12 months, Blake *et al.* (2016) reported high rates of stunting, wasting and underweight in Filipino children born SGA/IUGR, as well as in children born LBW. Padilla *et al.* (2010) observed perpetual lower weight, length, and HC in intrauterine growth-restricted children at 12 months in Spain. Padilla *et al.* (2010) pointed out that children with a history of IUGR had lower catch-up growth than their counterparts without a history of IUGR; this finding is contrary to the findings of Crume *et al.* (2014) discussed in this section. In the South African longitudinal study that included a comparison of anthropometry measurements between infants with abnormal RI vs normal RI, Feucht *et al.* (2021) observed lower fat-free mass (FFM) and FFM z-scores (FFMZ) at 6, 10, 14 weeks and six months in abnormal RI than normal RI infants, as well as in SGA vs AGA children at age six months. Additionally, lower mean WAZ, LAZ, and HCZ were found in SGA than in AGA infants at ages six weeks and ten weeks. On the other hand, Mazarico *et al.* (2016) reported similar postnatal growth outcomes (anthropometry and body composition) at 12 months of age between children with and without a history of abnormal Doppler measurements. Feucht *et al.* (2021) reported similar FFM and FFMZ in SGA vs AGA infants at age ten weeks.

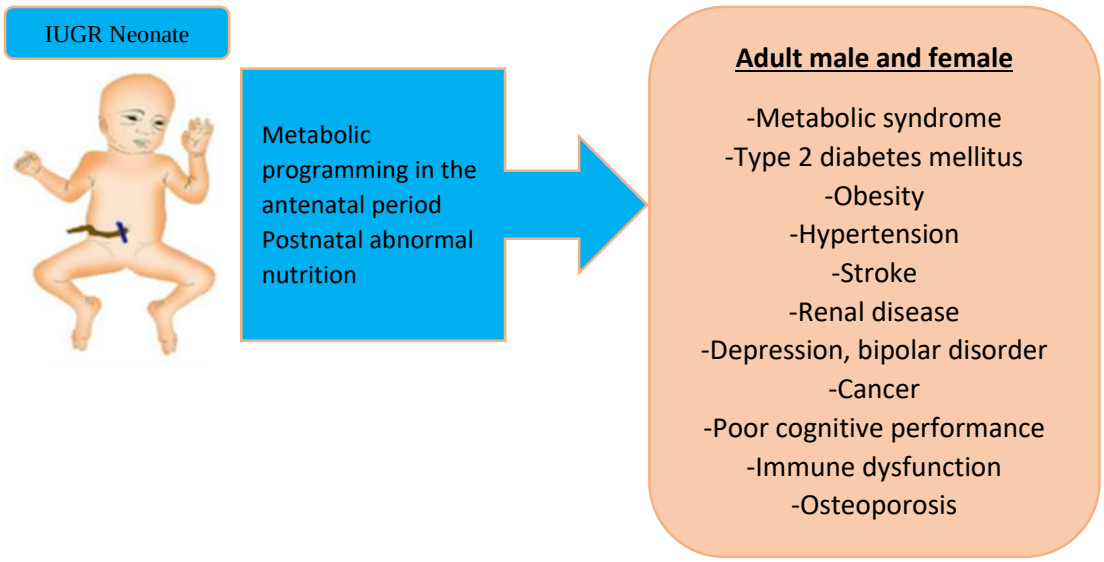

Figure 2.9: The long-term health implications that children born with IUGR may experience.

Adapted from Sharma *et al.* (2016)

2.7 POSTNATAL GROWTH OUTCOMES OF CHILDREN EXPOSED TO MATERNAL HIV INFECTION

Southern Africa is highly burdened with HIV infection, which is associated with the risk of suboptimal growth in CHEU (Fowler *et al.*, 2022). Several studies have reported poor growth outcomes among CHEU compared to their CHUU counterparts. A South African study that evaluated growth trajectories of normal breastfed children reported higher levels of stunting in CHEU than in CHUU at three months (9.0% vs 5.0%), six months (10.0% vs 6.0%), nine months (8.0% vs 2.0%) and 12 months (10.0% vs 4.0%) (le Roux *et al.*, 2019). The South African and Zambian studies that evaluated growth patterns of CHEU vs CHUU in these two countries also reported higher rates of stunting in CHEU (29.5%) than in CHUU (23.3%) at six months; the rates were higher in the South African than the Zambian cohort (Nyemba *et al.*, 2022). In their recent study that compared growth outcomes between CHEU and CHUU in Malawi and Uganda, Fowler *et al.* (2022) reported lower LAZ and WAZ among Ugandan CHEU at 12 and 24 months of age. Further, high stunting, underweight and wasting rates were also reported in Malawian CHEU and CHUU (Fowler *et al.*, 2022). A similar previous study by Aizire *et al.* (2020) reported lower LAZ, WAZ and HCZ at 24 months of age among Malawian and Ugandan CHEU compared to the CHUU. The stunting rate was high in Ugandan CHEU vs CHUU: 29.8% vs 13.3% at 12 months and 32.3% vs 18.2% at 24 months (Aizire *et al.*, 2020). Additionally, their longitudinal generalised estimating equations (GEE) model indicated that CHEU had a higher risk of stunting and HCZ compared to their CHUU control group (Aizire *et al.*, 2020). Another study in Uganda that determined the relationship between growth faltering and neurodevelopment in 170 CHEU from birth to 18 months of age reported 58.0% stunting and 15.0% underweight in CHEU at age 18 months (Sirajee *et al.*, 2021). A Nigerian study that compared the growth patterns of CHEU to CHUU reported higher percentages of stunting (28.4%) and wasting (7.8%) among the CHEU compared to the CHUU control group at birth (Jumare *et al.*, 2019). Jumare *et al.* (2019) further found that rates of stunting (44.3% vs 30.9%) and underweight (15.8% vs 6.2%) were higher in the first 18 months in the CHEU compared to the CHUU. The multivariable longitudinal analysis performed by Jumare *et al.* indicated that the odds of stunting and underweight were high in HIV-exposed settings. Further, a Kenyan study documented 20.0% stunting in CHEU vs 10.0% in the CHUU and lower HCZ in CHEU at age nine months (Neary *et al.*, 2021). Their multivariate analysis showed that maternal HIV exposure was highly associated with stunting at different time points and lower HCZ and microcephaly at nine months (Neary *et al.*, 2021). In Zimbabwe, Omoni

et al. (2017) observed that CHEU have poorer growth with higher odds of stunting and wasting than the CHUU counterparts in the first 12 months of life.

A pilot study in South Africa reported lower HC in CHEU compared to their CHUU counterparts at eight weeks but no significant differences in terms of length, weight and BMI between the groups (White *et al.*, 2019). Another South African study reported no significant growth differences between CHEU and CHUU, with a stunting rate of 6.7% in CHEU and 15.7% in CHUU and both stunting and underweight or wasting were observed in 3.4% and 1.7% of the CHEU population (Springer *et al.*, 2018). Figure 2.10 summarises the prevalence of stunting in four African countries: South Africa, Uganda, Nigeria, and Kenya, as discussed above.

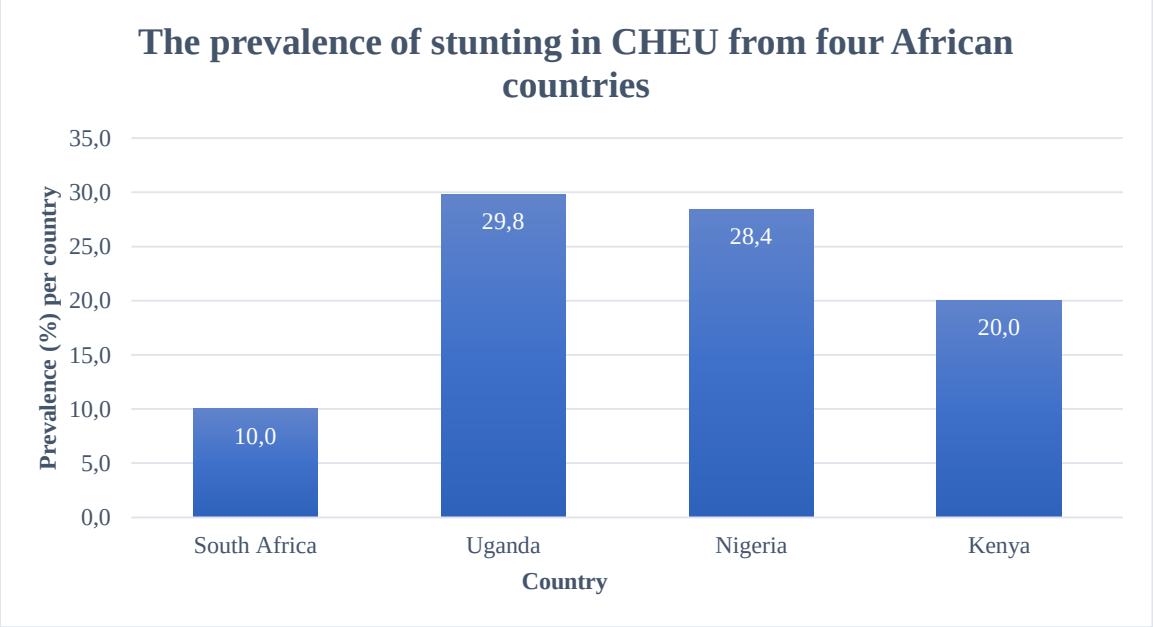

Figure 2.10: Stunting prevalence amongst HIV-exposed and –uninfected children aged under 24 months in four African nations.

Adapted from le Roux *et al.*, 2019; Aizire *et al.*, 2020; Jumare *et al.*, 2019; Neary *et al.*, 2021

The comparison between the four mentioned African countries shows that South Africa had the lowest prevalence of stunting in HIV settings in CHEU aged 12 months, while Uganda had the largest prevalence of stunting in CHEU at age 12 months. Numerous anthropometry measurements and their indices and z-scores are globally used in assessing the child's growth and development, which enable the determination of the prevalence of nutrition deficits and planning interventions. These are discussed in the next section.

2.8 ANTHROPOMETRIC INDICES AND Z-SCORES FOR ASSESSMENT OF GROWTH IN CHILDREN

Casadei and Kiel (2020) defined anthropometric measurements as a sequence of numerical body measurements, including bone, muscle, and adipose tissue, used to assess body growth and composition. The body measurements include weight, height, skinfold thickness and MUAC, and these anthropometric elements are used as indicators of nutritional status, more commonly in the form of indices: weight-for-age (WFA), weight-for-length (WFL), length-for-age (LFA), MUAC-for-age and body mass index-for-age (BMI-for-age) z-scores. These indices signify criteria for diagnosing stunting (<-2SD LFA), wasting (<-2SD WFL), underweight (<-2SD WFA), overweight (>+2SD WFA) and obesity (>+3SD WFA) in infants and young children (Casadei and Kiel, 2020; Sartorius *et al.*, 2020). Length is used in children from birth to two years of age as they cannot stand firmly, and BMI-for-age z-score (BAZ) is used for children two years of age and older (Gibson, 2005; Sartorius *et al.*, 2020). Child under- and over-nutrition are well defined using the WHO Child Growth Standards developed using data collected in the WHO Multicentre Growth Reference Study (MGRS) or WHO Anthro Survey Analyser to generate z-scores. The International Foetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) is a postnatal growth standard for term and preterm infants. The z-scores normalise the data for age and sex. The z-score cut-off point for overweight and obesity is WLZ ≥+2 for children under 24 months of age and a BAZ ≥+2 for children older than 24 months (Gibson, 2005; Sartorius *et al.*, 2020). Wasting is defined as a WLZ of <−2 for children under two years of age, thinness as BAZ of <−2 for children two years and older and stunting is described as an LAZ of <−2 (Sartorius *et al.*, 2020). HC is commonly used as an indicator of growth and by proxy for neurological development, in which HCZ less than –2 may indicate suboptimal brain growth and development.

Globally and in South Africa, anthropometric measurements and body composition are used to assess and monitor infants' and young children's growth and nutritional status. Koetaan *et al.* (2018) used WAZ, LAZ, and WLZ to assess the prevalence of underweight among children under the age of five years in the Free State Province of South Africa. The researchers pointed out that the indices LFA, WFL, HCZ and MUAC are critical anthropometric measurements that, when not documented in the Road to Health book (RTHB), result in undiagnosed child malnutrition (Koetaan *et al.*, 2018). In their study, Lesiapeto *et al.* (2010) utilised the anthropometric indices and the WHO Anthro software version 2® to determine risk factors of poor anthropometric status in children under five years in the Eastern Cape and KwaZulu-Natal

provinces. Another study by Maunder *et al.* (2015) generated height-for-age z-score (HAZ) scores and WAZ using the WHO Anthro software for 1 to 5-year-old children. Recently, anthropometric measurements and the WHO 2007 Child Growth Standards were used to determine stunting, wasting and obesity among children less than five years old (Sartorius *et al.*, 2020). A South African study by Nyati *et al.* (2019) investigated the prevalence of malnutrition and growth percentiles for urban South African children using measurements of the height, weight and z scores derived through WHO 2006 Child Growth Standards (0 - 5 years).

A study that evaluated the relationship between prenatal Doppler ultrasound measurements and postnatal anthropometric measurements in 12-month-old children with normal and abnormal Doppler made use of anthropometry and body composition measurements (Mazarico *et al.*, 2016). Beltrand *et al.* (2009) also used similar anthropometrical measurements, including BAZ, for investigating the postnatal growth of children born with a history of IUGR. Further, Blake *et al.* (2016) used LAZ, WLZ and WAZ to assess children's growth and nutritional status with SGA/IUGR. Lastly, HCZ, WLZ and LAZ were used in the assessment of growth and neurodevelopmental outcomes in children born with and without a history of IUGR (Padilla *et al.*, 2010), as well as in CHEU vs CHUU (Springer *et al.*, 2018; White *et al.*, 2019).

2.9 THE INFANT AND YOUNG CHILD FEEDING PRACTICES AND POSTNATAL GROWTH

According to the Convention on the Rights of the Child, every infant and child is entitled to the right to proper nutrition (WHO, 2021). Nonetheless, in many countries, children receive inadequate and unsafe complementary feeds and 45.0% of child deaths are associated with undernutrition globally (WHO, 2021). Optimal Infant and Young Child Feeding (IYCF) practices can potentially prevent and manage child malnutrition and reduce deaths of children under five years of age, consequently contributing to attaining SDG number 3 and its target of ending preventable deaths of children under five years by 2030. The WHO's 2016 recommendations on HIV and infant feeding advocate similar breastfeeding practices for WLWH, together with complete support for adherence to ART (WHO and UNICEF, 2016). Breastfeeding should begin within the first hour of life; infants should be exclusively breastfed for the first six months of life, then continued breastfeeding for two years or longer with the introduction of appropriate complementary foods at six months, and breastfeeding should be on demand (WHO and UNICEF, 2016). The optimum IYCF is attributed to the optimal growth and developmental outcomes. The present section covers IYCF and growth outcomes for

CHEU and CHUU, and children born with a history of IUGR, as well as the combination of the two groups. The South African study that evaluated the growth of CHEU vs CHUU who were fed an acidified starter formula in the first six months reported similar anthropometric measurements that demonstrated optimal growth rates of CHEU and CHUU (Cooper *et al.*, 2010). Another South African study by le Roux *et al.* (2019) reported low (45.0%) rates of EBF among CHEU and were also reported to have higher rates of stunting and underweight than their CHUU counterparts. In Uganda, a low rate of breastfeeding and a high rate of stunting and underweight were documented in CHEU at different time points until five years of age (Fowler *et al.*, 2022). The inadequate breastfeeding rates and poor growth outcomes with a high rate of stunting have been documented by Aizire *et al.* (2020). Poorer breastfeeding practices in CHEU compared with CHUU (1.0% vs 16.1%) have been documented in Nigeria, with CHEU having severely impaired linear and ponderal growth than CHUU at age 18 months (Jumare *et al.*, 2019). A study in Kenya observed lower rates of breastfeeding and poorer growth in CHEU than the CHUU controls, and suboptimal breastfeeding was associated with being underweight (Neary *et al.*, 2021).

In the Philippines, higher rates of EBF have been reported in non-SGA children (93.5%) compared to SGA children (86.6%); findings were similar for continued breastfeeding up to 12 months, and increased cases of stunting and underweight were observed in SGA children (Blake *et al.*, 2016). The assumption was that low maternal educational attainment was the contributing factor to low rates of EFB. In the same study, the univariable generalised estimating equations (GEE) analyses indicated that the odds of stunting during infancy were increased when infants were born SGA and fed with a bottle or were mixed-fed but decreased when infants were exclusively breastfed. Additionally, the multivariable GEE analyses showed a strong correlation between the odds of stunting and the type of feeding, as reported by Blake *et al.* (2016).

These findings indicate that there is an association between breastfeeding practices and postnatal growth of CHEU and children born with a history of IUGR/SGA. The breastfeeding practices in HIV settings seem to be inadequate; the next section highlights the breastfeeding statistics.

2.9.1 Prevalence of breastfeeding practices including in HIV settings

The benefits of breastfeeding to the mother and the child despite HIV infection are well documented in the literature (Binns *et al.*, 2016; Kornides and Kitsantas, 2013; Whitney and

Rolfes, 2015). Having to balance the risk of maternal HIV vertical transmission against the well-known and documented benefits of breastfeeding has made the ideal choice of feeding for CHEU complex in developing countries (Cooper *et al.*, 2010). Globally, the rate of EBF is estimated at 44.0% for infants 0 - 6 months of age over the period of 2015 to 2020 (WHO, 2021). The WHO has set goals for at least 50.0% and 70.0% of infants to be exclusively breastfed by 2025 and 2030, respectively (Masereka *et al.*, 2022; WHO and UNICEF, 2018). South Africa is also committed to this goal, as evidenced by government strategies supporting and promoting breastfeeding; these include the revised 2007 Baby Friendly Hospital Initiative (BFHI) or Mother Baby Friendly Initiative and the 2011 Tshwane declaration of support for breastfeeding by the Minister of Health.

The early initiation of breastfeeding has been documented to be high (75.0% to 100.0%) in South Africa since 2012 (Budree *et al.*, 2017; Doherty *et al.*, 2012; Goosen *et al.*, 2014; Siziba *et al.*, 2015). A South African study reported lower breastfeeding initiation (42.0% vs 97.0%) in WLWH than their HIV-uninfected counterparts (Doherty *et al.*, 2012). The low percentage of EBF has been reported globally and in South Africa. In the United States of America, the percentage of EBF at six months is reported to be 25.0%, and in the United Kingdom, it is below 1.0%, while 37.0% of infants aged below six months are being exclusively breastfed in SSA (Masereka *et al.*, 2022). In Uganda, the rate of EBF has been reported to be 36.0% (Nabunya *et al.*, 2020). A high percentage (91.0%) of continued breastfeeding at 12 months has been reported in Malawian CHEU, and 64.0% was noted in Uganda among CHEU (Fowler *et al.*, 2022). Lower rates of continued breastfeeding among CHEU than CHUU (49.0% vs 86.2%) were reported at age 12 months by Aizire *et al.* (2020) in their Malawian and Ugandan study. High breastfeeding rates in HIV settings were reported in Nigeria from birth up to 12 months, with EBF estimated at 80.3% in the first six months of life, although there was a decline to 1.0% at 18 months (Jumare *et al.*, 2019). Suboptimal rates of breastfeeding among CHEU (72.0%) vs CHUU groups (98.0%) were also documented in Kenya (Neary *et al.*, 2021). Chaudhury *et al.* (2017) observed that 99.5% of CHUU and 9.0% of CHEU were ever breastfed by the age of 24 months in Botswana.

In South Africa, the percentage of any EBF was estimated at 32.0%, which is considered superior to earlier statistics (Sayed and Schönfeldt, 2020; Shisana *et al.*, 2013). Nyemba *et al.* (2022) reported lower rates of EBF (32.0% vs 45.0%) and higher rates of formula feeding only (62.0% vs 40.0%) in the CHEU vs CHUU group in a combined study in South Africa and Zambia. Budree *et al.* (2017) reported that continued breastfeeding from 6 to 24 months is

estimated at 14.4% in South Africa. A South African study by le Roux *et al.* (2019) presented comprehensive findings on feeding practices in CHEU vs CHUU. The study reported higher rates of breastfeeding practices in CHEU than in CHUU: 54.0% vs 15.0% of EBF at six months; 93.0% vs 83.0% of ever exclusively breastfed, and the duration of EBF was a maximum of 6 months vs three months, respectively. Nonetheless, the predominant breastfeeding (9.0% vs 22.0%) and duration of any breastfeeding (duration: 6 months vs 11 months; rates: 41.0% vs 51.0% at 12 months) were lower in the CHEU vs CHUU. Lastly, partial breastfeeding and early weaning were also common in the CHEU. The suboptimal breastfeeding practices may translate into nutrition starvation and risk of malnutrition. The 2016 South African Demographic and Health Survey reported a high percentage (32.3%) of stunting in infants under the age of 6 months (National Department of Health (NDoH) *et al.*, 2018) and this finding may be due to the low rates of EBF in South Africa.

2.9.2 Complementary feeding practices of infants and young children

Complementary feeding involves introducing liquids other than breastmilk and appropriate and nourishing soft, semi-solid and solid foods at age six months, when the infant's gut has fully developed to handle foods additional to breastmilk. The WHO 2023 guideline for complementary feeding of infants and young children 6 - 23 months old recommends continued breastfeeding to 2 years or beyond, intake of milk formula or animal milk for non-breastfed infants and young children, consumption of diverse diets and avoidance of unhealthy foods and beverages, as well as nutrient supplements and fortified food products in settings where nutrient requirements are not met (WHO, 2023b). WHO recommends introducing complementary foods at six months, as the breastmilk alone is not enough anymore to supply adequate nutrition to the infants. Consumption of diverse diets includes daily intake of animal-source foods, fruits and vegetables and pulses. Infants and young children should not be given foods high in salt, sugar and trans fats. Child nutrition is a priority in South Africa, and the country has adopted the WHO recommendations on IYCF practices. Additionally, the South African government's strategies to improve infant and young child nutrition are in place and include the revised 2013 IYCF policy, the 2012 Regulations relating to Foodstuffs for Infants and Young Children and the 2012 Roadmap for Nutrition. This is a great step towards achieving improved child growth and development, as well as the SDG number 3 and the goal number 2 target of ending all forms of malnutrition by 2030. Nonetheless, Sayed and Schönfeldt (2020) pointed out that the necessity of complementary feeding has not received enough attention; this was also documented at the global level more than a decade ago by Piwoz *et al.* (2003). These

researchers stressed that efforts to promote and advocate for optimal complementary feeding have been insufficient as attention is given to the promotion and support of breastfeeding.

As children transition from breastmilk to a complementary diet, they are at a greater risk of malnutrition, including stunting, which necessitates promoting and advocating for appropriate complementary feeding practices (Black *et al.*, 2008). The inappropriate complementary feeding practices may include early or late introduction of foods and low nutrient-dense diets. Children introduced to complementary foods late in life are more likely to become obese as adults (Sayed and Schönfeldt, 2020). Also, in HIV settings, early introduction of complementary diets mixed with breastfeeding may increase the risk of maternal vertical transmission of HIV. In South Africa, it is usual to introduce foods or drinks other than breastmilk or other milk at an early age. The evidence from different studies showed that in the first month, 2.6% of infants stopped breastfeeding completely (Patil *et al.*, 2015), about 17.0% of infants were introduced to food (Siziba *et al.*, 2015) and CHEU were introduced to water (91.0%) and mabelle or maize meal soft porridge (83.7%) from as early as 16 weeks (Tshiambara *et al.*, 2023). A high percentage of infants (84.6%) are introduced to foodstuffs before age six months (Seonandan and McKerrow, 2016; Tshiambara *et al.*, 2023). The common food items introduced to South African children are maize meal porridge, commercial infant cereal, water, gripe water, rooibos tea, soft drinks and sugar water (Budree *et al.*, 2017; Faber *et al.*, 2016; Patil *et al.*, 2015; Tshiambara *et al.*, 2023). In their review, Sayed and Schönfeldt (2020) concluded that the diets of South African children are lacking in variety and do not match the standards for a minimally acceptable diet. These may be the leading cause of high (27.0%) rates of stunting for children under five years (National Department of Health (NDoH) *et al.*, 2018). Inadequate nutrition causes not only stunted growth but also neurodevelopmental impairments due to nutrient deficiencies.

2.10 CHILD NEURODEVELOPMENT

Neurodevelopment is defined as the development of the neurological pathways of the brain, which sway functioning or performance, including social skills, attention or focus skills, ability to read and intellectual functioning (Tognini, 2017). Tognini (2017) describes brain development as a complex process that starts in the early weeks of pregnancy. The first 1000 days, referred to as the critical period for neurodevelopment, offer the windows of opportunity for remodelling neural circuits based on response to experience (Tognini, 2017). Brain development and function involve learning, communication, relating to people, vision and

hearing, and the use of hands and fingers. These are also known as 1) motor development and skills, which relate to the development of muscles and bones of the child and the skills to move and coordinate the muscles and manipulate the environment. Motor development is divided into fine, referring to movements of small muscles such as hands and fingers (e.g. skills of holding a pencil), and gross motor development relating to movements of large muscles such as arms, trunk, and legs (e.g. kicking, throwing, walking and standing alone) (Wijnhoven *et al.*, 2004); 2) cognitive development involving memory, reasoning, intelligence, information processing, and sustained attention; 3) communication or language development involving sounds, gestures, words, and sentences. Language development is divided into expressive (involving asking questions and for objects, answering, and describing events) and receptive language (understanding spoken words), and 4) relating to responsive behaviour. Child neurodevelopment progresses throughout growth and development, and at each stage or age, there are developmental milestones that the child must perform. The inability to perform age-related activities may be an indicator of delayed neurodevelopment.

The literature describes IUGR, GA, HC, and abnormal Doppler measurements as the impartial contributing factors of child neurodevelopment (Baschat, 2011). Therefore, the neurodevelopment of children with aforesaid prenatal medical conditions or abnormalities should be prioritised. It is of great importance to detect neurodevelopmental deficits earlier in life, and there are validated methods for assessment and monitoring of early child neurodevelopment.

2.10.1 Neurodevelopment of children with a history of normal or abnormal Doppler and intrauterine growth restriction

Intrauterine growth restriction is an imperative risk factor for impaired child neurodevelopment (Baschat, 2011). It is mentioned that intrauterine growth-restricted children are predisposed to major and subtle neurodevelopmental limitations (Sharma *et al.*, 2016). According to Miller *et al.* (2016), IUGR leads to foetal hypoxia and subsequently brain sparing (Figure 2.11) and is associated with decreased volume of total brain and cerebral cortex, along with altered brain structure and function (Figure 2.12). Further, Miller and colleagues stated that the timing of the onset and magnitude of IUGR, as well as the GA at birth, determine the neurodevelopmental outcomes.

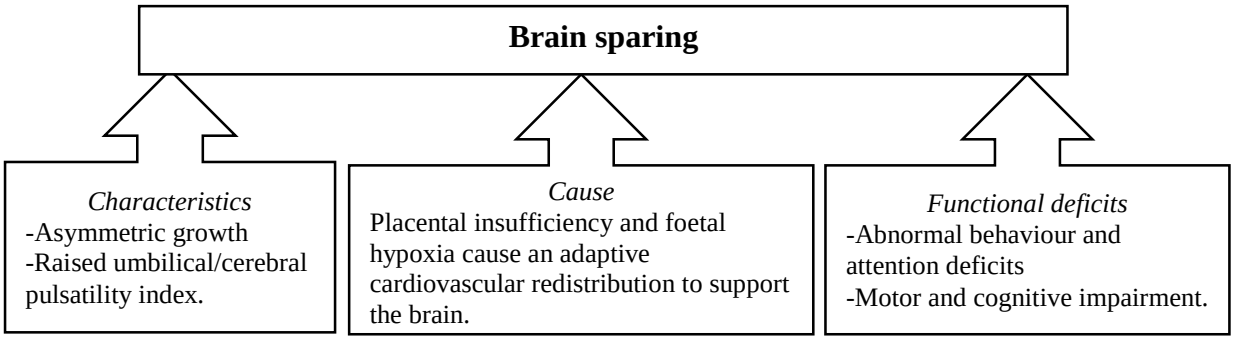

Figure 2.11: The characteristics, cause and functional deficits of brain sparing in children

Adapted from Miller *et al.* (2016)

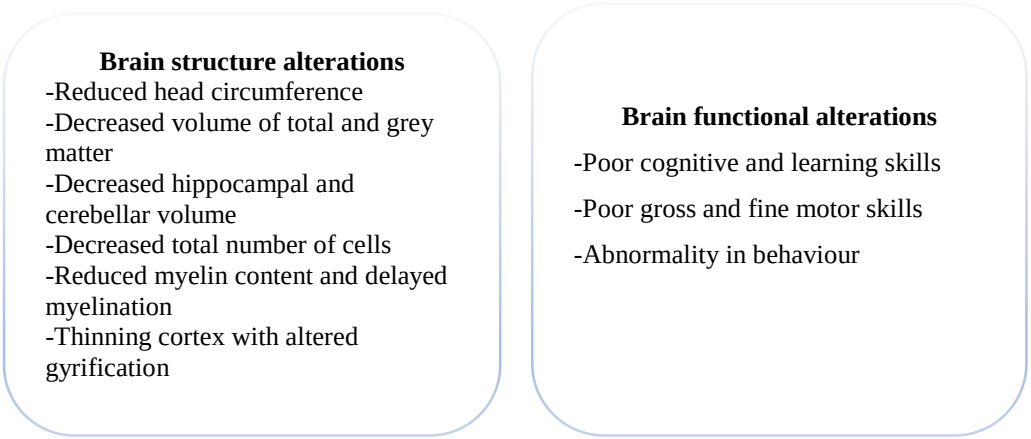

Figure 2.12: The common alterations in brain structure and function of intrauterine growth restricted children

Adapted from Miller *et al.* (2016)

The evidence based on the systematic review of early childhood neurodevelopment following IUGR indicated that children born with a history of IUGR were at risk for poorer neurodevelopmental outcomes from 6 months to 3 years of age (Levine *et al.*, 2015). Another systematic review by Murray *et al.* (2015) showed that intrauterine growth-restricted children had lower neurodevelopmental assessment scores than their non-IUGR counterparts between 1 month to 12 years of age across all neurodevelopmental domains; there was an indication that IUGR intensifies the risk of delayed development. The evidence for impaired neurodevelopment following placental insufficiency demonstrated abnormal motor developmental outcomes in children with a history of IUGR aged 24 months (Baschat, 2011; Bekedam *et al.*, 1985). Additionally, the literature showed that children who had an abnormal Doppler were known to have lower scores for attention capacity, motor skills and social interaction (Cruz-Martinez *et al.*, 2009). Children with a history of IUGR and born prematurely

are mostly affected by an abnormal delay in neurological and motor development (Baschat, 2011). Conversely, Padilla *et al.* (2010) assessed and compared neurodevelopmental outcomes among preterm children aged 12 months with and without a history of severe IUGR and reported no significant difference between these groups. As mentioned above, the determinants of neurological and developmental impairment in intrauterine growth-restricted children include body weight, HC, GA at birth and waveforms of the abnormal umbilical and middle cerebral artery blood flow (Baschat, 2011; von Beckerath *et al.*, 2013). One study observed that postpartum HC growth is a predictor of greater gross motor skills in male children aged 24 months but not for fine motor and cognitive scores (Dupont *et al.*, 2018). The cephalisation index (the ratio of the HC/brain to body weight) was reported to influence brain development. The high cephalisation index was reported to be common among children with a history of severe IUGR, and it was associated with brain vulnerability and severe psychomotor delay (Harel *et al.*, 1985).

2.10.2 Postpartum influence of maternal HIV infection on early childhood neurodevelopment

The findings of the South African study by Wedderburn *et al.* (2022) indicated the likelihood of negative effects of maternal HIV exposure on early structural brain development of CHEU. In the same study, small volumes of total grey matter and caudate were reported in CHEU compared to their CHUU counterparts (Figure 2.13) (Wedderburn *et al.*, 2022). Maternal HIV exposure puts children at risk of biological factors that ultimately affect their mental development (Sherr *et al.*, 2014). A South African study reported low neurobehavioral functioning among HIV-exposed new-borns (Rencken *et al.*, 2022). Further, South African CHEU and ART-exposed children were found to have high odds of receptive and expressive language delays at 24 months of age (Wedderburn *et al.*, 2019). Lower child developmental and vocabulary scores were reported in Zimbabwean CHEU compared to their CHUU counterparts at age 24 months who reside in rural areas (Ntozini *et al.*, 2020). A Kenyan study reported poorer long-term neurocognitive outcomes in CHEU than in CHUU (Benki-Nugent *et al.*, 2022). In Canada, Young *et al.* (2022) found that CHEU had lower neurodevelopmental scores than CHUU. In addition, CHEU exposed to ART were also found to have suboptimal cognitive and motor development compared with their ART-unexposed peers (McHenry *et al.*, 2018). Association between *in utero* ART exposure during pregnancy and higher risk of neurologic abnormalities in CHEU has been documented in the literature (Crowell *et al.*, 2020).

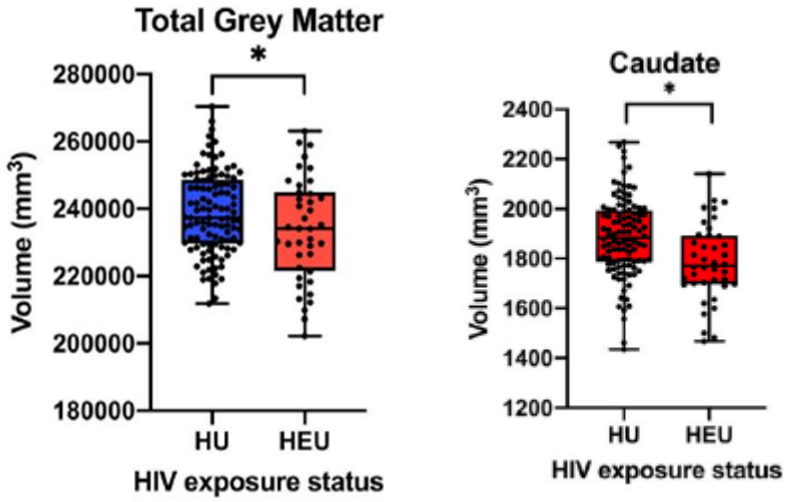

Figure 2.13: Comparison of total grey matter and caudate volumes between HIV-exposed-uninfected and HIV-unexposed-uninfected children in a South African birth cohort

Abbreviations: HEU: HIV-exposed and -uninfected; HU: HIV-unexposed

Source: Wedderburn *et al.* (2022)

One meta-analysis reported that CHEU and HIV-infected children had poorer neurodevelopmental outcomes, mainly cognitive and motor development, compared to their CHUU counterparts (McHenry *et al.*, 2018). A study on the assessment of cognitive, motor, and language in Indonesian HIV-infected children aged 0-36 months reported lower scores than the CHUU group (Apsari *et al.*, 2022). Budiapsari and Supadma (2022) showed that HIV-infected children's performance in cognitive, motor, and language domains is determined by their CD4 level, age, ART duration and household socioeconomic status. Other studies have indicated that HIV-infected children face a double rate of neurodevelopmental delays compared to their HIV-uninfected counterparts (Kandawasvika *et al.*, 2011; McHenry *et al.*, 2018).

Nevertheless, studies have reported similar neurodevelopmental outcomes between CHEU and CHUU. In Zimbabwe and Zambia, Kandawasvika *et al.* (2011) and Ngoma *et al.* (2014) observed no differences in motor, cognitive and language development between CHEU and CHUU at ages 12 and 15 to 36 months, respectively. A South African study reported that cognitive, motor and language development did not differ between CHEU and CHUU at 12 months; however, there were minor differences in vocalisation and language development, which decreased in most CHEU (Springer *et al.*, 2018). Springer and co-workers concluded that assessment of CHEU should be done routinely including at 18 months. Another South

African study by White *et al.* (2019) reported no differences between CHEU and CHUU at three months regarding expressive and receptive language, gross and fine motor skills, relating and response behaviour, and play activities. Many factors, other than HIV exposure, contribute to delayed child development and these include poor feeding practices and inadequate intakes of micronutrients.

2.10.3 Prevalence of neurodevelopment deficits in HIV-exposed children who had a history of intrauterine growth restriction

It has been established in the literature that pre- and postpartum HIV exposure and IUGR are independent risk factors for poor neurodevelopmental outcomes in early childhood and later life. The Canadian study that compared neurodevelopmental outcomes reported a higher (30.0%) prevalence of developmental delays in CHEU than CHUU group (13.4%) (Piske *et al.*, 2018). Severe delays in motor (14.3% and 28.6%) and cognitive development (40.0% and 60.0%) were observed in 18 to 72 months old CHEU and HIV-infected children in the Democratic Republic of Congo, respectively (Van Rie *et al.*, 2008). A Botswanan study found similar poor neurodevelopmental outcomes among 24-month-old CHEU vs CHUU, using the Bayley Scale for Infant and Toddler Development (BSITD-III) (Chaudhury *et al.*, 2017). In South Africa, the study that used BSITD-III to assess and compare developmental outcomes among Cape Town CHEU vs CHUU at age 12 months found that a higher percentage of CHEU had poorer language outcomes (28.0% vs 18.0%) than their counterparts, nonetheless, both groups had similar percentages with a similar poor motor outcome (6.9% vs 5.2%) (Springer *et al.*, 2018). The same study showed that more of CHEU had macrocephaly than CHUU (13.8% vs 5.5%) (Springer *et al.*, 2018). Nonetheless, the follow-up cohort study at ages 2 to 3 years by Springer et al. (2020) found no significant differences in language, motor and cognitive developmental domains between the CHEU and CHUU. Le Roux *et al.* (2018) observed delayed motor (9.0% vs 5.0%) and cognitive (10.0% vs 5.0%) development domains according to BSITD-III, among South African CHEU compared with CHUU aged 12 to 18 months, respectively. Additionally, delayed neurodevelopment in domains of socialisation (19.5%), language (9.8%) and motor skills (7.3%) were reported in South African CHEU with low-income backgrounds (De Beer *et al.*, 2020).

A study that investigated the associations between foetal Doppler parameters and neurodevelopmental delay reported that 52.8% of children with a history of IUGR had neurodevelopmental delay at two years of age (Baschat *et al.*, 2009). Von Beckerath *et al.* (2013) reported higher percentages (24.7%) of neurological impairment among children with

a history of IUGR; about 13.7% had a delay in psychomotor, 11.6% had delayed cognitive development, 15.1% and 8.9% had a delay in language and impaired vision, respectively. Lastly, the study that evaluated the impact of IUGR on early neurodevelopmental outcomes of 18 to 24-month-old children stated that 35.4% and 13.4% of children born with a history of IUGR had moderate and severe neurodevelopmental delays, respectively, in Saudi Arabia (Al-Qashar *et al.*, 2018).

2.10.4 Importance of early assessment of child neurodevelopment and assessing techniques

It is well noted that about 43% of children do not develop to their full potential globally (Chandna *et al.*, 2020). Early neurodevelopmental assessments, which diagnose developmental downsides earlier, can lead towards correction thereof through neurological physiotherapy-related interventions to ensure optimal development of the child. Late detection of neurodevelopmental impairment may result in permanent damage since some delays may be irreversible after the critical window period of neurodevelopment. This critical window period involves a rapid increase or growth of brain volume, which reaches adult size at about 24 months postpartum (Dupont *et al.*, 2018; Sacco *et al.*, 2015). Therefore, the assumption is that the external stimulus can promote brain development to achieve the desired child developmental outcomes. Padilla *et al.* (2010) stated that neurodevelopmental impairment in children with a history of IUGR, mainly those born premature, must be detected earlier in their lives to ascertain early interventions. In LMICs, early diagnosis of neurodevelopmental setbacks and appropriate intervention results in improved developmental outcomes in all young children, including those exposed to maternal HIV infection (Engle *et al.*, 2011).

The Bayley Scale for Infant Development second edition (BSID-II), BSITD-III and Griffiths scale and International Guide for Monitoring Child Development (GMCD) (screening tool) are tools commonly used for child neurological and developmental examinations (Bayley, 1993; Bayley, 2006a; Gortner *et al.*, 2003; Padilla *et al.*, 2010; White *et al.*, 2019). There are three editions of the Bayley test, namely BSID-I, BSID-II (Bayley, 1993) and BSITD-III (commonly known as Bayley-III) (Bayley, 2006b) editions and each edition is the revision of the previous version. The revision of BSITD-III included developing five specific tests: cognitive, language, motor, social-emotional and adaptive behaviour tests, while BSID-II only included three tests, viz. mental, motor and behaviour tests (Albers and Grieve, 2007; Bayley, 2006b). These tests have been positively used in many studies that have assessed and monitored infant development. Padilla *et al.* (2010) and von Beckerath *et al.* (2013) utilised BSID-II in their

study that assessed the infants' cognitive and psychomotor development and the Hammersmith Infant Neurological Examination (HINE) for neurological examination. It was noted that the BSID-II, Griffiths scale and HINE tests could not demonstrate the differences in neurodevelopmental outcomes in children with a history of IUGR (Padilla *et al.*, 2010). Dupont *et al.* (2018) employed BSITD-III in their study to evaluate infant cognitive and fine and gross motor development at 24 months of age in Canada. A South African study also used Bayley-III to assess and compare the cognitive, motor and language development of CHEU vs CHUU aged 12 months (Springer *et al.*, 2018). The literature underscores that cognitive testing skills of young children with Bayley-III were difficult as infants show a wide-ranging variability of behaviours; therefore, the tests are likely to give different results (Dupont *et al.*, 2018). Further, Reuner *et al.* (2013) noted that Bayley-III overestimated motor performance scores, particularly in young children. Bayley-III can be used together with GMCD, a novel method for monitoring and early detection of neurodevelopmental delays (Ertem *et al.*, 2008). The GMCD has been used in a South African pilot study amongst CHEU vs CHUU by White *et al.* (2019).

2.10.5 Correlations between child feeding practices, nutrient intakes and neurodevelopment

The human brain's structural and functional development is largely supported by nutrition; hence, in the first two years of life, a period of rapid growth and development, the brain is particularly susceptible to inadequate nutrition (Anjos *et al.*, 2013). Proper and appropriate feeding practices may ensure adequate child nutrition and, consequently, desired neurodevelopmental outcomes. The effects of infant feeding practices on child neurodevelopment have been scarcely documented in the literature. Eidelman (2013) showed that human breastmilk has a positive effect on child neurodevelopment probably due to its composition which includes thyroxine hormones and fat, mainly cholesterol (myelin), which are essential for neurodevelopment. Chandna *et al.* (2020) recently documented that an improved IYCF program was associated with better motor, language, and cognitive developmental outcomes when coupled with improved water, sanitation, and hygiene (WASH) among Zimbabwean CHEU aged 24 months. However, it was indicated that there was no evidence of the impacts of IYCF alone (Chandna *et al.*, 2020). Further, Rochat *et al.* (2016) reported that EBF and its duration were not associated with cognitive development in South African children aged 7 to 11 years. A systematic review by Mohammed *et al.* (2022) showed that there was no association between breastfeeding and cognitive development in sub-Saharan

African children. The South African study by le Roux *et al.* (2018) indicated that CHEU may be at high risk for cognitive and motor delay regardless of breastfeeding.

John *et al.* (2017) stated that nutritional deficiencies are the major contributors to delayed neurodevelopment in LMICs. The suboptimal infant feeding practices may be the leading cause of nutritional deficiencies, particularly iron deficiency, which has been documented to be common in HIV settings. Dewey and Vitta (2013) showed that the complementary diets of developing countries are poor in iron and zinc. The association between iron deficiency and neurodevelopment have been well documented in the literature (Engle *et al.*, 2007; Hamadani *et al.*, 2012; Juul *et al.*, 2019; Lozoff, 2007; Pala *et al.*, 2010). Inadequate iron and zinc intake, through either breastmilk or diet, during a critical period of rapid development adversely affect infant neurodevelopment by impairing cognitive and motor development. The evidence from animal models studies showed that during critical windows of development of the brain, lack or little iron results in alterations of neurotransmission, epigenetics, myelination brain metabolism (Tran *et al.*, 2009) and programming, neurotransmitter synthesis (Juul *et al.*, 2019) and neurotransmitter function (Soliman *et al.*, 2014). Zinc plays a central role in neuron formation, synapse generation, and migration, all involved in neurodevelopment; thus, zinc deficiency has been linked to poor cognitive and motor development in children (Anjos *et al.*, 2013). Trial studies on zinc supplementation provide evidence of a positive association between child zinc supplementation and motor skills and neuropsychological performance (John *et al.*, 2017). Bougma *et al.* (2013) showed that mild and moderate zinc deficiency leads to delayed cognitive development. Iodine deficiency has a negative outcome on the neurodevelopment (cognitive) of children through hypothyroidism effect on the neuron's structure and function (Anjos *et al.*, 2013). The negative impact of iodine deficiency on neurodevelopment is via interference with the synthesis of thyroxine and triiodothyronine hormones (John *et al.*, 2017).

The study that determined the associations between macronutrient intake of breastmilk and brain development among very preterm infants reported that infants who had high energy and protein intake had large total brain volume, cortical grey matter, and white matter volume (Bell *et al.*, 2022). Georgieff (2007) used experimental evidence to show a relationship between protein-energy intake and cortical grey matter, indicating that the cortex is sensitive to protein-energy malnutrition. It was also reported by Belfort *et al.* (2020) that high intakes of breastmilk fat and energy correlated with large weight, fat mass, and fat-free mass, and high breastmilk protein intake correlated with large length in preterm infants. Greater length and weight may

have a positive influence on neurodevelopment, and conversely, lower length or stunting may have a negative impact on neurodevelopment.

2.11 THE AETIOLOGY OF ANAEMIA

Anaemia remains a public health concern and the most common cause of illnesses and deaths among children (Osterbauer *et al.*, 2012). It is a haematological abnormality condition characterised by insufficient number of red blood cells or their oxygen carrying capacity to meet physiological needs (Newhall *et al.*, 2020). The depleted body stores, particularly during the period of intense growth in children, and inadequate nutrient intakes possibly due to suboptimal feeding practices, result in anaemia (Wong, 2017). Literature has shown that the aetiology of anaemia is multifactorial and uncertain, even in settings of HIV infection and exposure, as well as in backgrounds of poor nutrition (Balarajan *et al.*, 2011; Duffy *et al.*, 2020; Prendergast *et al.*, 2019). Findings of the study by Dryden-Peterson *et al.* (2011) showed that child *in utero* and postnatal exposure to maternal highly-active ART (HAART) and inadequate feeding practices strongly increased the risk of anaemia. Nutrient deficiencies, including vitamins A, C, B_{12}, iron and folate, resulting from an inadequate diet, cause nutritional anaemia. Iron deficiency is the common nutritional cause of anaemia in South Africa (Dorsamy *et al.*, 2022; Tunkyi and Moodley, 2018). Other risk factors of anaemia include infections such as HIV and tuberculosis, which cause anaemia of chronic diseases, as well as side effects of antiretroviral therapy (Tunkyi and Moodley, 2015). According to WHO (2023a), nutritional deficiencies, particularly iron deficiency and infectious diseases including HIV, are the most common causes of anaemia. Phiri *et al.* (2008) observed HIV as a crucial risk factor for anaemia in children aged 6 to 60 months. Additionally, other risk factors of severe anaemia in children are low GA at birth and low household income and maternal anaemia (Prieto-Patron *et al.*, 2018; Wong, 2017).

2.11.1 Prevalence of maternal and child anaemia

Anaemia is a major global health concern, particularly affecting women and young children. Globally, it affects 30.0% of women of childbearing age, 37.0% of pregnant women and 40.0% of young children (Juul *et al.*, 2019; WHO, 2023c). The LMICs are heavily affected, with 106 million women and 103 million children being anaemic in Africa (WHO, 2023c). The prevalence of anaemia was found to be higher in Indian WLWH than in HIV-uninfected mothers (Dadhwal *et al.*, 2017).

The highest prevalence of anaemia (76.0%) has been documented in Sub Saharan African children aged 6 to 23 months (Prieto-Patron *et al.*, 2018). In Brazil, Delicio *et al.* (2018) reported a 25.7% of the incidence of anaemia in HIV-unexposed-uninfected new-borns, which was associated with the use of nevirapine. Duffy *et al.* (2020) reported 53.0% and 21.0% of prevalence of moderate and severe anaemia, respectively, among 6 to 59-month-old HIV-infected and HIV-exposed Mozambican children. In Botswana, 7.4% of CHEU aged between 0 and 6 months were found to have severe anaemia, and six infants (0.3%) were assumed to have succumbed to severe anaemia (Dryden-Peterson *et al.*, 2011). In South Africa, the prevalence of anaemia in women and children has been reported as 28.1% and 61.3%, respectively (Tunkyi and Moodley, 2018; Turawa *et al.*, 2021). Also, as of 2019, WHO documented that 44.4% of South African children were anaemic (WHO, 2023a). Another study by Rothman *et al.* (2018) reported that 36.4% of children aged six months were anaemic in South African peri-urban areas. In contrast to the high prevalence of anaemia in CHEU, the Ugandan cross-sectional study findings indicated that CHEU had lower possibilities of anaemia than their CHUU counterparts (Osterbauer *et al.*, 2012).

2.11.2 Childhood anaemia and association with growth and neurodevelopment

Wong (2017) had stated that iron deficiency anaemia in children is a global health concern as it can lead to poor growth and neurodevelopmental impairment. Prendergast *et al.* (2019) noted that anaemia coincides with stunting, and the two are major risk factors for impaired neurodevelopment in children. They further articulated that cases of anaemia and stunting were higher in CHEU than in CHUU. Shorter length and reduced growth have been reported in children with iron deficiency anaemia, and improvements were observed following treatment with iron therapy (Soliman *et al.*, 2009). An animal study by Fretham *et al.* (2012) provided evidence that anaemia has an irreversible effect on neurological development. Anaemia in children has been positively associated with irreversible cognitive impairment (Prieto-Patron *et al.*, 2018). The study that assessed the effect of iron deficiency anaemia on child neurodevelopment using Bayley-II observed lower mean mental and psychomotor developmental indices among children with anaemia and those with a previous history of anaemia compared to their control counterpart group (Hokama *et al.*, 2005). In the same study, poor mean speech development quotients were observed in anaemic settings, using the Enjoji Scale of Infant Analytical development. An American study by Yang *et al.* (2021) that evaluated the association between anaemia and neurodevelopmental disorders among US children observed that anaemia was positively associated with developmental disorders among

anaemic children. In Uganda, Ssemata *et al.* (2020) reported deficits in cognitive, language and motor domains using Bayley-III in severely anaemic children under five years. Nevertheless, Rothman and colleagues (2018) found no associations between anaemia and psychomotor development scores in South African children.

2.12 CONCLUSION

South Africa has a high HIV prevalence among women of childbearing age and a high percentage of LBW and SGA new-borns. The present literature showed that IUGR and ante- and postnatal HIV exposure are independent risk factors for suboptimal growth and neurodevelopmental impairment. Many international and local studies have documented a high prevalence of poor growth and developmental delay in either IUGR or HIV-exposed children. Although IUGR resulting from placental insufficiency has been inadequately documented, and the subsequent adverse effects on postnatal growth and neurodevelopment, the available literature provides evidence that IUGR poses a threat to the child's early growth, development, health, and future complications related to non-communicable diseases originating from early deprivation of adequate nutrition. Low length measurement for age and delayed development were the most commonly reported deficits in children with an IUGR history, as the literature emphasised. Maternal HIV infection is thought to negatively influence prenatal and postnatal growth and neurodevelopment outcomes. Lastly, the prevalence of anaemia in children is reported to be high globally and in South African settings, with the CHEU group being at increased risk. Anaemia has been documented as a risk factor for stunting and poor neurodevelopment. The present literature indicates the need for the study on early assessment of growth, neurodevelopment and anaemia to inform clinicians and enable early detection and interventions.

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CHAPTER 3: METHODOLOGY

3.1 INTRODUCTION

This chapter covers a detailed description of the study setting, study population, study design, sample size, ethical considerations, and methods used for data collection and their validity and reliability. The information on the data analysis methods used and data management is also included.

3.2 STUDY SETTING AND POPULATION

The UmbiGodisa study is a follow-up to the Umbiflow International study, which determined the prevalence of abnormal UmA-RI, as well as AEDF, in unselected, low-risk obstetric populations at 28 - 34 weeks' gestation in Ghana, India, Kenya, Rwanda, and South Africa, using a single screening with the Umbiflow™ device. In South Africa, the pregnant women receiving antenatal care at Laudium Community Health Centre (CHC) were recruited from March 2019 to March 2020. The Umbiflow International study used a prospective cohort study design, and the local South African site recruited 1421 mothers during pregnancy, irrespective of the maternal HIV status, with subsequent grouping into low-risk Umbiflow (RI < 75^{th} centile) and high-risk Umbiflow (RI ≥ 75^{th} centile).

In order to expand the study population of CHEU with abnormal UmA-RI indicating placental insufficiency, the UmbiGodisa study also enrolled participants from the Siyakhula study for a once-off visit at the age of 18 months. The overall aim of the Siyakhula study was to assess factors affecting foetal and infant immunity, growth, and neurodevelopment in HIV- and antiretroviral-exposed uninfected children, with the study procedures very similar to the UmbiGodisa study and with pregnancy Doppler data also available.

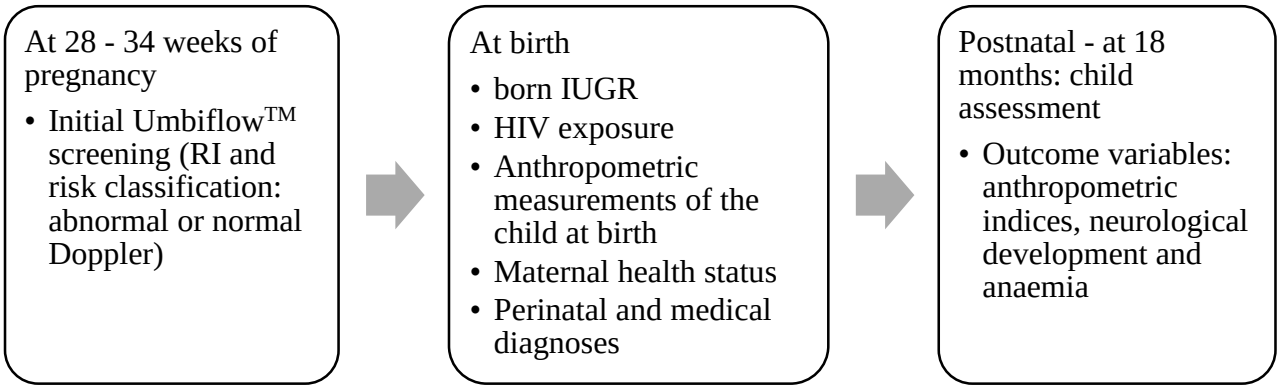
All participants from the two studies, now combined into the UmbiGodisa study, were followed up at the University of Pretoria's Research Centre for Maternal, Foetal, Newborn and Child Health Care Strategies and South African Medical Research Council (SAMRC) - Maternal and Infant Health Care Strategies Research Unit, located at Kalafong Provincial Tertiary Hospital, South-West Tshwane, Gauteng Province. The study population comprised CHUU and CHEU aged 18 months born with and without a history of IUGR as a result of placental insufficiency, as indicated by abnormal UmA-RI on Umbiflow™ Doppler screening during pregnancy.

The inclusion criteria of the Umbiflow International study were the low-risk singleton pregnancies (as per South African basic antenatal care (BANC-Plus) guidelines) between ≥28

weeks 0 days and ≤34 weeks 0 days' gestation at enrolment in women who were expected to give birth at Laudium CHC, Pretoria West Hospital or Kalafong Provincial Tertiary Hospital to facilitate follow-up of birth outcomes. Exclusion criteria included multiple pregnancies, women expected to give birth outside the study area, and lack of informed consent for any reason. The UmbiGodisa exclusion criteria had mothers with unknown HIV status, mothers under the age of 18 years, inability to obtain informed consent, children older than 21 months at the time of the study visit, and babies with chromosomal or structural abnormalities or other severe medical conditions. Lastly, Siyakhula WLWH, who had abnormal UmA-RI at 28 to 34 weeks of pregnancy, was included in the UmbiGodisa study. Written informed consent (Annex A) was obtained from the mothers before inclusion in the UmbiGodisa study.

3.3 STUDY DESIGN

The present descriptive cross-sectional study explored the growth and developmental outcomes of 18-month-old children with abnormal UmA-RI as a marker for IUGR compared to a similar group of children with normal UmA-RI, as mediated by maternal HIV infection (Figure 3.1). The study design was chosen because this was an observational study and mother-infant pairs were only followed up once at 18 months using the available pregnancy and birth information. The descriptive cross-sectional domain allowed the classification of the prevalence of growth and developmental outcomes between CHEU and CHUU populations with and without a history of IUGR, resulting in achieving the secondary study objectives.



Abbreviations: IUGR: Intrauterine growth restriction; RI: resistance index; SGA: small for gestational age

Figure 3.1: Flow diagram summarising the background of the present study

The double exposure group involved CHEU with abnormal UmA-RI (CHEU/AbN-RI) and CHUU with normal UmA-RI (CHUU/N-RI) served as the control group.

3.4 VARIABLES

The main outcome variables are shown in Figure 3.2.

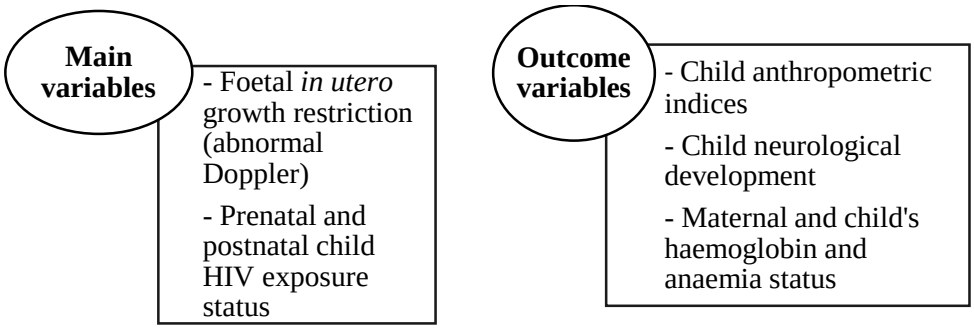


Figure 3.2: The main exposure and outcome variables measured in the study.

3.4.1 Modifiers

A. Infant feeding practices including breastfeeding over 18 months: duration of EBF, supplementary breastfeeding, cessation of breastfeeding, the timing of the introduction of complementary foods, and the child dietary intake of iron, zinc, and iodine at age 18 months.
B. Maternal obesity or undernutrition using BMI and MUAC at 18 months postpartum.

3.4.2 Covariates

As many factors contribute to child growth and development, data on potential confounders was collected, including maternal socio-demographic information, health and obstetric history, nutritional status, food security, and lifestyle factors (tobacco, alcohol, and drug use) (Section 3.6.1). Data on COVID-19 exposure was collected as well.

3.5 SAMPLE SIZE AND SAMPLE SIZE DETERMINATION

The sample size calculation using power analysis showed that a sample size of 280 was required overall with a split of 80%/20% for HIV-uninfected and HIV-infected women. The anticipated sample from the Umbiflow International study was 311, although 46 participants did not attend for their study visit. Four participants were subsequently excluded either because of age above the set upper limit of 21 months (n = 1), acute child illness at the study visit (n = 2) or parental choice to not complete the study visit (n = 1); therefore, this study included 261 participants from the Umbiflow International cohort and an additional 10 participants from the Siyakhula study, giving a total of 271 mother-child pairs. Figure 3.3 shows the number of investigated study participants.

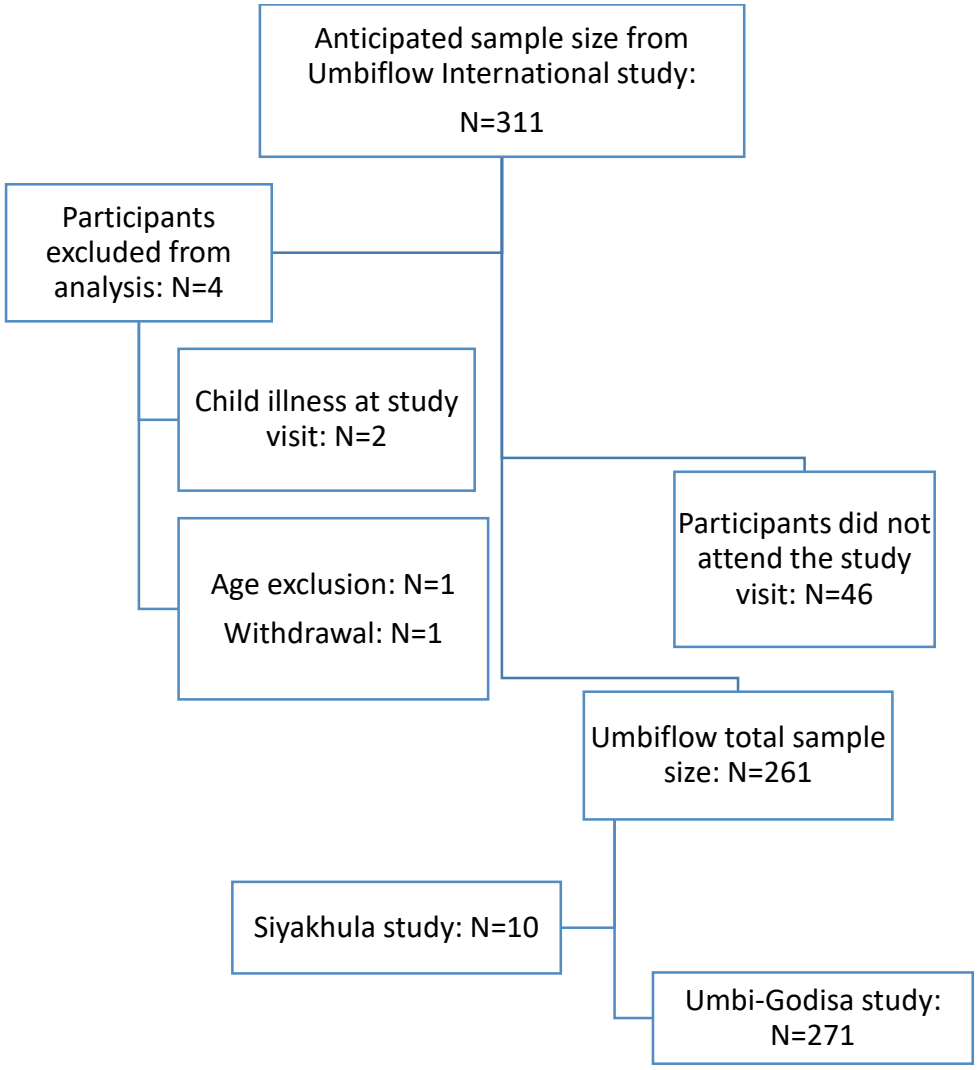
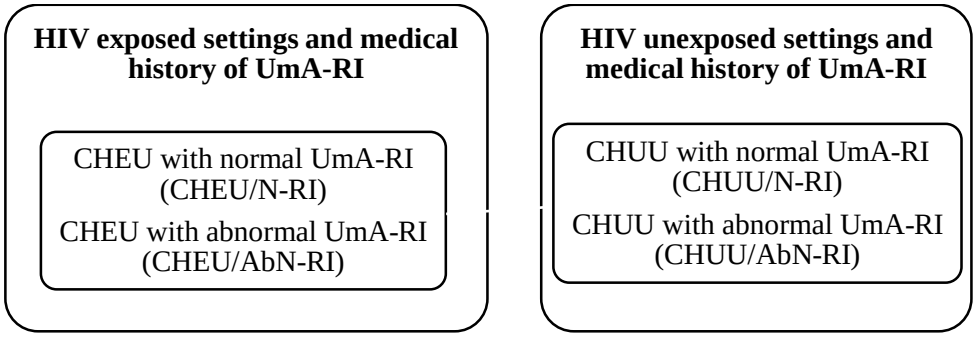


Figure 3.3: Flow diagram of study participants

The study population was grouped into four subgroups based on HIV exposure status and medical history of normal or abnormal UmA-RI (Figure 3.4).



Abbreviations: CHEU: HIV-exposed-uninfected children; CHUU: HIV-unexposed-uninfected children; UmA-RI: umbilical artery resistance index.

Figure 3.4: The four study groups based on HIV exposure and placental insufficiency, as measured by a normal or abnormal UmA-RI.

3.6 DATA COLLECTION METHODS

Mothers were contacted telephonically and booked for a study visit at the Research Centre. The researcher or trained study staff introduced the study to potential participants and administered the consent form. When conducting the study visit, the research staff were blinded to the Doppler category in which this mother-child pair falls. The researcher and trained study staff facilitated the data collection process using data collection sheets. The face-to-face interviews with the mother and the child development assessment were conducted by the researcher and trained study staff in either English, Pedi, Setswana, South Sotho, Venda, Zulu or Xhosa languages. The nurse and the researcher performed the mother and child's anthropometric measurements. The researcher performed data quality assessments of the completed data collection sheets.

3.6.1 Maternal socio-demographic information and impact of COVID-19

The participant identification questionnaire (Annex B) was used to identify eligible mother-child pairs and gathered maternal and child's date of birth, sex of the child, GA at birth and residential data. The information relating to maternal age, marital status, employment, pregnancy history, educational level, housing, access to water, internet, and electricity were collected using a structured questionnaire (Annex C). Furthermore, data on the impact of COVID-19 on health, income, and household food security were collected using a short structured self-designed COVID-19 questionnaire (Annex D). The maternal and infant postpartum questionnaire (Annex E) included assessment of the household food security status, using the United States Household Food Security Survey Module, which comprises a set of 10 questions asking on the perceptions of 1) inadequate household food budget or supply; 2) inadequate quality of food consumed and 3) occurrences of reduced food intake and consequences (Coleman-Jensen *et al.*, 2014). The limitation of this tool is that it does not provide specific information on food security of children.

3.6.2 Child feeding practices and dietary intake

The descriptive qualitative breastfeeding data over the first 18 months of life and data on the timing of the introduction of liquids, soft, semi-solids, and solids were collected using an adaptive standardised and previously used maternal and infant postpartum questionnaire (Annex F), based on adapted WHO questionnaires (WHO, 2010). The quantified single 24-hour dietary recall questionnaire (Annex F) based on previous studies was utilised to gather a child's dietary intake reported by the mother (Faber, 2005; Smuts *et al.*, 2005). The period covered by the 24-hour recall included Sunday, Tuesday and Thursday, as the interviews were

performed on Mondays, Wednesdays and Fridays. A detailed manual for administering a 24-hour dietary recall was available for guidance (Faber *et al.*, 2014). The standardised dietary kit containing samples of food and food containers, family unit utensils, and photographs were used to estimate and record the reported quantity of food consumed. Assessors were trained based on the Manual.

Additionally, for recording prepared food, dry oats were used to dish up and measure the estimated quantity consumed by the child. The portion of leftovers were noted and factored in the determination of the estimated actual consumption, and the breast milk substitutes (BMS) and infant cereals were recorded per dry amount and liquid in grams (g) and millilitres (mL) (Faber, 2005; Swanepoel *et al.*, 2019) (Faber, 2005). The intake of breastmilk was not quantified. The 24-hour recall was not done for children who were unwell in the past 24 hours.

The validity and reliability of 24-hr recall:

The 24-hour recall method of data collection is commonly used in nutrition surveys to gather dietary data of a particular population. The 24-hour recall tool has been validated for accurate measurement of energy intake in children, and interviewing parents to report a child's dietary intake is the most accurate procedure at the group level for children (Walker *et al.*, 2018). The 24-hour recall tool has been validated and adapted for use with mothers in LMICs to report their own and their child's intake (Arsenault *et al.*, 2020). The 24-hour recall has also been used to assess South African children's dietary intake (Faber, 2005; Faber *et al.*, 2016; Swanepoel *et al.*, 2019).

3.6.3 Maternal and child's medical history

The structured and pre infant follow-up questionnaire (Annex G) was used to gather PCR results and data for CHEU and current HIV status for mothers whose HIV status was negative during recruitment (WHO, 2010). In addition, maternal medical history relating to tuberculosis status and treatment, type of ART for WLWH and postpartum infections and treatment were also collected. This questionnaire also detailed the child's medical history, including diarrhoea, breathing difficulties, any chronic illness, and visits to health facilities due to illness.

3.6.4 Haemoglobin testing

The haemoglobin concentration is an indicator used in the biochemical assessment of anaemia and by proxy for iron deficiency (Institute of Medicine, 2001). The WHO defined anaemia as a haemoglobin concentration <11 g/dL in children aged 6 to 59 months, <11.5 g/dL in 5 to 11 years old children and <12 g/dL in 12 to 14 year old children (WHO, 2011). In non-pregnant

women, anaemia is defined as a haemoglobin level <12g/dL (Takuva *et al.*, 2013). Tunkyi and Moodley (2015) classified the haemoglobin levels into the following categories: severe anaemia (≤7 g/dL); moderate anaemia (7 - 9.9 g/dL); mild anaemia (10 - 10.9 g/dL) and normal (≥11 g/dL). The data regarding the maternal and child haemoglobin concentrations was collected using the HemoCue® Hb 201$^+$ System Analyzer. About 10µL of blood was drawn from the mother and child's middle finger using a HemoCue sterile safety lancet (2.25mL depth) and haemoglobin 201 microcuvettes. This system has been reported to be standard haemoglobin point-of-care testing and provides laboratory accuracy for determining haemoglobin in whole blood with a measuring range of 0 - 25.6 g/dL and provide results within 1 minute (Hemocue, 2021). Further, the system is easy to use and has outstanding lot-to-lot reproducibility. It is precisely calibrated from the factory in expectation of the International Council for Standardization in Haematology (ICSH) for determining haemoglobin (utilise a modification of Vanzetti's reagents, employing an azidemethemoglobin) and can store a maximum of 600 test results and their date and time (Hemocue, 2021). There are no further red cell indices available in the present study, which is a drawback, and in this it differs from a full blood count done at the laboratory.

3.6.5 Anthropometric measurements

In terms of the modifying factors, maternal weight and height were measured using a digital body weight scale (Mellerware Bodymax Body Scale, type: 20551) and stadiometer (Seca 216), respectively. The BMI was calculated as weight (in kilograms (kg)) / height^2 (in metres squared (m^2)), and the derived value was expressed in kg/m². The BMI classification used was as follows: severe malnutrition: ≤17.4kg/m^2; underweight: 17.5-18.4 kg/m^2; normal: 18.5-24.9 kg/m^2; overweight: 25.0-29.9 kg/m^2; obese: >30.0 kg/m^2 (WHO, 1995). Maternal MUAC was also measured (similar procedure as in children), with a MUAC of <23 cm used as the cut-off to identify wasting, which is also similar to BMI < 18.5 kg/m^2 (WHO, 1995).

Height was measured without shoes, bulky clothes, and hats. The patient stood against the stadiometer with eyes looking straight ahead, legs straight, heels together, arms at sides and shoulders relaxed, and the four points of contact being the head, shoulders, buttocks, and heels touching the surface of the stadiometer (Nieman, 2019). The measurement was taken to the nearest 0.1cm. The weight was measured with the scale placed on a hard surface, without shoes and bulky clothes. The patient looked straight ahead, arms at sides and stand on the scale until the weight stabilised. The reading was recorded to the nearest 0.01kg. Measurements were done twice and the mean was computed and recorded.

The child's length in centimetres (cm), weight (kg), HC in cm, MUAC and triceps skinfold (TSF) in cm were measured and recorded on the infant follow-up questionnaire (Annex G) and the Road-to-Health book. For intra- and inter-observer reliability, a complete set of anthropometric measurements were done two consecutive times (Stomfai *et al.*, 2011) and mean value was calculated and recorded. In the case where the two measurements differed by 0.5cm or 0.05kg, the third measurement was taken. The z-scores for birth anthropometry data were generated by the INTERGROWTH-21st (International Foetal and New-born Growth Consortium for the 21st Century) standard version 1.0.6257.25111, which is an international standard for size at birth (https://intergrowth21.tghn.org/). The INTERGROWTH-21st is limited to a maximum GA at birth of 42 weeks 6 days. At the age of 18 months, the WHO Anthro Survey Analyser software (http://www.who.int/childgrowth/software/en/), WHO, 2011, Geneva, Switzerland, was used to compute the z scores. These softwares provide sex- and age-normalised data for further analysis.

Length

Length was obtained using an infantometer (Seca 416, Birmingham, UK) to measure children. The measurement was taken with the child in the horizontal position, the head against the backboard, the crown securely against the headboard, and the Frankfort plane perpendicular to the backboard (Nieman, 2019). The child's body was kept aligned with the centreline of the backboard, the child's shoulders and buttocks securely touching the backboard, and the shoulders and hips at right angles to the long axis of the body. The child's legs were kept straight, and against the backboard, the footboard slides against the bottom of the feet (without shoes or socks) with the toes pointing upward and the measurement was read. Length was recorded to the nearest 0.1 cm and plotted on the WHO MGRS Growth Charts in the child's Road-to-Health book (Nieman, 2019). The LAZ scores less than -2 and -3 standard deviations (SD) were regarded as an indicator of poor linear growth (stunting) and severe stunting, respectively (De Onis *et al*., 2006).

Weight

The child's body weight was measured using a pan-type paediatric electronic scale; digital liquid-crystal display (LCD) baby weighing scale EBSA-20 (Zhongshan Jinli Electronic Weighing Equipment Co., Ltd). The child was weighed naked. The child was set lying down in the middle of the pan (Nieman, 2019). Weight was recorded to the nearest 0.01 kg and plotted on the WHO MGRS Growth Charts in the child's Road-to-Health book. A WLZ score below -

2 SD indicated wasting, while the WAZ scores below -2 and -3 SD indicated underweight and severe underweight, respectively (De Onis *et al*., 2006).

Head circumference

This is a crucial screening technique for detecting abnormalities of the head and brain growth (Nieman, 2019). The HC was measured using a flexible, non-stretchable measuring tape. The lower edge of the tape was positioned above the eyebrows, above (not over) the ears, and around the back of the head to measure the maximum circumference. The tape was pulled close to compress the hair, and the measurement was read to the nearest 0.1 cm (Nieman, 2019). An HCZ score below -1 SD may be a sign of developmental delays or impairment.

Mid upper arm circumference (fat-free mass/muscle mass)

Nieman (2019) defined MUAC as "the circumference of the upper arm at the midway between the shoulder tip and the elbow tip on the left arm". The MUAC was measured at the midpoint of the upper arm on the left side, with the arm relaxed and bent at 90º. The mid-arm point is determined by measuring the distance from the shoulder tip to the elbow and dividing it by two. A special tape was used for measuring the MUAC of a child. The cut-off reading measurements are <11.0 cm, which indicates severe acute malnutrition (SAM), <12.5 cm indicating moderate acute malnutrition (MAM), and ≥12.5 cm, indicating normal nutritional status. The MUAC is an easy to use screening tool for SAM and MAM among a large population size of children. MUAC-for-age z-scores (MUACZ) were determined (De Onis *et al*., 2006).

Triceps skinfold

The TSF measurement was done twice on the right arm using a Harpenden skinfold calliper (Baty International, Burgess Hill, UK). The measurement was taken with the child sitting on the mother's lap. The midpoint of the upper arm was marked on the posterior surface of the triceps during MUAC measurement. The skin and subcutaneous tissue were pinched 1cm from the marked site, and the tips of the callipers were applied to the marked area. The reading was taken after 3 seconds, and readings were recorded to the nearest 0.2mm. The triceps skinfold-for-age z-score (TSFZ) was determined.

3.6.6 Assessment of developmental indicators

The child's cognitive, motor, and language development was collected using the GMCD and the Bayley-III tools. The research staff were appropriately trained to test and assess a child's

development with the mentioned tools. The GMCD information was collected before Bayley-III by a different research staff to reduce bias.

The GMCD is an interview method designed to assess children's brain development, particularly in LMICs, and entails a series of open-ended questions that the child's caregiver answers about the child's development (White *et al.*, 2019). The GMCD assesses the postnatal brain development of children from age one month to three and a half years (Ertem, 2017). The functional domains assessed in GMCD involve expressive and receptive language, gross and fine motor skills, relating and response behaviour, play, and self-help activities (White *et al.*, 2019). The assessment was established with respect to the child age category, which includes milestones for each age category. The researcher recognises which specific standardised, pre-coded milestones the child has attained based on the mother's responses (Ertem, 2017). However, other examining questions were asked if the mother's feedback did not suffice to enable deducing attained milestones. The reliability and validity of GMCD have been reported by preliminary studies in Turkey (Ertem *et al.*, 2008). The GMCD is a standardised and validated method that has been used internationally to assess the brain development of about 12 000 children from countries such as Turkey, Argentina, India, and South Africa and has been reported to have appropriate accuracy for the identification of children with developmental delay (Ozturk Ertem *et al.*, 2019).

Bayley-III is a standardised and well-accepted tool, which was developed in 2006, and it is intended to assess the development of children from 1 month up to 42 months of age (Ballot *et al.*, 2017). It is an individually administered method designed to evaluate the functioning of developmental domains of infants and toddlers, thus detecting developmental delays (Albers and Grieve, 2007; Bayley, 2006; Dupont *et al.*, 2018). The method allows assessment of cognitive, language or communication (containing expressive and receptive communication subtests) and motor development (containing fine and gross motor subtests) (Bayley, 2006). The Bayley-III fine motor subtest comprises 66 items assessing skills related to eye movements, perceptual-motor integration, motor planning, and speed. The Bayley-III gross motor subtest encompasses 72 items measuring limbs and torso movements (Bayley, 2006). The Bayley-III cognitive scale covers 91 items gauging conceptual resources, information processing, and perceptual skills (Bayley, 2006). The Bayley-III receptive language subtest entails 49 items assessing preverbal behaviours involving recognising sounds and receptive vocabulary, morphological development and markers, and social referencing and verbal comprehension (Weiss *et al.*, 2010). The Bayley-III expressive language subtest contains 48

items assessing preverbal communication, vocabulary use, and morpho-syntactic development (Weiss *et al.*, 2010). There are milestones for each age category, and a child within a certain age category should attain all milestones of his or her age. The scale was administrated based on the manual's guidelines. A complete Bayley-III kit comprising technical and administrative manuals, a stimulus book, 25 record forms, three manipulative sets and baby-friendly, playful activities were used (Figure 3.5). The composite scores were obtained for the mentioned domains, which were then scaled to a metric that has a mean of 100, a standard deviation of 15, and a range of 40 – 160 (Ballot *et al.*, 2017). The scaled composite scores were interpreted based on Bayley-III guidelines. The method is easy to administer, allows caregiver involvement and data collected on the child have improved the incidence of clinical diagnosis; nevertheless, Bayley-III may be time-consuming, taking about 45 – 60 minutes to complete, and also it may overestimate, particularly, the motor performance among young children (Reuner *et al.*, 2013).

(Del Rosario *et al.*, 2021)

Figure 3.5: Bayley Scale for Infant and Toddler Development third edition kit

3.7 DATA MANAGEMENT

The study data was collected from the mother and the child by the researcher and study staff using standardised data collection sheets from February to December 2021, until no more eligible participants were available due to ageing out. The data collection sheets were filed, and data was managed and independently double entered on the online electronic platform, namely Research Electronic Data Capture (REDCap) v9.3.5, by the researcher and trained

study staff (Harris *et al.*, 2019; Harris *et al.*, 2009). REDCap is a secure web-based application for capturing data in clinical research areas and creating databases and projects (Patridge and Bardyn, 2018), developed by Vanderbilt University and hosted by SAMRC. REDCap is designed to offer a secure environment for researchers to collect and store highly sensitive information (Patridge and Bardyn, 2018); it offers an intuitive interface for validated data capture; automated data export procedures with downloads to commonly used statistical packages; audit trails for tracking data manipulation and export procedures and procedures for data integration and interoperability with external sources (Harris *et al.*, 2019; Harris *et al.*, 2009). The researcher ensured a smooth data capturing and cleaning process and ensured that data was of high quality. Checking and addressing missing or inaccurate data was dealt with immediately upon completing the interviews. Through comparing data entry 1 with data entry 2 (on REDCap), inconsistencies were resolved by referring to the original data collection sheet. Data was exported from REDCap to Microsoft Excel for further processing and analysis.

3.8 DATA PROCESSING AND STATISTICAL ANALYSIS

The study's main outcomes were 1) child anthropometric measurements: weight, length, HC, and MUAC and the indices WAZ, LAZ, WLZ, HCZ, MUACZ; 2) The neurodevelopmental domains: cognitive, motor and language composite scores; and 3) anaemia. The children born preterm were corrected for age using the following formula:

Anthro Preterm babies - correcting for age:

1. Actual age (AA) in days = Date of visit – Date of birth
2. Days premature (DP) = (40 weeks – GA in weeks) *7
3. Corrected Age (CA) in days = AA – DP
4. Corrected age in months = CA/365*12

Modifiers included phenotypes associated with poor growth and development, including infant feeding practices and dietary intake of iron, zinc, and iodine at 18 months; maternal over- and under-nutrition, maternal and child haemoglobin concentration levels; infant illness and socio-demographic factors. These clinical and phenotype data allowed for a comprehensive assessment of potential confounders, including, but not limited to, infant sex, maternal age, education, ethnicity, parity, smoking, and alcohol consumption at the time of the study visit. Statistical analyses to assess associations between IUGR and subsequent infant growth and developmental outcomes, as altered by maternal HIV exposure, were done using Spearman correlation.

Quality control was conducted to ensure data integrity. Outliers were reviewed and corrected in case of data capturing errors, including z-scores outside the reference population range (<-3 and >+3). Absolute measurements and z-scores that were clinically implausible were excluded from the analysis. As per WHO guidelines, z-scores below -5 and above 5 were also excluded from the analysis. The study utilised WHO guidelines to define stunting, underweight, wasting and moderate acute malnutrition as LAZ, WAZ, WLZ and MUACZ <-2 SD. Z-scores <-3 were classified as a severe condition. The birth anthropometry measurements included weight, length, HC, and the z-scores WLZ, LAZ and HCZ were determined using the INTERGROWTH-21st new-born size at birth standard. The highest GA that INTERGROWTH allows, which is 42 weeks 6 days, was used for the 12 participants with a recorded GA at birth from 43 to 46 weeks since a GA at birth of 43 weeks and above is unlikely. One child with a birth weight z-score above 3 was excluded from the analysis of birth anthropometry measurements but included in the analysis of the 18-month anthropometry measurements. Five mothers were pregnant and 2 children were brought to the study visit by the caregiver, hence maternal anthropometry was not collected on these participants.

In terms of neurodevelopmental outcomes, the composite score for Bayley-III is calculated based on a comparison of the child to a normative age-matched sample established by the assessment and is interpreted as follows for any of the five domains (Del Rosario *et al.*, 2021):

- Mid average functioning: composite score of 100 (15 SD standardised mean score)
- Mild impairment/at 'risk' of developmental delay: composite score of <85 (1 SD below the mean)
- Moderate impairment: composite score of <70 (2 SD below the mean)
- Severe impairment: composite score of <55

The reported household food intake measurements were converted to weight in grams using the SAMRC Food Quantities Manual published by SAFOODS (2018). The SAMRC FoodFinder™ 3.0 was used to analyse the food intake of groups of individuals, thereby quantifying the dietary intake of iron, zinc, and iodine. Nonetheless, SAMRC FoodFinder™ program has been recorded to have inadequate quality of iodine data and this may affect the iodine intake analysis results. The nutritional adequacy of the mentioned micronutrients intakes were assessed using Dietary Reference Intakes (DRI) published by the Institute of Medicine (2001). The Estimated Average Requirement (EAR) and adequate intake (AI) were used to estimate the prevalence of inadequate intakes within a group, and the dietary intake for the

group was considered nutritionally adequate if the median intake for the group was at or above the AI or EAR (Faber, 2005). The EARs for iodine, iron, and zinc for 1 to 3 years old children are 65 µg/day, 3.0 mg/day and 2.5 mg/day, respectively (Institute of Medicine, 2001). Anaemia was defined as discussed in section 3.6.4 (Tunkyi and Moodley, 2018; WHO, 2011).

The food security status was defined into four categories namely:

- Raw score zero - High food security among households
- Raw score 1-2 - Marginal food security among households
- Raw score 3-5 - Low food security among households
- Raw score 6-10 - Very low food security among households

The assessment of food security status was based on the sum of affirmative responses (raw score) to the 10 questions. Answers to "yes," "often," "sometimes," "almost every day," and "some days but not every day" are marked as affirmative (Coleman-Jensen *et al.*, 2014; Saediman *et al.*, 2019).

3.9 ETHICAL CONSIDERATION

Permissions for the Umbiflow International study were in place from the University of Pretoria-Ethics Committee (Ethics Reference Number 228/2018), Kalafong Provincial Tertiary Hospital, Pretoria West Hospital, and Laudium Community Health Centre. Similarly, permissions (including amendment for alignment of the 18-month study visit with the UmbiGodisa study) were in place for the longitudinal UmbiBaby and Siyakhula studies from the UP Ethics Committee (Ethics Reference Number 283/2019 and 294/2017, respectively), Kalafong Provincial Tertiary Hospital and Tshwane District Research Committee (all attached under Annex I). Further ethical approval was obtained from the Faculty of Natural and Agricultural Sciences and the Faculty of Health Sciences Ethics Committees for the study towards the degree of Doctor of Philosophy with Ethics Reference Number NAS259/2021.

3.9.1 Informed Consent

Mothers from the Umbiflow International study were given all the relevant information about the follow-up study by the researchers or study staff and adequate time to ask questions and discuss the study with significant others if they so wish. Mothers were asked to provide consent on behalf of themselves and their infants (Annex A: Participant information leaflet and informed consent form).

3.9.2 Potential Risks

Drawing a drop of blood for anaemia screening poses minimal risk to the study participants. However, some participants may have felt uncomfortable or distressed in response to some of the questions asked in the questionnaires. Study participants were free to refuse to answer any questions and were encouraged to contact the study team anytime. However, the study team had a lot of experience working with mothers and their babies and was on hand to offer counselling and referrals if needed. Clinical data extracted from medical records, delivery data and questionnaire data did not contain any personal identifiers. Data was identified only with the study identification number.

3.9.3 Potential Benefits

There was no direct benefit to participating in the proposed study. However, mothers benefited from the child's additional screening for growth and neurodevelopment and nutritional counselling and referrals for any further health care interventions, if needed. We diagnosed anaemia and any problems with development early and subsequently referred children for further assessment. Mothers were reimbursed for transport costs and given light refreshments. Knowledge gained from this study provided a better understanding of optimising the management of *in utero* growth-restricted infants postpartum in terms of their nutrition and neurological development.

3.9.4 Voluntary participation

Participants were recruited free of manipulation or coercion by trained research staff who carefully explained the study and gave potential participants time to consider their participation and ask any questions. Participation in the study was voluntary, and participants had the right to refuse to participate in any aspect of the study or withdraw from participation at any time. Refusal to participate did not have any negative consequences for the mother or the infant.

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CHAPTER 4: ARTICLE 1

The chapter is presented in article format per the author's guidelines of the Virus Journal, in which it was published in December 2022. The font, line spacing and spelling were kept the same as the rest of the thesis for uniformity. The published article is attached as annex H.

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EARLY CHILDHOOD GROWTH PARAMETERS IN SOUTH AFRICAN CHILDREN WITH EXPOSURE TO MATERNAL HIV INFECTION AND PLACENTAL INSUFFICIENCY

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ABSTRACT

Maternal HIV exposure and intrauterine growth restriction (IUGR) due to placental insufficiency both carry major risks to early child growth. We compared the growth outcomes of children aged 18 months who had abnormal umbilical artery resistance indices (UmA-RI), as a marker of placental insufficiency, with a comparator group of children with normal UmA-RI during pregnancy, as mediated by maternal HIV infection. The cross-sectional study included 271 children, grouped into four subgroups based on HIV exposure and history of normal/abnormal UmA-RI, using available pregnancy and birth information. Standard procedures were followed to collect anthropometric data, and z-scores computed as per World Health Organization growth standards. Lower length-for-age z-scores (LAZ) were observed in children who were HIV-exposed-uninfected (CHEU) (−0.71 ± 1.23; $p = 0.004$) and who had abnormal UmA-RI findings (−0.68 ± 1.53; $p < 0.001$). CHEU with abnormal UmA-RI had lower LAZ (−1.3 ± 1.3; $p < 0.001$) and weight-for-age z-scores (WAZ) (−0.64 ± 0.92; $p = 0.014$) compared to the control group. The prevalence of stunting was 40.0% in CHEU with abnormal UmA-RI and 16.0% in CHEU with normal UmA-RI ($p < 0.001$; $p = 0.016$, respectively). In conclusion, maternal HIV exposure and placental insufficiency are independent risk factors for childhood stunting, with this risk potentiated when these two risk factors overlap.

Keywords: children who are HIV-exposed-uninfected (CHEU); placental insufficiency; intrauterine growth restriction; child growth

1. INTRODUCTION

South Africa (SA) is burdened with a high prevalence (31.6%) of HIV infection in women of childbearing age [1] and pregnant women (30.0%) [2]. Nonetheless, access to antiretroviral therapy (ART) has increased over the years, with a >95% ART coverage during pregnancy and delivery [1], leading to an expanding population of children who are HIV-exposed-uninfected (CHEU). Adverse birth outcomes including intrauterine growth restriction (IUGR) and stillbirths have been documented in women living with HIV (WLHIV) compared to their HIV-negative counterparts, even when on ART [3–7]. IUGR is a clinical term describing a pathological inhibition of fetal growth preventing the foetus from attaining its genetic growth potential [8]. In SA, it is reported that CHEU have similar growth patterns with children who are HIV-unexposed-uninfected (CHUU); however, CHEU have been reported as a high-risk group due to *in utero* and postnatal ART exposure, as well as exposure to pathogens in immunocompromised family members [9,10].

International literature has indicated that CHEU are more likely than CHUU to experience impaired growth and neurodevelopmental outcomes, even in the context of high maternal ART coverage [11–14]. In the face of maternal HIV exposure as a risk for poor nutritional status, Black et al. stated that IUGR was associated with postnatal child wasting and stunting [15]. In agreement, Flores-Guillén et al. reported a 21% prevalence of stunting associated with IUGR in the HIV-negative population in Mexico [7].

Placental insufficiency is one immediate cause of IUGR, which in its extreme can lead to fetal demise/stillbirths [16,17]. IUGR, present in up to 30% of pregnancies, may be the most significant population-based attributable risk factor for preventable stillbirth [17,18]. Research has shown that pre-eclampsia, IUGR, and stillbirths linked to placental insufficiency complicate 10 to 15% of all pregnancies [16]. In developing countries, up to 24% of newborns, approximately 30 million, experience IUGR annually [19]. Low- and middle-income countries (LMICs) carry the highest burden of stillbirths (98%) and perinatal deaths [20]. Children born with IUGR are a high-risk group with short- and long-term morbidity and mortality. IUGR is a crucial risk factor for child undernutrition [21], coupled with an increased risk of overweight and obesity during adolescence, suboptimal intellectual and physical development, and other long-term chronic diseases in adult years [7,22].

The Umbiflow™ device is a low-cost continuous-wave Doppler screening tool for the detection of placental insufficiency in otherwise low-risk pregnancies [23]. Placental insufficiency is detected by an increased umbilical artery resistance index (UmA-RI), also known as an

abnormal RI. South African studies have reported a high prevalence of abnormal RI: 11.7% [24]; 13.0% [25]; 5.9% [26].

In view of the above, HIV exposure and placental insufficiency both carry major risks to early and late child growth and development, possibly compounding each other. However, the postnatal growth in children born to otherwise low-risk pregnancies with abnormal RI measurements, indicating IUGR, have not been intensively investigated, particularly in high HIV burden settings. Further, it is well known that adequate child nutrition is central for catch-up growth [27], with a positive correlation between breastfeeding and a child's growth and development. Exclusive breastfeeding (EBF) stimulates growth among CHEU and CHUU [13], as shown by Jumare *et al*. Additionally, there is still a lack of evidence on the nutritional management of children born with IUGR to optimize postnatal catch-up growth, including the impacts of feeding practices on the growth of children born with IUGR in the context of HIV exposure.

We therefore investigated and compared, at age 18 months, the growth outcomes of children born with and without IUGR due to placental insufficiency, as measured by an abnormal UmA-RI using Doppler screening during pregnancy, and as modified by maternal HIV status, in the Tshwane District in the Gauteng Province of SA.

2. MATERIALS AND METHODS

2.1. Study Settings and Participants

This study followed up participants from the SA arm of the Umbiflow International study, which studied the prevalence of raised UmA-RIs in low-risk pregnant women at 28–34 weeks' gestation in Ghana, India, Kenya, Rwanda and SA, using a single screening with the Umbiflow™ device between October 2018 and January 2020. Normal and abnormal UmA-RI was defined as <75th centile and ≥75th centile for the gestational age, respectively, as per Pattinson graphs [28]. The mother–child pairs were recruited at 18 months of age into the present study from a prospective longitudinal study and from an additional one-off follow-up from the Umbiflow International study, using the available pregnancy and birth information, at the University of Pretoria's Research Centre for Maternal, Fetal, Newborn and Child Health Care Strategies, located at Kalafong Provincial Tertiary Hospital, Tshwane District, Gauteng Province, SA. Additional participants were recruited from the Siyakhula study, a longitudinal study on the effects of maternal HIV infection on child outcomes, with an otherwise similar study design and at the same site, to increase the number in the CHEU group with an abnormal UmA-RI. The study population included children who had abnormal UmA-RIs during

pregnancy, as a marker for IUGR, compared to a similar group of children with normal UmA-RIs, as mediated by maternal HIV infection.

2.2. Study Design

This cross-sectional study explored the growth outcomes of children at the age of 18 months. Exclusion criteria included multiple pregnancies, inability to obtain informed consent, babies born to underage mothers, and babies with chromosomal or structural abnormalities or other severe medical conditions known to impact infant growth and development. The study population was grouped into four subgroups based on HIV exposure and history of normal or abnormal UmA-RI: (1) mothers who are HIV negative with normal UmA-RI (no exposure variable of interest; control group), (2) maternal HIV infection and normal UmA-RI (single exposure), (3) HIV-negative mothers with abnormal UmA-RI (single exposure), and (4) maternal HIV infection with abnormal UmA-RI (double exposure). The outcome variables were child growth parameters. The modifiers were infant feeding practices and other factors known to contribute to child growth; data on potential confounders was collected, including maternal socio-demographic information, medical and obstetric history, nutritional status and lifestyle factors.

2.3. Sample Size Determination

The sample size calculation using power analysis indicated that a sample size of 280 was required overall, with a split of 80/20% for CHUU and CHEU. The anticipated sample from the Umbiflow International cohort was 311; however, 46 participants did not attend their study visit, meaning 265 participants were enrolled. Four participants were subsequently excluded either because of age above the set upper limit of 21 months, acute child illness at the study visit or parental choice to not complete the study visit; therefore, this study included 261 participants from the Umbiflow International cohort and an additional 10 participants from the Siyakhula study. The sample size per group for the infant follow-ups was as follows: CHUU with normal UmA-RI: n = 186; CHEU with normal UmA-RI: n = 50; CHUU with abnormal UmA-RI: n = 20; and CHEU with abnormal UmA-RI: n = 15 (Figure 4.1).

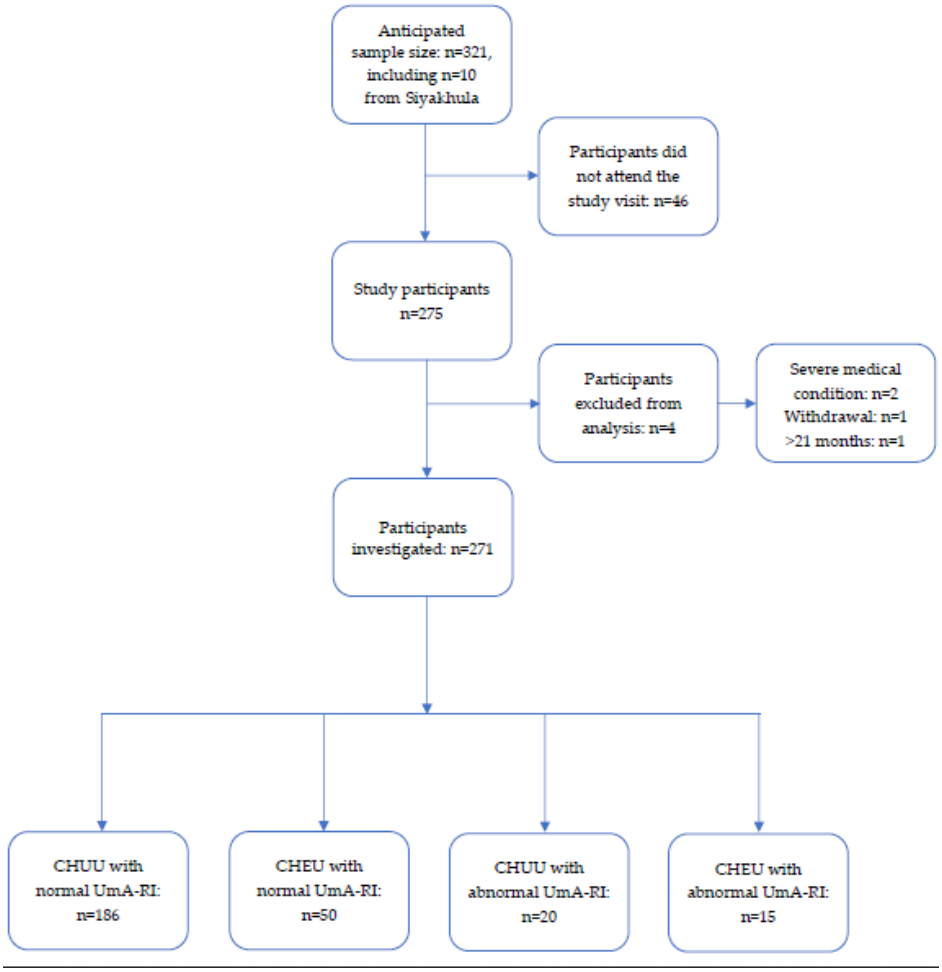

Figure 4.1: The flow diagram for study participants.

Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index.

2.4. Data Collection Methods

Mothers were contacted telephonically and invited to this study, after which written informed consent was obtained by the trained study staff. Data were collected between February and December 2021 using standardized data collection sheets, until no more eligible participants were available due to ageing out. The face-to-face interviews with the mothers were performed in either English or local languages. The child anthropometric measurements collected included weight, length and head circumference (HC), mid-upper arm circumference (MUAC) and triceps skinfold thickness (TSF); similar measurements were collected from the mother except for HC. For intra-observer reliability, the anthropometric measurements, performed as per standard procedures, were taken twice and the mean value was recorded [29,30]. Maternal socio-demographic information was collected using a structured questionnaire, while the descriptive qualitative feeding practices data were collected using a standardized maternal and infant postpartum questionnaire, which is based on adapted World Health Organization (WHO)

questionnaires. A structured infant follow-up questionnaire was used to collect maternal and child medical history, along with information obtained from the child's Road-to-Health booklet, which includes information from clinic visits. For CHEU, HIV antibody testing was performed as per national SA guidelines. One birthweight z-score of >+3 was excluded from the analysis of birth anthropometry measurements (n = 1). Five mothers were pregnant, and a child was brought to the study visit by the caregiver resulting in missing maternal anthropometry data for these mothers.

2.5. Data Processing and Statistical Analysis

The data were independently double entered on the online electronic platform, Research Electronic Data Capture (REDCap) v9.3.5, which is a secure web-based application for capturing data in clinical research projects [31]. The outliers were reviewed and corrected in case of error in data capturing, including z-scores outside the range of the reference population (<−3 and >+3). Absolute measurements and z-scores that were clinically implausible were excluded from the analysis. The z-scores for birth anthropometry data were generated using the INTERGROWTH-21st Newborn Size tool (International Fetal and Newborn Growth Consortium for the 21st Century, Oxford, UK) standard version 1.0.6257.25111. There were 12 participants with gestational age (GA) at birth ranging from 43 to 46 weeks, which is clinically unlikely; therefore, the highest GA of 42 weeks 6 days on the INTERGROWTH-21st tool was used for these birth anthropometry z-scores. WHO Anthro software was used to compute the z-scores for the 18-month anthropometric data and children born premature were corrected for gestational age. Both INTERGROWTH-21st and WHO Anthro provide sex- and age-normalized data. This study utilized WHO guidelines to define stunting, wasting, underweight, microcephaly and moderate/severe acute malnutrition as length-for-age z-score (LAZ), weight-for-length z-score (WLZ), weight-for-age z-score (WAZ), HC-for-age z-score (HCAZ), respectively. The z-scores <−3 were classified as severe suboptimal nutritional status. The BMI classification used was as follows: severe malnutrition: ≤17.4kg/m^2; underweight: 17.5-18.4 kg/m^2; normal: 18.5-24.9 kg/m^2; overweight: 25.0-29.9 kg/m^2; obese: >30.0 kg/m^2. The R statistical program was used for statistical analysis, for which each of the three test groups (with single and double exposure) was compared against the control group to determine if significant differences existed. In all instances, the Shapiro–Wilk test was used to determine if the data were normally distributed. For normally distributed data, the independent t-test was used to compare each test group against the control group, and a one-way ANOVA for comparing all four groups. For the non-normally distributed data, the Mann–Whitney U tests

was used to compare each test group against the control group, and the Kruskal–Wallis H test for comparing all four groups. All tests were performed at a 5% level of significance.

2.6. Ethical Considerations

Ethical approval for this study was obtained from the Faculty of Natural and Agricultural Sciences and Faculty of Health Sciences Ethics Committees of the University of Pretoria with reference number: NAS259/2021. The mothers were given all the relevant information about the follow-up study prior to recruitment. Mothers provided informed consent on behalf of themselves and their children.

3. RESULTS

3.1. The Socio-Demographic and Medical Characteristics of the Mothers of the Study Children

A total of 271 mothers were enrolled in this study, with anthropometric measurements not performed in 6 mothers due to repeat pregnancy (n = 5) or because the child was brought by a caregiver (n = 1). The maternal characteristics are presented in Table 4.1. WLHIV with an abnormal UmA-RI were significantly older (36.6 ± 6.1 years; $p < 0.001$). A high percentage of women had attained any secondary education (80.0%) and 66.7% of WLHIV with abnormal UmA-RI were unemployed. Half of the mothers had access to running water inside the yard and had flushing toilets. More mothers in the control group (29.6%) consumed alcohol at least once a month, compared to other groups. Cigarette smoking was uncommon in all groups. WLHIV with abnormal UmA-RI had lower weight, BMI and MUAC than their counterparts in the other three groups. Further, higher gravidity was reported in WLHIV than their HIV-uninfected counterparts ($p = 0.018$). Two-thirds of women delivered vaginally, but amongst women with history of an abnormal UmA-RI, caesarean section rates were high, with 50.0% of WLHIV and 46.7% of HIV-uninfected women requiring a caesarean section, respectively (Table 4.1).

Table 4.1: The maternal socio-demographic and medical characteristics

Variables		CHUU with Normal UmA-RI	CHEU with Normal UmA-RI	CHUU with Abnormal UmA-RI	CHEU with Abnormal UmA-RI	*p*-Value [a]
Sample size (n) (%)		186 (68.6%)	50 (18.5%)	20 (7.4%)	15 (5.5%)	
Mean age (years)		30.1 ± 5.1	31.3 ± 5.5	28.5 ± 4.5	36.6 ± 6.1	**<0.001**
Marital status	Single	79 (42.5%)	21 (42.0%)	6 (30.0%)	4 (26.7%)	n/a
	Married	69 (37.1%)	17 (34.0%)	10 (50.0%)	8 (53.3%)	
	Co-habiting	38 (20.4%)	12 (24.0%)	4 (20.0%)	3 (20.0%)	
Educational level	Any primary schooling	13 (7.0%)	4 (8.0%)	3 (15.0%)	2 (13.3%)	n/a
	Any secondary schooling	127 (68.3%)	40 (80.0%)	13 (65.0%)	12 (80.0%)	
	Post-school education	46 (24.7%)	6 (12.0%)	4 (20.0%)	1 (6.7%)	
Employment status	Unemployed	112 (60.2%)	29 (58.0%)	11 (55.0%)	10 (66.7%)	0.118
	Employed	74 (39.8%)	21 (42.0%)	9 (45.0%)	5 (33.3%)	
Monthly household income [b]	R 0–R 2000	37 (20.1%)	7 (14.0%)	0 (0%)	4 (26.7%)	n/a
	R 2001–R 4000	43 (23.4%)	15 (30.0%)	8 (40.0%)	0 (0%)	
	R 4001–R 6000	39 (21.2%)	13 (26.0%)	5 (25.0%)	6 (40.0%)	
	R 6001–R 8000	11 (6.0%)	5 (10.0%)	1 (5.0%)	2 (13.3%)	
	R 8000+	45 (24.5%)	9 (18.0%)	6 (30.0%)	1 (6.7%)	
	Don't know	9 (4.9%)	1 (2.0%)	0 (0%)	2 (13.3%)	
Access to running water	Inside house	68 (36.6%)	10 (20.0%)	7 (35.0%)	4 (26.7%)	n/a
	Inside yard	89 (47.8%)	27 (54.0%)	10 (50.0%)	8 (53.3%)	
	Communal tap	21 (11.3%)	9 (18.0%)	3 (15.0%)	3 (20.0%)	
	Water tank	8 (4.3%)	4 (8.0%)	0 (0%)	0 (0%)	
Access to toilet [c]	Flushing toilet	131 (70.4%)	30 (61.2%)	16 (80.0%)	9 (64.3%)	n/a
	Pit latrine/bucket	55 (29.6%)	19 (38.8%)	4 (20.0%)	5 (35.7%)	
Drinks alcohol [d]	Yes	54 (29.2%)	11 (22.0%)	2 (11.1%)	1 (7.1%)	n/a
Smokes cigarettes	Yes	3 (1.6%)	2 (4.0%)	0 (0%)	1 (6.7%)	n/a
Latest CD4 count	Cells/mm^3 [e]	N/A	463 ± 310	N/A	416 ± 295	0.147
Latest HIV viral load	Copies/mL (log)[f,g]	N/A	0.0 [0.0, 4.0]	N/A	0.0 [0.0, 0.0]	0.277
Current ART	TDF/FTC/EFV	N/A	31 (62.0%)	N/A	8 (53.3%)	n/a
	Other ART [h]	N/A	10 (20.0%)	N/A	6 (40.0%)	
	Not recorded	N/A	9 (18.0%)	N/A	1 (6.7%)	
Obstetric history	Parity [f]	2 [1, 3]	2 [1, 3]	2 [0, 2]	3 [3, 3]	**0.006**
	Gravidity [f]	2 [1, 3]	3 [2, 3]	2 [2, 3]	3 [3, 4]	**0.018**

	Previous pregnancy losses [f]	0 [0, 0]	0 [0, 1]	0 [0, 0]	0 [0, 1]	0.372
Umbilical artery Doppler	Preeclampsia/eclampsia	2 (13.3%)	0 (0%)	0 (0%)	0 (0%)	n/a
	UmA-RI value at 28–34 weeks' gestation [e]	0.64 ± 0.05	0.63 ± 0.04	0.74 ± 0.06	0.76 ± 0.04	**<0.001**
Mode of delivery	Vaginal delivery	127 (68.3%)	31 (62.0%)	10 (50.0%)	6 (40.0%)	
	Assisted delivery	3 (1.6%)	0 (0%)	0 (0%)	0 (0%)	**n/a**
	Caesarean section	56 (30.1%)	19 (38.0%)	10 (50.0%)	9 (60.0%)	
Body measurements and indices [e,i]	Weight (kg)	77.6 ± 19.6	77.5 ± 25.0	69.7 ± 20.2	63.1 ± 15.4	**0.016**
	Height (cm)	160.2 ± 6.2	161.3 ± 8.9	157.8 ± 5.3	158.3 ± 5.3	0.402
	BMI (kg/m²)	30.3 ± 7.7	29.5 ± 8.4	27.9 ± 7.4	25.1 ± 5.2	**0.043**
	MUAC (cm)	32.1 ± 5.0	31.8 ± 5.7	30.7 ± 5.0	28.0 ± 4.0	**0.025**
	TSF (mm)	19.9 ± 5.1	21.4 ± 7.4	20.0 ± 5.3	18.5 ± 3.6	0.243

Values in bold font indicate significant p-values. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index; CD4: clusters of differentiation 4; ART: antiretroviral therapy; TDF/FTC/EFV: tenofovir/emtricitabine/efavirenz; BMI: body mass index; MUAC: mid-upper-arm circumference; TSF: triceps skin fold; n/a: not applicable. [a] Comparisons involved all groups. Only variables with groups above 5 were included in these investigations as smaller groups lead to volatility of results. n/a implies no comparisons were performed due to low counts. [b] One South African Rand equates to 0.056 United States Dollars. [c] Missing information: CHEU with normal UmA-RI (n = 1); CHEU with abnormal UmA-RI (n = 1). [d] Mother drank alcohol at least once in a month, since the baby was born. [e] Mean and standard deviation (SD) reported. [f] Median and interquartile range [IQR] reported. [g] Undetectable viral load is reflected as zero. [h] Includes tenofovir (TDF), lamivudine (3TC) plus lopinavir/ritonavir or dolutegravir. [i] Total of 265 mothers were measured at the study visit, 5 were pregnant and 1 child was brought by the caregiver.

3.2. The Characteristics of the Study Children

A total of 271 CHUU and CHEU aged 18 months with and without a history of an abnormal UmA-RI *in utero* were investigated, and the findings are presented in Table 4.2. The study population had more females than males across the groups, except for CHEU with normal UmA-RI. The GA at birth was lower (36.8 ± 2.5 weeks) in CHUU with abnormal UmA-RI than in the other groups ($p < 0.001$). A history of malnutrition was reported in CHUU with normal UmA-RI (6.0%) and CHEU with normal UmA-RI (12.0%), and a history of diarrhea was common in the study population. Findings on feeding practices showed that CHEU with normal UmA-RI had a lower percentage of early initiation of breastfeeding (within one hour after birth) than the other groups ($p < 0.001$). Lastly, exclusive breastfeeding was lowest in WLHIV with abnormal UmA-RI, while mixed feeding (24.3%) was common in the control group. Percentages of current (supplementary) breastfeeding up to age 18 months were observed to be low in HIV-exposed settings.

Table 4.2: The medical background of children from birth to age 18 months

Variables		CHUU with Normal UmA-RI	CHEU with Normal UmA-RI	CHUU with Abnormal UmA-RI	CHEU with Abnormal UmA-RI	*p*-Value [a]
Sample size (n) (%)		186 (68.6%)	50 (18.5%)	20 (7.4%)	15 (5.5%)	
Mean age (months)		18.6 ± 0.9	18.5 ± 0.8	18.2 ± 0.2	18.8 ± 0.9	
Sex	Male	91 (48.9%)	28 (56.0%)	8 (40.0%)	4 (26.7%)	n/a
	Female	95 (51.1%)	22 (44.0%)	12 (60.0%)	11 (73.3%)	
Mean GA at birth (weeks)		39.3 ± 1.9	39.4 ± 1.3	36.8 ± 2.5	38.3 ± 1.0	**<0.001**
Born premature		15 (8.1%)	2 (4.0%)	7 (35.0%)	1 (6.7%)	n/a
APGAR score (5 min) [b]		10 [9, 10]	9 [9, 10]	9 [9, 10]	9 [9, 9]	0.069
Neonatal hospitalization		35 (18.9%)	6 (12.0%)	7 (35.0%)	1 (7.1%)	n/a
Neonatal diagnosis [c]	Respiratory distress	7 (3.8%)	3 (6.0%)	3 (15.0%)	0 (0%)	n/a
	Jaundice	18 (9.7%)	2 (4.0%)	2 (10.0%)	0 (0%)	
	Other	10 (5.4%)	1 (2.0%)	1 (5.0%)	1 (7.1%)	
Prevention of vertical HIV transmission	Single drug (NVP)	N/A	41 (82.0%)	N/A	7 (46.7%)	n/a
	Dual drug (NVP and AZT)	N/A	3 (6.0%)	N/A	6 (40.0%)	
History of childhood illnesses	Malnutrition	11 (6.0%)	6 (12.0%)	0 (0%)	0 (0%)	n/a
	Diarrhoea	57 (30.6%)	13 (26.0%)	4 (20.0%)	2 (13.3%)	
Hospital admission (post-neonatal)	Any illness	18 (9.7%)	5 (10.0%)	0 (0%)	1 (6.7%)	n/a
Breastfeeding	Ever breastfeed	178 (95.7%)	47 (94.0%)	20 (100.0%)	15 (100.0%)	
Early initiation of breastfeeding [d]	Within 1 h after birth	124 (78.5%)	21 (48.8%)	9 (52.9%)	8 (61.5%)	**<0.001**
	After 1 h of birth	34 (21.5%)	22 (51.2%)	8 (47.1%)	5 (38.5%)	
Infant feeding from birth until 6 months [e,f]	Exclusive breastfeeding	122 (65.9%)	32 (64.0%)	14 (70.0%)	8 (53.4%)	0.750
	Formula feeding	11 (5.9%)	3 (6.0%)	0 (0%)	0 (0%)	
	Mixed feeding	45 (24.3%)	8 (16.0%)	4 (20.0%)	2 (13.3%)	
	Formula feeding only at 6 months, but previous exclusive breastfeeding	7 (3.8%)	7 (14.0%)	2 (10.0%)	5 (33.3%)	
Current breastfeeding		50 (27.0%)	3 (6.1%)	4 (22.2%)	2 (14.3%)	n/a

Values in bold font indicate significant p-values. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index; GA: gestational age; IQR: interquartile range; NVP: nevirapine; AZT: Zidovudine; n/a: not applicable. [a] Comparisons involved all groups. Only variables with groups above 5 were included in these investigations as smaller groups lead to volatility of results. n/a implies no comparisons were made due to low counts. [b] Median interquartile range [IQR] reported. [c] Missing information: CHUU with abnormal UmA-RI (n = 1). [d] Unknown information for CHUU with normal UmA-RI (n = 28); CHEU with normal UmA-RI (n = 7); CHUU with abnormal UmA-RI (n = 3); CHEU with abnormal UmA-RI (n = 2). [e] Missing information: CHUU with normal UmA-RI (n = 1). [f] Comparison was performed for exclusive breastfeeding vs. all other feeding methods.

3.3. Growth Parameters of Study Children

Firstly, investigations involved comparisons of growth outcomes in HIV-exposed vs. unexposed and normal vs. abnormal UmA-RI settings for the entire study population of 271 children (Table 4.3). When comparing between CHEU and CHUU, lower LAZ was observed in CHEU (−0.73 ± 1.23; $p = 0.003$). Similar findings were observed in abnormal UmA-RI when compared to normal UmA-RI group (−0.68 ± 1.53; $p < 0.001$).

Table 4.3: The comparisons of mean growth outcomes at age 18 months in HIV-exposed vs. unexposed settings and normal vs. abnormal umbilical artery resistance index (UmA-RI) settings

Growth Indicator[a]	CHUU	CHEU	*p*-Value	Normal UmA-RI	Abnormal UmA-RI	*p*-Value
	n = 206 (76.0%)	n = 65 (24.0%)		n = 236 (87.1%)	n = 35 (12.9%)	
WAZ	0.04 ± 1.19	−0.24 ± 1.26	0.122	0.01 ± 1.19	−0.29 ± 1.32	0.122
LAZ	−0.05 ± 1.32	−0.73 ± 1.23	**0.003**	−0.14 ± 1.29	−0.68 ± 1.53	**<0.001**
WLZ	0.08 ± 1.21	0.14 ± 1.33	0.710	0.10 ± 1.26	0.04 ± 1.10	0.710
HCZ	0.93 ± 1.18	0.71 ± 1.15	0.198	0.88 ± 1.16	0.85 ± 1.27	0.141

Values in bold font indicate significant p-values. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index; WAZ: weight-for-age z-score; LAZ: length-for-age z-score; WLZ: weight-for-length z-score; HCZ: head circumference z-score. [a] Sex-normalized anthropometric indicators at age 18 months were computed using the World Health Organization (WHO) Anthro software of 2011, corrected for gestational age for preterm infants.

Further findings on growth outcomes across the four groups are reported as the means and standard deviations (SD) (Table 4.4). Investigations involved comparisons of the three test groups against the control group, respectively. CHEU with abnormal UmA-RI had lower WAZ at birth than the control group ($p = 0.003$). Additionally, lower LAZ was observed in CHEU with normal UmA-RI than the control group ($p = 0.023$). Findings at 18 months showed that when comparing CHEU with abnormal UmA-RI against the control group, there was a significant difference in weight, length and HC. Further, CHEU with abnormal UmA-RI had significantly lower LAZ ($p < 0.001$), as well as WAZ and HCZ ($p = 0.014$; $p = 0.016$, respectively) (Figure 2). Furthermore, the findings showed that there were no significant differences for growth outcomes in groups with single exposure, maternal HIV exposure or abnormal UmA-RI, respectively, against the control group. The prevalence of stunting was higher (40.0%) in CHEU with abnormal UmA-RI, than with single exposure, 16.0% in the HIV exposure and abnormal UmA-RI group, respectively ($p < 0.001$; $p = 0.016$). Wasting (8.0%) and underweight (6.0%) were observed in CHEU children with normal UmA-RI. The sensitivity analysis excluding children born preterm showed similar growth outcomes in which CHEU with abnormal UmA-RI had lower LAZ, WAZ and HCZ than the control group: $p = 0.003$; $p = 0.029$ and $p = 0.029$, respectively (data not shown). The rate of stunting remained high among the CHEU with abnormal UmA-RI group (35.7%).

Table 4.4: The mean anthropometric measurements and indicators of study children at birth and age 18 months, as per groups of control, single exposure and dual exposure to maternal HIV plus abnormal UmA-RI

Variables		CHUU with Normal UmA-RI	CHEU with Normal UmA-RI	CHUU with Abnormal UmA-RI	CHEU with Abnormal UmA-RI	*p*-Value [a]
Sample size (n) (%)		186 (68.6%)	50 (18.5%)	20 (7.4%)	15 (5.5%)	
At birth						
Anthropometry (mean ± SD)	Weight (g)	3187 ± 483	3108 ± 433	2649 ± 566	2704 ± 408	**<0.001** [b]
	Length (cm)	50.7 ± 3.0	49.9 ± 2.3	48.8 ± 2.9	49.1 ± 2.6	**0.005** [b]
	HC (cm)	34.5 ± 1.7	34.5 ± 1.5	33.0 ± 1.9	33.9 ± 1.5	**0.003** [b]
Indicators (mean ± SD) [d]	WAZ	−0.30 ± 1.16	−0.54 ± 0.96	−0.48 ± 1.07	−1.04 ± 0.76	**0.003**
	LAZ	0.68 ± 1.66	0.15 ± 1.32	0.64 ± 1.24	0.36 ± 1.28	**0.023**[c]
	HCZ	0.40 ± 1.34	0.35 ± 1.24	0.20 ± 0.95	0.48 ± 1.07	0.778
At age 18 months						
Anthropometry (mean ± SD)	Weight (kg)	10.9 ± 1.5	10.7 ± 1.8	10.8 ± 1.9	9.9 ± 1.0	**0.079**
	Length (cm)	81.9 ± 3.8	80.6 ± 3.3	81.1 ± 4.1	78.2 ± 3.5	**<0.001**
	HC (cm)	48.1 ± 1.6	48.1 ± 1.9	48.5 ± 1.9	47.1 ± 1.2	**0.011**
	MUAC (cm)	16.0 ± 1.4	16.2 ± 1.7	16.4 ± 1.7	16.0 ± 1.4	0.825
	TSF (mm)	8.7 ± 2.0	8.4 ± 2.2	8.7 ± 2.7	8.6 ± 1.3	0.869
Indicators (mean ± SD) [e]	WAZ	0.05 ± 1.15	−0.11 ± 1.32	−0.02 ± 1.52	−0.64 ± 0.92	**0.014**
	LAZ	−0.03 ± 1.30	−0.56 ± 1.16	−0.21 ± 1.53	−1.30 ± 1.32	**<0.001**
	WLZ	0.07 ± 1.20	0.19 ± 1.46	0.09 ± 1.32	−0.02 ± 0.76	0.662
	HCZ	0.89 ± 1.15	0.83 ± 1.23	1.26 ± 1.47	0.33 ± 0.73	**0.016**
Nutritional classifications (n; %)	Underweight	4 (2.2%)	3 (6.0%)	1 (5.0%)	0 (0.0%)	n/a
	Stunting [f]	9 (4.8%)	8 (16.0%)	2 (10.0%)	6 (40.0%)	**<0.001**
	Wasting	6 (3.2%)	4 (8.0%)	0 (0.0%)	0 (0.0%)	n/a

Values in bold font indicate significant p-values. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index; HC: head circumference; MUAC: mid-upper-arm circumference; TSF: triceps skinfold; WAZ: weight-for-age z-score; LAZ: length-for-age z-score; WLZ: weight-for-length z-score; HCZ: head circumference z-score; n/a: not applicable. [a] Comparisons between CHEU with abnormal UmA-RI group vs. control group. Only variables with groups above 5 were included in these investigations as smaller groups lead to volatility of results. n/a implies no comparisons were made due to low counts. [b] Comparisons were made between the four study groups. [c] Comparison between CHUU with normal UmA-RI vs. CHEU with normal UmA-RI groups. [d] The birth sex-normalized anthropometric indicators were computed using INTERGROWTH-21st software, using gestation-adjusted age for preterm infants. [e] The sex-normalized anthropometric indicators at age 18 months were computed using World Health Organization (WHO) Anthro software of 2011, using gestation-adjusted age for preterm infants. [f] Additionally, comparison between CHUU with normal UmA-RI vs. CHEU with normal UmA-RI: p = 0.016.

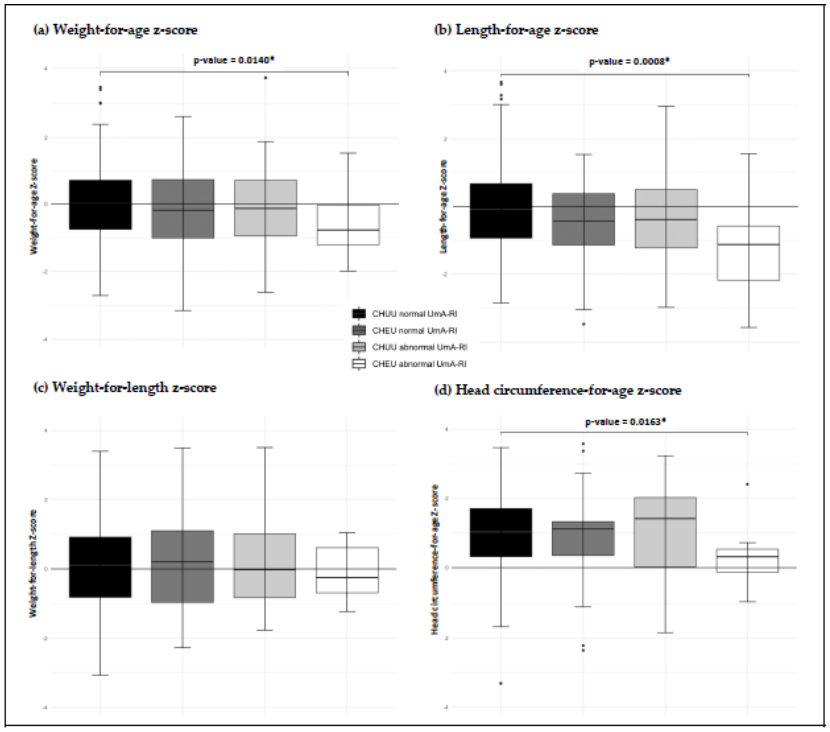

Figure 4.2: The box plots showing the significant differences for weight-for-age z-score, length-for-age z-score, weight-for-length z-score and HC-for-age z-score between the study groups.

The z-scores were computed using World Health Organization (WHO) Anthro software of 2011, using corrected age for premature children. (**a**) WAZ for CHEU abnormal UmA-RI is below the median line and lower than the three groups; (**b**) LAZ for CHEU abnormal UmA-RI is very far below the median and lower than the three groups; (**c**) WLZ for CHEU abnormal UmA-RI is the only group below the median line; (**d**) HCZ for CHEU abnormal UmA-RI is low compared to their counterparts in the different groups. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index; WAZ: weight-for-age z-score; LAZ: length-for-age z-score; HCZ: head circumference z-score; HC: head circumference.

Additional findings indicated that there were no significant differences between the growth indicators and child feeding practices during the first six months of life and up until 18 months of age (Table 4.5).

Table 4.5: Comparison of mean child growth indicators between breastfeeding practices

	Feeding Practices during the First Six Months of Life					Continued Breastfeeding at Age 18 Months		
	Exclusive Breastfeeding	**Formula Feeding**	**Mixed Feeding**	**Formula Feeding Only, but Previously Exclusive Breastfeeding**	***p*-Value [a]**	**Supplementary Breastfeeding**	**No Supplementary Breastfeeding**	***p*-Value [a]**
Sample size [b]	176 (65.2%)	14 (5.2%)	59 (21.9%)	21 (7.8%)		59 (21.8%)	212 (78.2%)	
WAZ [c]	−0.13 ± 1.18	0.03 ± 1.23	0.31 ± 1.25	−0.14 ± 1.24	0.105	0.03 ± 1.25	−0.04 ± 1.20	0.672
LAZ [c]	−0.34 ± 1.25	0.28 ± 1.33	0.10 ± 1.46	−0.32 ± 1.55	0.067	−0.01 ± 1.46	−0.27 ± 1.29	0.225
WLZ [c]	0.04 ± 1.19	−0.18 ± 1.78	0.34 ± 1.29	0.01 ± 1.13	0.335	0.05 ± 1.21	0.10 ± 1.25	0.768
HCZ [c]	0.86 ± 1.17	0.83 ± 1.12	1.07 ± 1.07	0.60 ± 1.39	0.506	1.04 ± 1.10	0.83 ± 1.19	0.220

Abbreviations: WAZ: weight-for-age z-score; LAZ: length-for-age z-score; WLZ: weight-for-length z-score; HCZ: head circumference z-score Sex-normalized anthropometric indicators at age 18 months were computed using World Health Organization (WHO) Anthro software of 2011, using gestation-adjusted age for preterm infants. [a] Comparison between the study groups. [b] Sample size in numbers and percentages (%). [c] Growth indicators are reported as the means and standard deviations (SD).

4. DISCUSSION

Our study shows that infants who had a dual *in utero* exposure, namely maternal HIV infection and placental insufficiency as measured by an abnormal UmA-RI, had a significantly lower LAZ and higher rates of stunting at 18 months (40.0%), compared to the control group. This finding indicates that maternal HIV infection compounded by unrelated placental insufficiency is an additive risk factor for stunting in SA children. The high percentage of stunting in CHEU contributes to the body of existing knowledge [13,14,32–36], and the finding regarding the high percentage of stunting in CHEU with a history of placental insufficiency and IUGR is novel in the SA context. The prevalence of placental insufficiency and abnormal UmA-RI in SA is high (12%), as reported by Nkosi *et al.* and Hlongwane *et al.*, and far exceeds the numbers previously reported in studies from high-income countries. The etiology is unknown, more so because women included in the SA studies were considered low-risk and healthy at the time of screening during their pregnancies.

Lower weight, length and HC reported in the present study among the CHEU-abnormal UmA-RI group at 18 months implies a high-risk group requiring closer follow-up and optimum nutrition care.

Advanced age in WLHIV with abnormal UmA-RI and maternal lifestyle behaviour such as use of alcohol were observed in the study population, which have been previously identified as risk factors for placental insufficiency and abnormal UmA-RIs by previous studies [16,37,38]. Adequate mean CD4 T cell counts and low viral loads were observed in the studied WLHIV. A high rate of caesarean section deliveries among the low-risk population has been reported in the Tshwane area, SA [39].

A history of diarrhea was common in the study children, even though most of the mothers self-reported having access to running water and flushing toilets, and diarrhea is known to be a common condition in early childhood. The reported high vertical HIV transmission prophylaxis in the CHEU group points to the success of the prevention of mother to child transmission of HIV (PMTCT) program in SA [40–42], with the high percentages of exclusive breastfeeding across the study groups showing improved support and promotion of breastfeeding in SA. Nonetheless, mixed feeding was observed in the present study population, including in CHEU, despite their risk of vertical HIV acquisition. Low adherence to breastfeeding guidelines have been documented in a South African cohort of CHEU and CHUU followed up until 18 months of age [40]. The WHO's 2016 recommendations on HIV and infant feeding advocate the same breastfeeding practices for all women irrespective of maternal HIV status, within the context of support for adherence to ART for WLHIV [43]. A high stunting rate was observed in the CHEU-abnormal UmA-RI group, with more than half being exclusively breastfed. A systematic review in LMICs stated that limited evidence exist between breastfeeding and growth outcomes [44]. Contrarily, previous studies, including studies from SA, have reported a positive association between exclusive breastfeeding and child growth [13,32,34,45].

Findings on low mean LAZ at 18 months of age in CHEU compared to CHUU counterparts were similar to reports from other African countries, with high stunting rates reported in Ethiopian (27.8%) [46], Nigerian (44.3%) [13] and Kenyan CHEU (20%) [14]. Many other studies have also reported suboptimal growth outcomes in CHEU compared to CHUU [32–36]. Szanyi et al., reported an association between stunting and maternal peri- and postnatal HIV exposure [46]. The present findings differed to those reported by Ramokolo et al. in SA [9] and in Malawi [47], as lower LAZ was observed in CHEU than CHUU. The findings on comparisons of growth outcomes between normal vs. abnormal UmA-RI were similar to those reported in Mexico, which showed a low mean LAZ (−1.22 ± 0.95) and a slightly higher percentage of stunting in participants born with IUGR [7,15,48]. Inadequate growth outcomes in children born with IUGR have been documented in the Philippines and Austria, with a reported association between stunting and IUGR [7,48,49]. According to Stranix-Chibanda et al. (2020), predictive factors for suboptimal growth trajectories, which are most pronounced for LAZ, include IUGR, low birth weight and length [50].

Research on the growth parameters of CHEU with abnormal UmA-RI is limited, with no published reports on the growth outcomes of children exposed to these dual insults. At 18 months of age, CHEU with abnormal UmA-RI had lower mean anthropometric measurements (weight, length and HC) and indicators (WAZ, LAZ and HCZ). The present study population's

normal WLZ and BMI suggest that CHEU with abnormal UmA-RI were symmetrically growth restricted and, as a result, would not be obviously visible within primary healthcare services if not well plotted on growth charts.

A lower mean weight, BMI and MUAC were also observed in the mothers of the CHEU with abnormal UmA-RI, implying that maternal nutrition may negatively influence the child's linear and ponderal growth. Previous findings indicated that maternal financial situation, BMI, nutrition, education, and age positively correlate with the health of their children [51]. The combination of the dual insult may carry a huge risk of suboptimal child growth, specifically length growth. Advanced age of WLHIV with abnormal UmA-RI may also be attributed to low growth indicators as it may influence childcare and feeding practices. Nevertheless, Fall and colleagues' findings in a normal population showed that children born to older mothers have less risk of stunting [52].

This study determined and compared the growth outcomes of CHEU with abnormal UmA-RI, a population that was previously unstudied. Study limitations include the small sample size of the subgroups of concern, particularly CHEU with abnormal UmA-RI (5.5%). Further, the high caesarean section rate for a low-risk pregnant population might indicate bias in obtaining a study population born to women with otherwise low-risk pregnancies. The caesarean section rate for the whole Umbiflow-International study (SA arm) group was 28%, which is similar to the rate of 30.1% in this subset. The fact that the children were investigated as a one-off at age 18 months meant that longitudinal data analysis was not possible; therefore, a lack of evaluation of growth over time was a drawback. Additionally, a history of childhood illnesses and breastfeeding practices were based on maternal recall. Future research should investigate CHEU with abnormal UmA-RI over a longer duration in order to better understand their long-term growth trajectories.

5. CONCLUSIONS

The present study determined and compared the growth outcomes of 18-month-old children born with and without IUGR due to placental insufficiency, modified by maternal HIV status. CHEU with abnormal UmA-RI had lower WAZ, LAZ and HCZ, and are especially at a significantly increased risk of stunting. Maternal HIV exposure and placental insufficiency are independent risk factors for childhood stunting, with this risk potentiated when these two risk factors are compounded.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee) of the University of Pretoria (protocol code NAS259/2021 on 12 November 2021).

Informed Consent Statement: Informed consent was obtained from all study participants.

Data Availability Statement: Data are available on request from the corresponding author, due to the University of Pretoria policy on data publication.

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CHAPTER 5: MANUSCRIPT 2

Article 1, discussed in Chapter 4, demonstrated that exposure to maternal HIV and placental insufficiency were risk factors for suboptimal growth, especially stunting, this then necessitated also investigating neurodevelopment, due to the known associations between childhood stunting and suboptimal development. Additionally, the study explored the dietary nutrient intake of these children, with a specific focus on micronutrients, to better understand whether the identified differences between the groups were possibly associated with differences in diet.

Therefore, the present chapter reports on child neurodevelopmental outcomes and micronutrient intakes, and the associations between micronutrient intakes and growth vs neurodevelopmental domains. The study population was also categorized into four subgroups as in Article 1 but with added abbreviations for group names: CHUU with normal UmA-RI (CHUU/N-RI; control group), CHEU with normal UmA-RI (CHEU/N-RI; single exposure), CHUU with abnormal UmA-RI (CHUU/AbN-RI; single exposure) and CHEU with abnormal UmA-RI (CHEU/AbN-RI; double exposure). The findings included comparisons between the groups.

The chapter is presented in article format as per the author's guidelines of the International Journal of Paediatrics, to which it has been submitted to be considered for publication. The font, line spacing and spelling were kept the same in the thesis for uniformity.

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GROWTH, NEURODEVELOPMENTAL OUTCOMES AND MICRONUTRIENT INTAKE IN 18-MONTH-OLD SOUTH AFRICAN CHILDREN WITH MATERNAL HIV EXPOSURE AND PLACENTAL INSUFFICIENCY: THE UMBIGODISA CROSS-SECTIONAL STUDY

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ABSTRACT

Background. Exposure to maternal HIV-infection and intrauterine growth restriction (IUGR) are both associated with suboptimal child growth and neurodevelopment. We assessed and compared growth and neurodevelopmental outcomes and micronutrient intakes in children who are HIV-exposed-uninfected (CHEU) and children who are HIV-unexposed-uninfected (CHUU), stratified by history of placental insufficiency. *Methods.* An abnormal umbilical artery resistance index (UmA-RI) on pregnancy Doppler ultrasound was used to detect placental insufficiency, as a proxy for IUGR. At 18-months postnatally, 264 mother-child pairs were followed-up and categorized into four subgroups: CHUU with normal UmA-RI (CHUU/N-RI; control group), CHEU with normal UmA-RI (CHEU/N-RI; single exposure), CHUU with abnormal UmA-RI (CHUU/AbN-RI; single exposure) and CHEU with abnormal UmA-RI (CHEU/AbN-RI; double exposure). A quantified single 24-hour dietary recall was used to collect dietary intake. Anthropometry measurements and z-scores were performed and computed according to standard procedures. Bayley-III scales to test for cognitive, language and motor development at corrected age. FoodFinder™ 3.0 was used for meal analysis, quantifying dietary intake of iron, zinc, and iodine. Comparisons were performed using the independent t-test and the Mann-Whitney U test. Associations were determined by Spearman's correlation. *Results.* CHEU/AbN-RI had significantly lower z-scores when compared with CHUU/N-RI: length-for-age z-score (-1.4±1.4 vs 0.0±1.3; p=0.001) and weight-for-age z-score (-0.6±1.0 vs 0.0±1.2; p=0.024). On Bayley tests, CHEU/AbN-RI had lower mean cognitive scores compared to CHUU/N-RI: 93.9±12.9 vs 100.1±10.8; p=0.042, with 21.4% of CHEU/AbN-RI having a mild delay in cognitive development. Overall, the mean language composite score was low in this population. Further, zinc intake and weight-for-age z-score were positively associated with language (r=0.10; p=0.042) and motor (r=0.10; p=0.028) developmental domains, respectively. Growth parameters of CHEU were positively associated with cognitive and motor developmental domains. *Conclusion.* CHEU/AbN-RI had suboptimal growth, particularly stunting, and delay in cognitive development compared to CHUU/N-RI, indicating a high-risk population in need of identification and appropriate interventions within Child Health/Nutrition programs.

Keywords: Growth, neurodevelopment, HIV-exposure, placental insufficiency, micronutrient intake

1. INTRODUCTION

Nutrition plays a critical role in early growth and brain development, and nutritional deficiencies are major contributors to delayed neurodevelopment in low- and middle-income countries (LMICs) [1, 2]. Suboptimal infant feeding practices are a leading cause of nutritional deficiencies [2]. The association between iron deficiency and neurodevelopment has been documented in the literature [3-8], with complementary diets fed to infants in developing countries previously found to contain suboptimal amounts of iron and zinc [9]. Inadequate iron, zinc and iodine intake during critical periods of rapid development is known to lead to impaired cognitive and motor development in animal model studies [5, 10, 11].

Child neurodevelopment is a global public health priority. Estimates are that almost half (43.0%) of children under five years are at risk of not fully attaining their developmental potential, particularly in LMICs [2, 12]. Child neurodevelopment is influenced by a multitude of pre- and post-natal maternal and child factors and exposures to environmental and biological risks, including HIV infection, placental insufficiency and suboptimal nutrition [4, 13].

In South Africa, the prevalence of HIV infection in pregnant women is approximately 30.0% [14]. The provision of antiretroviral therapy (ART) has resulted in improved maternal health, as well as the successful prevention of mother-to-child transmission (PMTCT) of HIV [12, 15, 16]. Therefore, the number of children who are HIV-exposed-uninfected (CHEU) is increasing, estimated at 15.4 million in 2020 globally [17] and 3.5 million in South Africa alone [18]. Pre- and postnatal exposure to maternal HIV infection and ART potentially affects growth and neurodevelopmental outcomes in CHEU [17], who have in several studies been shown to have poorer growth and neurodevelopmental outcomes, compared to children who are HIV-unexposed-uninfected (CHUU) [19-23]. Furthermore, Wedderburn and colleagues reported the risk of maternal HIV exposure on child's early structural brain development [24].

Placental insufficiency can be detected by an abnormal umbilical artery resistance index (UmA-RI) on Doppler ultrasound [25]. South African women, even those with seemingly low-risk pregnancies, have previously been found to have an unexpectedly high prevalence of abnormal UmA-RI of between 6% and 13% [26-28]. Placental insufficiency is associated with raised umbilical-placental vascular resistance and suboptimal blood flow, resulting in fetal starvation [29], and hence causes intrauterine growth restriction (IUGR) [25, 30], which in turn is strongly associated with suboptimal child neurodevelopment due to intrauterine hypoxia [26-28]. Additionally, maternal HIV infection has been reported as a risk factor for IUGR [31, 32],

due to inflammation and dysregulation of placental vasculogenesis and angiogenesis, resulting in altered placental function [33-35].

The first 1000 days of life are a period of incredible potential and enormous vulnerability [36-38]. Despite the huge risks posed by HIV infection and placental insufficiency to early and late child development, the postpartum neurodevelopmental outcomes of children with both of these risk factors have been overlooked. Additionally, there is a lack of information on the effects of feeding practices, particularly iron, zinc and iodine intakes on neurodevelopment, especially in the context of HIV exposure and placental insufficiency. This study therefore assessed, compared and determined associations between iron, zinc and iodine intakes and growth and neurodevelopmental outcomes in CHEU and CHUU born with and without IUGR in the Tshwane District in the Gauteng Province of South Africa.

2. MATERIALS AND METHODS

2.1. Study setting

The UmbiGodisa study followed up children born to mothers recruited from the South African arm of the Umbiflow International study, which determined the prevalence of abnormal UmA-RI in unselected low-risk women at 28 - 34 weeks' gestation through a single screening with the Umbiflow™ Doppler device in South Africa, India, Ghana, Kenya and Rwanda. The cut-off centiles for normal and abnormal UmA-RI were $<75^{th}$ and $\geq 75^{th}$ for the gestational age [39], respectively, previously linked to risk of suboptimal pregnancy outcomes like stillbirths. The South African study participants were screened from October 2018 to January 2020. To expand the study population of CHEU with abnormal UmA-RI, the current study enrolled additional participants from the Siyakhula study, carried out in the same locality, which assessed growth and neurodevelopment of CHEU, with similar study procedures and with pregnancy Doppler data available. The study participants were followed up at the University of Pretoria's Research Centre for Maternal, Fetal, Newborn and Child Health Care Strategies, located at Kalafong Provincial Tertiary Hospital in the Gauteng Province of South Africa.

2.2. Study design

UmbiGodisa was a descriptive cross-sectional study, which collected information on feeding practices, micronutrient intake and developmental outcomes of children with abnormal UmA-RI, as a marker for IUGR, compared to their counterparts with normal UmA-RI, mediated by maternal HIV infection. The study outcome for this manuscript was child neurodevelopment, namely cognitive, motor and language development, while the modifying factors included

infant growth parameters, dietary iron, zinc and iodine intake and feeding practices. The mother-child pairs were only assessed once, at 18 months postpartum. Participants were classified into four subgroups according to HIV exposure and normal/abnormal UmA-RI: CHUU with normal UmA-RI (CHUU/N-RI; control group), CHEU with normal UmA-RI (CHEU/N-RI; single exposure), CHUU with abnormal UmA-RI (CHUU/AbN-RI; single exposure) and CHEU with abnormal UmA-RI (CHEU/AbN-RI; double exposure).

2.3. Study participants

Mothers with low-risk singleton pregnancies, with known HIV status and UmA-RI at 28-34 weeks, who delivered in local facilities with available pregnancy and birth information, were included in the study. Exclusion criteria included multiple pregnancies, lack of informed consent for any reason, minors, children with abnormalities or severe medical conditions and children aged above 21 months at the time of study. Power analysis indicated that an overall sample size of 280 was sufficient with a split of 80/20% for CHUU and CHEU groups. The anticipated sample size was 311 from the Umbiflow International cohort, however, 46 participants were not interested to join the study, others had aged above the set upper limit (n=1), had severe medical conditions (n=2) or had withdrawn (n=1). Therefore, 261 participants were recruited but 7 participants were excluded from analysis due to obvious developmental abnormalities such as encephalopathy (n=3) and incomplete questionnaires or Bayley assessment (n=4). Overall, 264 participants were included in the analysis, which included the additional 10 participants from the Siyakhula study. The subgroups size: CHUU/N-RI: n = 181; CHEU/N-RI: n = 50; CHUU/AbN-RI: n = 19; and CHEU/AbN-RI: n = 14 (Figure 5.1).

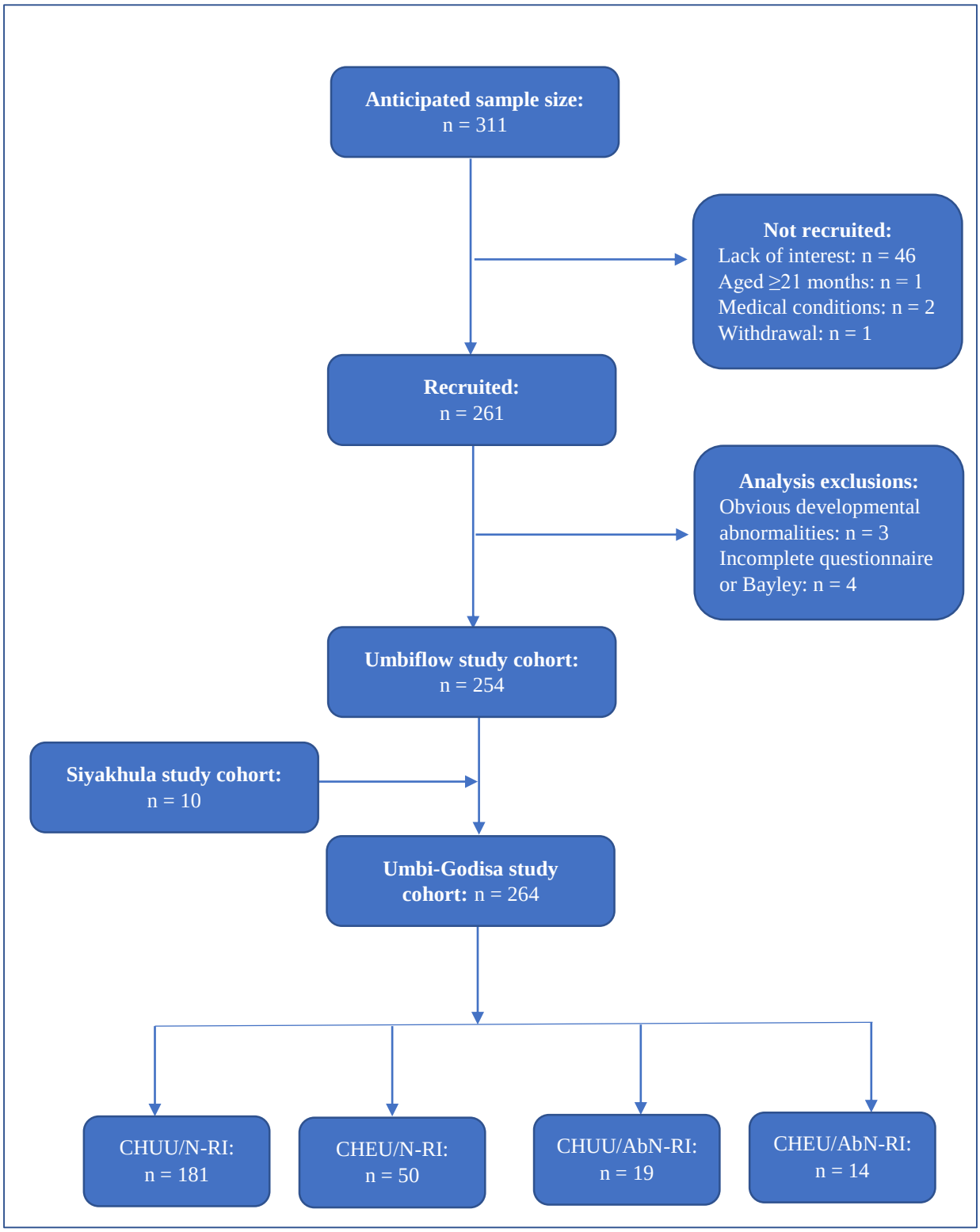

Figure 5.1: Study cohort

2.4. Data collection

Mothers were booked telephonically for a study visit. Trained study staff obtained the informed consent and facilitated the data collection process from February to December 2021. The face-

to-face maternal interviews and child growth and development assessments were conducted in local languages or English.

Sociodemographic, feeding practices and dietary intake data

A structured questionnaire was used to collect maternal-child sociodemographic variables and medical history. The household food security status was assessed using the adopted United States Household Food Security Survey Module [40]. The limitation of this tool is that it does not provide specific information on food security of children. The breastfeeding data over the first 6 months of life was collected using a standardized and previously used questionnaire based on maternal recall [41]. The validated and quantified single 24-hour dietary recall questionnaire was administered by trained study staff to gather the children's dietary intake, as reported by the mother [42-44]. A detailed manual for administering a 24-hour dietary recall was available for guidance [45] and a standardized dietary kit was utilized. Breast milk substitutes and infant cereals were recorded per dry amount of grams (g) [42, 46].

Growth and neurodevelopmental assessment

Child weight, length and head circumference (HC) measurements were performed according to standard procedures, and measured twice and the mean value was used. We used calibrated digital scale (Seca 354); mechanical infantometer (Seca 416) and non-stretchable tape measure for weight, length and HC measurements, respectively. The developmental screening was performed using the International Guide for Monitoring Child Development (GMCD) at the corrected age for prematurity, which entails a series of open-ended questions, according to the child's age, on functional domains including expressive and receptive language, gross and fine movements, relating and response behaviour, play, and self-help activities, that were answered by the child's caregiver [47]. The reliability and validity of GMCD have been reported by studies in Turkey and other LMIC settings [48]. An individually administered Bayley Scales of Infant and Toddler Development™ third edition (Bayley-III) was used to assess the cognitive, language (expressive and receptive) and motor (fine and gross) domains at the corrected age, using the Bayley-III kit. Bayley-III is a standardized and well-accepted tool that takes about 45 - 60 minutes to complete, and it may overestimate child performance [49, 50]. GMCD was performed first, then Bayley-III, and they were completed using different trained study staff to avoid bias. Bayley-III was assessed when the child was in an alert state.

2.5. Data processing and statistical analysis

All data was managed using the Research Electronic Data Capture database v9.3.5. Outliers were examined for plausible values and/or errors in data capturing. The reported household food intake measurements were converted to weight in grams [51]. South African based FoodFinder™ 3.0 was used for meal analysis of food intake, quantifying the dietary intake of iron, zinc, and iodine. FoodFinder™ 3.0 is programmed according to the use of iodized salt in South Africa. The Estimated Average Requirement (EAR) of 1 to 3 year old children for iodine, iron, and zinc are 65 mcg, 3.0 mg, and 2.5 mg per day, respectively [52]. The World Health Organization Anthro software was used to compute z-scores using corrected ages. Underweight, stunting, wasting and microcephaly were defined as weight-for-age z-score (WAZ), length-for-age z-score (LAZ), weight-for-length z-score (WLZ) and HC-for-age z-score (HCZ) of <-2, respectively, and the cut-off of <-3 was used as severe classifications. GMCD was classified as age-appropriate development if all milestones were attained; delay if one or more milestones were not attained in the appropriate age group and a significant delay if the child did not attain milestones in the appropriate age group and the previous age group. The Bayley-III composite score was calculated based on a comparison of the child to a normative age-matched sample established by the assessment and were interpreted as follows for any of the five domains, namely a) mid-average functioning: composite score of 100 (15 SD standardized mean score); b) mild impairment/at risk of developmental delay: <85 (1 SD < mean); c) moderate impairment: composite score of <70 (2 SD < mean); d) severe impairment: composite score of <55 [53].

All statistical analysis was performed using R Statistical Software version 4.3.0. The Shapiro Wilk test was used to determine if the data was normally distributed. The four groups were compared for descriptive statistics using ANOVA tests for the normally distributed data and the Kruskal-Wallis H test for the non-normally distributed data, for all continuous variables. For each of the variables which were significantly different, a posthoc analysis which included the Bonferroni correction was performed to determine which groups differed from which and the adjusted p-values were used. The Chi-squared test and Fisher's exact test were used for categorical variables. For the micronutrient intake, anthropometry measurements and Bayley-III, the three exposure groups were compared against the control group, where the independent t-test was used for normally distributed variables and the Mann-Whitney U test was used for variables with a non-normal distribution. Groups with very low cell counts were excluded in the analysis as smaller groups lead to volatile results (presented as "n/a" in tables). Spearman's correlation was used to determine associations and their significances between Bayley-III and

other variables. The correlation coefficient can be between -1 and +1, with stronger associations closer to the outer bounds (-1 and +1).

3. RESULTS

About 36.8% of CHUU/AbN-RI were born prematurely (<37 weeks' gestation) (Table 5.1). The mean birth weights were significantly different between the four groups (p<0.001). A posthoc analysis indicated that CHUU/N-RI had a higher mean birth weight than CHEU/AbN-RI (p=0.004) and CHUU/AbN-RI (p<0.001). Furthermore, CHEU/N-RI had a higher mean birth weight compared to CHUU/AbN-RI (p=0.002). A smaller mean HC was observed at birth in CHUU/AbN-RI when compared to CHUU/N-RI (p=0.004) and CHEU/N-RI (p=0.009). All CHEU were on ART prophylaxis and their mothers self-reported being on ART, with an overall latest mean CD4 count of 448 ± 298 cells/mm^3. Maternal mean age differed significantly between the four groups; p<0.001. A posthoc analysis showed that mothers of the CHEU/AbN-RI were the oldest (37.1 ± 5.9 years) compared to mothers of the CHUU/AbN-RI (28.8 ± 4.2 years; p<0.001), CHEU/N-RI (31.5 ± 5.4 years; p=0.007) and the control group (30.1 ± 5.1 years; p<0.001). Median gravidity and parity also differed significantly between the four groups; both p=0.007. Further analysis demonstrated that CHEU/AbN-RI had higher median gravidity (p = 0.028) and parity (p = 0.010) than the control group. More than 50% of mothers of CHEU/AbN-RI delivered by caesarean section. Further, most mothers in all groups were single, unemployed, resided in formal townships and had attained some form of secondary schooling. Overall, 48.8% of mothers were classified as being food insecure according to the US Household Food Security Survey Module. More than a quarter (30.0%) of mothers of CHUU/N-RI were drinking alcohol after pregnancy and 4.0% of mothers of CHEU/AbN-RI were smoking cigarettes after pregnancy.

Table 5.1: Participant and maternal characteristics and medical history

Measures	Subgroup	Statistic	CHUU/N-RI (control group)	CHEU/N-RI (single exposure)	CHUU/AbN-RI (single exposure)	CHEU/AbN-RI (double exposure)	P-value[a]
Sample size		N	181	50	19	14	
Age (months)		Mean ± SD	18.6 ± 0.9	18.5 ± 0.8	18.2 ± 0.2	18.8 ± 1.0	0.422
Sex	Female	*n* (%)	92 (50.8)	22 (44.0)	11 (57.9)	10 (71.4)	0.305
Premature birth		*n* (%)	15 (8.3)	2 (4.0)	7 (36.8)	1 (7.1)	n/a
Birth weight (g)		Mean ± SD	3194 ± 480	3108 ± 433	2650 ± 581	2742 ± 396	**<0.001**
Head circumference at birth (cm)			34.5 ± 1.7	34.5 ± 1.5	32.9 ± 1.9	34.0 ± 1.6	**0.006**
Birth weight z-score			-0.30 ± 1.16	-0.54 ± 0.96	-0.43 ± 1.08	-0.98 ± 0.75	0.105
Head circumference z-score			0.37 ± 1.32	0.35 ± 1.24	0.23 ± 0.97	0.50 ± 1.11	0.865
Apgar score at 5 minutes		Median [IQR]	10.0 [9.0, 10.0]	9.0 [9.0, 10.0]	9.0 [9.0, 9.0]	9.0 [9.0, 9.0]	**0.045**
Hospital admission during neonatal period		*n* (%)	33 (18.3)	6 (12.0)	6 (31.6)	1 (7.7)	n/a
HIV prophylaxis started [b]	NVP only	*n* (%)	N/A	41 (93.2)	N/A	6 (42.9)	n/a
	NVP and AZT		N/A	3 (6.8)	N/A	8 (57.1)	
Child ever had any form of malnutrition		*n* (%)	11 (6.1)	6 (12.0)	0 (0)	0 (0)	n/a
Child ever had diarrhoea			57 (31.5)	13 (26.0)	4 (21.1)	0 (0)	n/a
Child ever hospitalized for any illness			18 (9.9)	5 (10.0)	0 (0)	0 (0)	n/a
Maternal age (years)		Mean ± SD	30.1 ± 5.1	31.5 ± 5.4	28.8 ± 4.2	37.1 ± 5.9	**<0.001**
Obstetric history	Gravidity	Median [IQR]	2 [1, 3]	3 [2, 3]	2 [2, 3]	4 [3, 4]	**0.007**
	Parity		2 [1, 3]	2 [2, 3]	2 [2, 3]	3 [3, 3]	**0.007**
	Previous pregnancy losses		0 [0, 0]	0 [0, 1]	0 [0, 0]	0 [0, 1]	0.383
	Preeclampsia/ eclampsia	*n* (%)	2 (13.3)	0 (0)	0 (0)	0 (0)	n/a
	Postpartum complications		23 (12.7)	5 (10.0)	2 (10.5)	0 (0)	n/a
Umbilical artery Doppler	UmA-RI value at 28-34 weeks' gestation	Mean ± SD	0.64 ± 0.1	0.63 ± 0.0	0.75 ± 0.1	0.76 ± 0.0	**0.001**
	UmA-RI z-score		0.03 ± 0.7	-0.10 ± 0.6	1.81 ± 0.9	1.78 ± 0.7	**<0.001**
Mode of delivery	Vaginal delivery	*n* (%)	126 (69.2)	31 (62.0)	9 (47.4)	5 (35.7)	**<0.001**
	Caesarean section		54 (29.8)	19 (38.0)	10 (52.6)	9 (64.3)	
Latest CD4 count	cells/mm^3	Mean ± SD	N/A	463 ± 310	N/A	416 ± 295	0.965
Latest HIV viral load	copies/mL (log)	Median [IQR]	N/A	0.0 [0.0, 0.0]	N/A	0.0 [0.0, 0.0]	0.798
Current ART	TDF/FTC/EFV	*n* (%)	N/A	31 (62.0)	N/A	7 (50.0)	0.251
	Other ART		N/A	10 (20.0)	N/A	6 (42.9)	
	Not recorded		N/A	9 (18.0)	N/A	1 (7.1)	
Marital status	Single	*n* (%)	114 (63.0)	33 (66.0)	10 (52.6)	7 (50.0)	0.592
	Married/co-habiting		67 (37.0)	17 (34.0)	9 (47.4)	7 (50.0)	
Educational level	Any primary schooling	*n* (%)	13 (7.0)	4 (8.0)	3 (15.8)	2 (14.3)	0.221
	Any secondary schooling		124 (68.5)	40 (80.0)	12 (63.2)	11 (78.6)	
	Post-school education		44 (24.3)	6 (12.0)	4 (21.1)	1 (7.1)	
	Unemployed	*n* (%)	110 (60.8)	29 (58.0)	10 (52.6)	10 (71.4)	0.736

Maternal employment status	Employed		71 (39.2)	21 (42.0)	9 (47.4)	4 (28.6)	
Partner's employment status	Unemployed	*n* (%)	29 (16.9)	7 (14.3)	3 (15.8)	2 (14.3)	0.987
	Any type of employment		143 (83.1)	42 (85.7)	16 (84.2)	12 (85.7)	
Monthly household income (in ZAR) [c]	R 0 - R 2000	*n* (%)	36 (20.1)	7 (14.0)	0 (0)	4 (28.6)	n/a
	R 2001 - R 4000		42 (23.5)	15 (30.0)	8 (42.1)	0 (0)	
	R 4001 - R 6000		38 (21.2)	13 (26.0)	5 (26.3)	5 (35.7)	
	R 6001 - R 8000		11 (6.1)	5 (10.0)	1 (5.3)	2 (14.3)	
	R 8000 +		43 (24.0)	9 (18.0)	5 (26.3)	1 (7.1)	
	Don't know		9 (5.0)	1 (2.0)	0 (0)	2 (14.3)	
Household adult food security status	Food secure	*n* (%)	89 (49.2)	25 (50.0)	11 (57.9)	10 (71.4)	0.560
	Food insecure		92 (50.8)	25 (50.0)	8 (42.1)	4 (28.6)	
Description of neighbourhood	Formal township	*n* (%)	114 (63.0)	27 (54.0)	14 (73.7)	7 (50.0)	0.348
	Informal settlement		67 (37.0)	23 (46.0)	5 (26.3)	7 (50.0)	
Postnatal lifestyle behaviour	Any alcohol drinking[d]	*n* (%)	54 (30.0)	11 (22.4)	2 (12.2)	1 (7.7)	0.167
	Any cigarettes smoking [e]		3 (1.7)	2 (4.0)	0 (0.0)	1 (7.7)	n/a
Maternal mental health assessment [f]							
	Little interest or pleasure in doing things	*n* (%)	41 (22.9)	15 (30.0)	1 (5.9)	2 (15.4)	0.419
	Feeling down, depressed or hopeless		53 (29.6)	20 (40.0)	2 (11.8)	2 (15.4)	0.623
Maternal self-assessed general health rating							
	Well	*n* (%)	165 (91.1)	44 (88.0)	18 (94.7)	14 (100.0)	n/a
	Fair		13 (7.2)	6 (12.0)	0 (0)	0 (0)	
	Poor		3 (1.7)	0 (0)	1 (5.3)	0 (0)	

Notes: AbN-RI: abnormal umbilical artery resistance index; ART: antiretroviral therapy; AZT: zidovudine; CD4: a cluster of differentiation 4; CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; IQR: interquartile range; N-RI: normal umbilical artery resistance index; NVP: nevirapine; SD: standard deviation; TDF/FTC/EFV: tenofovir/ emtricitabine/ efavirenz; UmA-RI: umbilical artery resistance index; ZAR: South African Rand.

[a] Comparison was made between the four groups. The ANOVA test was used for the normally distributed data and the Kruskal Wallis H test was used for the data that was not normally distributed, for continuous variables. The Chi-squared test and Fisher's exact test were used for categorical variables. All tests were performed at a 5% level of significance. Significant difference is indicated by a bold p-value.
[b] CHEU/N-RI: n=44
[c] One South African Rand equates to 0.056 United States Dollars.
[d] Question asked: Since your baby was born, did you drink alcohol?
[e] Question asked: Since your baby was born, did you smoke cigarettes?
[f] CHUU/N-RI: n=179; CHUU/AbN-RI: n=17; CHEU/AbN-RI: n=13

On maternal recall, a high percentage of children (95.8%) were ever breastfed across the groups (Table 5.2). Early initiation of breastfeeding within one hour after birth was two-thirds or above in the CHEU/AbN-RI and CHUU/N-RI groups. The percentage of exclusive breastfeeding (EBF) in the first 6 months was high among CHEU/N-RI (40.0%) and CHEU/AbN-RI (42.9%) while percentage of mixed feeding was high in CHUU/N-RI (58.0%) and CHUU/AbN-RI

(52.6%). CHEU/N-RI stopped breastfeeding earlier at mean 7.1 ± 5.1 months than CHEU/AbN-RI (8.2 ± 5.2 months), CHUU/AbN-RI (8.8 ± 5.3 months) and CHUU/N-RI (11.4 ± 5.5 months); p<0.001. The mean introduction of cow's milk was earlier than the recommended twelve months of age in three of the groups (CHUU/N-RI: 11.6 ± 3.4, CHUU/AbN-RI: 9.6 ± 5.5 and CHEU/AbN-RI: 10.4 ± 2.9 months). CHEU/AbN-RI were introduced to protein-rich foods later at 11.2 ± 3.1 months than other groups; p=0.048. There were no significant differences for the intake of iron, zinc and iodine between CHUU/N-RI vs CHEU AbN-RI. Iodine intake was lower among CHUU/N-RI when compared to CHEU/N-RI (p=0.033) and CHUU/AbN-RI (p=0.005). Generally, the iodine intake was below the EAR across all the groups.

Table 5.2: Child feeding practices based on maternal recall and micronutrient intake

Measures	Subgroup	Statistic	CHUU/N-RI (control group)	CHEU/N-RI (single exposure)	CHUU/AbN-RI (single exposure)	CHEU/AbN-RI (double exposure)	P-value[a]
Sample size		N	181	50	19	14	
Ever breastfed	Yes	*n* (%)	173 (95.6)	47 (94.0)	19 (100.0)	14 (100.0)	0.601
Early initiation of breastfeeding after birth [b]	Within 1 hour	*n* (%)	121 (78.1)	21 (48.8)	8 (50.0)	8 (66.7)	**0.001**
	After 1 hour		34 (21.9)	22 (51.2)	8 (50.0)	4 (33.3)	
Feeding practices in the first 6 months	EBF	*n* (%)	58 (32.0)	20 (40.0)	7 (36.8)	6 (42.9)	n/a
	Formula feeding		11 (6.1)	3 (6.0)	0 (0)	0 (0)	
	Mixed feeding		105 (58.0)	20 (40.0)	10 (52.6)	3 (21.4)	
	Initially EBF, then formula feeding only		6 (3.3)	7 (14.0)	2 (10.5)	5 (35.7)	
Currently breastfeeding	Yes	*n* (%)	48 (26.7)	3 (6.1)	4 (23.5)	2 (15.4)	n/a
Age at weaning (in months)		Mean ± SD	11.4 ± 5.5	7.1 ± 5.1	8.8 ± 5.3	8.2 ± 5.2	**<0.001**
Introduction of food and timing of introduction							
Formula milk	Age (months)	Mean ± SD	5.6 ± 5.2	5.1 ± 3.8	6.1 ± 4.0	7.6 ± 8.3	0.815
Water			4.1 ± 2.1	4.6 ± 2.2	4.4 ± 1.8	4.6 ± 2.2	0.318
Tea, juice			9.7 ± 3.9	9.0 ± 4.0	8.9 ± 3.6	11.4 ± 4.3	0.235
Cow's milk			11.6 ± 3.4	12.7 ± 2.7	9.6 ± 5.5	10.4 ± 2.9	0.150
Semi-solids, e.g. cereals, porridge			5.2 ± 1.6	5.2 ± 1.7	5.5 ± 1.0	5.7 ± 1.7	0.388
Solids, e.g. vegetables, fruit			8.8 ± 3.5	8.3 ± 3.3	7.7 ± 2.2	10.1 ± 4.4	0.474
Protein rich foods, e.g. eggs			9.7 ± 3.4	8.9 ± 3.7	8.5 ± 2.6	11.2 ± 3.1	**0.048**
Dietary nutrients intake[c]	Iron (mg)	Mean ± SD	6.4 ± 5.1	6.9 ± 4.3	7.8 ± 7.3	6.3 ± 3.3	0.510
	Zinc (mg)		5.1 ± 5.2	5.5 ± 3.4	5.5 ± 2.8	4.6 ± 2.6	0.871
	Iodine (mcg)		26.2 ± 15.3	41.7 ± 23.9	45.4 ± 16.4	36.0 ± 18.5	0.094

Notes: AbN-RI: abnormal umbilical artery resistance index; EBF: exclusive breastfeeding; CHUU: children who are HIV-unexposed-uninfected; CHEU: children who are HIV-exposed-uninfected; mcg: micrograms; mg: milligrams; N-RI: normal umbilical artery resistance index; SD: standard deviation.

[a] Comparison was made between the four groups. The ANOVA tests was used for the normally distributed data and the Kruskal-Wallis H test was used for the data that was not normally distributed. The Chi-squared test and

Fisher's exact test were used for categorical variables. All tests were performed at a 5% level of significance. Significant difference is indicated by a bold p value.
[b] CHUU/N-RI: n=155; CHEU/N-RI: n=43; CHUU/AbN-RI: n=16; CHEU/AbN-RI: n=12
[c] Comparison was made between CHUU/N-RI (control group) vs CHEU/AbN-RI (double exposure group). The Mann-Whitney U test was used. All tests were performed at a 5% level of significance. Dietary nutrient intake reported excludes any intake via breastmilk for the children who were still breastfed at the 18-month study visit.

In general, the CHEU/AbN-RI (double exposure) group had significantly lower anthropometric measurements and growth indices at age 18 months when compared to the CHUU/N-RI (control) group (Table 3). These include weight (kg) (9.9 ± 1.1 vs 10.9 ± 1.6; p=0.015), length (cm) (78.1 ± 3.7 vs 81.9 ± 3.8; p=0.001) and HC (cm) (47.3 ± 1.1 vs 48.1 ± 1.6; p=0.024) and z-scores: WAZ (-0.6 ± 1.0 vs 0.0 ± 1.2; p=0.024), LAZ (-1.4 ± 1.4 vs 0.0 ± 1.3; p=0.001) and HCZ (0.4 ± 0.7 vs 0.9 ± 1.2; p=0.035), while the WLZ were similar (0.0 ± 0.8 vs 0.1 ± 1.2; p=0.843).

The findings of the GMCD screening indicated that 21.4% of CHEU/AbN-RI (double exposure) and 10.0% of CHEU/N-RI (CHEU single exposure) had a delay in gross movements. The Bayley-III assessment showed that the CHEU/AbN-RI (double exposure) group had a lower mean cognitive composite score than the control group: 93.9±12.9 vs 100.1±10.8; p=0.042, and lower motor composite score compared to the control group, but this was not significant. Further, 21.4% of CHEU/AbN-RI showed a mild delay in the cognitive developmental domain. Overall, the mean language composite score was low in this population (overall mean was 89.4 ± 12.3) (Table 5.3). In the total study population, zinc intake was positively associated with the language domain (r=0.10; p=0.042), while WAZ was positively associated with the motor (r=0.10; p=0.028) developmental domain, and while statistically significant the correlations were not strong (Table 5.4). In this cohort, iron intake was not significantly associated with any developmental domains. In the CHEU group we found that cognitive development was significantly positively correlated with WAZ (r=0.15; p=0.021) and LAZ (r=0.35; p=0.009), while motor development was significantly positively correlated with WAZ (r=0.32; p=0.007), LAZ (r=0.26; p=0.017), WLZ (r=0.27; p=0.044) and HCZ (r=0.24; p=0.021). In the abnormal UmA-RI group, the motor developmental domain was significantly positively correlated with the HCZ (r=0.43; p=0.038).

Table 5.3: Anthropometric measurements, GMCD screening and Bayley-III results at age 18 months

Measures	Subgroup	Statistic	CHUU/N-RI (control group)	CHEU/N-RI (single exposure)	CHUU/AbN-RI (single exposure)	CHEU/AbN-RI (double exposure)	P-value[a]
Sample size		N	181	50	19	14	
Anthropometric measurements	Weight (kg)	Mean (SD)	10.9 ± 1.6	10.7 ± 1.8	10.8 ± 2.0	9.9 ± 1.1	**0.015**
	Length (cm)		81.9 ± 3.8	80.6 ± 3.3	81.0 ± 4.2	78.1 ± 3.7	**0.001**
	Head circumference (cm)		48.1 ± 1.6	48.1 ± 1.9	48.7 ± 1.7	47.3 ± 1.1	**0.024**
Growth indices [b]	Weight-for-age z-score	Mean (SD)	0.0 ± 1.2	-0.1 ± 1.3	0.0 ± 1.6	-0.6 ± 1.0	**0.024**
	Length-for-age z-score		0.0 ± 1.3	-0.6 ± 1.2	-0.2 ± 1.6	-1.4 ± 1.4	**0.001**
	Weight-for-length z-score		0.1 ± 1.2	0.2 ± 1.5	0.2 ± 1.3	0.0 ± 0.8	0.843
	Head circumference-for-age z-score		0.9 ± 1.2	0.8 ± 1.2	1.4 ± 1.4	0.4 ± 0.7	**0.035**
GMCD Screening results, as reported by the caregiver [c]							
Had concerns about child's development		*n* (%)	6 (3.3)	4 (8.0)	3 (15.8)	2 (14.3)	n/a
Relating	Delay	*n* (%)	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)	n/a
	Significant delay		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Play activities	Delay	*n* (%)	2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	n/a
	Significant delay		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Self-help activities	Delay	*n* (%)	3 (1.7)	0 (0.0)	1 (5.6)	0 (0.0)	n/a
	Significant delay		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Expressive language	Delay	*n* (%)	5 (2.8)	0 (0.0)	1 (5.6)	1 (7.1)	n/a
	Significant delay		5 (2.8)	1 (2.0)	0 (0)	1 (7.1)	
Receptive language	Delay	*n* (%)	0 (0.0)	0 (0.0)	1 (5.6)	0 (0.0)	n/a
	Significant delay		0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)	
Gross movement	Delay	*n* (%)	12 (6.8)	5 (10.0)	1 (5.6)	3 (21.4)	n/a
	Significant delay		1 (0.6)	1 (2.0)	1 (5.6)	0 (0.0)	
Fine movement	Delay	*n* (%)	6 (3.6)	1 (2.2)	1 (5.6)	0 (0.0)	n/a
	Significant delay		2 (1.2)	2 (4.3)	0 (0.0)	0 (0.0)	
Bayley-III composite scores	Cognitive composite scores	Mean (SD)	100.1 ± 10.8	100.4 ± 10.3	98.2 ± 11.7	93.9 ± 12.9	**0.042**
	Language composite scores[d]		89.4 ± 12.5	88.7 ± 11.4	90.6 ± 10.6	90.9 ± 15.8	0.327
	Motor composite scores[d]		100.0 ± 11.7	99.4 ± 12.8	97.9 ± 11.6	95.6 ± 14.2	0.189
Cognitive domain	Normal development	*n* (%)	175 (97.2)	46 (92.0)	18 (94.7)	11 (78.6)	n/a
	Mild delay		4 (2.2)	4 (8.0)	0 (0)	3 (21.4)	
	Moderate delay		1 (0.6)	0 (0)	1 (5.3)	0 (0)	
Language domain[d]	Normal development	*n* (%)	108 (60.0)	31 (62.0)	14 (73.7)	10 (71.4)	n/a
	Mild delay		67 (37.2)	18 (36.0)	5 (26.3)	3 (21.4)	
	Moderate delay		5 (2.8)	1 (2.0)	0 (0)	1 (7.1)	
Motor domain[d]	Normal development	*n* (%)	170 (94.4)	44 (88.0)	18 (94.7)	13 (92.9)	n/a
	Mild delay		10 (5.6)	6 (12.0)	0 (0)	0 (0)	
	Moderate delay		0 (0)	0 (0)	1 (5.3)	1 (7.1)	

Notes: AbN-RI: abnormal umbilical artery resistance index; CHUU: children who are HIV-unexposed-uninfected; CHEU: children who are HIV-exposed-uninfected; cm: centimetre; GMCD: International Guide for Monitoring Child Development; kg: kilograms N-RI: normal umbilical artery resistance index; SD: standard deviation.

[a] The results presented are for the comparison between CHUU/N-RI (control group) vs CHEU/AbN-RI (double exposure group). For normally distributed data, the independent t-test was used, and the Mann-Whitney U tests was used in the non-normally distributed data. All tests were performed at a 5% level of significance. Significant differences are indicated by bolded p-values.
[b] Corrected for prematurity.
[c] No significance tests could be performed due to small cell counts.
[d] Language developmental measure comprises of expressive and receptive subdomains. Motor developmental measure includes fine and gross motor subdomains.

Table 5.4: Associations between different measurements and Bayley-III composite scores

Measures	**Cognitive developmental domain**		**Language developmental domain**		**Motor developmental domain**	
	Association[a]	P-value[b]	Association[a]	P-value[b]	Association[a]	P-value[b]
Total study population						
Daily iron intake (mg)	0.05	0.472	0.05	0.973	0.02	0.777
Daily zinc intake (mg)	0.08	0.062	0.10	**0.042**	0.08	0.128
Daily iodine intake (mcg)	-0.02	0.944	-0.05	0.400	0.01	0.567
Weight-for-age z-score	0.04	0.426	-0.05	0.759	0.10	**0.028**
Length-for-age z-score	0.05	0.284	0.00	0.797	0.07	0.133
Weight-for-length z-score	0.02	0.756	-0.05	0.596	0.10	0.063
Head circumference-for-age z-score	0.03	0.928	0.00	0.974	0.09	0.137
CHUU group						
Daily iron intake (mg)	0.03	0.844	0.00	0.679	0.01	0.726
Daily zinc intake (mg)	0.06	0.174	0.05	0.093	0.06	0.153
Daily iodine intake (mcg)	-0.05	0.177	-0.06	0.130	0.04	0.530
Weight-for-age z-score	-0.04	0.598	-0.11	0.247	0.02	0.426
Length-for-age z-score	-0.04	0.789	-0.03	0.919	-0.01	0.821
Weight-for-length z-score	-0.04	0.627	-0.11	0.148	0.06	0.376
Head circumference-for-age z-score	-0.02	0.499	-0.03	0.614	0.03	0.776
CHEU group						
Daily iron intake (mg)	0.12	0.088	0.19	0.389	0.04	0.977
Daily zinc intake (mg)	0.18	0.019	0.22	0.190	0.10	0.549
Daily iodine intake (mcg)	0.13	0.796	0.02	0.410	-0.04	0.801
Weight-for-age z-score	0.15	**0.021**	0.04	0.196	0.32	**0.007**
Length-for-age z-score	0.35	**0.009**	0.07	0.510	0.26	**0.017**
Weight-for-length z-score	0.21	0.166	0.14	0.185	0.27	**0.044**
Head circumference-for-age z-score	0.32	0.359	0.12	0.429	0.24	**0.021**
Normal UmA-RI group						
Daily iron intake (mg)	0.07	0.125	0.04	0.766	0.00	0.745
Daily zinc intake (mg)	0.11	**0.035**	0.08	**0.044**	0.06	0.123
Daily iodine intake (mcg)	0.00	0.598	-0.08	0.241	0.01	0.746
Weight-for-age z-score	0.01	0.407	-0.06	0.707	0.07	0.129
Length-for-age z-score	0.01	0.747	-0.01	0.738	0.02	0.458
Weight-for-length z-score	0.01	0.993	-0.06	0.497	0.10	0.152
Head circumference-for-age z-score	-0.01	0.879	-0.01	0.811	0.04	0.441
Abnormal UmA-RI group						
Daily iron intake (mg)	-0.23	0.203	0.03	0.441	0.04	0.935
Daily zinc intake (mg)	-0.13	0.488	0.14	0.737	0.15	0.931

Daily iodine intake (mcg)	-0.10	0.630	0.16	0.435	0.09	0.894
Weight-for-age z-score	0.16	0.237	0.01	0.837	0.26	0.077
Length-for-age z-score	0.16	0.252	0.06	0.916	0.35	0.126
Weight-for-length z-score	0.11	0.367	0.02	0.737	0.17	0.121
Head circumference-for-age z-score	0.31	0.093	0.03	0.657	0.43	**0.038**

Notes: CHUU: children who are HIV-unexposed-uninfected; CHEU: children who are HIV-exposed-uninfected; mcg: microgram; mg: milligram; UmA-RI: umbilical artery resistance index.
[a] The Spearman's correlation measure was used. The correlation can be between -1 and +1 with stronger associations closer to the outer bounds (-1 and +1).
[b] The significance test was performed to determine if the association was significantly different from 0, if this was not the case then there was no significant association. The p-values that are <0.05 have significant associations, indicated in bold font.

4. DISCUSSION

The present study successfully investigated the overlooked population of CHEU children who additionally had been exposed to placental insufficiency *in utero*, and found that comparative to their unaffected counterparts, these children had lower growth indices and deficits in cognitive development, as well as risk of delayed motor development. This was a low-risk population in terms of the pregnancy history, apart from the exposure to maternal HIV in the CHEU group. Over a third of CHUU who had IUGR (as measured by an abnormal UmA-RI) were born premature, this being a risk factor for suboptimal growth and development. At birth, children who had IUGR had relatively lower mean birthweights, which is a well-known contributing factor for further poor growth and development. The higher maternal age in the CHEU/AbN-RI group may also have impacted their growth outcomes as advanced maternal age has previously been related to childhood stunting [54]. The percentage of EBF in the first six months reported in HIV exposed groups is better than the previously reported percentage of 37.0% in South African women living with HIV and this may indicate the success of ART programs in protecting, supporting and promoting breastfeeding [55]. In this cohort, water was, on average, given to children from as early as four months, similar to the finding reported in a South African review [56]. Also, cow's milk was introduced to children from nine months. Although the WHO 2023 guideline for complementary feeding of infants and young children recommends the intake of animal milk for non-breastfed infants aged 6 – 11 months, cow's milk interferes with iron absorption due to the high calcium content, increasing the risk of iron deficiency anaemia [57]. Protein-rich foods, crucial for a children's optimal growth, were introduced late in the CHEU/AbN-RI group, at eleven months, placing these children at even higher risk of suboptimal growth and health. In the present study, the dietary intake of iron and zinc were adequate, while the iodine intake was lower than the EAR. Iodine deficiency is the preventable cause of irreversible neurodevelopmental deficits in children [58]. Salt fortification with iodine is the most effective intervention for addressing iodine deficiency. In South Africa,

iodization of table salt is mandatory, however, iodization of salt used in food production is voluntary [59]. Therefore, with increasing nutrition transition (shifts in diet), the low intake of iodine in this study population may be due to the declining consumption of discretionary salt and increasing consumption of non-discretionary salt [60].

In this cohort, the double exposure (CHEU/AbN-RI) group had suboptimal growth indicated by the lowest mean LAZ, WAZ and HCZ, with stunting predominating at 18 months of age. Similar findings for CHEU were reported in previous studies in Ethiopia, Malawi and Uganda that determined the growth of children exposed to maternal HIV infection and ART [61-63], although IUGR caused by placental insufficiency was not examined as a covariate in these studies.

In general, the findings from GMCD screening showed a cause for concern in children in terms of the gross movement subdomain and that the CHEU/AbN-RI group was most affected (21.4%). Similarly, CHEU/AbN-RI had lower mean cognitive composite scores than the control group on the Bayley test. However, all groups had mean composite scores that were within the normal functioning range in the three developmental domains, indicating optimal neurodevelopment. These findings were in line with the Sacchi et al. report that children who had IUGR had lower cognitive scores [64], and differed from De Beer et al. who found that CHUU and CHEU had similar developmental outcomes [65]. Nonetheless, additional categorical analysis of the Bayley test revealed that 21.4% of CHEU/AbN-RI had mild delays in the cognitive domain. Moreover, we observed that mild delay in the language domain was common across the groups. Similar findings on language delays in CHEU have been reported in South Africa [19, 66], potentially linked to the fact that South Africa is a multilingual country. Other causes of developmental delays include preventable causes such as inadequate nutrition and lack of an adequate care environment and stimulation [67]. Our findings on low cognitive and motor composite scores among CHEU and children who had IUGR were in line with the previously reported neurodevelopmental outcomes of CHEU vs CHUU [20, 22, 23, 66, 68] and children with a history of abnormal Doppler [69-71].

Additionally, our study determined associations between several variables and three neurodevelopmental domains, although only weak positive associations were found, likely because childhood growth and development are multifactorial. Zinc intake was significantly associated with language development in the total study population, in line with previously reported positive associations between zinc and neurodevelopment [2]. Furthermore, we observed positive associations between growth parameters and cognitive and motor

development in CHEU. Similarly, studies have shown that stunting is associated with poor cognitive development [2, 72]. Also, associations between LAZ and motor development were reported in South African children [73].

This study investigated the growth and neurodevelopment of an overlooked at-risk population born with a history of placental insufficiency and exposure to maternal HIV infection, also taking into account their micronutrient intake. The once-off investigation at 18 months is a limitation of the study as we could not determine changes in growth and neurodevelopmental outcomes over time for our study population. Also, the small sample size of children who had placental insufficiency (abnormal UmA-RI cohort as a proxy for IUGR) was a drawback.

5. CONCLUSION

CHEU are at risk of suboptimal growth outcomes, particularly stunting, which are worse when HIV exposure is compounded by IUGR attributed to placental insufficiency, as measured by an abnormal UmA-RI in pregnancy. This group of children is furthermore at risk of deficits in cognitive development. The study findings advocate for the large-scale implementation of antenatal Doppler screening, including on low-risk pregnant mothers at the primary health care level, to identify foetuses at risk of IUGR, in order to implement strategies, including early nutritional intervention and follow-up care, to enhance catch-up growth and address cognitive delays, especially in geographical areas with high maternal HIV prevalence. Future research should include large-scale longitudinal studies for a better understanding of the growth and neurodevelopmental trajectories in this vulnerable population. Also, we recommend the determination of iron, zinc and iodine status of children using quantified food frequency questionnaire.

List of Abbreviations

AbN-RI : Abnormal resistance index
ART : Antiretroviral therapy
CHEU : Children who are HIV-exposed-uninfected
CHUU : Children who are HIV-unexposed-uninfected
EAR : Estimated Average Requirement
GMCD : International Guide for Monitoring Child Development
HC : Head circumference
HCZ : Head circumference-for-age z-score

HIV : Human immunodeficiency virus
IUGR : Intrauterine growth restriction
LAZ : Length-for-age z-score
LMICs : Low-and middle-income countries
N-RI : Normal resistance index
PMTCT : Prevention of mother-to-child HIV transmission
UmA-RI : Umbilical artery resistance index
WAZ : Weight-for-age z-score
WLZ : Weight-for-length z-score

Ethics Approval and Consent to Participate

Ethical approval for the study was obtained from the University of Pretoria Faculty of Natural and Agricultural Sciences and Faculty of Health Sciences Ethics Committees with reference number: NAS259/2021. The study was conducted in accordance with the Declaration of Helsinki. Informed and written consent was obtained from all study participants.

Consent for Publication

Not applicable

Data Availability

The datasets generated during and/or analysed during the current study are available in the University of Pretoria repository, https://doi.org/10.25403/UPresearchdata.24754485.v1.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' Contributions

UF, RP, HM and MN contributed to project design and administration. HM and MN facilitated data collection. HM, TB and MN validated data for analysis. TB performed all data analysis and visualizations. MN and HM performed data curation. MN wrote the first draft of the manuscript. MN, UF, MH, HM, RP and VV contributed to reviewing and editing of the manuscript. All authors read and approved the final manuscript.

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CHAPTER 6: MANUSCRIPT 3

In the previous chapter, the findings of manuscript 2 indicated that children with dual exposure to maternal HIV and placental insufficiency were at risk of cognitive deficits, in addition to the risk of stunting reported in Article 1. However, there were no associations found between growth parameters and micronutrient intake vs neurodevelopment in children with dual exposure. This stimulated an investigation of the prevalence of maternal and child anaemia and the impact of maternal anaemia on child neurodevelopment and anaemia.

Thus, this chapter reports on the prevalence of anaemia among mother-child pairs, and associations between maternal and child haemoglobin vs neurodevelopment. The present chapter sought to establish whether maternal haemoglobin has impact on child haemoglobin and neurodevelopmental outcomes. Additionally, the manuscript reported associations between neurodevelopmental outcomes and stunting. The study population was categorized into four subgroups based on HIV exposure and history of placental insufficiency (to find the condition that poses a risk to children): HIV-unexposed-uninfected children (CHUU) vs HIV-exposed-uninfected children (CHEU) and normal UmA-RI (N-RI) children vs abnormal UmA-RI (AbN-RI) children. Also, as in Chapters 4 and 5, CHUU/N-RI (control) vs CHEU/AbN-RI (dual exposure).

The chapter is presented in article format as per the author's guidelines of the journal Anaemia, to which it has been submitted to be considered for publication. The font, line spacing and spelling were kept the same in the thesis for uniformity.

Hindawi

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THE IMPACT OF MATERNAL HIV INFECTION AND ANAEMIA TOGETHER WITH PLACENTAL INSUFFICIENCY ON NEURODEVELOPMENT AND ANAEMIA IN SOUTH AFRICAN CHILDREN

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ABSTRACT

Anaemia is a primary global health concern affecting women and children, and is known to be associated with delayed neurodevelopment in children. We compared and determined associations between maternal anaemia and child neurodevelopment and anaemia among 18-month-old children exposed to maternal HIV-infection and placental insufficiency. Placental insufficiency was detected by an abnormal umbilical artery resistance index (UmA-RI) on Doppler ultrasound during gestation. The cross-sectional study was conducted among 260 mother-child pairs grouped into HIV-unexposed-uninfected children (CHUU): n=198 vs HIV-exposed-uninfected children (CHEU): n=62 and normal UmA-RI (N-RI): n=225 vs abnormal UmA-RI (AbN-RI): n=35. Also, CHUU/N-RI (control): n=178 was compared to CHEU/AbN-RI (dual exposure): n=15. Both maternal and children's haemoglobin concentrations were tested using the HemoCue® Hb 201^{+}. Bayley-III assessed children's cognitive, motor, and language development at the corrected age. Chi-squared and Fisher exact tests were used to compare outcomes between groups, and regression models were used to determine associations. Above one-third of children across the groups were mildly anaemic: CHUU: 43.9%, CHEU: 41.9%, N-RI children: 44.4%, and AbN-RI children: 37.1%, and CHUU/N-RI: 44.4% and CHEU/AbN-RI: 33.3%. About 25.7% of mothers in the AbN-RI group were mildly anaemic, significantly more than the N-RI mothers (9.8%); p=0.027. In the CHEU group, maternal haemoglobin concentrations were associated with child haemoglobin concentrations: β=0.19, 95% confidence interval (CI) (0.02,0.36); p=0.028. Bayley-III assessment demonstrated significantly lower mean cognitive composite scores among the AbN-RI group compared to the N-RI group: 96.4±12.2 vs 100.0±10.5; p=0.017. Also, significantly lower mean cognitive scores were observed in CHEU/AbN-RI compared to CHUU/N-RI: 93.9±12.9 vs 100.0±10.6; p=0.045. There was no evidence to suggest an association between haemoglobin concentration and child neurodevelopment, however, in CHEU, cognitive development was associated with LAZ: β=3.34, 95% CI (1.13,5.54), p=0.004. Child health and nutrition-sensitive programs need to prioritize CHEU and children with placental insufficiency as at-risk groups for cognitive delays.

Keywords: maternal HIV exposure; anaemia; placental insufficiency; neurodevelopment

1. INTRODUCTION

Anaemia is a primary global health concern, particularly affecting women and young children. Globally, it affects 30.0% of women of childbearing age, 37.0% of pregnant women and 40.0% of young children [1, 2]. Low- and middle-income countries (LMICs) are heavily affected, with 106 million women and 103 million children being anaemic in Africa [2]. In South Africa, the prevalence of anaemia in women and children has been reported as 28.1% and 61.3%, respectively [3, 4]. It has been associated with impaired physical health, stunting, and delayed neurodevelopment in 6- to 24-month-old children [1, 4-7].

Nutrient deficiencies including vitamin A, B_{12}, C, iron, and folate, resulting from inadequate diet, are causes of nutritional anaemia. In South Africa iron deficiency is a common nutritional cause of anaemia [3, 8]. Other risk factors of anaemia include infections such as HIV and tuberculosis, which cause anaemia of chronic diseases, as well as side effects of therapy [9].

Nutrient deficiencies are most common in people living with HIV as they are prone to food and nutrition insecurity. South Africa is a country highly burdened by HIV infection, particularly among women and girls (53.0%) [4]. Estimates showed that 30.0% of South African pregnant women are living with HIV, with an anaemia prevalence of 60.6% to 71.3% [4, 10]. Nonetheless, these women give birth to HIV-free infants due to the successes of the prevention of vertical transmission of HIV programs [11, 12]. Notwithstanding the fact that the population of HIV-free children born to women living with HIV, referred to as children who are HIV-exposed-uninfected (CHEU), are classified as at-risk for inadequate nutrition, poor growth and neurodevelopment. Delicio et al. [13] reported a significant prevalence of anaemia (25.7%) in CHEU in Brazil.

Another factor affecting child neurodevelopment and growth is placental insufficiency. Placental insufficiency is one of the immediate causes of intrauterine growth restriction (IUGR) and can be detected by an abnormal umbilical artery resistance index (UmA-RI) on Doppler ultrasound during pregnancy [14]. Lower neurodevelopmental scores have previously been described in children with a history of IUGR [15-18]. Child neurodevelopment is a public health concern; estimates are that over one-third of children under five years are at risk of not attaining their full developmental potential, particularly in LMICs [19]. Placental insufficiency may additionally expose children to anaemia.

In view of the above, it is of great importance to better understand the associations between maternal and child anaemia and neurodevelopmental outcomes of children who had placental insufficiency in the context of high maternal HIV prevalence in the LMIC setting.

2. MATERIALS AND METHODS

2.1. Study Setting, Design and Population

The study is a follow-up to the South African arm of the Umbiflow International study that determined the prevalence of abnormal UmA-RIs as a marker for placental insufficiency in unselected, low-risk obstetric populations at 28 - 34 weeks' gestation in Ghana, India, Kenya, Rwanda, and South Africa [20]. Abnormal UmA-RIs were defined as resistance indices ≥75th centile-for-gestational age, previously described to indicate a high risk for suboptimal clinical outcomes, including stillbirths and lower birth weights-for-gestational age, linked to placental insufficiency [21]. To expand the study population of children with both placental insufficiency and maternal HIV exposure, the present study also enrolled participants from the Siyakhula study, which investigated growth and neurodevelopment in CHEU from the same geographical area and similar study population.

The cross-sectional UmbiGodisa study was conducted in 2021 among 18-month-old CHEU and HIV-unexposed-uninfected children (CHUU) with normal or abnormal UmA-RI, with available pregnancy and birth data, at the University of Pretoria's Research Centre for Maternal, Fetal, Newborn and Child Health Care Strategies at Kalafong Provincial Tertiary Hospital. Children with chromosomal or structural abnormalities or other severe medical conditions known to impact child neurodevelopment were excluded. The anticipated sample was 311 from the Umbiflow International study. However, 46 participants did not attend the planned study visit, two participants subsequently withdrew, and two children had severe medical conditions. A total of 271 participants were recruited, including 10 participants from the Siyakhula study. Eleven participants were excluded from the current analysis, as they had incomplete questionnaires (n=4), had an obvious developmental disability (n=1), had acute medical conditions resulting in an inadequately alert state (n=2) or had missing haemoglobin concentrations as they were not tested due to the temporary malfunctioning of the instrument on the day of their study visit (n=4). We, therefore, investigated 260 mother-child pairs, grouped based on HIV-exposure status (CHUU: n=198; CHEU: n=62) and normal (n=225) or abnormal UmA-RI (n=35) during pregnancy. In further analysis, the grouping was as follows: CHUU with normal UmA-RI (CHUU/N-RI; control group; n=178), CHEU with normal UmA-

RI (CHEU/N-RI; single exposure; n=47), CHUU with abnormal UmA-RI (CHUU/AbN-RI; single exposure; n=20) and CHEU with abnormal UmA-RI (CHEU/AbN-RI; dual exposure; n=15).

2.2. Questionnaires

The face-to-face maternal interviews were conducted by trained study staff in either English or local languages, using structured and previously used questionnaires to collect maternal and child sociodemographic data and medical history, as well as descriptive qualitative breastfeeding data over the first 18 months of life, which was based on maternal recall.

2.3. Measurements

The haemoglobin concentration is used as an indicator of anaemia and by proxy for iron deficiency, with anaemia defined by the World Health Organization (WHO) as a haemoglobin concentration <11 g/dL in children aged 6 to 59 months and <12 g/dL in non-pregnant women [22, 23]. Maternal and child haemoglobin concentrations were measured using the HemoCue® Hb 201^{+} System Analyzer. About 10µL of blood was drawn using a HemoCue sterile safety lancet (2.25mm depth) and Hb 201 micro cuvettes. This system has been reported to be the standard haemoglobin point-of-care test, which provides laboratory accuracy for determining haemoglobin in whole blood with a measuring range of 0 - 25.6 g/dL and delivers results within 1 minute [24]. Further, the system is easy to use, has been adjusted for up to 2000 m height above the sea level and has outstanding lot-to-lot reproducibility. The International Council for Standardization in Hematology (ICSH) calibrates it from the factory for determining haemoglobin [24].

Child neurodevelopment outcomes were measured using the Bayley Scales of Infant and Toddler Development™ third edition (Bayley-III), which is a standardized and well-accepted tool for developmental assessment of children aged 1 to 42 months and assesses children in terms of cognitive, motor, and language domains [25]. The Bayley-III assessment was conducted at the corrected age, with the motor domain including the fine and gross motor domains and expressive and receptive language included in the language domain.

The anthropometric measurements of the mother-child pairs were performed following standardized WHO procedures [26]. Two trained assessors carried out measurements two consecutive times, and if the measurements were similar, the mean value was recorded [27]. A third measurement was done if two measurements differed by 0.5cm or 0.05kg. There were

missing anthropometric measurements for seven mothers because of repeat pregnancies (n=5) or children were brought by the caregivers (n=2).

2.4. Data Processing and Analysis

Data was managed and independently double-entered on the online electronic platform, Research Electronic Data Capture (REDCap) v9.3.5. Missing or inaccurate data was dealt with immediately upon completing the interviews. The study classified the haemoglobin concentration into the following categories for children: severe anaemia (<7.0 g/dL); moderate anaemia (7.0-9.9 g/dL); mild anaemia (10.0-10.9 g/dL) and normal (≥11.0 g/dL). Haemoglobin concentration classification for non-pregnant women was as follows: severe anaemia (< 8.0 g/dL); moderate anaemia (8.0-10.9 g/dL); mild anaemia (11.0-11.9 g/dL) and normal (≥12.0 g/dL) [9]. Bayley-III composite scores were defined as 100 for mid-average functioning, <85 for mild impairment, <70 for moderate impairment and <55 for severe impairment. Maternal body mass index (BMI) was computed, with subsequent classification as follows: underweight: <18.5 kg/m^2; normal: 18.5-24.9 kg/m^2; overweight: 25-29.9 kg/m^2; obese: ≥30 kg/m^2. The child birth anthropometric z-scores were computed using the INTERGROWTH-21st Newborn Size tool (International Fetal and Newborn Growth Consortium for the 21st Century, Oxford, UK), and the WHO Anthro Survey Analyzer was used to compute childhood z-scores: weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ), head circumference-for-age (HCZ), and mid-upper arm circumference-for-age (MUACZ). Premature births were corrected for age. The study utilized WHO guidelines to define stunting, underweight, wasting and moderate acute malnutrition as LAZ, WAZ, WLZ and MUACZ <-2 SD. The z-scores <-3 were classified as a severe condition. The anthropometrical z-scores outside the range of the reference population (<-3 and >+3) were reviewed and corrected in the event of an error in data capturing. R statistical software (version 4.3.0) was used for descriptive statistical analysis. The variables were assessed for normality using the Shapiro-Wilk test. Frequencies and percentages were reported for categorical data and significances were determined using the chi-squared test, and Fishers exact test as an alternative where group counts were less than five. For continuous data, means and standard deviations were reported and significances were determined using the independent t test. Associations were determined using univariable and multivariable linear regression models. Multivariable models were adjusted for UmA-RI status, maternal HIV status, sex, LAZ and WLZ. P-values < 0.05 were considered to be statistically significant.

2.5. Ethical Consideration

Ethical approvals were obtained from the Faculty of Natural and Agricultural Sciences and Faculty of Health Sciences Ethics Committees with ethics reference numbers NAS259/2021 and 283/2019, respectively, in accordance with Declaration of Helsinki guidelines. Participants were given relevant information about the follow-up study, and participation was voluntary. Mothers provided consent on behalf of themselves and their infants. There were minimal risks for participation in the study. Mothers were reimbursed for transport costs and given light refreshments.

3. RESULTS

3.1. Baseline characteristics

A total of 260 mother/caregiver-child pairs participated in this study, and their baseline characteristics are presented in Table 6.1. In terms of the birth outcomes, we observed that the percentage of premature births was significantly higher among children who had abnormal UmA-RI (AbN-RI), as a proxy for IUGR (22.9%), in comparison to their normal UmA-RI (N-RI) counterparts (7.1%); p = 0.007. Also, children who had AbN-RI had significantly lower mean length (p = 0.049) and mean head circumference (HC) at birth (p = 0.034) than N-RI children. Further, CHEU had a lower mean birth length (p = 0.037) and birthweight z-score (p = 0.031) than CHUU. For the feeding practices during the first six months, percentages of exclusive breastfeeding were significantly higher in CHEU than in CHUU (43.5% vs 34.3%; p = 0.004) and in children who had AbN-RI than in N-RI children (42.9% vs 36.0%; p = 0.015). More than 50% of CHUU and N-RI children were mixed-fed. Current or continued breastfeeding was found in 26.8% of CHUU and 11.3% in CHEU; p = 0.038. When comparing maternal factors between the groups, it was observed that mothers of CHEU (p = 0.004) and children who had AbN-RI (p = 0.015) were significantly older than their counterparts. Mothers of CHEU had higher median gravidity (3 [2, 3] vs 2 [2, 3]; p = 0.054), and significantly higher median parity (2 [2, 3] vs 2 [1, 3]; p = 0.040), antenatal iron (38.7% vs 12.1%; p <0.001) and folic acid supplementation (30.6% vs 9.1%; p <0.001) than mothers of CHUU. Further, it was found that mothers of children who had AbN-RI had significantly lower body weight, BMI and MUAC compared to their N-RI counterparts, and significantly higher (28.6% vs 12.0%; p = 0.022) percentage of antenatal folic acid supplementation than mothers of N-RI children. A significantly higher percentage (28.9%) of mothers of N-RI children drank alcohol since giving birth than AbN-RI counterparts (5.7%); p = 0.008. Across all the groups, most mothers were married or cohabiting, had attained secondary schooling, and were unemployed.

Table 6.1: Participants' baseline characteristics and birth information based on HIV exposure and normal or abnormal umbilical artery resistance index

Variables	CHUU	CHEU	P-value	N-RI	AbN-RI	P-value
Sample size (n)	198	62		225	35	
Child factors						
Child age (months), mean ±SD	18.5 ± 0.8	18.6 ± 0.8	0.180	18.6 ± 0.9	18.5 ± 0.7	0.930
Child sex: Female, n (%)	101 (51.0)	33 (53.2)	0.770	114 (50.7)	20 (57.1)	0.590
Premature birth, n (%)	20 (10.1)	4 (6.5)	0.460	16 (7.1)	8 (22.9)	**0.007**
GA at birth (weeks), mean ±SD	39.0 ± 2.0	39.2 ± 1.7	0.720	39.2 ± 1.8	38.3 ± 2.5	**0.045**
Birthweight (g), mean ± SD	3073 ± 482	3183 ± 558	0.090	3107 ± 469	3048 ± 682	0.860
Birth length (cm), mean ± SD	50.4 ± 3.1	49.6 ± 2.3	**0.037**	50.4 ± 2.9	49.2 ± 3.1	**0.049**
Birth HC (cm), mean ± SD	34.3 ± 1.7	34.3 ± 1.5	0.780	34.4 ± 1.6	33.5 ± 2.2	**0.034**
Birthweight z-score, mean ± SD	-0.4 ± 1.1	-0.7 ± 1.0	**0.031**	-0.4 ± 1.1	-0.7 ± 1.0	0.420
Birth length z-score, mean ± SD	0.6 ± 1.6	0.2 ± 1.3	0.058	0.5 ± 1.6	0.5 ± 1.2	0.150
Birth HC z-score, mean ± SD	0.4 ± 1.3	0.3 ± 1.2	0.440	0.4 ± 1.3	0.4 ± 1.0	0.240
Child received multivitamins or supplements, n (%)	42 (21.2)	9 (14.5)	0.350	47 (20.9)	4 (11.4)	0.300
The child was ever breastfed, n (%)	190 (96.0)	59 (95.2)	0.730	214 (95.1)	35 (100.0)	0.370
Infant feeding from birth to 6 months, n (%)			**0.004**			**0.015**
EBF	68 (34.3)	27 (43.5)		81 (36.0)	15 (42.9)	
Formula feeding	10 (5.1)	4 (6.6)		14 (6.2)	0 (0.0)	
Mixed feeding	112 (56.6)	20 (32.3)		118 (52.4)	14 (40.0)	
Formula feeding but previously EBF	8 (4.0)	11 (17.7)		12 (5.3)	6 (17.1)	
Continued breastfeeding	53 (26.8)	7 (11.3)	**0.038**	53 (23.6)	7 (20.0)	0.900
Maternal factors at 18 months postpartum						
Age (years), mean ± SD	30.0 ± 5.1	32.5 ± 5.8	**0.004**	30.3 ± 5.2	32.1 ± 6.5	**0.015**
UmA-RI z-score, mean ± SD	0.2 ± 0.9	0.3 ± 1.0	0.320	0.0 ± 0.6	1.7 ± 0.8	**0.012**
Gravidity, median [IQR]	2 [2, 3]	3 [2, 3]	0.054	2 [2, 3]	2 [2, 3]	0.770
Parity, median [IQR]	2 [1, 3]	2 [2, 3]	**0.040**	2 [2, 3]	2 [2, 3]	0.830
Previous pregnancy losses, n (%)	40 (20.2)	19 (30.6)	0.110	54 (24.0)	5 (14.3)	0.440
Weight (kg), mean ± SD [a]	76.9 ± 19.8	75.6 ± 23.9	0.430	78.1 ± 20.8	67.1 ± 18.1	**0.002**
Height (cm), mean ± SD [a]	159.9 ± 6.1	161.3 ± 8.1	0.200	160.6 ± 6.8	158.0 ± 5.2	0.090
BMI (kg/m²), mean ± SD [a]	30.1 ± 7.7	28.8 ± 8.1	0.470	30.3 ± 7.9	26.8 ± 6.5	**0.009**
MUAC (cm), mean ± SD [a]	32.0 ± 5.0	31.1 ± 5.7	0.530	32.1 ± 5.2	29.6 ± 4.6	**0.009**
Iron supplementation [b], n (%)	24 (12.1)	24 (38.7)	**<0.001**	34 (15.1)	10 (28.6)	0.096
Folic acid supplementation [c], n (%)	18 (9.1)	19 (30.6)	**<0.001**	27 (12.0)	10 (28.6)	**0.022**
Maternal TB status, n (%)			0.410			0.580
Previously had TB	2 (1.0)	2 (3.2)		4 (1.8)	0 (0.0)	
Never had TB	187 (94.4)	53 (85.5)		208 (92.4)	32 (91.4)	
Unknown	9 (4.6)	7 (11.3)		13 (5.8)	3 (8.6)	
Latest CD4 count (cells/mm³), mean ± SD	n/a	429 ± 294	n/a	455 ± 311	336 ± 232	0.510
Latest viral load (copies/mL),median [IQR]	n/a	0 [0, 0]	n/a	0 [0, 0]	0 [0, 0]	0.540
Drinks alcohol [d], n (%)	51 (25.8)	16 (25.8)	>0.999	65 (28.9)	2 (5.7)	**0.008**
Smokes cigarettes [e], n (%)	4 (2.0)	2 (3.2)	0.950	6 (2.7)	0 (0.0)	0.720
Marital status, n (%)			0.490			0.260

Single	82 (41.4)	23 (37.1)		95 (42.2)	10 (28.6)	
Married/cohabiting	116 (58.6)	39 (62.9)		130 (57.8)	25 (71.4)	
Education level, n (%)			0.570			0.310
Any primary level	19 (9.6)	5 (8.1)		22 (9.8)	2 (5.7)	
Any secondary level	133 (67.2)	46 (74.2)		151 (67.1)	28 (80.0)	
Any tertiary level	46 (23.2)	11 (17.7)		52 (23.1)	5 (14.3)	
Employment status, n (%)			0.880			>0.999
Any employment	79 (39.9)	24 (38.7)		89 (39.6)	14 (40.0)	
Unemployed	119 (60.1)	38 (61.3)		136 (60.4)	21 (60.0)	

Abbreviations: AbN-RI: abnormal umbilical artery resistance index; BMI: body mass index; CHUU: HIV-unexposed-uninfected children; CHEU: HIV-exposed-uninfected children; cm: centimeter; EBF: exclusive breastfeeding; HC: head circumference; IQR: interquartile range; GA: gestational age; kg: kilogram; n/a: not applicable; N-RI: normal umbilical artery resistance index; MUAC: mid-upper arm circumference; SD: standard deviation; TB: tuberculosis.

Notes: A P-value less than 0.05 indicates that the results were statistically significant. [a] Anthropometry measurements were carried out on 253 mothers: CHUU: n = 192; CHEU: n = 61; N-RI: n = 219; and AbN-RI: n = 34. [b] Antenatal supplementation of iron supplements. [c] Antenatal supplementation of folic acid. [d] Question asked: Since your baby was born, did you drink alcohol? [e] Question asked: Since your baby was born, did you smoke cigarettes?

3.2. Haemoglobin concentrations and Bayley-III developmental composite scores

Table 6.2 presents the findings on child anthropometric outcomes, maternal and child haemoglobin and Bayley-III findings between the CHUU vs CHEU as well as the N-RI vs AbN-RI groups. The comparisons between groups indicated significantly lower LAZ among CHEU vs CHUU (-0.7 ± 1.2 vs -0.1 ± 1.3; p = 0.014) and in children who had AbN-RI vs N-RI children (-0.7 ± 1.5 vs -0.1 ± 1.3; p = 0.020). Significantly lower cognitive composite scores were observed among children who had AbN-RI when compared to their N-RI counterparts: 96.4 ± 12.2 vs 100.0 ± 10.5; p = 0.017. More than a third of children across the compared groups presented with mild anaemia, while around one quarter (25.7%) of mothers of AbN-RI children were mildly anaemic when compared to mothers of N-RI children (9.8%); p = 0.027.

Table 6.2: Comparisons of gestational age-corrected anthropometry and Bayley-III mean composite scores as well as haemoglobin concentrations in HIV-exposed vs HIV-unexposed children and children with normal vs abnormal umbilical artery resistance index in utero, and their mothers

Measures, mean ± SD	CHUU	CHEU	P-value	N-RI	AbN-RI	P-value
Sample size (n)	198	62		225	35	
Children's anthropometric measurements and indices						
Weight (kg)	10.9 ± 1.6	10.5 ± 1.7	0.130	10.8 ± 1.6	10.4 ± 1.7	**0.045**
Length (cm)	81.8 ± 3.8	80.2 ± 3.4	**0.023**	81.7 ± 3.7	79.8 ± 4.1	**0.006**
MUAC (cm)	16.1 ± 1.5	16.2 ± 1.6	0.900	16.1 ± 1.5	16.2 ± 1.6	0.740
HC (cm)	48.1 ± 1.6	47.9 ± 1.8	0.240	48.1 ± 1.7	47.9 ± 1.7	0.460
Weight-for-age z-score	0.0 ± 1.2	-0.2 ± 1.3	0.170	0.0 ± 1.2	-0.3 ± 1.3	0.110
Length-for-age z-score	-0.1 ± 1.3	-0.7 ± 1.2	**0.014**	-0.1 ± 1.3	-0.7 ± 1.5	**0.020**
Weight-for-length z-score	0.1 ± 1.2	0.1 ± 1.3	0.950	0.1 ± 1.3	0.0 ± 1.1	0.770
MUAC-for-age z-score	1.1 ± 1.1	1.1 ± 1.2	0.790	1.1 ± 1.1	1.2 ± 1.2	0.570
HC-for-age z-score	0.9 ± 1.2	0.7 ± 1.2	0.230	0.9 ± 1.2	0.8 ± 1.3	0.700
Children's Bayley composite scores, mean ± SD						
Cognitive development	99.8 ± 10.7	98.5 ± 11.1	0.910	100.0 ± 10.5	96.4 ± 12.2	**0.017**
Language development	89.5 ± 12.2	88.8 ± 12.4	0.120	89.1 ± 12.2	90.7 ± 12.8	0.660
Motor development	99.7 ± 11.7	98.4 ± 13.5	0.180	99.7 ± 12.0	96.9 ± 12.6	0.530
Children's haemoglobin concentration and classification						
Haemoglobin value (g/dL)	11.0 ± 1.2	11.0 ± 1.1	0.800	11.0 ± 1.2	11.0 ± 1.3	0.170
Haemoglobin level classification n (%)						
Normal haemoglobin concentration (≥11.0 g/dL)	100 (50.5)	33 (53.2)	0.920	115 (51.1)	18 (51.4)	0.210
Mild anaemia (10.0 - 10.9 g/dL)	87 (43.9)	26 (41.9)		100 (44.4)	13 (37.1)	
Moderate anaemia (7.0-9.9 g/dL)	11 (5.6)	3 (4.8)		10 (4.4)	4 (11.4)	
Maternal haemoglobin concentration and classification						
Haemoglobin value (g/dL)	12.8 ± 1.5	12.3 ± 1.6	0.081	12.7 ± 1.5	12.4 ± 1.4	0.430
Haemoglobin level classification, n (%)						
Normal haemoglobin concentration (≥12.0 g/dL)	152 (76.8)	40 (64.5)	0.100	171 (76.0)	21 (60.0)	**0.027**
Mild anaemia (11.0-11.9 g/dL)	22 (11.1)	9 (14.5)		22 (9.8)	9 (25.7)	
Moderate anaemia (8.0-10.9 g/dL)	24 (12.1)	13 (21.0)		32 (14.2)	5 (14.3)	

Abbreviations: AbN-RI: abnormal umbilical artery resistance index; CHUU: HIV-unexposed-uninfected children; CHEU: HIV-exposed-uninfected children; g/dL: gram per decilitre; HC: head circumference; MUAC: mid-upper arm circumference; N-RI: normal umbilical artery resistance index; SD: standard deviation.

Note: A P-value less than 0.05 indicates that results were statistically significant, and such P-values are in bold.

Further analysis included participants grouped into CHUU/N-RI, CHEU/N-RI, CHUU/AbN-RI and CHEU/AbN-RI. Table 6.3 presents child anthropometric outcomes, indices, haemoglobin and neurodevelopment findings among CHUU/N-RI (control) vs CHEU/AbN-RI (dual exposure) groups. Results on anthropometric measurements and indices showed that the CHEU/AbN-RI (dual exposure) group had lower anthropometric measurements and z-scores than the control group. Findings of the Bayley-III test demonstrated that CHEU/AbN-RI had a significantly lower mean cognitive composite score than the control group (93.9 ± 12.9 vs

100.0 ± 10.6; p = 0.045). About 44.7% of CHEU/N-RI and 40.0% of CHUU/AbN-RI were mildly anaemic.

No statistically significant differences existed between the mean child haemoglobin values and haemoglobin classifications when comparing the exposure groups to the control group. However, CHUU/AbN-RI had a mean haemoglobin value of 10.9 ± 1.4 g/dL, indicating mild anaemia in these children. Mothers of CHEU/AbN-RI had significantly lower mean haemoglobin concentrations than mothers of CHUU/N-RI (12.0 ± 1.3 vs 12.8 ± 1.5; p = 0.024). The findings on the maternal haemoglobin classifications differences between the control and dual exposure groups were not statistically significant. However, it was observed that more mothers of CHEU/AbN-RI were mildly (20.0%) or moderately anaemic (20.0%), compared to mothers of the control group (9.6% and 12.4%, respectively). Further comparative analysis showed that a significantly higher percentage (30.0%) of mothers of CHUU/AbN-RI were mildly anaemic compared with control group mothers (9.1%); p = 0.020). Also, mothers of CHEU/N-RI had significantly lower mean haemoglobin values than their counterparts in the control group: 12.4 ± 1.7 g/dL vs 12.8 ± 1.5 g/dL; p = 0.022, and more (21.7%) of these mothers were moderately anaemic compared to mothers in the control group (12.0%), although not statistically significant.

Table 6.3: Gestational age-corrected anthropometry and Bayley-III composite scores as well as haemoglobin concentrations and classifications at the 18-month study visit: control vs dual exposure infant groups, as well as their mothers

Measures, mean ± SD	**CHUU/N-RI (control)**	**CHEU/AbN- RI (dual exposure)**	**P-value**
Sample size (n)	178	15	
Children's anthropometric measurements and indices			
Weight (kg)	10.9 ± 1.6	9.9 ± 1.0	**0.009**
Length (cm)	81.9 ± 3.8	78.2 ± 3.5	**0.017**
MUAC (cm)	16.1 ± 1.4	16.0 ± 1.4	0.540
HC (cm)	48.1 ± 1.6	47.1 ± 1.2	**0.011**
Weight-for-age z-score	0.0 ± 1.2	-0.6 ± 0.9	**0.019**
Length-for-age z-score	-0.1 ± 1.3	-1.3 ± 1.3	**0.001**
Weight-for-length z-score	0.1 ± 1.2	0.0 ± 0.8	0.800
MUAC-for-age z-score	1.1 ± 1.1	1.0 ± 1.0	0.330
HC-for-age z-score	0.9 ± 1.2	0.3 ± 0.7	**0.020**
Maternal anthropometric measurements			
Age (years)	30.1 ± 5.1	36.6 ± 6.1	**<0.001**
Weight (kg)	77.5 ± 19.7	63.1 ± 15.4	**0.004**
Height (cm)	160.1 ± 6.2	158.3 ± 5.3	0.490
BMI (kg/m²)	30.3 ± 7.7	25.1 ± 5.2	**0.009**
MUAC (cm)	32.0 ± 5.1	28.0 ± 4.0	**0.003**
Children's Bayley composite scores			

Cognitive development	100.0 ±10.6	93.9 ± 12.9	**0.045**
Language development	89.4 ± 12.4	90.9 ± 15.8	0.352
Motor development	99.9 ± 11.7	95.6 ± 14.2	0.205
Children's haemoglobin concentration and classification			
Haemoglobin value (g/dL)	11.0 ± 1.2	11.1 ±1.1	0.350
Haemoglobin level classification n (%)			
Normal haemoglobin concentration (≥11.0 g/dL)	91 (51.1)	9 (60.0)	0.690
Mild anaemia (10.0-10.9 g/dL)	79 (44.4)	5 (33.3)	
Moderate anaemia (7.0-9.9 g/dL)	8 (4.5)	1 (6.7)	
Maternal haemoglobin concentration and classification			
Haemoglobin value (g/dL)	12.8 ± 1.5	12.0 ±1.3	**0.024**
Haemoglobin level classification, n (%)			
Normal haemoglobin concentration (≥12.0 g/dL)	139 (78.0)	9 (60.0)	0.230
Mild anaemia (11.0-11.9 g/dL)	17 (9.6)	3 (20.0)	
Moderate anaemia (8.0-10.9 g/dL)	22 (12.4)	3 (20.0)	

Abbreviations: AbN-RI: abnormal umbilical artery resistance index; BMI: body mass index; CHEU: HIV-exposed-uninfected children; CHUU: HIV-unexposed-uninfected children; cm: centimetre; HC: head circumference; kg: kilogram; MUAC: mid-upper arm circumference; N-RI: normal umbilical artery resistance index; g/dL: gram per decilitre; SD: standard deviation.

Note: A P-value less than 0.05 indicates that results were statistically significant, and such P-values are in bold.

3.3. Association between maternal and child haemoglobin concentration and child neurodevelopment

In univariable regression analysis of the total population, maternal haemoglobin was not significantly associated with child haemoglobin (β = 0.08 (95% confidence interval (CI): -0.02, 0.17); p = 0.113), but further analysis showed that in the CHEU group, a significant association was found (β = 0.19 (0.02, 0.36); p = 0.028). This significant association remained on multivariable regression analysis, adjusted for umbilical artery resistance index status, maternal HIV status, sex, LAZ and WLZ (β = 0.19 (0.01, 0.37); p = 0.035).

In univariable and multivariable regression analysis, we found no indications to suggest an association between the child or maternal haemoglobin and child neurodevelopment. In particular, we observed no evidence of the association between haemoglobin concentration and cognitive development in the AbN-RI and CHEU/AbN-RI groups. As a result, we further determined the association between child neurodevelopment, LAZ and stunting. In the CHEU group, the univariable and multivariable regression analysis indicated a significant positive association between cognitive and motor development and increasing LAZ, and a significant negative association between cognitive development and stunting (Table 6.4 and Table 6.5).

Table 6.4: Univariable linear regression findings for the association between child LAZ and stunting and Bayley-III composite scores at 18 months

	LAZ		Stunting	
	Univariable Model β (95% CI)	**P-value**	**Univariable Model β (95% CI)**	**P-value**
Total population				
Cognitive domain	0.67 (-0.33, 1.67)	0.190	-6.15 (-10.75, -1.55)	**0.009**
Language domain	0.30 (-0.84, 1.44)	0.608	-2.83 (-8.10, 2.43)	0.290
Motor domain	0.57 (-0.09, 2.16)	0.072	-7.26 (-12.43, -2.10)	**0.006**
CHUU				
Cognitive domain	-0.13 (-1.29, 1.03)	0.830	-1.92 (-8.83, 4.99)	0.585
Language domain	0.13 (-1.18, 1.44)	0.840	-0.34 (-8.16, 7.48)	0.931
Motor domain	0.21 (-1.05, 1.47)	0.739	-2.23 (-9.74, 5.28)	0.559
CHEU				
Cognitive domain	3.34 (1.13, 5.54)	**0.004**	-9.10 (-16.78, -3.20)	**0.005**
Language domain	0.82 (-1.84, 3.47)	0.541	-4.87 (-12.95, 3.20)	0.232
Motor domain	3.53 (0.80, 6.26)	**0.012**	-10.64 (-19.04, -2.23)	**0.014**
N-RI				
Cognitive domain	0.32 (-0.77, 1.42)	0.560	-6.05 (-11.55, -0.55)	**0.031**
Language domain	0.40 (-0.86, 1.66)	0.531	-2.51 (-8.92, 3.91)	0.442
Motor domain	0.68 (-0.57, 1.93)	0.284	-6.74 (-13.05, -0.44)	**0.036**
AbN-RI				
Cognitive domain	1.60 (-1.19, 4.40)	0.252	-4.28 (-14.42, 5.87)	0.397
Language domain	0.16 (-2.85, 3.16)	0.916	-5.25 (-15.88, 5.38)	0.321
Motor domain	2.20 (-0.66, 5.04)	0.126	-7.18 (-17.49, 3.13)	0.166
CHEU/AbN-RI				
Cognitive domain	1.44 (-4.48, 7.36)	0.605	-1.04 (-16.81, 14.73)	0.888
Language domain	-2.49 (-9.65, 4.68)	0.464	-1.63 (-20.93, 17.68)	0.858
Motor domain	1.34 (-5.23, 7.90)	0.665	-6.08 (-23.10, 10.93)	0.451

Abbreviations: AbN-RI: abnormal umbilical artery resistance index; CHUU: HIV-unexposed and uninfected children; CHEU: HIV-exposed-uninfected children; n/a: not applicable; CI: confidence interval; LAZ: length-for-age z-score; N-RI: normal umbilical artery resistance index.

Note: A P-value less than 0.05 indicates that results were statistically significant, and such P-values are in bold.

Table 6. 5: Multivariable linear regression findings for the association between child LAZ and stunting and Bayley-III composite scores at 18 months

	LAZ		Stunting	
	Multivariable Model β (95% CI)	**P-value**	**Multivariable Model β (95% CI)**	**P-value**
Total population				
Cognitive domain	0.26 (-0.93, 1.45)	0.667	-4.40 (-9.37, 0.57)	0.083
Language domain	0.43 (-0.62, 1.47)	0.421	-2.04 (-7.72, 3.65)	0.481
Motor domain	0.72 (-0.44, 1.88)	0.221	-4.62 (-10.16, 0.92)	0.101
CHUU				
Cognitive domain	-0.19 (-1.34, 0.97)	0.751	-1.08 (-7.95, 5.79)	0.757
Language domain	0.14 (-1.18, 1.47)	0.830	0.03 (-7.84, 7.90)	0.994
Motor domain	0.08 (-1.17, 1.34)	0.895	-1.16 (-8.61, 6.29)	0.760
CHEU				
Cognitive domain	2.93 (0.49, 5.37)	**0.019**	-9.53 (-17.36, -1.69)	**0.018**
Language domain	0.92 (-1.10, 3.84)	0.529	-5.25 (-14.55, 4.06)	0.263
Motor domain	3.39 (0.41, 6.38)	**0.027**	-9.23 (-18.97, 0.52)	0.062
N-RI				
Cognitive domain	0.22 (-0.90, 1.35)	0.698	-5.19 (-11.09, 0.70)	0.084
Language domain	0.30 (-0.99, 1.59)	0.647	-0.92 (-7.70, 5.87)	0.790
Motor domain	0.41 (-0.86, 1.68)	0.527	-3.92 (-10.60, 2.75)	0.248
AbN-RI				
Cognitive domain	1.02 (-1.93, 3.98)	0.484	0.48 (-10.32, 11.29)	0.928
Language domain	0.07 (-3.38, 3.51)	0.969	-6.22 (-18.47, 6.03)	0.307
Motor domain	2.28 (-0.80, 5.35)	0.141	-5.58 (-16.99, 5.83)	0.324
CHEU/AbN-RI				
Cognitive domain	0.26 (-8.96, 9.48)	0.951	1.49 (-18.41, 21.39)	0.869
Language domain	-4.67 (-16.82, 7.49)	0.408	-3.64 (-30.87, 23.58)	0.769
Motor domain	2.30 (-9.38, 13.98)	0.667	-7.84 (-32.67, 17.00)	0.494

Abbreviations: AbN-RI: abnormal umbilical artery resistance index; CHUU: HIV-unexposed and uninfected children; CHEU: HIV-exposed-uninfected children; n/a: not applicable; CI: confidence interval; LAZ: length-for-age z-score; N-RI: normal umbilical artery resistance index.

Note: Multivariable models were adjusted for umbilical artery resistance index status/placental insufficiency, HIV status, child sex, prematurity and maternal hemoglobin. A P-value less than 0.05 indicates that the results were statistically significant, and such P-values are in bold.

4. DISCUSSION

Our study aimed to determine if there was an association between maternal/child haemoglobin levels, exposure to maternal HIV infection and placental insufficiency *in utero* and child neurodevelopment. Findings indicated lower cognitive scores among children with a history of placental insufficiency, as a marker for IUGR, and even lower cognitive scores in the dual exposure group (CHEU/AbN-RI). However, there was no evidence that lower cognitive scores were associated with either child or maternal hemoglobin. Nearly a quarter of children who had AbN-RI were born premature; a similar finding has been previously reported [15]. The high number of premature births may be attributed to the fact that once the clinicians had

identified the abnormal Doppler, the mothers were referred for obstetric management, and if the risk of stillbirth was deemed to be too high then the pregnancy was delivered. Many of these premature births were therefore iatrogenic. Regarding breastfeeding practices, a high percentage of CHEU and children who had AbN-RI were reported to be exclusively breastfed during the first six months of life. In children who had AbN-RI, a high percentage of exclusive breastfeeding may be due to the fact that many of these children were born prematurely, and their mothers were counselled and supported on breastfeeding through the Kangaroo Mother Care programme in the local geographic area. The high percentage of exclusive breastfeeding in CHEU on the other hand may be attributed to the success in the promotion of breastfeeding in the context of HIV [28, 29]. Furthermore, a similar high percentage of exclusive breastfeeding has been recently reported in South African CHEU [30]. On the other hand, current or continued breastfeeding percentages were low in all groups, despite the known importance of promoting growth and development during the critical period of transitioning from exclusive breastfeeding to complementary feeding [30]. Across all the groups, the reported percentage of mothers who received antenatal iron and folic acid supplementation was very low. Nonetheless, a significant percentage of mothers of CHEU had antenatal iron and folic acid supplementation. Micronutrient deficiencies, including iron deficiency, are known to be common in pregnant women living with HIV due to increased requirements as a result of pregnancy and HIV infection. Still, irrespective of maternal HIV status, all pregnant women should be given multiple micronutrient supplementations as a standard of care for prevention and treatment [31].

The study found that there were no statistical significances in terms of haemoglobin levels (except for mothers of CHEU/AbN-RI vs CHUU/N-RI) and classification between groups for mothers (except for mothers in N-RI vs AbN-RI groups) and their children. Observations were that across the groups, mothers and children had mean haemoglobin values on or above the standard cut-off values except for CHUU/AbN-RI children, who were mildly anaemic. Also, above a quarter of mothers of AbN-RI were mildly anaemic. These findings of mild anaemia in children who had AbN-RI and their mothers were in line with the literature that maternal anaemia is related to the occurrence of childhood anaemia [32]. Another possible risk factor for mild anaemia in children with previous in utero growth restriction may be a high percentage of premature birth in this group, with prematurity a known risk factor for childhood anaemia [32]. Further, more than a quarter of children across all the groups were mildly anaemic.

However, the reported percentages were lower than the 61.3% prevalence of childhood anaemia previously reported in a South African systematic review by Turawa et al. [4].

CHEU who had AbN-RI had low anthropometric measurements and indices, particularly mean LAZ, indicating a risk of stunting. Similar poorer growth parameters have been reported in South African CHEU compared to CHUU [30]. Other researchers have shown that IUGR does not impact anthropometric measurements [33], perhaps these may depend on the type of anthropometric measurement used. Furthermore, children who had AbN-RI and CHEU/AbN-RI had significantly lower Bayley-III mean cognitive composite scores. Lower cognitive scores were reported among children who had IUGR by Sacchi et al. [18] in their systematic review and meta-analysis involving 60 studies that included 52,822 children, as well as by von Beckerath et al. [15]. The was no significant difference in mean cognitive scores between CHEU vs CHUU, similar to previous studies in South Africa and Botswana, in which similar cognitive development was described in CHEU and CHUU [34-36].

Our univariable and multivariable regression analyses indicated that no associations existed between maternal or child haemoglobin and child neurodevelopment. These findings align with many reports in the literature [37-39]. However, the findings differed from those of Olney et al. [40] and a systematic review and meta-analysis by Larson et al. [41], which showed an association between child haemoglobin and motor development. Nonetheless, studies in the literature did not focus on the dual exposures of HIV and placental insufficiency.

The lower cognitive scores among children who had AbN-RI and CHEU/AbN-RI were not associated with child or maternal haemoglobin concentrations, and the findings differed from another report, although HIV exposure and UmA-RI were not investigated [42]. Observations were that these children who were HIV-exposed and had AbN-RI also had lower LAZ; however, further investigations showed a significant association between cognitive, and motor development and LAZ among CHEU only. This may suggest that poor linear growth is not a risk factor for cognitive deficit in children who had UmA-RI.

Limitations of the study include the lack of a full blood count for assessing anaemia, such as ferritin values which will determine iron deficiency anaemia. Also, the maternal haemoglobin at 18-month postpartum may not fully indicate the maternal haemoglobin during pregnancy/early postpartum, which is likely more important in terms of its impact on child neurodevelopment. Another limitation was that we had a small dual exposure group sample

size. Nonetheless, this study investigated the overlooked population of children exposed to HIV and placental insufficiency.

5. CONCLUSIONS

The prevalence of anaemia remains alarmingly high among South African children irrespective of their medical backgrounds. This study's findings add to the existing knowledge that children who had IUGR, as measured by Doppler ultrasound in pregnancy in otherwise low-risk pregnancies, have lower cognitive scores, with the dual exposure group with added in utero HIV exposure, having the lowest cognitive scores. The findings indicate that maternal HIV exposure and placental insufficiency leading to IUGR are risk factors for impaired cognitive neurodevelopment. However, there was no evidence to suggest that child neurodevelopment was associated with maternal haemoglobin concentration in HIV- and placental insufficiency-exposed groups. Child health and nutrition-sensitive programmes should prioritize CHEU and children who had placental insufficiency as at-risk groups for cognitive delays.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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CHAPTER 7: GENERAL DISCUSSION AND CONCLUSION

General discussion, limitations, conclusions and recommendations

7.1 INTRODUCTION

In the present chapter, the summary of the key findings from the literature review and research articles related to this thesis are discussed. The literature review explored in Chapter 2 covered the areas of placental insufficiency and IUGR, maternal HIV infection and child HIV exposure, growth and neurodevelopment, anaemia and feeding practices. The findings of the UmbiGodisa study have been presented in chapters (Chapter 4, Chapter 5 and Chapter 6), as per study objectives. The summary of study limitations, public health perspective, conclusions and future research recommendations are presented in this chapter.

The present literature showed that IUGR and maternal HIV exposure are risk factors for suboptimal growth and neurodevelopmental impairment in children. Many studies have reported poor growth and/or neurodevelopmental delay in either children who had IUGR (Blake *et al.*, 2016; Feucht *et al.*, 2021; Levine *et al.*, 2015; Salam *et al.*, 2014; von Beckerath *et al.*, 2013) or children exposed to maternal HIV (Aizire *et al.*, 2020; Fowler *et al.*, 2022; le Roux *et al.*, 2019; Nyemba *et al.*, 2022; Springer *et al.*, 2018; Wedderburn *et al.*, 2019; Young *et al.*, 2022). Low length-for-age z-score and delayed development were the most reported deficits in children who had IUGR emphasised by the literature. However, IUGR resulting from placental insufficiency has been inadequately documented. There were no studies reporting growth and neurodevelopmental outcomes in children who had IUGR compounded by maternal HIV exposure. Maternal HIV infection is thought to negatively influence prenatal and postnatal growth and neurodevelopment outcomes. Furthermore, the prevalence of maternal and childhood anaemia has previously been reported to be alarmingly high, both globally and in South Africa (Juul *et al.*, 2019; Tunkyi and Moodley, 2015; Turawa *et al.*, 2021; WHO, 2023). Various studies have shown poor growth and neurodevelopment associated with anaemia (Soliman *et al.*, 2009; Ssemata *et al.*, 2020; Yang *et al.*, 2021). Studies reporting on anaemia in children who also had IUGR are lacking.

For that reason, the present study investigated how IUGR, resulting from placental insufficiency, in addition to *in utero* and early postnatal environments altered by maternal HIV infection, influences the child's growth and neurodevelopment, as well as anaemia, at age 18 months. The specific objectives were as follows:

A. To assess and compare the anthropometric indices and z-scores at age 18 months in children with normal or abnormal UmA-RI, between CHEU and CHUU.
B. To assess and compare neurodevelopment at age 18 months, including cognitive, motor and language development of children with normal or abnormal UmA-RI, between CHEU and CHUU.
C. To compare haemoglobin concentrations, and determine prevalence and the impact of maternal anaemia on child neurodevelopment and anaemia in children with normal or abnormal UmA-RI between CHEU and CHUU.
D. To determine and compare the feeding practices over 18 months and the dietary intake of iron, zinc, and iodine at age 18 months in CHUU and CHEU with normal or abnormal UmA-RI, and relate this to neurodevelopmental outcomes.

The key findings are discussed below as per the research manuscripts. Findings on feeding practices were reported within the papers' background characteristics for the study population.

7.2 EARLY CHILDHOOD GROWTH PARAMETERS IN SOUTH AFRICAN CHILDREN WITH EXPOSURE TO MATERNAL HIV INFECTION AND PLACENTAL INSUFFICIENCY

The study investigated and compared, at age 18 months, the growth outcomes of children born with and without IUGR due to placental insufficiency, as measured by an abnormal UmA-RI using Doppler screening during pregnancy, and as modified by maternal HIV status. The study population was grouped based on HIV exposure and normal or abnormal UmA-RI (as a marker of IUGR). When comparing growth parameters between CHEU and CHUU, significantly lower LAZ was observed in CHEU (-0.73 ± 1.23 vs -0.05 ± 1.32; $p = 0.003$). Many other studies have reported suboptimal growth in CHEU compared to CHUU (Aizire et al., 2020; Fowler et al., 2022; le Roux et al., 2019; Nyemba et al., 2022; Sirajee et al., 2021). Also, significantly lower LAZ was observed in abnormal UmA-RI compared to the normal UmA-RI group (-0.68 ± 1.53 vs -0.14 ± 1.29; $p < 0.001$). Further, CHEU/AbN-RI had significantly lower LAZ (-1.30 ± 1.32 vs -0.03 ± 1.30; $p < 0.001$), WAZ (-0.64 ± 0.92 vs 0.05 ± 1.15; $p = 0.014$) and HCZ (0.33 ± 0.73 vs 0.89 ± 1.15; $p = 0.016$) than the control group (CHUU/N-RI). A higher percentage of CHEU/AbN-RI (40.0%) and CHEU/N-RI (16.0%) were stunted than the control group (4.8%); $p < 0.001$ and $p = 0.016$, respectively. These findings indicate that maternal HIV infection compounded by unrelated placental insufficiency are additive risk factors for stunting among South African children. The high percentage of stunting in CHEU

adds to the body of existing knowledge (Aizire et al., 2020; Fowler et al., 2022; Jumare et al., 2019; le Roux et al., 2019; Neary et al., 2021; Nyemba et al., 2022; Sirajee et al., 2021), and the finding regarding the high percentage of stunting in CHEU with a history of IUGR due to placental insufficiency is novel in the South African context. Other possible causes of stunting may be suboptimal feeding practices, inadequate nutrition and care and social stimulation.

7.3 GROWTH, NEURODEVELOPMENTAL OUTCOMES AND MICRONUTRIENT INTAKE IN 18-MONTH-OLD SOUTH AFRICAN CHILDREN WITH MATERNAL HIV EXPOSURE AND PLACENTAL INSUFFICIENCY: THE UMBIGODISA CROSS-SECTIONAL STUDY

The study assessed, compared and determined associations between iron, zinc and iodine intakes and growth and neurodevelopmental outcomes in CHEU and CHUU born with and without IUGR. The percentage of EBF in the first six months reported in HIV-exposed groups (CHEU/N-RI: 40%; CHEU/AbN-RI: 42.9%) is better than the previously reported percentage of 37.0% in South African women living with HIV and this may indicate the success of ART programmes in better protecting, supporting and promoting breastfeeding. Nonetheless, the rates of EBF are still below the 50% average. Breastmilk intake promotes optimal growth and development. CHEU/AbN-RI (the double exposure group) had lower LAZ than CHUU/N-RI, as also reported in article 1. Across all the groups, iodine intakes were below EAR. This finding was unexpected, as in South Africa, the policy on iodization of table salt has been in place for years with the iodised salt available in markets. The possible reason for low iodine intake in children may be the consumption of small quantities of food with added salt. Iodine is essential for the growth, development, health and well-being of children. The persistent low iodine intake may result in iodine deficiency and ultimately, growth and developmental abnormalities (Pem, 2015).

The findings of the GMCD screening indicated that 21.4% of CHEU/AbN-RI and 10.0% of CHEU/N-RI (CHEU single exposure) had a delay in gross movements. Bayley-III assessment demonstrated that the CHEU/AbN-RI had lower mean cognitive composite scores compared to the control group: 93.9 ± 12.9 vs 100.1 ± 10.8; $p = 0.042$. Further, the Bayley-III assessment indicated that 21.4% of CHEU/AbN-RI had a mild delay in cognitive development. This finding was similar to the Sacchi et al. report that children who had IUGR had lower cognitive scores (Sacchi *et al.*, 2020). The findings suggest that CHEU/AbN-RI are at risk of cognitive deficits. The lower cognitive development may be related to lower LAZ and a high prevalence

of stunting (as reported in article 1) in CHEU/AbN-RI. Overall, the mean language composite score was low in this population (overall mean was 89.4 ± 12.3), potentially linked to the fact that South Africa is a multilingual country. Children growing up in a multilingual community or family learn and use several languages, so some of their language development aspects or awareness differs from monolingual children (Atagi, 2017), mainly because their learning experience is divided between diverse languages. Thus, triggers mental stress and consequently, challenges of language processing and representation (Bosworth *et al.*, 2021; Shook and Marian, 2012). Factors that influence child neurodevelopmental delay in the first 1000 days may be multifactorial and include poverty, poor nutrition (suboptimal breastfeeding practices and inadequate complementary feeding) lack of stimulation and care and maternal stress (Pem, 2015).

In the CHEU group, it was found that cognitive development was significantly positively correlated with WAZ (r = 0.15; p = 0.021) and LAZ (r = 0.35; p = 0.009). It is well known that lower LAZ or stunting is linked to deficits in cognitive development. In the same group, motor development was significantly positively correlated with WAZ (r = 0.32; p = 0.007), LAZ (r = 0.26; p = 0.017), WLZ (r = 0.27; p = 0.044) and HCZ (r = 0.24; p = 0.021). Similarly, studies have shown that stunting is associated with suboptimal cognitive development (John *et al.*, 2017; Sudfeld *et al.*, 2016). These findings of CHEU may suggest that child HIV exposure influences growth and motor development. Also, associations between LAZ and motor development were reported in South African children (Rothman *et al.*, 2018). Zinc intake was significantly associated with language development in the total study population (r = 0.10; p = 0.042), in line with previously reported positive associations between zinc and neurodevelopment (John *et al*., 2017).

7.4 THE IMPACT OF MATERNAL HIV INFECTION AND ANAEMIA TOGETHER WITH PLACENTAL INSUFFICIENCY ON NEURODEVELOPMENT AND ANAEMIA IN SOUTH AFRICAN CHILDREN.

The aim was to determine the prevalence of anaemia and associations between maternal anaemia and child neurodevelopment and anaemia among 18-month-old children exposed to maternal HIV infection and placental insufficiency. For breastfeeding practices, a high percentage of CHEU and children who had AbN-RI were reported to be exclusively breastfed during the first six months of life. In children who had AbN-RI, a high percentage of exclusive

breastfeeding may be because many of these children were born prematurely, and their mothers were counselled and supported on breastfeeding through the Kangaroo Mother Care programme in the local geographic area. Lower rates of continued breastfeeding were reported in CHEU (11.3%) than in CHUU (26.8); p = 0.038. This may be due to the perceived risk of vertical transmission of HIV. Continued breastfeeding supplement the child diet, ensuring adequate nutrient intake. It may be even more important in the context of HIV; as nutrient deficiencies are more common. A higher percentage (30.0%) of mothers of CHUU/AbN-RI were mildly anaemic compared to mothers of the control group [(9.1%); p = 0.020]. Further, findings showed that mothers of CHEU/N-RI and CHEU/AbN-RI had lower mean haemoglobin values than their counterparts in the control group: 12.4 ± 1.7 g/dL vs 12.8 ± 1.5 g/dL; p = 0.022 and 12.0 ± 1.3 g/dL vs 12.8 ± 1.5 g/dL; p = 0.024, respectively. Above one-third of children across the groups were mildly anaemic: CHUU/N-RI (control): 44.4%, CHEU/N-RI (CHEU single exposure): 44.7%, CHUU/AbN-RI (AbN-RI single exposure): 40.0% and CHEU/AbN-RI (dual exposure): 33.3%; furthermore, CHUU: 43.9%, CHEU: 41.9%, N-RI children: 44.4% and children who had AbN-RI: 37.1%. These findings, although alarmingly high, were lower than the 61.3% prevalence of childhood anaemia previously reported in a South African study (Turawa *et al.*, 2021). Contrarily, in article 2, the findings indicated that dietary intake of iron was adequate and above EAR among the study population, but in this article, a high prevalence of mild anaemia was observed. The possible cause of mild anaemia in this study population may be nutrient deficiencies other than iron, such as vitamin B_{12} (causing pernicious anaemia) and vitamin C (which improves non-haem iron absorption), resulting from inadequate feeding practices. Another possible cause of mild anaemia in this study may be iron malabsorption, which can be influenced by various factors, including high calcium intake from milk feeds.

The Bayley-III assessment showed that children who had IUGR resulting from placental insufficiency (AbN-RI group) and CHEU/AbN-RI (dual exposure) had deficits in cognitive development (as reported in manuscript 2 among CHEU/AbN-RI). These findings indicate that child HIV exposure and IUGR resulting from placental insufficiency are independent risk factors for deficits in cognitive development. The *in utero* and postnatal exposure to ART and maternal HIV infection may influence cognitive development. There were no significant associations between maternal haemoglobin and child neurodevelopment in CHEU and abnormal UmA-RI groups, differing from other previous findings (Wainstock *et al.*, 2019). In particular, we observed no evidence for the association between maternal and child

haemoglobin concentration and the reported lower cognitive development in the AbN-RI group and CHEU/AbN-RI. In the similar groups, lower LAZ was found, however, further investigations showed a significant association between cognitive, and motor development and LAZ in CHEU only. This may suggest that poor linear growth is not a risk factor for cognitive deficit in children who had AbN-RI. Observations were that many (22.9%) of these AbN-RI children were also born premature, so the possibility is that cognitive deficits may be attributed to premature births.

7.5 LIMITATIONS OF THE STUDY

The limitations of the study included a small population size of the subgroup of high-risk children, particularly the dual exposure group of CHEU with abnormal UmA-RI (5.5%). The Umbiflow International study found 5.9% and 0.7% of high-risk foetuses determined by the abnormal UmA-RI and absent end-diastolic flow (AEDF), respectively in South Africa (Vannevel *et al.*, 2022). Since children were investigated only once-off at age 18 months, this meant that longitudinal data analysis was not possible; therefore, a lack of evaluation of growth and neurodevelopment over time was a drawback, on the other hand, this also meant that the study staff did not intervene in any of the measures of growth and developmental outcomes. Other study limitations included unmeasured confounders, the potential maternal memory biases, underreporting and over reporting of the 24-hour recall. However, previous studies showed that in children younger than 23 months, a single quantified 24-hour recall had sufficient correlation with typical intakes of micronutrients such as zinc and iron (Murphy *et al.*, 2006; Padilha *et al.*, 2017). Also, the feeding practices including breastfeeding were based on maternal recall, which has a limitation in accuracy, also due to the possibility of social desirability bias. Further, the lack of full blood counts; including ferritin and C-reactive protein, for the comprehensive assessment of anaemia was a limitation of the study. Additionally, the maternal haemoglobin at 18 months postpartum may not be fully indicative of the maternal haemoglobin during pregnancy or early postpartum, which is likely more important in terms of its impact on child neurodevelopment. Lastly, delimitations of the study included only children who were aged 18 months at the time of the study and those who had a medical history of placental insufficiency resulting from blood flow resistance in the umbilical artery

7.6 CONCLUSIONS

Compared to the normal population, both groups of CHEU and AbN-RI children are high-risk populations for suboptimal growth outcomes, mainly stunting. The risk is worse when the

exposure to maternal HIV is compounded by IUGR attributed to placental insufficiency, as measured by an abnormal UmA-RI during pregnancy. Furthermore, both groups of children are also at risk of deficits in cognitive development. On the other hand, the findings were reassuring and suggested that child neurodevelopment was not associated with maternal haemoglobin concentration in children who had IUGR. Nevertheless, the prevalence of mild anaemia in children remained high in South Africa regardless of the reported adequate dietary iron and zinc intake. Additionally, even though breastfeeding practices were found to be better as compared to previous percentages, they were still below the 50% average. The adverse effect of maternal HIV exposure and IUGR resulting from placental insufficiency on child growth and development has the potential to be irreversible, especially in the absence of early intervention during the first golden 1000 days of life. The study findings advocate for the large-scale implementation of antenatal Doppler screening, including on low-risk pregnant mothers at the primary health care level, to identify foetuses at risk of IUGR, in order to implement strategies, including early nutritional intervention and follow-up care, to enhance catch-up growth and better address cognitive delays, especially in geographical areas with high maternal HIV prevalence.

7.7 PUBLIC HEALTH VIEWPOINT

HIV infection among childbearing and pregnant women is a global public health concern because of its negative impact on maternal nutrition, health, well-being and related suboptimal pregnancy outcomes, including IUGR. Co-infections such as tuberculosis are also common in HIV settings. Maternal HIV infection unfavourably affects the growth and development of CHEU possibly due to direct contact with a close family member (i.e. the mother) with a compromised immune system, potential pathogens and *in utero* exposure to the virus itself as well as to ART. Also, HIV infection is often linked with poverty and food insecurity, thus the risk of poor maternal nutrition, including nutrient deficiencies, leading to conditions such as anaemia. Child HIV exposure and poverty are detrimental to a child's growth and development because of possible food and nutrition insecurities. Poor feeding practices, including short duration of breastfeeding, are common in WLWH. Breastmilk provides infants with optimal nutrition for growth and development, therefore, poor breastfeeding practices increase the risk of suboptimal growth and development. The overall population of CHEU is increasing, but it is an overlooked group, despite their vulnerability to inadequate nutrition, suboptimal growth, particularly stunting, and neurodevelopment.

Maternal HIV infection and undernutrition are also linked to IUGR, which is considered an important determinant of child stunting and developmental delay (Bhutta *et al.*, 2013; Black *et al.*, 2013). IUGR is relevant to public health because of its adverse effects on child growth and development. Stunting, developmental delay and anaemia in children remain significant public health concerns (John *et al.*, 2017; Juul *et al.*, 2019; WHO, 2023). Stunted children are likely not to reach their potential physical growth and cognitive development. In fact, after the first 1000 days of life, stunting and neurodevelopmental impairment may be irreversible. The present study reported associations between child growth parameters and neurodevelopment. Stunting has short- and long-term effects, including poor neurocognitive development, learning and working capacity (productivity), risk of obesity and chronic diseases, particularly non-communicable diseases, which ultimately impact human lives and the economy negatively. Similarly, childhood anaemia can adversely affect neurodevelopment, leading to reduced learning capacity and low productivity during adulthood. Also, anaemia, particularly iron deficiency anaemia, is a known risk factor for stunting.

The high prevalence of stunting, mild anaemia and lower cognitive developmental scores reported in this study among South African children is alarming and signifies a need for public health action to improve maternal and child health and nutrition during the first 1000 days. Also, low iodine intake reported in this study population is relevant to public health nutrition. Children have the right to develop to their full physical, emotional and cognitive potential. Child health and nutrition programmes need to identify and prioritize CHEU and children who have placental insufficiency as at-risk populations needing interventions tailored to ameliorate potential stunting, anaemia and cognitive delays.

For South Africa to achieve and sustain improved maternal and child nutrition, the focus should be broadened to both nutrition-specific and nutrition-sensitive programmes. Reinforcement of IYCF, micronutrient supplementation and fortification (home fortification with micronutrient powders and industrial food fortification, particularly policy on iodisation of table salt) programmes, as well as promoting dietary diversity have the potential to improve low iodine intake, stunting and anaemia rates in children. Optimal feeding practices ensure adequate nutrition pivotal for catch-up growth. Besides, South Africa has implemented social safety net programmes for children (social grants) to protect children against poverty and food insecurity and alleviate malnutrition. However, child undernutrition is still a challenge, this may signify the need for reinforcement and regular evaluation of this programme. Moreover, there is a need

for consolidation of early childhood development policy to ensure that all children develop to their full cognitive and physical potential.

Interventions aimed at reducing new HIV infections among women and adolescent girls should also consider strengthening women's empowerment component. Empowering women is paramount to combating the HIV epidemic because it stimulates and builds confidence, and enables participation in informed decision-making regarding their sexual behaviour, HIV testing, management and prevention from mother to child (Bashemera *et al.*, 2013; Schierl *et al.*, 2023).

7.8 RECOMMENDATIONS FOR FUTURE STUDIES

In the context of our study findings, future studies should consider the following recommendations:

- Large-scale longitudinal studies to develop a better understanding of the growth and neurodevelopmental trajectories of CHEU and children who had placental insufficiency. Considering the large-scale study will ensure adequate representativity of subgroups, particularly groups of children who had placental insufficiency. Also, a large-scale study has the capacity to reveal even minor effects or differences between control and test groups. Investigating childhood growth and neurodevelopment over time on a large sample size will provide insightful and generalizable findings that will better inform policy-making and nutrition-sensitive programmes.
- Investigate the biochemical assessment of iron, zinc and iodine status of children and its correlation with neurodevelopment. Information on full blood count will include ferritin levels that will be used to determine iron deficiency anaemia, as well as markers of inflammation such as C-reactive protein. Assessing nutrient intake through only taking a dietary history may not reflect the actual amount of nutrients absorbed and utilised by the body, due to various physiological factors affecting nutrient absorption. Therefore, including biochemical testing in the research can provide the actual status or levels of nutrients in the body which are bioavailable for utilisation. This can then be correlated with a child's neurodevelopmental outcomes, developing evidence of the relationships.
- Investigate the barriers to exclusive breastfeeding practices among all mothers, with a special focus on mothers of N-RI children and CHUU, to inform the national policy on

infant and young child feeding practices. The present study findings showed poor exclusive breastfeeding and mixed feeding practices among groups of N-RI children and CHUU. Therefore, this calls for research to investigate the barriers to optimal feeding practices.

- The findings of the present study showed a high percentage of premature births among children who had IUGR. For future studies, it will be of importance to determine the association between premature birth, placental insufficiency/IUGR and maternal HIV infection, as there is limited literature on this topic.
- The literature overview shows that the aetiology of placental insufficiency is not clearly understood. However, assumptions are that lifestyle habits during pregnancy, such as smoking and alcohol consumption, may be important risk factors for placental insufficiency. In the present study, the percentage of mothers who smoked (cigarettes to be specific) and drank alcohol during pregnancy was very low; particularly in groups of children born with placental insufficiency, therefore this study could not associate the mentioned lifestyle habits with the risk of placental insufficiency. Future studies should thus consider exploring maternal nutrition and lifestyle behaviour and habits, other than smoking and alcohol, during pregnancy and their relationship with placental insufficiency.

The findings of this cross-sectional study were, and will continue to be, disseminated to policymakers, health professionals and science and related communities through conference presentations and proceedings, and publication in peer-reviewed journals accredited by DHET: *Viruses, International Journal of Paediatrics* and *Anaemia.* Presentations were made at the conferences and workshops and the abstracts were published, these include (1) a poster presentation at the International Workshop on HIV & Paediatrics 2023, on 21 to 22 July in Brisbane, Australia (attended in person), (2) oral presentation at South African Paediatric Association Conference 2023 on 8 – 10 September, Sandton, Johannesburg and (3) oral presentation at the 5th International Developmental Paediatrics Association Congress 2023, on 28 November to 1 December, Johannesburg (in person). The published abstracts have been included as Annexes. Also, the abstract has been accepted for poster presentation at the International Workshop on HIV & Paediatrics 2024 to be held in Munich, Germany in July 2024. Lastly, an abstract has been accepted for oral presentation at the Nutrition 2024 to be held in Durban, South Africa in October 2024.

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ANNEX A: INFORMED CONSENT FORM

PARENTAL OR LEGAL GUARDIAN INFORMATION & INFORMED CONSENT

STUDY TITLE: **Early child outcomes of *in utero* growth restricted and premature babies - a prospective cohort study in South Africa**

Principal Investigator: Dr Helen Mulol

Co-investigators: Prof. Ute Feucht, Dr Valerie Vannevel, Prof. Robert Pattinson

Institution: University of Pretoria

DAYTIME AND AFTERHOURS TELEPHONE NUMBER(S):

Daytime number/s: 012 945 2015

Afterhours number: 083 7062543

DATE AND TIME OF FIRST INFORMED CONSENT DISCUSSION:

date	**month**	**year**

:
Time

Dear Parent or Legal Guardian
Dear Ms. /Mrs. ……...

1) INTRODUCTION

We invite your child to participate in a research study. This information document will help you to decide if your child may want to participate. Before you agree that your child may take part, you should fully understand what is involved. If you have any questions that this document does not fully explain, please do not hesitate to ask the researcher.

2) THE NATURE AND PURPOSE OF THIS STUDY

The aim of this study is to continue to follow up on your baby who was part of the Umbiflow study so that we can monitor the growth and development of your baby at the age of 18 months.

3) EXPLANATION OF PROCEDURES AND WHAT WILL BE EXPECTED FROM PARTICIPANTS.

We are inviting women from the Umbiflow study to partake in this follow-up study. We will pay for your transportation to Kalafong Hospital for the study. If you agree to take part in the research we will ask you to come for one visit when your infant is 18 months old at Kalafong Hospital. We would like permission to take a photograph of your child's Road to Health book in order to have a record of the measurements of your child from birth. The following procedures will be done:

3.1 Procedures for the mother

- We will ask you to come to Kalafong Hospital with your baby for one visit when your baby is 18 months old.
- At the visit we will ask you questions about how you are feeling and if you are coping with the infant.
- At the visit we will ask you if Covid-19 has affected you or your family.
- At the visit we will ask you to answer some questions about you and your infant's health, diet and how you feed your infant.
- We will look at your fat and muscle mass which is measured by a machine which passes a very small amount of electricity through your body and will not hurt or harm you in any way.
- If your answers to the questions show that you are not coping well with your infant, we will refer you to Kalafong Hospital Family Medicine, who will give you further guidance.

3.2 Procedures for the child

- The child visit will be when the baby is 18 months old.
- At this visit we will weigh and measure your infant's length and head size and look at his / her Road to Health chart.
- We will check to see if your infant can do the usual things expected of a infant at that age, for example, walking.
- At the study visit we will check for iron levels by taking a drop of blood from your infant's finger which will give you the result immediately.
- We will also determine the amount of fat and muscle mass in your child's body at this visit. In order to do this we use a test, which is used in many countries. This test involves the child drinking a few teaspoons of heavy water, which is safe for your child. It is found in small amounts already in your child's body and will not increase your child's weight. This heavy water mixes with the normal water in your body and we will take a saliva sample using some cotton wool from your child before and 2.5 hours after drinking the heavy water. These saliva samples are then taken to a laboratory where the amount of heavy water in the saliva samples is measured which tells us the amount of fat and muscle mass in your child's body.
- If there are any problems with the child's development or anaemia, we will send your child for further care to a specialist doctor.

3.3 Testing of Samples

- Most of the tests will be done at the University of Pretoria. We also ask your permission to store all left-over samples that we have collected, free of cost, for future testing. We will first get approval from the Faculty of Health Science Research Ethics Committee, University of Pretoria before doing any more tests on these samples.

4) POSSIBLE RISK AND DISCOMFORT INVOLVED

There is only minimal risk or possible discomfort involved with providing a blood or saliva sample and measuring your child's growth and development.

5) POSSIBLE BENEFITS OF THIS STUDY

The benefits for your child is that the research team will give you an assessment on the development of your child based on the measurements performed at the 18-month visit. Your child will have additional checks on his / her growth and brain development. We will be able to see if your child's iron levels are low and any problems with development early and your child can be referred for treatment.

6) YOUR CHILD'S RIGHTS AS A PARTICIPANT?

Your child's participation in this study is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the necessary services at this clinic or hospital will continue and nothing will change. If you choose not to participate in this research project you will be offered the treatment that is routinely offered in this clinic or hospital. You are allowed to withdraw from the study at any time and you will still receive the treatment that is routinely offered in this clinic or hospital. Any information or samples we collect from you as part of this study before you withdraw will remain part of the study. There will be no further information or samples collected from you once you withdraw from the study.

7) ETHICS APPROVAL

Approvals will be obtained from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, Medical Campus, Tswelopele Building, Level 4-59, Telephone numbers 012 356 3084 / 012 356 3085 and written approval will be granted by that committee. The study will be structured in accordance with the Declaration of Helsinki (last update: October 2013), which deals with the recommendations guiding doctors in biomedical research involving humans. A copy of the Declaration may be obtained from the investigator should you wish to review it.

8) INFORMATION AND CONTACT PERSONS

If you have any questions concerning this study, you should contact:

1. Dr Helen Mulol: 083 706 2543
2. Prof Ute Feucht: 072 428 0465 or Dr Valerie Vannevel: 072 998 8704

9) COMPENSATION

You child will not be paid to take part in the study. However, any costs you and your child have because of taking part in the study, for example, transport costs to Kalafong Hospital for your follow-up visit, will be paid back to you.

10) CONFIDENTIALITY

All information about your child will be kept strictly confidential. Participants will be identified for study purposes with a unique study number. Your personal identifying information will not be connected to the information collected for this research study. Information collected about you and your child during the research will be stored safely and will only be available to the approved researchers. Research reports and articles in scientific journals will not include any information that may identify your child.

11) CONSENT TO PARTICIPATE IN THIS STUDY

- I confirm that the person requesting my consent for my child to take part in this study has told me about the nature and process, any risks or discomforts, and the benefits of the study.
- I have also received, read and understood the above written information about the study.
- I have had adequate time to ask questions and I have no objections for my child to participate in this study.
- I am aware that the information obtained in the study, including personal details, will be anonymously processed and presented in the reporting of results.
- I understand that my child will not be penalised in any way should I wish to withdraw my child from the study and that the withdrawal will not affect my child's management.
- I hererby volunteer to take part in this study.
- I have received a signed copy of this informed consent agreement.

_____ _____

Parent/Legal Guardian's name (Please print) Date

_____

Parent/Legal Guardian's signature

_____ _____

Researcher's name (Please print) Date

_____

Researcher's signature

_____ _____

Witness name (Please print) Date

_____

Witness signature

AFFIRMATION OF INFORMED CONSENT BY AN ILLITERATE PARTICIPANT (if suitable)

I, the undersigned, ……, have read and have explained fully to the participant, named …… , the participant information document, which describes the nature and purpose of the study in which I have asked the child's parent/legal guardian to participate. The explanation I have given has mentioned both the possible risks and benefits of the study and the alternative treatments available for the child's illness. The participant indicated that she understands that she will be free to withdraw from the study at any time for any reason and without jeopardizing the child's standard care.

I hereby certify that the patient has agreed to participate in this study.

_____ _____

Parent/Legal Guardian's name (Please print) Date

_____

Parent/Legal Guardian's signature

_____ _____

Researcher's Name (Please print) Date

_____

Researcher's Signature

_____ _____

Name of the person who witnessed
the informed consent (Please print) Date

_____

Signature of the Witness

ANNEX B: PARTICIPANT INDENTIFICATION QUESTIONNAIRE

Before enrolment, please confirm the following: **YES** NO

1. Baby has no severe medical conditions or chromosomal or structural abnormalities ☐ ☐

Participant identification **Study number: UMB __ __ __**

Mother's name*	
Date of Birth	
Area of residence*	
Phone number*	
Hospital Number	
Clinic*	
Home language	
Physical address*	
Postal address*	
Work address*	
Email address*	
Partner's name*	
Cell number*	
Work telephone*	
Email address *	
Next of kin's name*	
Cell number*	

After delivery of infant:

Baby's name*	
Date of Birth	
Sex	
Hospital Number	
Place of birth*	

Gestational age according to Road to Health Book	__ __ weeks

Form completed by: _____ **Form checked by**: _____

ANNEX C: POSTNATAL FORM FIRST VISIT

Postnatal form: First Visit

UmbiBaby Study no: _____

Date: First postnatal visit _____ (DD MM YYYY if unknown 01-01-1900)

MATERNAL CHARACTERISTICS

Mother: Date of Birth _____ (DD MM YYYY if unknown 01-01-1900)

Mother: Age _____ (00 (y) if unknown)

Gravidity (number of pregnancies including current pregnancy) _____ (0; 99 if unknown)

Parity (number of all previous births ≥ 28 weeks) _____ (0; 99 if unknown)

Abortions, miscarriages, TOP _____ (0; 99 if unknown)

Comment: Previous pregnancy losses _____

DEMOGRAPHIC CHARACTERISTICS

Mother: Home language

- ◯ Afrikaans
- ◯ English
- ◯ Ndebele
- ◯ Northern Sotho/Sepedi
- ◯ South Sotho
- ◯ Swazi
- ◯ Tsonga
- ◯ Tswana
- ◯ Venda
- ◯ Xhosa
- ◯ Zulu
- ◯ Other
- ◯ Unknown

If Other, please specify: _____ (Specify other home language)

Mother: Population group	○ Black/African ○ Indian/Asian ○ Coloured ○ White ○ Other ○ Not recorded
If Other, please specify	_____
Mother: Highest level of education (completed)	○ No schooling ○ Grade 1 ○ Grade 2 ○ Grade 3 ○ Grade 4 ○ Grade 5 ○ Grade 6 ○ Grade 7 ○ Grade 8 ○ Grade 9 ○ Grade 10 ○ Grade 11 ○ Grade 12 ○ Certificate ○ Diploma ○ Degree ○ Not recorded
Mother: Marital status	○ Single ○ Married ○ Co-habiting ○ Widowed ○ Divorced ○ No partner ○ Other ○ Not recorded
If Other, please specify	_____
Mother: Current employment	○ No ○ Yes ○ Not recorded
Mother: Type of employment	○ Full-time ○ Part-time ○ Piece jobs ○ Unknown ○ Self-Employed
Mother: Social grant (Multiple answers possible)	☐ None ☐ Medical grant ☐ Foster care ☐ Child support ☐ Care dependency ☐ Pension ☐ Not recorded
Partner: Highest level of education	○ No schooling ○ Grade 1 ○ Grade 2 ○ Grade 3 ○ Grade 4 ○ Grade 5 ○ Grade 6 ○ Grade 7 ○ Grade 8 ○ Grade 9 ○ Grade 10 ○ Grade 11 ○ Grade 12 ○ Certificate ○ Diploma ○ Degree ○ Unknown ○ No partner ○ Not recorded
Partner: Current employment	○ No ○ Yes ○ No partner ○ Not recorded

Partner: Type of employment ◯ Full-time ◯ Part-time ◯ Piece jobs ◯ Not recorded ◯ Self-employed	
How much is the income in your household each month?	◯ R 0 - R 2000 ◯ R 2001 - R 4000 ◯ R 4001 - R 6000 ◯ R 6001 - R 8000 ◯ R 8000 + ◯ Don't know ◯ Prefer not to answer
Mother: HIV status	◯ Positive ◯ Negative ◯ Unknown
Partner: HIV status	◯ Positive ◯ Negative ◯ Unknown

HOUSEHOLD

Description of neighbourhood:	◯ Urban/City ◯ Formal township ◯ Informal settlement/ Squatter ◯ Rural ◯ Other ◯ Not recorded
If Other, please specify	_____
Adults (18 years and over) living in home (including participant):	_____ (999 if unknown)
Children (under 18 years) living in home (including infant in this study)	_____ (999 if unknown)
Access to running water:	◯ None ◯ Communal tap ◯ Inside yard ◯ Inside house ◯ Other ◯ Not recorded
If Other, please specify:	_____
Access to toilet	◯ None ◯ Pit latrine ◯ Flushing toilet ◯ Other ◯ Not recorded
If Other, please specify:	_____
Electricity at home:	◯ No ◯ Yes ◯ Not recorded
Functional fridge at home:	◯ No ◯ Yes ◯ Not recorded

Television at home:	○ No ○ Yes ○ Not recorded
Telephone available at home:	○ No ○ Yes ○ Not recorded
Do you have a cell phone?	○ No ○ Yes ○ Not recorded
Computer at home:	○ No ○ Yes ○ Not recorded

Do you have access to the internet:

☐ No ☐ Yes, at home ☐ Yes, at other places ☐ Not recorded ☐ Yes, on my phone

Do you/ your family own a car?	○ No ○ Yes ○ Not recorded
Is your house made of brick & cement?	○ No ○ Yes ○ Not recorded
Do you rent?	○ No ○ Yes ○ Not recorded
Do you stay in a RDP house	○ No ○ Yes ○ Not recorded

Date of next appointment

_____
(DD MM YYYY if unknown 01-01-1900)

FOOD COST QUESTIONNAIRE

What is the average income for the household per month?

□ R0-2000
□ R2000 – R4000
□ R4000 – R6000
□ R6000 – R8000
□ More than R8000

What is the average income of the main source of income of the household per month?

□ R0-2000
□ R2000 – R4000
□ R4000 – R6000
□ R6000 – R8000
□ More than R8000

Does your household receive any income from grants?

□ Yes
□ No

What kind of grants? (Please answer the question by ticking in the brackets, tick all grants your household receive and the number of each.

□ Child Support Grant

□ Grant-in-Aid

□ Care Dependency Grant

□ Foster Child Grant

□ Social Relief of Distress

□ Grant for older persons

□ Disability grant

□ War veterans grant

□ Pension

□ Other:

_____

How many people are receiving grants in your household?_ _____

How many people are earning an income per month in your household?

□ One
□ Two
□ More than two
□ None
□ Other:_____

What is your partnership status?

□ Living with a partner
□ Not living with a partner

How many children (under 18 years) are residing in your household?_____

How many are your own children?_____

Do you have any dependent individuals (above 18 years) living with you?

□ Yes, have dependent individuals living with me
□ No dependent individuals living with me.

How many dependent individuals are living with you?_____
Who usually does the grocery shopping in the house?

□ Me
□ My partner
□ Children
□ Other, specify:_____

Do you buy in bulk or on a daily basis when food is needed?

□ Bulk
□ Daily
□ Other:_____

Do you only buy food when the food is on special?

□ Yes
□ No
□ Sometimes

To which of these shops do you go to buy food?

□ Local producer (on street)
□ Big Hypermarket
□ Local Shop (e.g. OK/Shoprite/Boxer)
□Other:_____
□ Not recorded

Why do you mostly choose these shops?

□ Distance from home
□ Prices
□ Quality
□ Other:_____
□ Not recorded

What can you say about the freshness of products i.e. fruits and vegetables at a local producer on the streets?

□ Fresh most of the times
□ Sometimes
□ Never
□ Other:_____

What is your main mode of transport?

□ Walking
□ Public transport
□ Own car
□ Other:_____

How often do you use public transport (e.g. taxi) to the shops to buy food?

□ Most of the times
□ Sometimes
□ Never
□ Other:_____

What is the estimate taxi fare you pay to buy food?_____
Do you give your child any milk feeds?

□ Yes
□ No
□ Sometimes

What kind of milk feeds do you give to your child?

□ Cow's milk only
□ Formula milk only
□ Cow's milk and formula milk

□ Other:_____

What is the name of the formula milk you give to your child?

□ Nan pelargon
□ Infacare classic
□ Lactogen
□ Nan
□ NIDO
□ Milk powder (Klim)
□ Other:_____

Why do you choose this kind of formula milk?

_____

How much are you spending on (any) milk, either for the child or for the household per month?

□ Below R50.00
□ R100.00 to R150.00
□ R150.00 to R200.00
□ Above R200.00

Do you buy the formula in bulk, on a daily basis when needed, when on special?

□ Bulk
□ Daily
□ When on special

ANNEX D: COVID-19 QUESTIONNAIRE

Impact of COVID-19 Questionnaire

1. Thinking from the time of the start of COVID-19, have you, the study child, or other household member tested positive for COVID-19?
   - ☐ Yes
   - ☐ No → Skip to question 5
   - ☐ Prefer not to answer → Skip to question 5
2. How many household members tested positive for COVID-19?
   - ☐ One member
   - ☐ Two members
   - ☐ Three members
   - ☐ More than three members
   - ☐ Prefer not to answer
3. If yes, which person(s) tested positive for COVID-19? (Multiple answers possible)
   - ☐ The study child
   - ☐ Mother of the study child
   - ☐ Father of the study child
   - ☐ A grandparent
   - ☐ Other child
   - ☐ Other household member(s) – please specify: _____
   - ☐ Prefer not to answer
4. For each of the persons who tested positive for COVID-19, please complete the following table:

Household member (please identify, e.g. grandparent, tenant, etc.)	**Age**	**When did the household member test COVID-positive?**	**Was the person hospitalized with COVID?**	**Did the person fully recover from COVID?**
A. Study child	_____ Years _____ Months	☐ In last 3 months ☐ 3-6 months ☐ 6-9 months ☐ >9 months ☐ Other (specify): _____ ☐ Prefer not to answer	☐ Yes ☐ No ☐ Unsure ☐ Prefer not to answer	☐ Yes ☐ No, not yet ☐ No, long-term complications ☐ No, demised ☐ Other, please specify:_____ ☐ Prefer not to answer
B. Mother of study child	_____ Years	☐ In last 3 months ☐ 3-6 months ☐ 6-9 months ☐ >9 months ☐ Other (specify): _____ ☐ Prefer not to answer	☐ Yes ☐ No ☐ Unsure ☐ Prefer not to answer	☐ Yes ☐ No, not yet ☐ No, long-term complications ☐ No, demised ☐ Other, please specify:_____ ☐ Prefer not to answer

C. Father of study child	_____ Years ☐ Unsure	☐ In last 3 months ☐ 3 -6 months ☐ 6-9 months ☐ >9 months ☐ Other (specify): _____ ☐ Prefer not to answer	☐ Yes ☐ No ☐ Unsure ☐ Prefer not to answer	☐ Yes ☐ No, not yet ☐ No, long-term complications ☐ No, demised ☐ Other, please specify:_____ ☐ Prefer not to answer
D. Other child 1	_____ Years __Months ☐ Unsure	☐ In last 3 months ☐ 3-6 months ☐ 6-9 months ☐ >9 months ☐ Other (specify): _____ ☐ Prefer not to answer	☐ Yes ☐ No ☐ Unsure ☐ Prefer not to answer	☐ Yes ☐ No, not yet ☐ No, long-term complications ☐ No, demised ☐ Other, please specify:_____ ☐ Prefer not to answer
E. Other child 2	_____ Years ___Months ☐ Unsure	☐ In last 3 months ☐ 3-6 months ☐ 6-9 months ☐ >9 months ☐ Other (specify): _____ ☐ Prefer not to answer	☐ Yes ☐ No ☐ Unsure ☐ Prefer not to answer	☐ Yes ☐ No, not yet ☐ No, long-term complications ☐ No, demised ☐ Other, please specify:_____ ☐ Prefer not to answer
F. Other child 3	_____ Years ___Months ☐ Unsure	☐ In last 3 months ☐ 3-6 months ☐ 6-9 months ☐ >9 months ☐ Other (specify): _____ ☐ Prefer not to answer	☐ Yes ☐ No ☐ Unsure ☐ Prefer not to answer	☐ Yes ☐ No, not yet ☐ No, long-term complications ☐ No, demised ☐ Other, please specify:_____ ☐ Prefer not to answer
G. Other adult household member 1 - please specify: _____	_____ Years ☐ Unsure	☐ In last 3 months ☐ 3-6 months ☐ 6-9 months ☐ >9 months ☐ Other (specify): _____ ☐ Prefer not to answer	☐ Yes ☐ No ☐ Unsure ☐ Prefer not to answer	☐ Yes ☐ No, not yet ☐ No, long-term complications ☐ No, demised ☐ Other, please specify:_____ ☐ Prefer not to answer

H. Other adult household member 2 - please specify: _____	_____ Years ☐ Unsure	☐ In last 3 months ☐ 3-6 months ☐ 6-9 months ☐ >9 months ☐ Other (specify): _____ ☐ Prefer not to answer	☐ Yes ☐ No ☐ Unsure ☐ Prefer not to answer	☐ Yes ☐ No, not yet ☐ No, long-term complications ☐ No, demised ☐ Other, please specify:_____ ☐ Prefer not to answer
I. Other adult household member 3 - please specify: _____	_____ Years ☐ Unsure	☐ In last 3 months ☐ 3-6 months ☐ 6-9 months ☐ >9 months ☐ Other (specify): _____ ☐ Prefer not to answer	☐ Yes ☐ No ☐ Unsure ☐ Prefer not to answer	☐ Yes ☐ No, not yet ☐ No, long-term complications ☐ No, demised ☐ Other, please specify:_____ ☐ Prefer not to answer
J. Other adult household member 4 - please specify: _____	_____ Years ☐ Unsure	☐ In last 3 months ☐ 3-6 months ☐ 6-9 months ☐ >9 months ☐ Other (specify): _____ ☐ Prefer not to answer	☐ Yes ☐ No ☐ Unsure ☐ Prefer not to answer	☐ Yes ☐ No, not yet ☐ No, long-term complications ☐ No, demised ☐ Other, please specify:_____ ☐ Prefer not to answer

5. Did you / another household member lose your / their job due to COVID-19?
   - ☐ Yes → Continue to question 6
   - ☐ No → Skip to question 7
   - ☐ Prefer not to answer
6. Who was the person(s) who lost the job? (Multiple answers possible)
   - ☐ Mother
   - ☐ Father
   - ☐ Other household member (s): _____
   - ☐ Prefer not to answer
7. What impact did COVID-19 have on your household income?
   - ☐ Household income reduced to zero
   - ☐ Reduced household income
   - ☐ Increased household income
   - ☐ No impact on the household income
   - ☐ Prefer not to answer
8. What COVID-19 related assistance did your household receive (or currently)? ☐ COVID-grant
   - ☐ Increased child support grant due to COVID
   - ☐ UIF
   - ☐ Food parcels
   - ☐ Other COVID relief: specify _____
   - ☐ None
   - ☐ Prefer not to answer

9. Thinking of the period of the COVID-19 pandemic, did you and your household members have enough food to eat?

- ☐ Always
- ☐ Most of the time
- ☐ Sometimes
- ☐ Never
- ☐ Prefer not to answer

10. How often are you and your family members eating food that you used to eat before the COVID 19 pandemic?

- ☐ Always
- ☐ Most of the time
- ☐ Sometimes
- ☐ Never
- ☐ Prefer not to answer

ANNEX E: MATERNAL AND INFANT POSTPARTUM QUESTIONNAIRE

Maternal and Infant Postpartum Questionnaire: First Visit

UmbiBaby Study No.: U M B __ __ __

Visit Date (DD/MM/YYYY): _____ / _____ / _____

Infant Date of Birth (DD/MM/YYYY): _____ / _____ / _____ Infant Gender: _____

INFANT FEEDING AND PERINATAL HISTORY

1. Did you ever breastfeed or try to breastfeed your baby, even if only for a single feed?

□ Yes → Skip to Question 3 □ No □ Prefer not to answer → Skip to Question 5

2. If No, why was this? Select all that apply

□ Personal choice → Skip to Question 5
□ Personal circumstances (e.g., other demands, return to work) → Skip to Question 5
□ You were unwell → Skip to Question 5
□ Baby was too small or unwell → Skip to Question 5
□ You didn't think you had enough milk → Skip to Question 5
□ Lack of support / resources → Skip to Question 5
□ Other reason. Please specify:_____ → Skip to Question 5
□ Prefer not to answer → Skip to Question 7

3. How soon after birth was your baby first put to the breast? *(Skip if NO to Question 1)*

□ Immediately → please specify the time since birth: _____ minutes or _____ hours
□ Never (baby was fed pumped milk)
□ Prefer not to answer

4. Has your baby ever been fed breast milk from a bottle or a cup?

□ Yes □ No □ Prefer not to answer

5. At how many weeks or months postnatal (after birth) is this first visit occurring?

□ 6 weeks □ 10 weeks □ 14 weeks □ 6 months □ 9 months □ 12 months □ 18 months □ 24 months

6. How did you feed your baby in the first 6 months?

□ Breast milk only directly from the breast (*no* expressed breast milk and *no* formula feeding) from birth to baby's current age
□ Breast milk only with some feeding directly from the breast and some expressed breast milk , but *no* formula feeding up to baby's current age
□ Breast milk (directly from the breast/ expressed breast milk **and donor** breast milk), but *no* formula feeding up to baby's current age
□ Breast milk and formula feeding (baby received some formula before current age but also received direct/expressed breast milk **and donor** milk)
□ Breast milk and formula feeding (baby received some formula before his/her current age but still received some direct or expressed breast milk at his/her current age)
□ Formula milk only, but previously also breast milk
□ Formula feeding only (baby did not receive any breast milk between birth and his/her current age)

7. Has your baby had any liquids other than breast milk or formula since his/her birth (even if it was a temporary supplement)? Other liquids include water, glucose water, donor breast milk, evaporated milks, goat's milk, cow's milk, tea, rooibos tea or any other drink (including muthi). Any semi-solids like yoghurt, cerelas. Any solids, like porridge, vegetables?

□ Yes → If Yes, please specify: _____
□ No
□ Prefer not to answer

8. Was your baby admitted to the neonatal unit in the first week of life?
   □ Yes
   □ No → Skip to Question 12
9. How old was your baby in days? _____
10. What was the diagnosis?
   □ Respiratory Distress Syndrome (RDS) / Hyaline Membrane Disease (HMD)
   □ Congenital pneumonia
   □ Neonatal sepsis
   □ Jaundice
   □ Convulsions
   □ Other reason. Please specify:_____
11. Information from Hospital File: If neonate was admitted, which of the following interventions did the infant receive?
   □ Continuous Positive Airway Pressure (CPAP)
   □ Ventilation
   □ Surfactant
   □ Cooling
   □ Other Please specify: _____
   □ Unknown

11.1 If Ventilation was used, for how many days: _____

12. Has your baby ever taken any prescribed medications?

   □ Yes → If Yes, please specify: _____
   □ No
   □ Prefer not to answer
13. The following questions are only to be asked if the mother is HIV-positive. Since birth:

NVP started?	Yes	No	Dose:	Still given?	Yes	No
AZT started?	Yes	No	Dose:	Still given?	Yes	No
Other drugs started?	Yes	No	Dose:	Still given?	Yes	No
				Time/s given:		
Birth PCR Result	Positive		Negative	Indeterminate		

MOTHER'S HEALTH AND DIETARY SUPPLEMENTS

Now we would like to ask some general questions about your health and lifestyle since your baby was born.

14. How would you currently rate your general health?
   □ Excellent
   □ Very good
   □ Good
   □ Fair
   □ Poor
   □ Prefer not to answer
15. What is your current weight? _____kilograms
   □ Don't know
   □ Prefer not to answer

16. Did you have any infections post partum?

□ Yes

□ No → Skip to Question 17

If Yes: Please specify: _____

Date (DD/MM/YYYY): _____

Treatment given: _____

17. Did you have any complications / illnesses post partum?

□ Yes

□ No → Skip to Question 18

If Yes: Please specify: _____

Date (DD/MM/YYYY): _____

Treatment given: _____

18. Are you taking any prescribed medication?

□ Yes

□ No → Skip to Question 19

If Yes: Please specify: _____

Date (DD/MM/YYYY): _____

19. Since your baby was born, have you taken any vitamins, minerals or other dietary supplements?

□ Yes

□ No → Skip to Question 20

□ Prefer not to say → Skip to Question 20

<table>
<tr><td colspan="3">How often have you used the following since you gave birth?</td></tr>
<tr><td>Prenatal vitamin (before baby is born and possibly during breastfeeding)</td><td>□ Never
□ Less than 1 per month
□ 1-3 days per month
□ 1-3 days per week
□ 4-6 days per week
□ Every day
□ Don’t know
□ Prefer not to say</td><td>If Yes, please list the brand name and specific type:
_____
_____</td></tr>
<tr><td>Note: Some pregnant women are prescribed a Pregamal Tablet and asked to take 2 tablets per day during pregnancy and breastfeeding.</td><td colspan="2">□ Has this dose and intake routine been prescribed by your health care provider? □ Yes □ No

Are you adhering to this prescription (are you following the instructions)? □ Yes □ No □ Don’t know □ Prefer not to say</td></tr>
<tr><td>Multivitamin</td><td>□ Never
□ Less than 1 per month
□ 1-3 days per month
□ 1-3 days per week
□ 4-6 days per week
□ Every day
□ Don’t know
□ Prefer not to say</td><td>If Yes, please list the brand name and specific type:
_____
Does your multivitamin contain minerals (like iron, zinc, etc.)?
□ Yes □ No
□ Don’t know
□ Prefer not to say</td></tr>
</table>

Folic acid or folate (NOT as part of a multivitamin or prenatal multivitamin)	□ Never □ Less than 1 per month □ 1-3 days per month □ 1-3 days per week □ 4-6 days per week □ Every day □ Don't know □ Prefer not to say	Dose: □ less than 400 mcg (0.4mg) □ 400-799 mcg □ 800-999 mcg □ 1000 (1mg) or more, up to 4000 mcg (4 mg) □ 5000 (5mg) □ Don't know □ Prefer not to say
Iron (NOT as part of a multivitamin or prenatal multivitamin)	□ Never □ Less than 1 per month □ 1-3 days per month □1-3 days per week □ 4-6 days per week □ Every day □ Don't know □ Prefer not to say	Dose: □ Less than 10 mg □ 10-14 mg □ 15-39 mg □ 40 mg or more □ Don't know □ Prefer not to say
Note: Some pregnant women are prescribed a Gulf Ferrous Sulphate Compound Tablet and asked to take 2 tablets per day during pregnancy and breastfeeding.	□ Has this dose and intake routine been prescribed by your health care provider? □ Yes □ No Are you adhering to this prescription (are you following the instructions)? □ Yes □ No □ Don't know □ Prefer not to say	
Calcium supplements or calcium containing antacids (NOT as part of a multivitamin or prenatal multivitamin)	□ Never □ Less than 1 per month □ 1-3 days per month □ 1-3 days per week □ 4-6 days per week □ Every day □ Don't know □ Prefer not to say	Dose: □ less than 500 mg □ 500-599 mg □ 600-999 mg □ 1000 mg or more □ Don't know □ Prefer not to say
Other, Please specify: (Examples Vitamin C, Zinc, Vitamin D, Probiotics, Cod liver oil, Other Fish oil or Omega 3 fatty acids Name: _____	□ Never □ Less than 1 per month □ 1-3 days per month □ 1-3 days per week □ 4-6 days per week □ Every day □ Don't know □ Prefer not to say	Dose: _____

FOOD SECURITY

The following questions ask about your access to food over the past 12 months.

20. Which of the following statements best describes the food eaten in your household in the past 12 months?

□ You and other household members always had enough of the kinds of food you wanted to eat.
□ You and other household members had enough to eat, but not always the kinds of food you wanted.
□ **Sometimes** you and other household members did not have enough to eat.
□ **Often** you and other household members didn't have enough to eat.

□ Don't know
□ Prefer not to answer

The following statements may be used to describe the food situation for a household. Please indicate if the statement was often true, sometimes true, or never true for you and other household members in the past 12 months.

21. You and other household members worried that food would run out before you got money to buy more. Was that often true, sometimes true, or never true in the past 12 months?
- □ Often true
- □ Sometimes true
- □ Never true
- □ Don't know
- □ Prefer not to answer

22. The food that you and other household members bought just didn't last, and there wasn't any money to get more. Was that often true, sometimes true, or never true in the past 12 months?
- □ Often true
- □ Sometimes true
- □ Never true
- □ Don't know
- □ Prefer not to answer

23. You and other household members couldn't afford to eat balanced meals. In the past 12 months was that often true, sometimes true, or never true?
- □ Often true
- □ Sometimes true
- □ Never true
- □ Don't know
- □ Prefer not to answer

If the participant responds "Sometimes" or "Often" to Question 20 OR "Often true" or "Sometimes true" to ANY ONE of Questions 21-23 then continue to Question 24; otherwise, skip to the next section (Question 30).

The following questions are about the food situation in the past 12 months for you or any other adults in your household.

24. In the past 12 months, did you or other adults in your household ever cut the size of your meals or skip meals because there wasn't enough money for food?
- □ Yes
- □ No → Skip to Question 26
- □ Don't know → Skip to Question 26
- □ Prefer not to answer → Skip to Question 26

25. How often did this happen?
- □ Almost every month
- □ Some months but not every month
- □ Only 1 or 2 months
- □ Don't know
- □ Prefer not to answer

26. In the past 12 months, did you personally ever eat less than you felt you should have because there wasn't enough money to buy food?

□ Yes
□ No
□ Don't know
□ Prefer not to answer

27. In the past 12 months, did you personally lose weight because you didn't have enough money for food?

□ Yes
□ No
□ Don't know
□ Prefer not to answer

*If the participant responded **"Yes"** to Questions 24, 26 or 27, continue to Question 28; otherwise, skip to the next section (Question 30).*

28. In the past 12 months, did you or other adults in your household ever not eat for a whole day because there wasn't enough money for food?

□ Yes
□ No → Skip to Question 30
□ Don't know → Skip to Question 30
□ Prefer not to answer → Skip to Question 30

29. How often did this happen?

□ Almost every month
□ Some months but not every month
□ Only 1 or 2 months
□ Don't know
□ Prefer not to answer

SMOKING AND ALCOHOL

30. Do you smoke cigarettes?

□ Yes
□ Do not smoke → Skip to Question 32
□ Prefer not to answer → Skip to Question 32

If Yes, how many cigarettes do you smoke each day now? _____

31. Do you smoke inside your home?

□ Yes
□ No
□ Prefer not to answer

32. Does any member of your household smoke cigarettes (even if not inside your home)?

□ Yes □ No
□ Prefer not to answer

33. How often are you usually exposed to other people's tobacco smoke inside your home?

□ Every day
□ Almost every day
□ At least once a week
□ At least once a month

□ Less than once a month
□ Never
□ Don't know
□ Prefer not to answer

34. During leisure time outside of your home, how often are you usually exposed to other people's tobacco smoke?

□ Every day
□ Almost every day
□ At least once a week
□ At least once a month
□ Less than once a month
□ Never
□ Don't know
□ Prefer not to answer

35. Since your baby was born, how often do you drink alcohol?

□ 6 to 7 times a week
□ 4 to 5 times a week
□ 2 to 3 times a week
□ Once a week
□ 2 to 3 times a month → Skip to Question 37
□ About once a month → Skip to Question 37
□ Less than monthly → Skip to Question 37
□ Never → Skip to Question 37
□ Don't know → Skip to Question 37
□ Prefer not to answer → Skip to Question 37

36. Since your baby was born, how often did you have four or more alcoholic drinks at the same sitting or occasion?

□ 6 to 7 times a week
□ 4 to 5 times a week
□ 2 to 3 times a week
□ Once a week
□ 2 to 3 times a month
□ About once a month
□ 6 to 11 times a year
□ 1 to 5 times a year
□ Never
□ Don't know
□ Prefer not to answer

Form completed by: _____ **Form checked by**: _____

ANNEX F: 24 HOUR DIETARY RECALL

Infant 24-Hour Dietary Recall

Study No.: U M B __ __ __ Visit Date: DD/MM/YYYY

Infant Visit:

6 weeks	10 weeks	14 weeks	6 months
9 months	12 months	18 months	24 months

What day of the week does this recall refer to?

Sunday	Monday	Tuesday	Wednesday	Thursday

Please recall what your infant ate and drank yesterday, including breast milk and water:

Time of Day	**What food and drink?**	**How was it prepared? What was added?**	**How much was eaten?**	**How much was left?**
Waking up to about 9 o'clock (breakfast time)				
Mid-morning (09h00-12h00)				
Lunch time (12h00-14h00)				
Afternoon (14h00-17h00)				

Supper time (17h00-sunset)				
After supper; during the night				

Would you describe the food that your infant ate yesterday as typical of his/her usual food intake?	Yes	No
If NO, please give the reason:		

Form completed by: _____ Form checked by: _____

ANNEX G: INFANT FOLLOW-UP QUESTIONNAIRE

Infant Follow-Up Form (9 - 24 months)

Date: _____ Study No.: UMB __ __ __

Infant Date of Birth: DD/MM/YYYY

Infant visit (months)	9		12		18		24	
Immunization given today	Measles		PCV		Hexaxim		None	
Infant study blood done?	YES	NO	Not indicated		Comment:			
Breast milk sample taken?	YES	NO	Not indicated (not breastfeeding)					
MATERNAL INFORMATION								
Maternal Date of birth & age	DOB: DD/MM/YYYY						Age (years):	
Maternal Anthropometry:	Weight (kg):		Height(cm):				MUAC (cm):	
	Maternal triceps skin fold thickness (mm):							
Maternal Hb Value:	Anaemia (Hb < 10 g/dL):				YES		NO	
Maternal HIV-status:	POSITIVE	NEGATIVE			UNKNOWN		Date:	
If positive - Latest CD4:							Date	
- Latest Viral load:							Date	
- Current ART:							Date:	
In the last week has she missed any dose?	YES	NO	If YES how many doses		____	Not sure		
Maternal TB status:	CURRENT	PREVIOUS	NEVER	UNKNOWN	Date:			
- Current INH prophylaxis:	YES		NO		Date:			
- Current TB treatment:	YES		NO		Date:			
- Type TB								
Other infections since the last visit	YES	NO	(If YES, what, when, treatment & results)					
What	Date:		Treatment		Results			
-								
-								
Medication use since the last visit	YES	NO	UNSURE	(If YES: What & when)				
- Antibiotics				Date:				
- Iron supplement/ferrous sulphate				Date:				
-				Date:				
-				Date:				
PHQ-2 Now I want to ask you specifically about how you have been feeling. Over the past two weeks, how often have you been bothered by any of the following problems?								
Little interest or pleasure in doing things?	Not at all	Several days	More than half the days	Nearly every day				
Feeling down, depressed or hopeless?	Not at all	Several days	More than half the days	Nearly every day				
Have you ever received treatment (psychotherapy or medication) for depression? If yes, when was this?	No	Yes – after index child was born	Yes – before index child was born					

INFANT INFORMATION:				
Infant Anthropometry				
Weight (kg):	Length (cm):		MUAC (cm):	
Head Circumference (cm):	Triceps skinfold thickness (mm):			
Weight-for-age: z-score < -2	YES	NO	Comment:	
Height-for-age : z-score < -2	YES	NO	Comment:	
Weight-for-height: z-score < -2	YES	NO	Comment:	
Hb Value:	Anaemia (Hb < 10 g/dL):		YES	NO
Infant clinical examination comments, referrals.				

Infant Feeding			
1. Are you currently breastfeeding your baby or giving your baby expressed breast milk?	YES	NO - skip to Question 2	Prefer not to answer – skip to Question 3
2. How old was your baby when you stopped breastfeeding?	_____ days , _____ weeks, _____ months		Prefer not to answer
3. How old was your baby when you introduced formula?	_____ days, _____ weeks, _____ months		Prefer not to answer
4.What was the main reason for introducing formula?	□ Breastfeeding took too long or was too tiring □ Needed to return to work □ Convenience or to allow others to feed □ To try and get baby to sleep through the night □ Insufficient milk to satisfy the baby □ Baby wouldn't suck because unwell or low birth weight □ Baby wouldn't suck for no apparent reason □ Baby irritable or colicky □ Baby not gaining weight □ Painful breasts or sore nipples □ Mastitis or breast abscess □ Milk dried up □ The right time/age to change □ Other reason → Please specify: _____ □ Prefer not to answer		
5. What type of formula do you usually feed your baby	□ Cow's milk-based formula □ Lactose-free cow's milk-based formula □ Soy-based formula □ Other → Please specify:_____ □ Prefer not to answer		
6. What is the specific brand and type of formula do you usually feed your baby?			
7. What form of formula do you usually use?	□ Liquid ready-to-use □ Powder concentrate (add water) □ Prefer not to answer		
8.1 What was the <u>first</u> drink other than breastmilk that your baby received? How old was your baby when you gave this drink for the first time (in months)		_____ months	
8.2 What was the <u>first</u> solid, semi-solid or soft foods (with a spoon) that your baby received? How old was your baby when you gave this drink for the first time (in months)		_____ months	
9. Did you introduce the following into your infant's diet? If YES, when was this?			
9a. Water or glucose water	NO	YES	_____ months
9b. Other liquids eg tea, juice	NO	YES	_____ months
9c. Cow's milk or Klim, Nespray etc	NO	YES	_____ months
9d. Semi-solids eg cereals, porridge	NO	YES	_____ months
9e. Solids eg vegetables, fruit	NO	YES	_____ months
9f. Protein eg meat, eggs, peanut butter, cheese, yoghurt, fish	NO	YES	_____ months
9g. Traditional medicines	NO	YES	_____ months

Infant Illness and Medications - Illnesses **since the last visit** (this information is collected from mother's history and also information documented on RTHB, or any other patient health record like discharge summaries)		
10. Has your child had malnutrition/Kwashiorkor or has a health provider told you that he/she was not growing as well as expected.	YES	NO
11. Has your child had diarrhea Date……	YES	NO
12. Has your child had difficulty in breathing? Date……	YES	NO

13. Was your child admitted for any illness in the hospital? If Yes, describe …… When ? Month / year: ……	YES	NO
14. Did you visit any health care facility because your child was ill? If Yes, describe ……Date……	YES	NO
15. Does your child currently need or use medicine prescribed by a doctor or nurse? If Yes, describe ……	YES	NO
16. Does your child have any chronic illness or medical condition? If Yes, describe ……	YES	NO
17. Is your child limited in any way in his or her ability to do the things most children of the same age can do? If Yes, describe ……	YES	NO
18. Does your child have any kind of developmental problem, disability for which he/she needs or gets special treatment or stimulation? If Yes, describe ……	YES	NO
19. Do you currently access any child care grant assistance because of your child's condition/illness (Care dependency grant)	YES	NO
20. Do you currently receive child social grant for this child?	YES	NO
21. Do you receive a foster care grant for this child?	YES	NO

22. Does your baby receive any vitamins or supplement drops?	YES	NO	Prefer not to answer	Don't know

23. If YES, which vitamins or supplements are you giving the infant and how often?	**Vitamin / Supplement**	**Dosage**	**Number of times (specify per day / week / month)**
	□ Vitamin D drops		
	□ Iron supplement		
	□ Other - please specify type: _____		
	□ Prefer not to answer		

24. The following questions are asked only if the mother is HIV positive:				
Last Infant PCR Result	POSITIVE	NEGATIVE	INDETERMINATE	DATE: DD/MM/YYYY
If infant is Positive	Date mother informed of result		DD/MM/YYYY	
	Date & time of ARV initiation		DD/MM/YYYY	_____H _____

Drugs and doses used for initiation:	1			Dose:
	2			Dose:
	3			Dose:
Infant PCR taken at this visit	YES	NO	N/A	Lab Reference No.

GMCD (scores 0-3):	
Expressive Language:	
Receptive Language:	
Gross Movements:	
Fine Movements:	
Relating:	
Play activities:	
Self-help activities (from 12 months):	

BAYLEY SCORE:

CHECKLIST			
Infant Developmental Screening done? (GMCD)	YES	NO	
Bayley Developmental Screening done?	YES	NO	
Infant Body Composition done?	YES	NO	
Infant Dietary Recall done?	YES	NO	
Maternal Dietary Recall done?	YES	NO	

Maternal Body Composition done?	YES	NO	
Infant Food Frequency Questionnaire done?	YES	NO	
Food Cost Questionnaire done?	YES	NO	
NEXT APPOINTMENT DATE	DD/MM/YYYY		

Form completed by: _____ **Form checked by**: _____

ANNEX H: PUBLISHED ARTICLE 1 AND CONFERENCE PROCEEDINGS

viruses

MDPI

Artide

Early Childhood Growth Parameters in South African Children with Exposure to Maternal HIV Infection and Placental Insufficiency

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Abstract: Maternal HIV exposure and intrauterine growth restriction (IUGR) due to placental insufficiency both carry major risks to early child growth. We compared the growth outcomes of children aged 18 months who had abnormal umbilical artery resistance indices (UmA-RI), as a marker of placental insufficiency, with a comparator group of children with normal UmA-RI during pregnancy, as mediated by maternal HIV infection. The cross-sectional study included 271 children, grouped into four subgroups based on HIV exposure and history of normal/abnormal UmA-RI, using available pregnancy and birth information. Standard procedures were followed to collect anthropometric data, and z-scores computed as per World Health Organization growth standards. Lower length-for-age z-scores (LAZ) were observed in children who were HIV-exposed-uninfected (CHEU) (-0.71 ± 1.23; $p = 0.004$) and who had abnormal UmA-RI findings (-0.68 ± 1.53; $p < 0.001$). CHEU with abnormal UmA-RI had lower LAZ (-1.3 ± 1.3; $p < 0.001$) and weight-for-age z-scores (WAZ) (-0.64 ± 0.92; $p = 0.014$) compared to the control group. The prevalence of stunting was 40.0% in CHEU with abnormal UmA-RI and 16.0% in CHEU with normal UmA-RI ($p < 0.001$; $p = 0.016$, respectively). In conclusion, maternal HIV exposure and placental insufficiency are independent risk factors for childhood stunting, with this risk potentiated when these two risk factors overlap.

Keywords: children who are HIV-exposed-uninfected (CHEU); placental insufficiency; intrauterine growth restriction; child growth

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1. Introduction

South Africa (SA) is burdened with a high prevalence (31.6%) of HIV infection in women of childbearing age [1] and pregnant women (30.0%) [2]. Nonetheless, access to antiretroviral therapy (ART) has increased over the years, with a >95% ART coverage during pregnancy and delivery [1], leading to an expanding population of children who are HIV-exposed-uninfected (CHEU). Adverse birth outcomes including intrauterine growth restriction (IUGR) and stillbirths have been documented in women living with HIV (WLHIV) compared to their HIV-negative counterparts, even when on ART [3–7]. IUGR is a clinical term describing a pathological inhibition of fetal growth preventing the fetus from attaining its genetic growth potential [8]. In SA, it is reported that CHEU have similar growth patterns with children who are HIV-unexposed-uninfected (CHUU); however, CHEU have been reported as a high-risk group due to in utero and postnatal ART exposure, as well as exposure to pathogens in immunocompromised family members [9,10].

International literature has indicated that CHEU are more likely than CHUU to experience impaired growth and neurodevelopmental outcomes, even in the context of high maternal ART coverage [11–14]. In the face of maternal HIV exposure as a risk for poor nutritional status, Black et al. stated that IUGR was associated with postnatal child wasting and stunting [15]. In agreement, Flores-Guillén et al. reported a 21% prevalence of stunting associated with IUGR in the HIV-negative population [7].

Placental insufficiency is one immediate cause of IUGR, which in its extreme can lead to fetal demise/stillbirths [16,17]. IUGR, present in up to 30% of pregnancies, may be the most significant population-based attributable risk factor for preventable stillbirth [17,18]. Research has shown that pre-eclampsia, IUGR, and stillbirths linked to placental insufficiency complicate 10 to 15% of all pregnancies [16]. In developing countries, up to 24% of newborns, approximately 30 million, experience IUGR annually [19]. Low- and middle-income countries (LMICs) carry the highest burden of stillbirths (98%) and perinatal deaths [20]. Children born with IUGR are a high-risk group with short- and long-term morbidity and mortality. IUGR is a crucial risk factor for child undernutrition [21], coupled with an increased risk of overweight and obesity during adolescence, suboptimal intellectual and physical development, and other long-term chronic diseases in adult years [7,22].

The Umbiflow™ device is a low-cost continuous-wave Doppler screening tool for the detection of placental insufficiency in otherwise low-risk pregnancies [23]. Placental insufficiency is detected by an increased umbilical artery resistance index (UmA-RI), also known as an abnormal RI. South African studies have reported a high prevalence of abnormal RI: 11.7% [24]; 13.0% [25]; 5.9% [26].

In view of the above, HIV exposure and placental insufficiency both carry major risks to early and late child growth and development, possibly compounding each other. However, the postnatal growth in children born to otherwise low-risk pregnancies with abnormal RI measurements, indicating IUGR, have not been intensively investigated, particularly in high HIV burden settings. Further, it is well known that adequate child nutrition is central for catch-up growth [27], with a positive correlation between breastfeeding and a child's growth and development. Exclusive breastfeeding (EBF) stimulates growth among CHEU and CHUU [13], as shown by Jumare et al. Additionally, there is still a lack of evidence on the nutritional management of children born with IUGR to optimize postnatal catch-up growth, including the impacts of feeding practices on the growth of children born with IUGR in the context of HIV exposure.

We therefore investigated and compared, at age 18 months, the growth outcomes of children born with and without IUGR due to placental insufficiency, as measured by an abnormal UmA-RI using Doppler screening during pregnancy, and as modified by maternal HIV status, in the Tshwane District in the Gauteng Province of SA.

2. Materials and Methods

2.1. Study Settings and Participants

This study followed up participants from the SA arm of the Umbiflow International study, which studied the prevalence of raised UmA-RIs in low-risk pregnant women at 28–34 weeks' gestation in Ghana, India, Kenya, Rwanda and SA, using a single screening with the Umbiflow™ device between October 2018 and January 2020. Normal and abnormal UmA-RI was defined as <75th centile and ≥75th centile for the gestational age, respectively, as per Pattinson graphs [28]. The mother–child pairs were recruited at 18 months of age into the present study from a prospective longitudinal study and from an additional one-off follow-up from the Umbiflow International study, using the available pregnancy and birth information, at the University of Pretoria's Research Centre for Maternal, Fetal, Newborn and Child Health Care Strategies, located at Kalafong Provincial Tertiary Hospital, Tshwane District, Gauteng Province, SA. Additional participants were recruited from the Siyakhula study, a longitudinal study on the effects of maternal HIV infection on child outcomes, with an otherwise similar study design and at the same site, to increase the number in the CHEU group with an abnormal UmA-RI. The study population included children who had

abnormal UmA-RIs during pregnancy, as a marker for IUGR, compared to a similar group of children with normal UmA-RIs, as mediated by maternal HIV infection.

2.2. Study Design

This cross-sectional study explored the growth outcomes of children at the age of 18 months. Exclusion criteria included multiple pregnancies, inability to obtain informed consent, babies born to underage mothers, and babies with chromosomal or structural abnormalities or other severe medical conditions known to impact infant growth and development. The study population was grouped into four subgroups based on HIV exposure and history of normal or abnormal UmA-RI: (1) mothers who are HIV negative with normal UmA-RI (no exposure variable of interest; control group), (2) maternal HIV infection and normal UmA-RI (single exposure), (3) HIV-negative mothers with abnormal UmA-RI (single exposure), and (4) maternal HIV infection with abnormal UmA-RI (double exposure). The outcome variables were child growth parameters. The modifiers were infant feeding practices and other factors known to contribute to child growth; data on potential confounders was collected, including maternal socio-demographic information, medical and obstetric history, nutritional status and lifestyle factors.

2.3. Sample Size Determination

The sample size calculation using power analysis indicated that a sample size of 280 was required overall, with a split of 80/20% for CHEU and CHUU. The anticipated sample from the Umbiflow International cohort was 311; however, 46 participants did not attend their study visit, meaning 265 participants were enrolled. Four participants were subsequently excluded either because of age above the set upper limit of 21 months, acute child illness at the study visit or parental choice to not complete the study visit; therefore, this study included 261 participants from the Umbiflow International cohort and an additional 10 participants from the Siyakhula study. The sample size per group for the infant follow-ups was as follows: CHUU with normal UmA-RI: $n = 186$; CHEU with normal UmA-RI: $n = 50$; CHUU with abnormal UmA-RI: $n = 20$; and CHEU with abnormal UmA-RI: $n = 15$ (Figure 1).

2.4. Data Collection Methods

Mothers were contacted telephonically and invited to this study, after which written informed consent was obtained by the trained study staff. Data were collected between February and December 2021 using standardized data collection sheets, until no more eligible participants were available due to ageing out. The face-to-face interviews with the mothers were performed in either English or local languages. The child anthropometric measurements collected included weight, length and head circumference (HC), mid-upper arm circumference (MUAC) and triceps skinfold thickness (TSF); similar measurements were collected from the mother except for HC. For intra-observer reliability, the anthropometric measurements, performed as per standard procedures, were taken twice and the mean value was recorded [29,30]. Maternal socio-demographic information was collected using a structured questionnaire, while the descriptive qualitative feeding practices data were collected using a standardized maternal and infant postpartum questionnaire, which is based on adapted World Health Organization (WHO) questionnaires. A structured infant follow-up questionnaire was used to collect maternal and child medical history, along with information obtained from the child's Road-to-Health booklet, which includes information from clinic visits. For CHEU, HIV antibody testing was performed as per national SA guidelines. One birthweight z-score of >+3 was excluded from the analysis of birth anthropometry measurements (n = 1). Five mothers were pregnant, and a child was brought to the study visit by the caregiver resulting in missing maternal anthropometry data for these mothers.

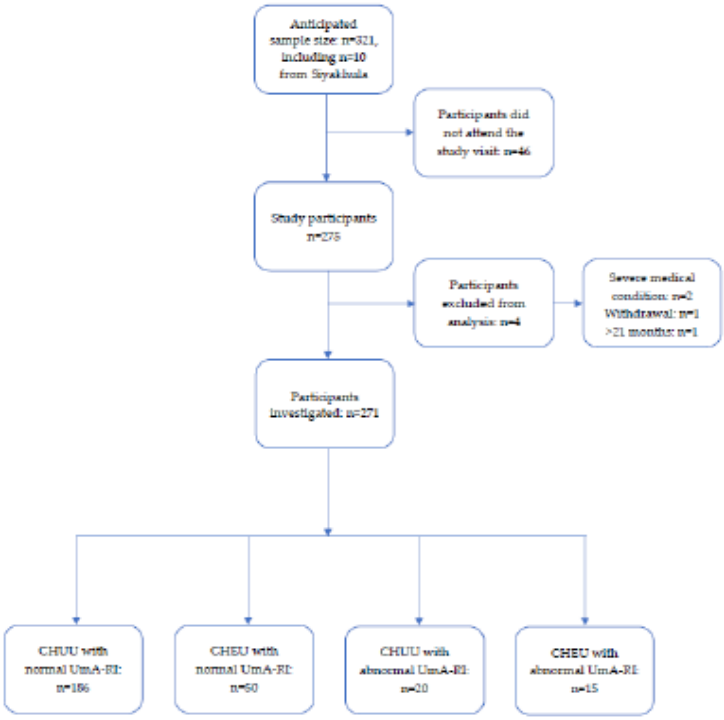

Figure 1. The flow diagram for study participants. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index.

2.5. Data Processing and Statistical Analysis

The data were independently double entered on the online electronic platform, Research Electronic Data Capture (REDCap) v9.3.5, which is a secure web-based application for capturing data in clinical research projects [31]. The outliers were reviewed and corrected in case of error in data capturing, including z-scores outside the range of the reference population (<−3 and >+3). Absolute measurements and z-scores that were clinically implausible were excluded from the analysis. The z-scores for birth anthropometry data were generated using the INTERGROWTH-21st Newborn Size tool (International Fetal and Newborn Growth Consortium for the 21st Century, Oxford, UK) standard version 1.0.6257.25111. There were 12 participants with gestational age (GA) at birth ranging from 43 to 46 weeks, which is clinically unlikely; therefore, the highest GA of 42 weeks 6 days on the INTERGROWTH-21st tool was used for these birth anthropometry z-scores. WHO Anthro software was used to compute the z-scores for the 18-month anthropometric data and children born premature were corrected for gestational age. Both INTERGROWTH-21st and WHO Anthro provide sex- and age-normalized data. This study utilized WHO guidelines to define stunting, wasting, underweight, microcephaly and moderate/severe acute malnutrition as length-for-age z-score (LAZ), weight-for-length z-score (WLZ), weight-for-age z-score (WAZ), HC-for-age z-score (HCAZ), respectively. The z-scores <−3 were classified as severe suboptimal nutritional status. The R statistical program was used for statistical analysis, for which each of the three test groups (with single and double exposure) was compared against the control group to determine if significant differences

existed. In all instances, the Shapiro–Wilk test was used to determine if the data were normally distributed. For normally distributed data, the independent t-test was used to compare each test group against the control group, and a one-way ANOVA for comparing all four groups. For the non-normally distributed data, the Mann–Whitney U tests was used to compare each test group against the control group, and the Kruskal–Wallis H test for comparing all four groups. All tests were performed at a 5% level of significance.

2.6. Ethical Considerations

Ethical approval for this study was obtained from the Faculty of Natural and Agricultural Sciences and Faculty of Health Sciences Ethics Committees of the University of Pretoria with reference number: NAS259/2021. The mothers were given all the relevant information about the follow-up study prior to recruitment. Mothers provided informed consent on behalf of themselves and their children.

3. Results

3.1. The Socio-Demographic and Medical Characteristics of the Mothers of the Study Children

A total of 271 mothers were enrolled in this study, with anthropometric measurements not performed in 6 mothers due to repeat pregnancy (n = 5) or because the child was brought by a caregiver (n = 1). The maternal characteristics are presented in Table 1. WLHIV with an abnormal UmA-RI were significantly older (36.6 ± 6.1 years; $p < 0.001$). A high percentage of women had attained any secondary education (80.0%) and 66.7% of WLHIV with abnormal UmA-RI were unemployed. Half of the mothers had access to running water inside the yard and had flushing toilets. More mothers in the control group (29.6%) consumed alcohol at least once a month, compared to other groups. Cigarette smoking was uncommon in all groups. WLHIV with abnormal UmA-RI had lower weight, BMI and MUAC than their counterparts in the other three groups. Further, higher gravidity was reported in WLHIV than their HIV-uninfected counterparts ($p = 0.018$). Two-thirds of women delivered vaginally, but amongst women with history of an abnormal UmA-RI, caesarean section rates were high, with 50.0% of WLHIV and 46.7% of HIV-uninfected women requiring a caesarean section, respectively (Table 1).

Table 1. The maternal socio-demographic and medical characteristics.

Variables		CHUU with Normal UmA-RI	CHEU with Normal UmA-RI	CHUU with Abnormal UmA-RI	CHEU with Abnormal UmA-RI	*p*-Value [a]
Sample size (n) (%)		186 (68.6%)	50 (18.5%)	20 (7.4%)	15 (5.5%)	
Mean age (years)		30.1 ± 5.1	31.3 ± 5.5	28.5 ± 4.5	36.6 ± 6.1	**<0.001**
Marital status	Single	79 (42.5%)	21 (42.0%)	6 (30.0%)	4 (26.7%)	n/a
	Married	69 (37.1%)	17 (34.0%)	10 (50.0%)	8 (53.3%)	
	Co-habiting	38 (20.4%)	12 (24.0%)	4 (20.0%)	3 (20.0%)	
Educational level	Any primary schooling	13 (7.0%)	4 (8.0%)	3 (15.0%)	2 (13.3%)	n/a
	Any secondary schooling	127 (68.3%)	40 (80.0%)	13 (65.0%)	12 (80.0%)	
	Post-school education	46 (24.7%)	6 (12.0%)	4 (20.0%)	1 (6.7%)	
Employment status	Unemployed	112 (60.2%)	29 (58.0%)	11 (55.0%)	10 (66.7%)	0.118
	Employed	74 (39.8%)	21 (42.0%)	9 (45.0%)	5 (33.3%)	
Monthly household income [b]	R 0–R 2000	37 (20.1%)	7 (14.0%)	0 (0%)	4 (26.7%)	n/a
	R 2001–R 4000	43 (23.4%)	15 (30.0%)	8 (40.0%)	0 (0%)	
	R 4001–R 6000	39 (21.2%)	13 (26.0%)	5 (25.0%)	6 (40.0%)	
	R 6001–R 8000	11 (6.0%)	5 (10.0%)	1 (5.0%)	2 (13.3%)	
	R 8000+	45 (24.5%)	9 (18.0%)	6 (30.0%)	1 (6.7%)	
	Don't know	9 (4.9%)	1 (2.0%)	0 (0%)	2 (13.3%)	
Access to running water	Inside house	68 (36.6%)	10 (20.0%)	7 (35.0%)	4 (26.7%)	n/a
	Inside yard	89 (47.8%)	27 (54.0%)	10 (50.0%)	8 (53.3%)	
	Communal tap	21 (11.3%)	9 (18.0%)	3 (15.0%)	3 (20.0%)	
	Water tank	8 (4.3%)	4 (8.0%)	0 (0%)	0 (0%)	

Table 1. *Cont.*

Variables		CHUU with Normal UmA-RI	CHEU with Normal UmA-RI	CHUU with Abnormal UmA-RI	CHEU with Abnormal UmA-RI	*p*-Value [a]
Access to toilet [c]	Flushing toilet Pit latrine/bucket	131 (70.4%) 55 (29.6%)	30 (61.2%) 19 (38.8%)	16 (80.0%) 4 (20.0%)	9 (64.3%) 5 (35.7%)	n/a
Drinks alcohol [d]	Yes	54 (29.2%)	11 (22.0%)	2 (11.1%)	1 (7.1%)	n/a
Smokes cigarettes	Yes	3 (1.6%)	2 (4.0%)	0 (0%)	1 (6.7%)	n/a
Latest CD4 count	Cells/mm^3 [e]	N/A	463 ± 310	N/A	416 ± 295	0.147
Latest HIV viral load	Copies/mL (log) [f,g]	N/A	0.0 [0.0, 4.0]	N/A	0.0 [0.0, 0.0]	0.277
Current ART	TDF/FTC/EFV Other ART [h] Not recorded	N/A N/A N/A	31 (62.0%) 10 (20.0%) 9 (18.0%)	N/A N/A N/A	8 (53.3%) 6 (40.0%) 1 (6.7%)	n/a
Obstetric history	Parity [f]	2 [1, 3]	2 [1, 3]	2 [0, 2]	3 [3, 3]	**0.006**
	Gravidity [f]	2 [1, 3]	3 [2, 3]	2 [2, 3]	3 [3, 4]	**0.018**
	Previous pregnancy losses [f]	0 [0, 0]	0 [0, 1]	0 [0, 0]	0 [0, 1]	0.372
Umbilical artery Doppler	Preeclampsia/eclampsia UmA-RI value at 28–34 weeks' gestation [g]	2 (13.3%) 0.64 ± 0.05	0 (0%) 0.63 ± 0.04	0 (0%) 0.74 ± 0.06	0 (0%) 0.76 ± 0.04	n/a **<0.001**
Mode of delivery	Vaginal delivery Assisted delivery Caesarean section	127 (68.3%) 3 (1.6%) 56 (30.1%)	31 (62.0%) 0 (0%) 19 (38.0%)	10 (50.0%) 0 (0%) 10 (50.0%)	6 (40.0%) 0 (0%) 9 (60.0%)	n/a
Body measurements and indices [g,i]	Weight (kg) Height (cm) BMI (kg/m^2) MUAC (cm) TSF (mm)	77.6 ± 19.6 160.2 ± 6.2 30.3 ± 7.7 32.1 ± 5.0 19.9 ± 5.1	77.5 ± 25.0 161.3 ± 8.9 29.5 ± 8.4 31.8 ± 5.7 21.4 ± 7.4	69.7 ± 20.2 157.8 ± 5.3 27.9 ± 7.4 30.7 ± 5.0 20.0 ± 5.3	63.1 ± 15.4 158.3 ± 5.3 25.1 ± 5.2 28.0 ± 4.0 18.5 ± 3.6	**0.016** 0.402 **0.043** **0.025** 0.243

Values in bold font indicate significant *p*-values. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index; CD4: clusters of differentiation 4; ART: antiretroviral therapy; TDF/FTC/EFV: tenofovir/emtricitabine/efavirenz; BMI: body mass index; MUAC: mid-upper-arm circumference; TSF: triceps skin fold; n/a: not applicable. [a] Comparisons involved all groups. Only variables with groups above 5 were included in these investigations as smaller groups lead to volatility of results. n/a implies no comparisons were performed due to low counts. [b] One South African Rand equates to 0.056 United States Dollars. [c] Missing information: CHEU with normal UmA-RI (n = 1); CHEU with abnormal UmA-RI (n = 1). [d] Mother drank alcohol at least once in a month, since the baby was born. [e] Mean and standard deviation (SD) reported. [f] Median and interquartile range [IQR] reported. [g] Undetectable viral load is reflected as zero. [h] Includes tenofovir (TDF), lamivudine (3TC) plus lopinavir/ritonavir or dolutegravir. [i] Total of 265 mothers were measured at the study visit, 5 were pregnant and 1 child was brought by the caregiver.

3.2. The Characteristics of the Study Children

A total of 271 CHUU and CHEU aged 18 months with and without a history of an abnormal UmA-RI in utero were investigated, and the findings are presented in Table 2. The study population had more females than males across the groups, except for CHEU with normal UmA-RI. The GA at birth was lower (36.8 ± 2.5 weeks) in CHUU with abnormal UmA-RI than in the other groups ($p < 0.001$). A history of malnutrition was reported in CHUU with normal UmA-RI (6.0%) and CHEU with normal UmA-RI (12.0%), and a history of diarrhea was common in the study population. Findings on feeding practices showed that CHEU with normal UmA-RI had a lower percentage of early initiation of breastfeeding (within one hour after birth) than the other groups ($p < 0.001$). Lastly, exclusive breastfeeding was lowest in WLHIV with abnormal UmA-RI, while mixed feeding (24.3%) was common in the control group. Percentages of current (supplementary) breastfeeding up to age 18 months were observed to be low in HIV-exposed settings.

Table 2. The medical background of children from birth to age 18 months.

Variables		CHUU with Normal UmA-RI	CHEU with Normal UmA-RI	CHUU with Abnormal UmA-RI	CHEU with Abnormal UmA-RI	*p*-Value [a]
Sample size (n) (%)		186 (68.6%)	50 (18.5%)	20 (7.4%)	15 (5.5%)	
Mean age (months)		18.6 ± 0.9	18.5 ± 0.8	18.2 ± 0.2	18.8 ± 0.9	
Sex	Male Female	91 (48.9%) 95 (51.1%)	28 (56.0%) 22 (44.0%)	8 (40.0%) 12 (60.0%)	4 (26.7%) 11 (73.3%)	n/a
Mean GA at birth (weeks)		39.3 ± 1.9	39.4 ± 1.3	36.8 ± 2.5	38.3 ± 1.0	**<0.001**
Born premature		15 (8.1%)	2 (4.0%)	7 (35.0%)	1 (6.7%)	n/a
APGAR score (5 min) [b]		10 [9, 10]	9 [9, 10]	9 [9, 10]	9 [9, 9]	0.069
Neonatal hospitalization		35 (18.9%)	6 (12.0%)	7 (35.0%)	1 (7.1%)	n/a
Neonatal diagnosis [c]	Respiratory distress Jaundice Other	7 (3.8%) 18 (9.7%) 10 (5.4%)	3 (6.0%) 2 (4.0%) 1 (2.0%)	3 (15.0%) 2 (10.0%) 1 (5.0%)	0 (0%) 0 (0%) 1 (7.1%)	n/a
Prevention of vertical HIV transmission	Single drug (NVP) Dual drug (NVP and AZT)	N/A N/A	41 (82.0%) 3 (6.0%)	N/A N/A	7 (46.7%) 6 (40.0%)	n/a
History of childhood illnesses	Malnutrition Diarrhea	11 (6.0%) 57 (30.6%)	6 (12.0%) 13 (26.0%)	0 (0%) 4 (20.0%)	0 (0%) 2 (13.3%)	n/a
Hospital admission (post-neonatal)	Any illness	18 (9.7%)	5 (10.0%)	0 (0%)	1 (6.7%)	n/a
Breastfeeding	Ever breastfeed	178 (95.7%)	47 (94.0%)	20 (100.0%)	15 (100.0%)	
Early initiation of breastfeeding [d]	Within 1 h after birth After 1 h of birth	124 (78.5%) 34 (21.5%)	21 (48.8%) 22 (51.2%)	9 (52.9%) 8 (47.1%)	8 (61.5%) 5 (38.5%)	**<0.001**
Infant feeding from birth until 6 months [e,f]	Exclusive breastfeeding Formula feeding Mixed feeding Formula feeding only at 6 months, but previous exclusive breastfeeding	122 (65.9%) 11 (5.9%) 45 (24.3%) 7 (3.8%)	32 (64.0%) 3 (6.0%) 8 (16.0%) 7 (14.0%)	14 (70.0%) 0 (0%) 4 (20.0%) 2 (10.0%)	8 (53.4%) 0 (0%) 2 (13.3%) 5 (33.3%)	0.750
Current breastfeeding		50 (27.0%)	3 (6.1%)	4 (22.2%)	2 (14.3%)	n/a

Values in bold font indicate significant *p*-values. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index; GA: gestational age; IQR: interquartile range; NVP: nevirapine; AZT: Zidovudine; n/a: not applicable. [a] Comparisons involved all groups. Only variables with groups above 5 were included in these investigations as smaller groups lead to volatility of results. n/a implies no comparisons were made due to low counts. [b] Median interquartile range [IQR] reported. [c] Missing information: CHUU with abnormal UmA-RI (n = 1). [d] Unknown information for CHUU with normal UmA-RI (n = 28); CHEU with normal UmA-RI (n = 7); CHUU with abnormal UmA-RI (n = 3); CHEU with abnormal UmA-RI (n = 2). [e] Missing information: CHUU with normal UmA-RI (n = 1). [f] Comparison was performed for exclusive breastfeeding vs. all other feeding methods.

3.3. Growth Parameters of Study Children

Firstly, investigations involved comparisons of growth outcomes in HIV-exposed vs. unexposed and normal vs. abnormal UmA-RI settings for the entire study population of 271 children (Table 3). When comparing between CHEU and CHUU, lower LAZ was observed in CHEU (-0.73 ± 1.23; $p = 0.003$). Similar findings were observed in abnormal UmA-RI when compared to normal UmA-RI group (-0.68 ± 1.53; $p < 0.001$).

Further findings on growth outcomes across the four groups are reported as the means and standard deviations (SD) (Table 4). Investigations involved comparisons of the three test groups against the control group, respectively. CHEU with abnormal UmA-RI had lower WAZ at birth than the control group ($p = 0.003$). Additionally, lower LAZ was observed in CHEU with normal UmA-RI than the control group ($p = 0.023$). Findings

at 18 months showed that when comparing CHEU with abnormal UmA-RI against the control group, there was a significant difference in weight, length and HC. Further, CHEU with abnormal UmA-RI had significantly lower LAZ ($p < 0.001$), as well as WAZ and HCZ ($p = 0.014$; $p = 0.016$, respectively) (Figure 2). Furthermore, the findings showed that there were no significant differences for growth outcomes in groups with single exposure, maternal HIV exposure or abnormal UmA-RI, respectively, against the control group. The prevalence of stunting was higher (40.0%) in CHEU with abnormal UmA-RI, than with single exposure, 16.0% in the HIV exposure and abnormal UmA-RI group, respectively ($p < 0.001$; $p = 0.016$). Wasting (8.0%) and underweight (6.0%) were observed in CHEU children with normal UmA-RI. The sensitivity analysis excluding children born preterm showed similar growth outcomes in which CHEU with abnormal UmA-RI had lower LAZ, WAZ and HCZ than the control group: $p = 0.003$; $p = 0.029$ and $p = 0.029$, respectively (data not shown). The rate of stunting remained high among the CHEU with abnormal UmA-RI group (35.7%).

Table 3. The comparisons of mean growth outcomes at age 18 months in HIV-exposed vs. unexposed settings and normal vs. abnormal umbilical artery resistance index (UmA-RI) settings.

Growth Indicators [a]	CHUU	CHEU	*p*-Value	Normal UmA-RI	Abnormal UmA-RI	*p*-Value
	n = 206 (76.0%)	n = 65 (24.0%)		n = 236 (87.1%)	n = 35 (12.9%)	
WAZ	0.04 ± 1.19	−0.24 ± 1.26	0.122	0.01 ± 1.19	−0.29 ± 1.32	0.122
LAZ	−0.05 ± 1.32	−0.73 ± 1.23	**0.003**	−0.14 ± 1.29	−0.68 ± 1.53	**<0.001**
WLZ	0.08 ± 1.21	0.14 ± 1.33	0.710	0.10 ± 1.26	0.04 ± 1.10	0.710
HCZ	0.93 ± 1.18	0.71 ± 1.15	0.198	0.88 ± 1.16	0.85 ± 1.27	0.141

Values in bold font indicate significant *p*-values. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index; WAZ: weight-for-age z-score; LAZ: length-for-age z-score; WLZ: weight-for-length z-score; HCZ: head circumference z-score. [a] Sex-normalized anthropometric indicators at age 18 months were computed using the World Health Organization (WHO) Anthro software of 2011, corrected for gestational age for preterm infants.

Table 4. The mean anthropometric measurements and indicators of study children at birth and age 18 months, as per groups of control, single exposure and dual exposure to maternal HIV plus abnormal UmA-RI.

Variables		CHUU with Normal UmA-RI	CHEU with Normal UmA-RI	CHUU with Abnormal UmA-RI	CHEU with Abnormal UmA-RI	*p*-Value [a]
Sample size (n) (%)		186 (68.6%)	50 (18.5%)	20 (7.4%)	15 (5.5%)	
At birth						
Anthropometry (mean ± SD)	Weight (g)	3187 ± 483	3108 ± 433	2649 ± 566	2704 ± 408	**<0.001** [b]
	Length (cm)	50.7 ± 3.0	49.9 ± 2.3	48.8 ± 2.9	49.1 ± 2.6	**0.005** [b]
	HC (cm)	34.5 ± 1.7	34.5 ± 1.5	33.0 ± 1.9	33.9 ± 1.5	**0.003** [b]
Indicators (mean ± SD) [d]	WAZ	−0.30 ± 1.16	−0.54 ± 0.96	−0.48 ± 1.07	−1.04 ± 0.76	**0.003**
	LAZ	0.68 ± 1.66	0.15 ± 1.32	0.64 ± 1.24	0.36 ± 1.28	**0.023** [c]
	HCZ	0.40 ± 1.34	0.35 ± 1.24	0.20 ± 0.95	0.48 ± 1.07	0.778
At age 18 months						
Anthropometry (mean ± SD)	Weight (kg)	10.9 ± 1.5	10.7 ± 1.8	10.8 ± 1.9	9.9 ± 1.0	**0.079**
	Length (cm)	81.9 ± 3.8	80.6 ± 3.3	81.1 ± 4.1	78.2 ± 3.5	**<0.001**
	HC (cm)	48.1 ± 1.6	48.1 ± 1.9	48.5 ± 1.9	47.1 ± 1.2	**0.011**
	MUAC (cm)	16.0 ± 1.4	16.2 ± 1.7	16.4 ± 1.7	16.0 ± 1.4	0.825
	TSF (mm)	8.7 ± 2.0	8.4 ± 2.2	8.7 ± 2.7	8.6 ± 1.3	0.869
Indicators (mean ± SD) [e]	WAZ	0.05 ± 1.15	−0.11 ± 1.32	−0.02 ± 1.52	−0.64 ± 0.92	**0.014**
	LAZ	−0.03 ± 1.30	−0.56 ± 1.16	−0.21 ± 1.53	−1.30 ± 1.32	**<0.001**
	WLZ	0.07 ± 1.20	0.19 ± 1.46	0.09 ± 1.32	−0.02 ± 0.76	0.662
	HCZ	0.89 ± 1.15	0.83 ± 1.23	1.26 ± 1.47	0.33 ± 0.73	**0.016**

Table 4. *Cont.*

Variables		CHUU with Normal UmA-RI	CHEU with Normal UmA-RI	CHUU with Abnormal UmA-RI	CHEU with Abnormal UmA-RI	*p*-Value [a]
Nutritional classifications (n; %)	Underweight	4 (2.2%)	3 (6.0%)	1 (5.0%)	0 (0.0%)	n/a
	Stunting [f]	9 (4.8%)	8 (16.0%)	2 (10.0%)	6 (40.0%)	**<0.001**
	Wasting	6 (3.2%)	4 (8.0%)	0 (0.0%)	0 (0.0%)	n/a

Values in bold font indicate significant *p*-values. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index; HC: head circumference; MUAC: mid-upper-arm circumference; TSF: triceps skinfold; WAZ: weight-for-age z-score; LAZ: length-for-age z-score; WLZ: weight-for-length z-score; HCZ: head circumference z-score; n/a: not applicable. [a] Comparisons between CHEU with abnormal UmA-RI group vs. control group. Only variables with groups above 5 were included in these investigations as smaller groups lead to volatility of results. n/a implies no comparisons were made due to low counts. [b] Comparisons were made between the four study groups. [c] Comparison between CHUU with normal UmA-RI vs. CHEU with normal UmA-RI groups. [d] The birth sex-normalized anthropometric indicators were computed using INTERGROWTH-21st software, using gestation-adjusted age for preterm infants. [e] The sex-normalized anthropometric indicators at age 18 months were computed using World Health Organization (WHO) Anthro software of 2011, using gestation-adjusted age for preterm infants. [f] Additionally, comparison between CHUU with normal UmA-RI vs. CHEU with normal UmA-RI: $p = 0.016$.

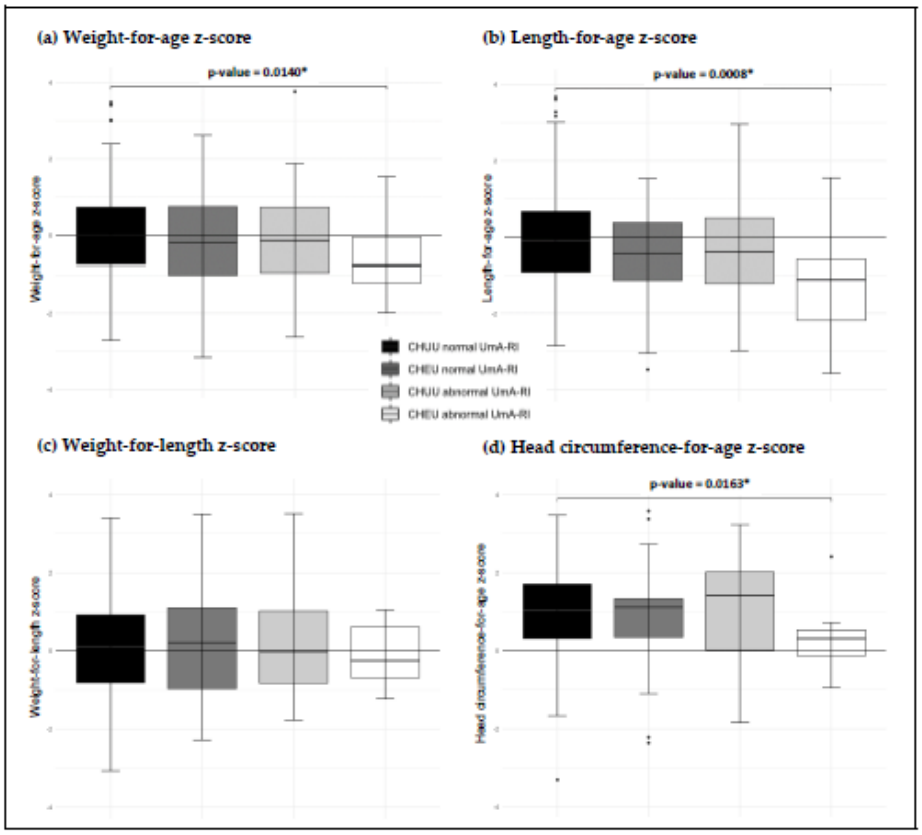

Figure 2. The box plots showing the significant differences for weight-for-age z-score, length-for-age z-score, weight-for-length z-score and HC-for-age z-score between the study groups. The z-scores

were computed using World Health Organization (WHO) Anthro software of 2011, using corrected age for premature children. (**a**) WAZ for CHEU abnormal UmA-RI is below the median line and lower than the three groups; (**b**) LAZ for CHEU abnormal UmA-RI is very far below the median and lower than the three groups; (**c**) WLZ for CHEU abnormal UmA-RI is the only group below the median line; (**d**) HCZ for CHEU abnormal UmA-RI is low compared to their counterparts in the different groups. Abbreviations: CHEU: children who are HIV-exposed-uninfected; CHUU: children who are HIV-unexposed-uninfected; UmA-RI: umbilical artery resistance index; WAZ: weight-for-age z-score; LAZ: length-for-age z-score; HCZ: head circumference z-score; HC: head circumference.

Additional findings indicated that there were no significant differences between the growth indicators and child feeding practices during the first six months of life and up until 18 months of age (Table 5).

Table 5. Comparison of mean child growth indicators between breastfeeding practices.

	Feeding Practices during the First Six Months of Life					Continued Breastfeeding at Age 18 Months		
	Exclusive Breastfeeding	Formula Feeding	Mixed Feeding	Formula Feeding Only, but Previously Exclusive Breastfeeding	*p*-Value [a]	Supplementary Breastfeeding	No Supplementary Breastfeeding	*p*-Value [a]
Sample size [b]	176 (65.2%)	14 (5.2%)	59 (21.9%)	21 (7.8%)		59 (21.8%)	212 (78.2%)	
WAZ [c]	−0.13 ± 1.18	0.03 ± 1.23	0.31 ± 1.25	−0.14 ± 1.24	0.105	0.03 ± 1.25	−0.04 ± 1.20	0.672
LAZ [c]	−0.34 ± 1.25	0.28 ± 1.33	0.10 ± 1.46	−0.32 ± 1.55	0.067	−0.01 ± 1.46	−0.27 ± 1.29	0.225
WLZ [c]	0.04 ± 1.19	−0.18 ± 1.78	0.34 ± 1.29	0.01 ± 1.13	0.335	0.05 ± 1.21	0.10 ± 1.25	0.768
HCZ [c]	0.86 ± 1.17	0.83 ± 1.12	1.07 ± 1.07	0.60 ± 1.39	0.506	1.04 ± 1.10	0.83 ± 1.19	0.220

Abbreviations: WAZ: weight-for-age z-score; LAZ: length-for-age z-score; WLZ: weight-for-length z-score; HCZ: head circumference z-score Sex-normalized anthropometric indicators at age 18 months were computed using World Health Organization (WHO) Anthro software of 2011, using gestation-adjusted age for preterm infants. [a] Comparison between the study groups. [b] Sample size in numbers and percentages (%). [c] Growth indicators are reported as the means and standard deviations (SD).

4. Discussion

Our study shows that infants who had a dual in utero exposure, namely maternal HIV infection and placental insufficiency as measured by an abnormal UmA-RI, had a significantly lower LAZ and higher rates of stunting at 18 months (40.0%), compared to the control group. This finding indicates that maternal HIV infection compounded by unrelated placental insufficiency is an additive risk factor for stunting in SA children. The high percentage of stunting in CHEU contributes to the body of existing knowledge [13,14,32–36], and the finding regarding the high percentage of stunting in CHEU with a history of placental insufficiency and IUGR is novel in the SA context. The prevalence of placental insufficiency and abnormal UmA-RI in SA is high (12%), as reported by Nkosi et al. and Hlongwane et al., and far exceeds numbers previously reported in studies from high-income countries. The etiology is unknown, more so because women included in the SA studies were considered low risk and healthy at the time of screening during their pregnancies.

Lower weight, length and HC reported in the present study among the CHEU-abnormal UmA-RI group at 18 months implies a high-risk group requiring closer follow-up and optimum nutrition care.

Advanced age in WLHIV with abnormal UmA-RI and maternal lifestyle behavior such as use of alcohol were observed in the study population, which have been previously identified as risk factors for placental insufficiency and abnormal UmA-RIs by previous studies [16,37,38]. Adequate mean CD4 T cell counts and low viral loads were observed in the studied WLHIV. A high rate of caesarean section deliveries among the low-risk population has been reported in the Tshwane area, SA [39].

A history of diarrhea was common in the study children, even though most of the mothers self-reported having access to running water and flushing toilets, and diarrhea is known to be a common condition in early childhood. The reported high vertical HIV

transmission prophylaxis in the CHEU group points to the success of the prevention of mother to child transmission of HIV (PMTCT) program in SA [40–42], with the high percentages of exclusive breastfeeding across the study groups showing improved support and promotion of breastfeeding in SA. Nonetheless, mixed feeding was observed in the present study population, including in CHEU, despite their risk of vertical HIV acquisition. Low adherence to breastfeeding guidelines have been documented in a South African cohort of CHEU and CHUU followed up until 18 months of age [40]. The WHO's 2016 recommendations on HIV and infant feeding advocate the same breastfeeding practices for all women irrespective of maternal HIV status, within the context of support for adherence to ART for WLHIV [43]. A high stunting rate was observed in the CHEU-abnormal UmA-RI group, with more than half being exclusively breastfed. A systematic review in LMICs stated that limited evidence exist between breastfeeding and growth outcomes [44]. Contrarily, previous studies, including studies from SA, have reported a positive association between exclusive breastfeeding and child growth [13,32,34,45].

Findings on low mean LAZ at 18 months of age in CHEU compared to CHUU counterparts were similar to reports from other African countries, with high stunting rates reported in Ethiopian (27.8%) [46], Nigerian (44.3%) [13] and Kenyan CHEU (20%) [14]. Many other studies have also reported suboptimal growth outcomes in CHEU compared to CHUU [32–36]. Szanyi et al., reported an association between stunting and maternal peri- and postnatal HIV exposure [46]. The present findings differed to those reported by Ramokolo et al. in SA [9] and in Malawi [47], as lower LAZ was observed in CHEU than CHUU. The findings on comparisons of growth outcomes between normal vs. abnormal UmA-RI were similar to those reported in Mexico, which showed a low mean LAZ (-1.22 ± 0.95) and a slightly higher percentage of stunting in participants born with IUGR [7,15,48]. Inadequate growth outcomes in children born with IUGR have been documented in the Philippines and Austria, with a reported association between stunting and IUGR [7,48,49]. According to Stranix-Chibanda et al. (2020), predictive factors for suboptimal growth trajectories, which are most pronounced for LAZ, include IUGR, low birth weight and length [50].

Research on the growth parameters of CHEU with abnormal UmA-RI is limited, with no published reports on the growth outcomes of children exposed to these dual insults. At 18 months of age, CHEU with abnormal UmA-RI had lower mean anthropometric measurements (weight, length and HC) and indicators (WAZ, LAZ and HCZ). The present study population's normal WLZ and BMI suggest that CHEU with abnormal UmA-RI were symmetrically growth restricted and, as a result, would not be obviously visible within primary healthcare services if not well plotted on growth charts.

A lower mean weight, BMI and MUAC were also observed in the mothers of the CHEU with abnormal UmA-RI, implying that maternal nutrition may negatively influence the child's linear and ponderal growth. Previous findings indicated that maternal financial situation, BMI, nutrition, education, and age positively correlate with the health of their children [51]. The combination of the dual insult may carry a huge risk of suboptimal child growth, specifically length growth. Advanced age of WLHIV with abnormal UmA-RI may also be attributed to low growth indicators as it may influence childcare and feeding practices. Nevertheless, Fall and colleagues' findings in a normal population showed that children born to older mothers have less risk of stunting [52].

This study determined and compared the growth outcomes of CHEU with abnormal UmA-RI, a population that was previously unstudied. Study limitations include the small sample size of the subgroups of concern, particularly CHEU with abnormal UmA-RI (5.5%). Further, the high caesarean section rate for a low-risk pregnant population might indicate bias in obtaining a study population born to women with otherwise low-risk pregnancies. The caesarean section rate for the whole Umbiflow-International study (SA arm) group was 28%, which is similar to the rate of 30.1% in this subset. The fact that the children were investigated as a one-off at age 18 months meant that longitudinal data analysis was not possible; therefore, a lack of evaluation of growth over time was a drawback. Additionally,

a history of childhood illnesses and breastfeeding practices were based on maternal recall. Future research should investigate CHEU with abnormal UmA-RI over a longer duration in order to better understand their long-term growth trajectories.

5. Conclusions

The present study determined and compared the growth outcomes of 18-month-old children born with and without IUGR due to placental insufficiency, modified by maternal HIV status. CHEU with abnormal UmA-RI had lower WAZ, LAZ and HCZ, and are especially at a significantly increased risk of stunting. Maternal HIV exposure and placental insufficiency are independent risk factors for childhood stunting, with this risk potentiated when these two risk factors are compounded.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee) of the University of Pretoria (protocol code NAS259/2021 on 12 November 2021).

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Data Availability Statement: Data are available on request from the corresponding author, due to the University of Pretoria policy on data publication.

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ABSTRACT BOOK

5TH INTERNATIONAL DEVELOPMENTAL PEDIATRICS ASSOCIATION (IDPA) CONGRESS 2023

OUR CHILDREN OUR FUTURE, FROM VULNERABILITY TO RESILIENCE

28 November 2023 - 1 December 2023
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INTERNATIONAL DEVELOPMENTAL PEDIATRICS ASSOCIATION CONGRESS
Indaba Hotel & Conference Centre, Fourways, Johannesburg
28 NOVEMBER TO 1 DECEMBER 2023

Abstract ID Number: 9

GROWTH AND NEURODEVELOPMENT OF CHILDREN EXPOSED TO MATERNAL HIV AND PLACENTAL INSUFFICIENCY IN A PERI-URBAN AREA OF SOUTH AFRICA

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OBJECTİVES
Children who are HIV-exposed-and-uninfected (CHEU) and those who are growth restricted in utero due to placental insufficiency are both regarded as high-risk populations, which can impact growth and neurodevelopment. Nevertheless, the growth and neurodevelopmental outcomes of CHEU who have also experienced growth restriction in-utero have not been researched. The study compared and determined the association between growth and neurodevelopmental outcomes of 18-month-old children with in-utero HIV exposure and abnormal umbilical artery resistance indices (UmA-RI), indicating placental insufficiency.

METHODS
In this cross-sectional study, we investigated 264 mother-child pairs, who were grouped into four subgroups based on HIV exposure and history of normal/abnormal UmA-RI, using available pregnancy and birth information. The World Health Organization standard procedures were used for anthropometric measurements and z-score calculations, and Bayley III to test child development.

RESULTS
CHEU with abnormal UmA-RI (n=14) had lower length-for-age z-scores (-1.40 ± 1.40 vs -0.04 ± 1.31; p=0.001) and weight-for-age z-scores (-0.60 ± 0.96 vs 0.04 ± 1.16; p=0.02) compared to children who are HIV-unexposed-and-uninfected (CHUU) with normal UmA-RI (n=181). Nearly a quarter (21.4%) of CHEU with abnormal UmA-RIs had a mild delay in cognitive development, 7.1% had a moderate delay in language and 7.1% had a moderate delay in motor development compared to CHUU with normal UmA-RI: 2.2%, 2.8% and 0.0%, respectively. Weight-for-age z-scores had a positive significant association with motor development: 0.10; p=0.027.

CONCLUSION
Exposure to both maternal HIV infection and placental insufficiency is linked with stunting, underweight and cognitive developmental delay. Underweight children are likely to have delayed motor development.

TOPIC CATEGORY
2 Early Childhood Development

SOUTH AFRICAN PAEDIATRIC ASSOCIATION CONFERENCE 2023

**ABSTRACTS OF THE SAPA CONFERENCE
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ORAL PRESENTATIONS

ABSTRACTS

Objective. The current study examines the clinical effects of using ES by measuring the users' lung function. This study also aims to quantify the quality of life (QoL) of the participants using ES.
Methods. In this cross-over study, we recruited 65 children, between 5 and 12 years of age, with asthma and used a pMDI for more than 3 months. Participants were randomised into ES and pMDI groups and their baseline FEV1, FEV1/FVC ratio, and percentage difference pre- and post-bronchodilator FEV1 (%Diff FEV1). After 6 weeks the participants returned to the clinic and their lung function was measured. Along with that, their QoL data were captured using the Paediatric Asthma Quality of Life Questionnaire (PAQLQ), and asthma control was assessed using Childhood Asthma Control Test (C-ACT). For the following 6 weeks, participants using ES were crossed over into the pMDI group and vice-versa. After these 6 weeks, participants' data were collected again. Data between these two groups were statistically compared using the Mann-Whitney *U* test with the statistical significance criteria $p<0.05$.
Results. The bronchodilator reversibility was significantly lower in the ES group. The differences in lung function from previous measurements was significantly better in ES. Asthma was controlled better in the ES group.
Conclusion. The Easy Squeezy improves lung function and aids in controlling asthma better.

Feeding practices and growth outcomes of 18-month-old children with exposure to maternal HIV and placental insufficiency in South-West Tshwane: a community-based cross-sectional study

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Introduction. Nutrition plays a critical role in child growth and development. Children who are HIV-exposed-and-uninfected (CHEU) and those who had fetal growth restriction due to placental insufficiency are both regarded as high-risk populations. Suboptimal feeding practices may exacerbate the risk of poor growth in these populations. We investigated the feeding practices and growth outcomes of children with in-*utero* HIV exposure and abnormal umbilical artery resistance indices (UmA-RI), indicating placental insufficiency.
Objective. To determine and compare feeding practices and growth outcomes of children aged 18 months, with and without in-*utero* HIV exposure and abnormal UmA-RI.
Methods. A descriptive cross-sectional study was conducted on 264 children: grouped into four subgroups based on HIV exposure and normal/abnormal UmA-RI, using available pregnancy and birth information. The World Health Organization (WHO) standard procedures were followed for anthropometric measurements and *z*-score calculations, and a standardized questionnaire based on WHO was used for feeding practices.
Results. CHEU with abnormal UmA-RI (n=14) were stopped breastfeeding earlier (median (interquartile range (IQR)) 4.0 months (1.0, 15.8) v. 13.0 months (6.0 – 16.0); p=0.0002) and were introduced to protein-rich foods later (median (IQR) 12.0 months (9.0 – 12.0) v. 9.0 months (7.0 – 12.0); p=0.05) compared with children who are HIV-unexposed-and-uninfected (CHUU) with normal UmA-RI (n=181). CHEU with abnormal UmA-RI had lower length-for-age *z*-scores (-1.40±1.40 v. -0.04±1.31; p=0.001), weight-for-age *z*-scores (-0.60±0.96 v. 0.04±1.16; p=0.02) and head-circumference-for-age *z*-scores (0.42±0.66 v. 0.90±1.15; p=0.04) compared to CHUU with normal UmA-RI.
Conclusion. Early cessation of breastfeeding, late introduction of protein-rich foods, and exposure to both maternal HIV infection and placental insufficiency are associated with stunting and underweight.

Laryngomalacia: When to refer for surgery?

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Introduction. Laryngomalacia (LM) is the most common cause of stridor in infants, presenting shortly after birth with variable severity. The noisy breathing is created by shortened aryepiglottic folds and excess arytenoid mucosa that manifests as inspiratory stridor. In majority of children, treatment is conservative with a few symptomatic cases receiving reflux medication. In 20% of children with severe laryngomalacia, surgical intervention is necessary to alleviate symptoms. Knowing which children need more than medical management is key to best practice. Which children will benefit from otolaryngology (ENT) referral, and what are the factors that determine the need for supraglottoplasty?
Objective. To determine the clinical factors in symptomatic children with laryngomalacia that led up to supraglottoplasty, and response to therapy thereof. To identify those children who require a tracheostomy and whether supraglottoplasty was still indicated to decannulate (remove tracheostomy).
Methods. A retrospective review of medical folders for all children who underwent supraglottoplasty, by a single surgeon in South Africa over 7 years.
Results. 42 patients were included in this study: n=40/42 underwent supraglottoplasty (SGP), n=34/42 underwent SGP alone, n=2/42 required a tracheostomy post SGP, n=8/42 underwent tracheostomy, n=6/42 required a tracheostomy


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Methods: The ACTG questionnaire was cross culturally adapted to Uganda. Consenting postpartum women were assigned to Audio Computerized self –Assisted (ACASI) and Provider Assisted Interviews (PAIs) in a nested study within the PROMOTE Cohort Study in Uganda. Questionnaire construct scores for self-efficacy, social support, anxiety and depression plus viral load, patient demographics and clinical predictors of ART adherence were modelled using a mixed effects logistic model, with repeated measures over a period of one year.

Results: Of 166 women, 21 had the questionnaire administered via ACASI while 145 via PAIs. 4.2% (7/166) were not virally suppressed at baseline and their level of non-suppression was consistent throughout one year of follow up. High self-efficacy scores were associated with 27% lower odds of viral non-suppression (Odds Ratio [OR] 0.73;95% CI:0.54 - 0.98). High depression scores were associated with 22% higher odds of non-suppression (OR 1.22;95% CI:1.01- 1.49) by 22%. Other variables like Age, Body Mass Index, Duration on ART, Marital status, Employment, Education level, Electricity in premises, Tap water and Travel time to clinic from home were not associated with viral suppression in the covariate-adjusted analyses. Median Self efficacy and depression scores were 8 (IQR 1-9) and 1.2 (IQR 0-16) respectively. Focussed group discussion data showed high acceptability and feasibility of using the ACTG questionnaire in Ugandan settings.

Conclusion: Lower Self efficacy and higher depression scores on the ACTG adherence questionnaire could be used to identify Ugandan women at risk of viral non-suppression in HIV program settings.

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Growth and Neurodevelopmental Outcomes of 18-Month-Old Children With Exposure to Maternal HIV and Placental Insufficiency in a Peri-Urban Area of South Africa

Nyofane M[1,2,3,4], Hoffman M[1,3,4], Mulol H[1,3,4], Botha T[1,3,4], Pattinson R[1,3,4], Feucht U[1,3,4]

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Background: Children who are HIV-exposed-and-uninfected (CHEU) and those who are growth restricted in utero due to placental insufficiency are both regarded as high risk populations, which can impact growth and neurodevelopment as well as short- and long-term complications in terms of morbidity and mortality. However, the growth and neurodevelopmental outcomes of CHEU who have also experienced growth restriction in utero have not been researched. We therefore compared the growth and neurodevelopment outcomes of children aged 18 months with in utero HIV exposure and abnormal umbilical artery resistance indices (UmA-RI), indicating placental insufficiency.

Methods: The cross-sectional study investigated 264 mother-child pairs, who were grouped into four subgroups based on HIV exposure and history of normal/abnormal UmA-RI, using available pregnancy and birth information. The World Health Organization standard procedures were used for anthropometric measurements and z-score calculations, and Bayley III to test child development.

Results: CHEU with abnormal UmA-RI (n=14) had lower length-for-age z-scores (-1.40 ± 1.40 vs -0.04 ± 1.31; p=0.001), weight-for-age z-scores (-0.60 ± 0.96 vs 0.04 ± 1.16; p=0.02) and head-circumference-for-age z-scores (0.42 ± 0.66 vs 0.90 ± 1.15; p=0.04) compared to children who are HIV-unexposed-and-uninfected (CHUU) with normal UmA-RI (n=181). Nearly a quarter (21.4%) of CHEU

ANNEX I: ETHICS APPROVAL LETTERS

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Natural and Agricultural Sciences
Ethics Committee
E-mail: ethics.nas@up.ac.za

8 September 2021

ETHICS SUBMISSION: LETTER OF APPROVAL

Mr M Nyofane
Department of Consumer and Food Sciences
Faculty of Natural and Agricultural Science
University of Pretoria

Reference number: NAS259/2021
Project title: Growth and neurodevelopmental outcomes of 18-month-old children with in utero growth restriction due to placental insufficiency as measured by umbilical artery Doppler examination during pregnancy and as modified by maternal HIV status

Dear Mr M Nyofane,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Natural and Agricultural Sciences Research Ethics Committee.

Please note the following about your ethics approval:

- Please use your reference number (NAS259/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
- Please note that ethical approval is granted for the duration of the research (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
- The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
- **Applications using GM permits**: If the GM permit expires before the end of the study, please make an amendment to the application with the new GM permit before the old one expires
- **Applications using Animals**: NAS ethics recommendation does not imply that Animal Ethics Committee (AEC) approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

The application meets the ethics requirements set by the NAS ethics committee for dealing with human participants.

This study is related to another study and ethical approval has been granted. There is preference that the study is also approved by Health Sciences. Please note the Ethics clearance number is 283/2019 and not UMBIBABY.

Post approval submissions including application for ethics extension and amendments to the approved application should be submitted online via the Ethics work centre.

We wish you the best with your research.

Yours sincerely,

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

Faculty of Health Sciences **Research Ethics Committee**

12 November 2021

Endorsement Notice

Dear Dr HM Mulol

Ethics Reference No: NAS259/2021
Title: Growth and neurodevelopmental outcomes of 18-month-old children with in utero growth restriction due to placental insufficiency as measured by umbilical artery Doppler examination during pregnancy and as modified by maternal HIV status

The **New Application** as supported by documents received between 2021-09-15 and 2021-11-10 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2021-11-10 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Ethics Approval is valid for 1 year and needs to be renewed annually by 2022-11-12.
- Please remember to use your protocol number (NAS259/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPhamMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

11 February 2021

Approval Certificate
Amendment

Ethics Reference No.: 283/2019
Title: Early child outcomes of in utero growth restricted and premature babies - a prospective cohort study in South Africa (the UmbiBaby study)

Dear Dr UD Feucht

The **Amendment** as supported by documents received between 2021-01-27 and 2021-02-10 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2021-02-10 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Please remember to use your protocol number (283/2019) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2011 and Expires 03/14/2021

17 May 2019

Approval Certificate
Amendment

Ethics Reference No.: 294-2017
Title: Assessment of factors impacting on foetal and infant immunity, growth, and neurodevelopment in HIV- and antiretroviral-exposed uninfected children (the Siyakhula study)

Dear Dr UD Feucht

The **Amendment** as supported by documents received between 2019-05-02 and 2019-05-17 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 2019-05-15.

Please note the following about your ethics approval:

- Please remember to use your protocol number (294-2017) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

KALAFONG HOSPITAL
PRIVATE BAG X396
PRETORIA
0001

ENQUIRIES : MS P MONYEPAO
TEL : 012 318 6929
FAX : 012 373 6791
EMAIL : Patricia.Monyepao@gauteng.gov.za
REF : KPTH 31/2019

TO: Dr UD Feucht

RE: PERMISSION TO CONDUCT RESEARCH

TITLE: EARLY CHILD OUTCOMES OF IN UTERO GRWTH RESTRICTED AND PREMATURE BABIES - A PROSECTIVES COHORT STUD IN SOUTH AFRICA

Permission is hereby granted for the research to be conducted at **Kalafong Provincial Tertiary Hospital.**

This is done in accordance to the "Promotion of Access to Information Act. No 2 of 2000".

Please note that in addition to receiving approval from the hospital research committee, you are still required to seek permission from the relevant departments.

Furthermore, collecting of data and consent for participation remains the responsibility of the researcher.

You are also required to submit your final report or summary of your findings and recommendations to the office of the CEO.

Approved:

DR K.E LETEBELE-HARTELL
SENIOR MANAGER: MEDICAL SERVICES
DATE: 09/01/2019

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Enquiries: Dr. Lufuno Razwiedani
Tel: +27 12 451 9036
E-mail: lufuno.razwiedani@gauteng.gov.za

TSHWANE RESEARCH COMMITTEE: CLEARANCE CERTIFICATE

MEETING: 06/2017
PROJECT NUMBER: 93/2017
NHRD REFERENCE NUMBER: GP_201710_002

TOPIC: Assessment of factors impacting on foetal and infant immunity, growth, and neurodevelopment in HIV- and antiretroviral-exposed uninfected children

Name of the Researcher:

Felicia Molokoane	Andrea Prinsloo
Ameena Goga	Louise du Toit
Mphele Mulaudzi	Helen Steel
Robert Pattinson	Marlene Gilfillan
Theuns Avenant	Ute Feucht
Jennifer Makin	Theresa Rossouw

Facility: Kalafong Tertiary Hospital
Pretoria West Hospital

Name of the Department: University of Pretoria

NB: THIS OFFICE REQUEST A FULL REPORT ON THE OUTCOME OF THE RESEARCH DONE AND

NOTE THAT RESUBMISSION OF THE PROTOCOL BY RESEARCHER(S) IS REQUIRED IF THERE IS DEPARTURE FROM THE PROTOCOL PROCEDURES AS APPROVED BY THE COMMITTEE.

DECISION OF THE COMMITTEE: APPROVED

.....
Dr. R. Oyedipe
Acting Chairperson: Tshwane Research Committee
Date: 09/11/2017

.....
Ms. M. Lerutla
Acting Chief Director: Tshwane District Health
Date: 13/11/17

ANNEX J: AUTHOR GUIDELINES

Manuscript Submission Overview

Types of Publications

Full experimental details must be provided so that the results can be reproduced. *Viruses* requires that authors publish all experimental controls and make full datasets available where possible (see the guidelines on **Supplementary Materials** and references to unpublished data).

Manuscripts submitted to *Viruses* should neither be published previously nor be under consideration for publication in another journal. The main article types are listed below and a comprehensive list of article types can be found **here.**

- *Article:* These are original research manuscripts. The work should report scientifically sound experiments and provide a substantial amount of new information. The article should include the most recent and relevant references in the field. The structure should include an Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion, and Conclusions (optional) sections, with a suggested minimum word count of 4000 words.

- *Review:* Reviews offer a comprehensive analysis of the existing literature within a field of study, identifying current gaps or problems. They should be critical and constructive and provide recommendations for future research. No new, unpublished data should be presented. The structure can include an Abstract, Keywords, Introduction, Relevant Sections, Discussion, Conclusions, and Future Directions, with a suggested minimum word count of 4000 words.

Submission Process

Manuscripts for *Viruses* should be submitted online at **susy.mdpi.com**. The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the **criteria to qualify for authorship**) and that they have all read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the **submission website**. Once you have registered, **click here to go to the submission form for *Viruses***. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

Accepted File Formats

Authors are encouraged to use the **Microsoft Word template** or **LaTeX template** to prepare their manuscript. Using the template file will substantially shorten the time to complete copy-editing and publication of accepted manuscripts. The total amount of data for all files must not exceed 120 MB. If this is a problem, please contact the Editorial Office **viruses@mdpi.com**. Accepted file formats are:

- *Microsoft Word:* Manuscripts prepared in Microsoft Word must be converted into a single file before submission. When preparing manuscripts in Microsoft Word, we encourage you to use the ***Viruses* Microsoft Word template file**. Please insert your graphics (schemes, figures, *etc.*) in the main text after the paragraph of its first citation.

- *LaTeX:* Manuscripts prepared in LaTeX must be collated into one ZIP folder (including all source files and images, so that the Editorial Office can recompile the submitted PDF). When preparing manuscripts in LaTeX, we encourage you to use the ***Viruses* LaTeX template files**. You can now also use the online application **writeLaTeX** to submit articles directly to *Viruses*. The MDPI LaTeX template file should be selected from the **writeLaTeX template gallery**.

- *Supplementary files:* May be any format, but it is recommended that you use common, non-proprietary formats where possible (see **below** for further details).

Disclaimer: Usage of these templates is exclusively intended for submission to the journal for peer-review, and strictly limited to this purpose and it cannot be used for posting online on preprint servers or other websites.

Free Format Submission

Viruses now accepts free format submission:

- We do not have strict formatting requirements, but all manuscripts must contain the required sections: Author Information, Abstract, Keywords, Introduction, Materials & Methods, Results, Conclusions, Figures and Tables with Captions, Funding Information, Author Contributions, Conflict of Interest and other Ethics Statements. Check the Journal ***Instructions for Authors*** for more details.

- Your references may be in any style, provided that you use the consistent formatting throughout. It is essential to include author(s) name(s), journal or book title, article or chapter title (where required), year of publication, volume and issue (where appropriate) and pagination. DOI numbers (Digital Object Identifier) are not mandatory but highly encouraged. The bibliography software package *EndNote*, ***Zotero***, *Mendeley*, *Reference Manager* are recommended.

- When your manuscript reaches the revision stage, you will be requested to format the manuscript according to the journal guidelines.

Cover Letter

A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work. It should explain why the manuscript fits the scope of the journal.

Any prior submissions of the manuscript to MDPI journals must be acknowledged. If this is the case, it is strongly recommended that the previous manuscript ID is provided in the submission system, which will ease your current submission process. The names of proposed and excluded reviewers should be provided in the submission system, not in the cover letter.

All cover letters are required to include the statements:

- We confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal.

- All authors have approved the manuscript and agree with its submission to (journal name).

Author Identification

Authors are encouraged to add a biography (300–1500 characters) to the submission and upload it to **SciProfiles**. This should be a single paragraph and should contain the following points:

1. Authors' full names followed by current positions;
2. Education background including institution information and year of graduation (type and level of degree received);
3. Work experience;
4. Current and previous research interests;
5. Memberships of professional societies and awards received.

If a manuscript is accepted for publication, we will add an icon linking to your online **ORCID** profile in the final version of the published paper.

Author Affiliation

All authors should list their current affiliation and the affiliation where most research was carried out for the preparation of their manuscript. We recommend adding as primary the affiliation where most of the research was conducted or supported, but please check with your institution for any contractual agreement requirements.

It is very important that author names and affiliations are correct. Incorrect information can mean a lack of proper attribution or incorrect citation and can even lead to problems with promotion or funding. After the publication of an article, updates or corrections to the author's address or affiliation may not be permitted.

Independent Researcher

If one or all the authors are not currently affiliated with a university, institution or company, or have not been during the development of the manuscript, they should list themselves as an "Independent Researcher".

Manuscript Preparation

General Considerations

- **Research manuscripts** should comprise:
    - **Front matter**: Title, Author list, Affiliations, Abstract, Keywords.
    - **Research manuscript sections**: Introduction, Materials and Methods, Results, Discussion, Conclusions (optional).
    - **Back matter**: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, **References**.
- **Review manuscripts** should comprise the **front matter**, literature review sections and the **back matter**. The template file can also be used to prepare the front and back matter of your review manuscript. It is not necessary to follow the remaining structure. Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the **PRISMA** guidelines.
- **Graphical Abstract:**

  A graphical abstract (GA) is an image that appears alongside the text abstract in the Table of Contents. In addition to summarizing the content, it should represent the topic of the article in an attention-grabbing way. Moreover, it should not be exactly the same as the Figure in the paper or just a simple superposition of several subfigures. Note that the GA must be original and unpublished artwork. Any postage stamps, currency from any country, or trademarked items should not be included in it.

  The GA should be a high-quality illustration or diagram in any of the following formats: PNG, JPEG, or TIFF. Written text in a GA should be clear and easy to read, using one of the following fonts: Times, Arial, Courier, Helvetica, Ubuntu or Calibri.

  The minimum required size for the GA is 560 × 1100 pixels (height × width). The size should be of high quality in order to reproduce well.
- **Acronyms/Abbreviations/Initialisms** should be defined the first time they appear in each of three sections: the abstract; the main text; the first figure or table. When defined for the first time, the acronym/abbreviation/initialism should be added in parentheses after the written-out form.
- **SI Units** (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.
- **Virus nomenclature:** Read the instructions concerning viruses nomenclature **here**.
- **Accession numbers** of RNA, DNA and protein sequences used in the manuscript should be provided in the Materials and Methods section. Also see the section on **Deposition of Sequences and Expression Data**.
- **Equations:** If you are using Word, please use either the Microsoft Equation Editor or the MathType add-on. Equations should be editable by the editorial office and not appear in a picture format.
- **Research Data and supplementary materials:** Note that publication of your manuscript implies that you must make all materials, data, and protocols associated with the publication available to readers. Disclose at the submission stage any restrictions on the availability of materials or information. Read the information about **Supplementary Materials** and Data Deposit for additional guidelines.
- **Preregistration:** Where authors have preregistered studies or analysis plans, links to the preregistration must be provided in the manuscript.
- **Guidelines and standards:** MDPI follows standards and guidelines for certain types of research. See **https://www.mdpi.com/editorial_process** for further information.

Front Matter

These sections should appear in all manuscript types

- **Title:** The title of your manuscript should be concise, specific and relevant. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used. Please do not include abbreviated or short forms of the title, such as a running title or head. These will be removed by our Editorial Office.

- **Author List and Affiliations:** Authors' full first and last names must be provided. The initials of any middle names can be added. The PubMed/MEDLINE standard format is used for affiliations: complete address information including city, zip code, state/province, and country. At least one author should be designated as the corresponding author. The email addresses of all authors will be displayed on published papers, and hidden by Captcha on the website as standard. It is the responsibility of the corresponding author to ensure that consent for the display of email addresses is obtained from all authors. If an author (other than the corresponding author) does not wish to have their email addresses displayed in this way, the corresponding author must indicate as such during proofreading. After acceptance, updates to author names or affiliations may not be permitted. Equal Contributions: authors who have contributed equally should be marked with a superscript symbol (†). The symbol must be included below the affiliations, and the following statement added: “These authors contributed equally to this work”. The equal roles of authors should also be adequately disclosed in the author contributions statement. Please read the criteria to qualify for authorship.

- **Abstract:** The abstract should be a total of about 200 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts, but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used; 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article: it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.

- **Keywords:** Three to ten pertinent keywords need to be added after the abstract. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Research Manuscript Sections

- **Introduction:** The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance, including specific hypotheses being tested. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the main conclusions. Keep the introduction comprehensible to scientists working outside the topic of the paper.

- **Materials and Methods:** They should be described with sufficient detail to allow others to replicate and build on published results. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited. Give the name and version of any software used and make clear whether computer code used is available. Include any pre-registration codes.

- **Results:** Provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

- **Discussion:** Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible and limitations of the work highlighted. Future research directions may also be mentioned. This section may be combined with Results.

- **Conclusions:** This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

- **Patents:** This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

Back Matter

- **Supplementary Materials:** Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.

- **Author Contributions:** Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it; AND has approved the submitted version (and version substantially edited by journal staff that involves the author’s contribution to the study); AND agrees to be personally accountable for the author’s own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in

which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.
For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project Administration, X.X.; Funding Acquisition, Y.Y.", please turn to the **CRediT taxonomy** for the term explanation. For more background on CRediT, see **here**. "**Authorship must include and be limited to those who have contributed substantially to the work. Please read the section concerning the criteria to qualify for authorship carefully**".

- **Funding:** All sources of funding of the study should be disclosed. Clearly indicate grants that you have received in support of your research work and if you received funds to cover publication costs. Note that some funders will not refund article processing charges (APC) if the funder and grant number are not clearly and correctly identified in the paper. Funding information can be entered separately into the submission system by the authors during submission of their manuscript. Such funding information, if available, will be deposited to FundRef if the manuscript is finally published.
Please add: "This research received no external funding" or "This research was funded by [name of funder] grant number [xxx]" and "The APC was funded by [XXX]" in this section. Check carefully that the details given are accurate and use the standard spelling of funding agency names at https://search.crossref.org/funding, any errors may affect your future funding.

- **Institutional Review Board Statement:** In this section, please add the Institutional Review Board Statement and approval number for studies involving humans or animals. Please note that the Editorial Office might ask you for further information. Please add "The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of NAME OF INSTITUTE (protocol code XXX and date of approval)." OR "Ethical review and approval were waived for this study, due to REASON (please provide a detailed justification)." OR "Not applicable" for studies not involving humans or animals. You might also choose to exclude this statement if the study did not involve humans or animals.

- **Informed Consent Statement:** Any research article describing a study involving humans should contain this statement. Please add "Informed consent was obtained from all subjects involved in the study." OR "Patient consent was waived due to REASON (please provide a detailed justification)." OR "Not applicable." for studies not involving humans. You might also choose to exclude this statement if the study did not involve humans.
Written informed consent for publication must be obtained from participating patients who can be identified (including by the patients themselves). Please state "Written informed consent has been obtained from the patient(s) to publish this paper" if applicable.

- **Data Availability Statement:** In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Please refer to suggested Data Availability Statements in section "**MDPI Research Data Policies**". You might choose to exclude this statement if the study did not report any data.

- **Acknowledgments:** In this section you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

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