# Immune Reconstitution Inflammatory Syndrome-Associated Graves Disease in HIV-Infected Patients: Clinical Characteristics and Response to Radioactive Iodine Therapy

IO Lawal[1,2,*], AT Orunmuyi[3], GO Popoola[4], KMG Mokoala[1,2], T Lengana[1], MM Sathekge[1,2]

1. Department of Nuclear Medicine, University of Pretoria & Steve Biko Academic Hospital, Pretoria 0001, South Africa
2. Nuclear Medicine Research Infrastructure (NuMeRI), Steve Biko Academic Hospital, Pretoria 0001, South Africa
3. Department of Nuclear Medicine, University of Ibadan & University College Hospital, Ibadan, Nigeria
4. Department of Epidemiology and Community Health, University of Ilorin, Ilorin, Nigeria.

*Correspondence to: Ismaheel O. Lawal, Department of Nuclear Medicine, University of Pretoria, Pretoria 0001, South Africa. Telephone: +27 12 354 2302. Fax: +27 12 354 1219. Email: ismaheellawal@gmail.com

## Abstract

**Objectives**: We aimed to describe the clinical characteristics and the response to radioactive iodine (RAI) treatment of immune reconstitution inflammatory syndrome-associated Graves disease (IRIS-GD) in comparison to Graves disease (GD) seen in HIV-uninfected patients.

**Methods**: We retrospectively reviewed the medical records of patients treated with RAI for GD. We obtained clinical, biochemical, and HIV-related information of patients from their medical records. We compared patient characteristics and response to RAI treatment between patients with IRIS-GD and GD seen in HIV-uninfected patients.

**Results**: A total of 253 GD patients, including 51 patients with IRIS-GD, were included. Among IRIS-GD patients, CD4 cell nadir was 66 cells/µL (range=37-103) with a peak HIV viral load of 60,900 copies/mL (range=36,542-64,500). At the time of diagnosis of IRIS-GD, all patients had a completely suppressed HIV viremia with a CD4 cell count of 729 cells/µL (range=350 - 1279). The median interval between the commencement of HIV treatment and the onset of GD was 63 months. At 3-months follow-up, the proportion of patients with IRIS-GD achieving successful RAI treatment outcome (euthyroid/hypothyroid state) was lower than HIV-uninfected patients (35.3% versus 63.4%, respectively, $p<0.001$). The response rate remained lower (60.8%) among patients with IRIS GD compared with HIV-uninfected GD patients (80.2%), $p=0.004$ at 6-month follow-up. After correcting for differences in age, gender, and pretreatment thyroid-stimulating hormone level, there was no significant difference in RAI treatment response between the two groups.

**Conclusions**: After correcting for possible confounders, the response to RAI treatment is not different between patients with IRIS-GD and GD in HIV-uninfected patients.

**Keywords**: Immune reconstitution inflammatory syndrome, Graves disease, HIV, Radioactive iodine treatment

## Introduction

Immune reconstitution inflammatory syndrome (IRIS) is a new-onset or worsening of symptoms of diseases in a human immunodeficiency virus (HIV)-infected patient starting effective antiretroviral therapy (ART) [1]. IRIS is most common in the setting of severe immunosuppression (low plasma level of CD4 cells) and high HIV viral load at the time of commencing ART [2-4]. Despite the widespread availability of safe and effective ART and the current recommendation of treating all HIV-infected patients regardless of their CD4 cell level, a significant proportion of patients are diagnosed with HIV and commenced on ART at a very advanced disease stage [5]. This makes IRIS to remain a condition of clinical significance even in the ART era [4]. In IRIS, immune reconstitution manifesting as a rise in CD4 cell level that occurs in response to effective ART leads to host recognition of foreign antigen (from microbial agents and tumors), causing antigen-specific immune responses. The intense inflammatory reaction that accompanies this host immune response to foreign antigens is responsible for worsening clinical and radiological manifestations of diseases that characterize IRIS. IRIS is best described in the setting of opportunistic infections such as tuberculosis and tumors, including Kaposi sarcoma [2-4]. IRIS resulting from an immune response against self-antigens leading to autoimmune disorders is less well-characterized. Autoimmune diseases occurring in the setting of IRIS include Graves disease (GD) and rheumatic diseases [6,7].

GD is an autoimmune disease characterized by the development of autoantibodies against the thyroid-stimulating hormone (TSH) receptor expressed on thyroid follicular cells. This leads to stimulation of the TSH receptors and an increased in thyroid hormone production. High circulating levels of free thyroid hormones, free thyroxine (fT4), and free triiodothyronine (fT3) have multisystemic effects causing severe patient debilitation and may impair working ability in up to a third of affected patients [8]. The exact pathophysiology of GD seen in IRIS is still not completely known. IRIS-associated GD (IRIS-GD) occurs in the context of immunoregulatory imbalance characterized by thymic enlargement, persistence of TREC (T-cell receptor excision circle) levels in CD4 and CD8 cells, and high circulating levels of naïve CD8 cell. There is also a failure of thymic deletion of autoreactive T-cells [9,10].

The first-line treatment of GD is with anti-thyroid medications (ATD) that act to reduce hormone production by the thyroid gland. Thyroidectomy is reserved for patients with contraindications to other treatment modalities, those with large goiters, and for patients with suspicion of co-existing thyroid malignancy [11]. Treatment of GD with radioactive iodine (RAI) is increasingly being applied either as first-line treatment or as an alternative treatment in patients who do not tolerate or respond to ATD. The thyroid gland traps iodine, which it uses to iodinate tyrosyl residues in thyroid hormone production. When trapped in the thyroid follicular cells, RAI releases beta particles that are energetic and lead to the destruction of the follicular cells [12]. RAI is a safe, potent, and cost-effective modality for GD therapy [13]. A 2011 survey among clinical endocrinologists in the United States showed that 59.7% would use RAI as the first-line GD therapy [14].

Published reports on IRIS-GD are scanty in the literature, limited to case reports and small series [15-21]. Also, no study has reported the effectiveness of RAI in the treatment of IRIS-GD. In this study, we aimed to report the clinical characteristics of HIV-infected patients who developed GD as IRIS during ART. We also aimed to report on the effectiveness of RAI in treating IRIS-GD seen in people living with HIV (PLHIV) infection compared with GD seen in the non-HIV-infected population.

## Methods

We performed a retrospective review of the medical records of all patients 18 years and above with clinical and biochemically confirmed Graves disease referred to the Department of Nuclear Medicine at Steve Biko academic hospital for RAI therapy between 01 January 2015 and 31 December 2019. Our department serves as a referral center and provides nuclear medicine services for regions within two of nine South African provinces. Graves disease patients are referred to our department for RAI therapy after failing ATD, develop intolerable side effects to ATD, or as newly diagnosed treatment-naïve Graves disease. We included in this study all patients treated with 15mCi empirical dosing of radioactive I-131 for Graves disease. Our exclusion criteria in patient selection were previous history of thyroid surgery, concomitant thyroid pathologies such as thyroid nodules, previous history of RAI treatment, unknown HIV serostatus, and treatment with RAI dosing other than 15mCi. The human research ethics committee of the Faculty of Health Sciences at the University of Pretoria approved this study and waived the need for patient consent due to the retrospective design of this study.

### *Definitions*

All patients had a Tc-99m pertechnetate thyroid scintigraphy at first presentation at our department. We defined Graves disease in any patient with all the following criteria: (1) clinical features suggestive of thyrotoxicosis, (2) elevated serum levels of thyroid-stimulating hormone antibody (TSHRAb), (3) elevated levels of fT4 and fT3 and suppressed thyroid-stimulating hormone (TSH) levels, and (4) diffusely intense tracer uptake in the thyroid gland with no associated hot or cold nodule on thyroid scintigraphy.

We defined IRIS-GD in a patient that commenced ART at a very low CD4 cell level (<200 cell/μL), achieved immune restoration and complete suppression of plasma HIV viremia on ART, and developed features of Graves disease as defined above. Patients with GD preceding the acquisition of HIV infection do not satisfy the criteria for IRIS-GD.

We followed up all patients treated with RAI for a minimum of six months. We obtained repeat thyroid function tests at three- and six-months post-RAI treatment. We defined successful RAI treatment as any RAI-treated patients who achieved euthyroid or hypothyroid state at six-month post RAI treatment. We considered RAI treatment to have failed in any patients who were still hyperthyroid at 6-month post RAI treatment.

### *Data collection*

We retrieved the medical records of all patients treated for GD in our department during the study period. We extracted the following data from the records of all qualifying patients:

- Epidemiological variables including age and gender
- Clinical variables including HIV serostatus, duration of GD symptoms prior to RAI treatment, use of ATD and beta-blockers prior to RAI therapy, associated thyroid ophthalmopathy, family history of GD, and history of any chronic medical conditions
- Biochemical variables including TSH, fT4, fT3 with four weeks prior to RAI treatment and at three- and six-months post RAI treatment. TSHRAb and thyroid peroxidase antibody (TPOAb) levels prior to RAI treatment.
- HIV-specific variables obtained from HIV-infected patients, including CD4 cell count nadir, highest HIV viral load prior to commencing ART, CD4 cell count and HIV viral load at the time of GD diagnosis, duration of HIV infection at the time of GD diagnosis.

***Statistical analysis***

Qualitative data were expressed as frequency (percentage). Quantitative variables were expressed as mean ± standard deviation (SD) if normally distributed or as median and interquartile range (IQR)/range if not normally distributed. We used the $\chi^2$ test (for qualitative variables) and the Mann-Whitney U test (for quantitative variables) to test the association between the clinical and biochemical variables versus RAI treatment outcome at six-month post-therapy. We compared patients with IRIS-GD with GD patients without HIV infection with respect to clinical and biochemical variables using the $\chi^2$ test (for qualitative variables) and Independent Samples T-test or Mann-Whitney U test (for quantitative variables that are normally distributed or skewed variables, respectively). We performed matching based on age and gender of patients with IRIS-GD with undetectable serum TSH levels (≤0.01 mIU/L) prior to RAI therapy versus patients with GD and undetectable serum level of TSH but without HIV infection. The matched pair were compared regarding the outcome of RAI using the $\chi^2$ test. We performed a binary logistic regression analysis to identify factors that predict the RAI treatment outcome. We set statistical significance at a *P*-value of $< 0.05$. We performed statistical analysis using SPSS statistics version 21.0 (IBM Corp., Armonk, NW, USA).

## Results

Out of 427 patients treated for GD in our department during the study period, 253 patients met the criteria for inclusion into this study (supplementary figure). Fifty-one of these patients were HIV-infected and met the clinical definition of IRIS-GD. There were 214 females (84.6%), and the mean age of the study population was 39.80 ± 13.10 years. Among patients diagnosed with IRIS-GD, median CD4 cell nadir prior to commencement of ART was 66 cells/µL (range=37-103) with a median peak HIV viral load of 60,900 copies/mL (range=36,542-64,500). All patients with IRIS-GD had achieved completely suppressed plasma HIV viremia (<40 copies/mL) at the time of GD diagnosis and had a median CD4 cell count of 729 cells/µL (range=350 - 1279). The median interval between HIV diagnosis and the diagnosis of GD was 63 months. Table 1 shows the detailed baseline demographic and clinical characteristics of the study population. There was associated eye signs in 46.2% of patients, and only 9.9% of the study population had a family history of GD. About a fifth of patients (22.1%) had no history of treatment with ATD prior to referral for RAI treatment. The majority of patients had uncontrolled hyperthyroidism evident by a suppressed TSH level (median TSH of 0.01 mIU/L) at the time of RAI treatment. Table 2 shows the detailed clinical and biochemical characteristics of the study population at the time of RAI treatment.

**Table 1:** Demographic and baseline HIV-related clinical variables of the study population

| Variable | Frequency | Percent |
|---|---|---|
| **Age, N=253 (years)** | | |
| Mean ± SD | 39.8 ± 13.1 | |
| Range | 18 – 91 | |
| **Gender, N=253** | | |
| Male | 39 | 15.4 |
| Female | 214 | 84.6 |
| **HIV infection, N=253** | | |
| Yes | 51 | 20.2 |
| No | 202 | 79.8 |
| **CD4 cell count nadir before ART, N=32 (cells/µL)** | | |
| Median (IQR) | 66 (42 – 96) | |
| Range | 37 – 103 | |
| **Highest viral load before ART, N=41 (copies/mL)** | | |
| Median (IQR) | 60900 (36542) | |
| Range | 36542 – 64500 | |
| **Interval between HIV diagnosis and the diagnosis of Graves disease, N=51 (months)** | | |
| Median (IQR) | 63 (39 – 82) | |
| Range | 22 - 148 | |
| **CD4 cell count at Graves diagnosis, N=51 (cells/µL)** | | |
| Median (IQR) | 729 (597 – 914) | |
| Range | 350 – 1279 | |

**HIV**: Human Immunodeficiency Virus; **IQR**: Interquartile range; **CD4**: Cluster of Differentiation 4

**Table 2:** Clinical and laboratory parameters of the study population

| Variable | Frequency | Percent |
|---|---|---|
| **Eye signs, N=253** | | |
| Yes | 117 | 46.2 |
| No | 136 | 53.8 |
| **Family history of GD, N=253** | | |
| Yes | 25 | 9.9 |
| No | 228 | 90.1 |
| **Diabetes mellitus, N=253** | | |
| Yes | 16 | 6.3 |
| No | 237 | 93.7 |
| **Hypertension, N=253** | | |
| Yes | 78 | 30.8 |
| No | 175 | 69.2 |
| **Use of ATD, N=253** | | |
| Yes | 197 | 77.9 |
| No | 56 | 22.1 |
| **Use of Beta-blockers, N=253** | | |
| Yes | 140 | 55.3 |
| No | 113 | 44.7 |
| **TPOAb before RAIT, N=113** | | |
| Positive | 68 | 26.9 |
| Negative | 45 | 17.8 |
| **Duration of GD symptoms before RAIT, N=253 (months)** | | |
| Median (IQR) | 8.00 (4.00 – 22.0) | |
| Range | 1.00 – 152.00 | |
| **Duration of ATD use, N=253 (months)** | | |
| Median (IQR) | 6.00 (3.00 – 12.00) | |
| Range | 0 – 146 | |
| **TSH prior to RAIT, N=253 (mIU/L)** | | |
| Median (IQR) | 0.01 (0.01 – 0.01) | |
| Range | 0.01 – 10.65 | |
| **fT3 prior to RAIT, N=253 (pmol/L)** | | |
| Median (IQR) | 23.00 (10.53 – 37.45) | |
| Range | 2.80 – 150.50 | |
| **fT4 prior to RAIT, N=253 (pmol/L)** | | |
| Median (IQR) | 35.90 (21.45 – 55.80) | |
| Range | 3.90 – 140.40 | |
| **TSHRAb prior to RAIT, N=253 (U/L)** | | |
| Median (IQR) | 16.88 (4.95 – 33.30) | |
| Range | 1.00 – 46.00 | |

**GD**: Graves disease; **ATD**: Anti-Thyroid Medications; **TPOAb**: Thyroid Peroxidase Antibody; **RAIT**: Radioactive Iodine Treatment; **TSH**: Thyroid Stimulating Hormone; **fT4**: free Thyroxine; **fT3**: free triiodothyronine; **TSHRAb**: Thyroid Stimulating Hormone Antibody

### *Radioactive iodine treatment treatment outcome and the factors associated with a successful treatment outcome*

At 3-month post RAI treatment, 146 patients (57.7%) had achieved successful treatment defined as a euthyroid or hypothyroid state. The proportion of patients who achieved successful RAI treatment

improved to 193 patients (76.3%) at 6-month follow-up (figure 1, table 3). Among the several factors tested for their association with a successful RAI treatment outcome at 3-month follow-up, we found the presence of thyroid eye signs (p=0.018), HIV-infection, a high pretreatment fT4 (p=0.048), and a longer duration of GD symptoms prior to RAI treatment (p=0.004) to be significantly associated with RAI treatment outcome. At 6-month post RAI treatment, the presence of thyroid eye signs, HIV infection, and a longer duration of GD symptoms were significantly associated with treatment outcome. Table 3 shows the details of the association of different parameters with RAI treatment outcome assessed at 6-month post-treatment.

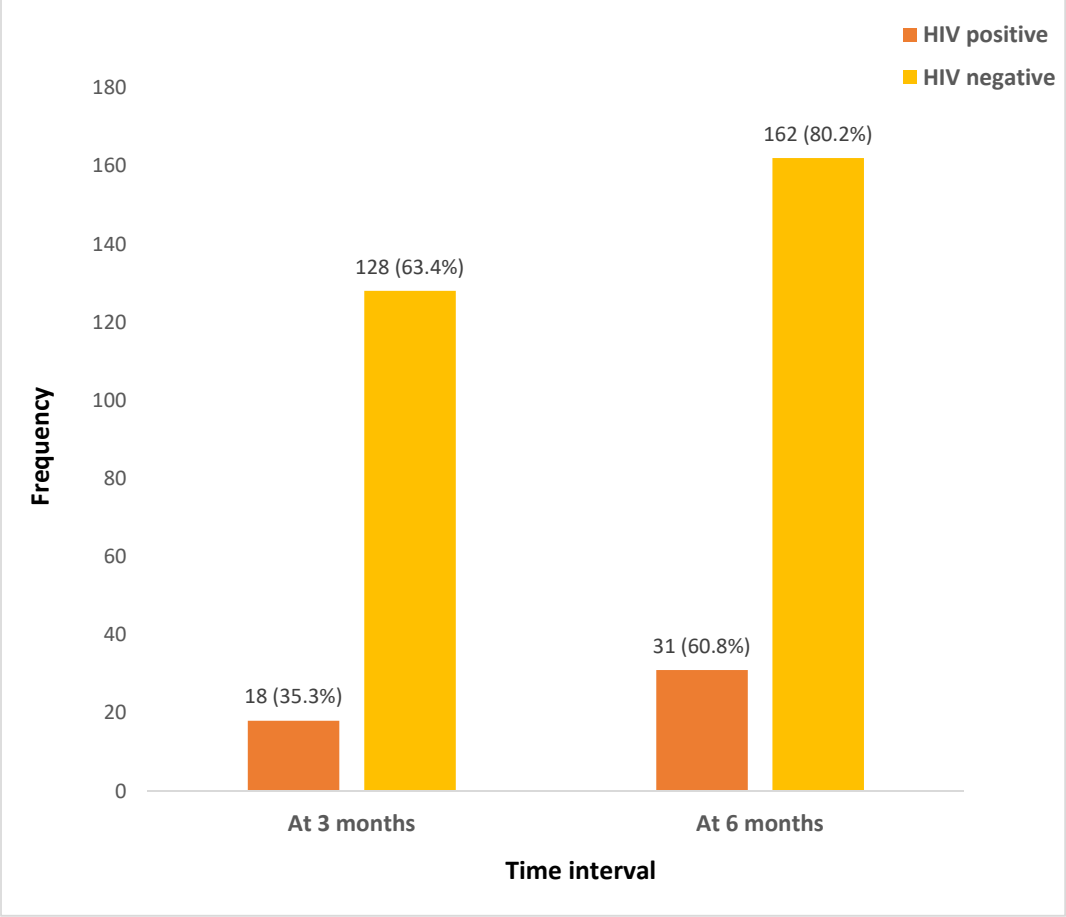


**Figure 1**. Proportion of patients with and without HIV infection with successful treatment outcomes at 3 and 6 months after radioactive iodine treatment. Successful treatment is defined as patients who achieved a euthyroid or hypothyroid state after radioactive iodine treatment

**Table 3**: Association between effectiveness of RAI treatment at 6 months versus demographic and clinical variables

| Variable | Positive treatment outcome at 6 months<br>Yes<br>n (%) | <br>No<br>n (%) | $\chi^2$ | *p*-value |
|---|---|---|---|---|
| **Age, N=253** | | | | |
| Mean ± SD | 40.20 ± 13.37 | 39.27 ± 12.41 | 0.447[t] | 0.655 |
| **Gender, N=253** | | | | |
| Male | 28 (71.8) | 11 (28.2) | 0.514 | 0.474 |
| Female | 165 (77.1) | 49 (22.9) | | |
| **HIV, N=253** | | | | |
| Yes | 31 (60.8) | 20 (39.2) | 8.433 | **0.004*** |
| No | 162 (80.2) | 40 (19.8) | | |
| **Use of beta blockers, N=253** | | | | |
| Yes | 104 (74.3) | 36 (25.7) | 0.692 | 0.405 |
| No | 89 (78.8) | 24 (21.2) | | |
| **Use of ATD, N=253** | | | | |
| Yes | 149 (75.6) | 48 (24.4) | 0.208 | 0.648 |
| No | 44 (78.6) | 12 (21.4) | | |
| **TPOAb, N=113** | | | | |
| Positive | 57 (83.8) | 11 (16.2) | 1.181 | 0.277 |
| Negative | 34 (75.6) | 11 (24.4) | | |
| **Eye signs, N=253** | | | | |
| Yes | 82 (70.1) | 35 (29.9) | 4.623 | **0.032*** |
| No | 111 (81.6) | 25 (18.4) | | |
| **Family history of GD, N=253** | | | | |
| Yes | 22 (88.0) | 3 (12.0) | 2.105 | 0.147 |
| No | 171 (75.0) | 57 (25.0) | | |
| **DM, N=253** | | | | |
| Yes | 10 (62.5) | 6 (37.5) | 1.794[F] | 0.222 |
| No | 183 (77.2) | 54 (22.8) | | |
| **HTN, N=253** | | | | |
| Yes | 57 (73.1) | 21 (26.9) | 0.641 | 0.423 |
| No | 136 (77.7) | 39 (22.3) | | |
| | **Median (IQR)** | **Median (IQR)** | **U** | ***p* value** |
| **Pre-treatment TSH, N=253 (mIU/L)** | | | | |
| Median (range) | 0.01 (0.01 – 10.65) | 0.01 (0.01 – 0.88) | 4764.000 | 0.578 |
| **Pre-treatment fT4 level, N=253 (pmol/L)** | 34.05 (20.55 – 51.55) | 39.40 (22.30 – 68.90) | 4148.000 | 0.115 |
| **Pre-treatment TSHRAb level, N=253 (U/L)** | 16.74 (4.52 – 33.63) | 17.80 (7.33 – 40.00) | 388.000 | 0.701 |
| **Duration of ATD use, N=253 (months)** | 6.00 (3.00 – 12.00) | 7.00 (3.00 – 21.00) | 1946.000 | 0.259 |
| **Duration of GD symptoms prior to RAIT, N=253 (months)** | 8.00 (4.00 – 18.00) | 13.00 (6.00 – 31.00) | 3547.500 | **0.040*** |

**$\chi^2$**: Chi square test; **t**: Independent Samples T test; **U**: Mann-Whitney U test; *****: *p* value <0.05; **HIV**: Human Immunodeficiency Virus; **ATD**: Anti-Thyroid Medication; **TPOAb**: Thyroid Peroxidase Antibody; **GD**: Graves disease; **DM**: Diabetes Mellitus; **HTN:** Hypertension; **TSH**: Thyroid Stimulating Hormone**; fT4**: free thyroxine; **RAIT**: Radioactive Iodine Treatment

We performed binary logistic regression analysis to further assess the strength of the presence of HIV infection, pretreatment TSH level, pretreatment fT4 level, and the duration of symptoms of GD as predictors RAI treatment outcome assessed at 3-month post RAI therapy. Presence of HIV infection (Odds ratio, OR=0.276; 95% CI:0.132-0.577; p=0.001), pre-treatment fT4 (OR=0.039; 95%CI:0.078-0.999; p=0.039) and duration of GD symptoms before RAI treatment (OR=0.981; 95%CI:0.967-0.995; p=0.009) all had a significant negative association with successful RAI treatment outcome assessed at 3-months post-treatment. In a binary logistic regression analysis of factors predictive of successful treatment outcome assessed at 6-month post-therapy, presence of thyroid eye signs (OR=0.494; 95%CI:0.256-0.954; p=0.036) and the duration of GD symptoms prior to RAI treatment both had a negative significant association with RAI treatment outcome assessed at 6-month follow-up.

***Comparison of outcome of RAI treatment between IRIS-GD and GD in patients without HIV infection***

At three-months follow-up, 35.3% of patients with IRIS-GD had achieved a successful treatment outcome (euthyroid/hypothyroid state), which was significantly lower than 63.4% of patients with GD but without HIV-infection who achieved same, p<0.001. The proportion of patients with successful treatment outcome had increased to 60.8% among the IRIS-GD cohort compared with 80.2% among cohort without HIV-infection, p=0.004 (table 4).

**Table 4:** Comparison of treatment outcome at 3- and 6-months post RAIT between patients with IRIS-GD and with HIV-uninfected patients with GD

| Successful treatment outcome | IRIS-GD n (%) | HIV-uninfected GD n (%) | Total N (%) | $\chi^2$ | *p*-value |
|---|---|---|---|---|---|
| **At 3 months, N=253** | | | | | |
| Yes | 18 (35.3) | 128 (63.4) | 146 (57.7) | 13.148 | **<0.001*** |
| No | 33 (64.7) | 74 (36.6) | 107 (42.3) | | |
| **At 6 months, N=253** | | | | | |
| Yes | 31 (60.8) | 162 (80.2) | 193 (76.3) | 8.483 | **0.004*** |
| No | 20 (39.2) | 40 (19.8) | 60 (23.7) | | |

**$\chi^2$**: Chi square test; ***: *p* value <0.05; HIV**: Human Immunodeficiency Virus; **IRIS-GD**: Immune Reconstitution Inflammatory Syndrome-associated Graves Disease; **RAIT**: Radioactive Iodine Treatment; **GD**: Graves Disease; Successful treatment outcome represent patients who achieved euthyroid or hypothyroid state after radioactive iodine therapy

We compared patients with IRIS-GD and HIV-uninfected patients with GD with respect to epidemiological, clinical, and biochemical characteristics. There was a higher proportion of females, lower pretreatment serum TSH, and shorter duration of ATD use prior to RAI treatment among the cohort of patients with IRIS-GD. Other variables such as age, prior use of beta-blockers, TPOAb positivity, family history of GD, and presence of co-morbid conditions were not significantly different between the two groups (Table 5).

**Table 5**: Association between HIV infection and demographic and clinical variables

| Variable | IRIS-GD n (%) | HIV-uninfected GD n (%) | Total N (%) | $\chi^2$ | *p*-value |
|---|---|---|---|---|---|
| **Age, N=253 (years)** | | | | | |
| Mean ± SD | 38.53 ± 8.44 | 40.12 ± 14.01 | | -0.775[t] | 0.439 |
| **Gender, N=253** | | | | | |
| Male | 3(5.9) | 36(17.8) | 39(15.4) | 4.452 | **0.035*** |
| Female | 48(94.1) | 166(82.2) | 214(84.6) | | |
| **Use of beta blockers, N=253** | | | | | |
| Yes | 28(54.9) | 112(55.4) | 140(55.3) | 0.005 | 0.944 |
| No | 23(45.1) | 90(44.6) | 113(44.7) | | |
| **Use of ATD, N=253** | | | | | |
| Yes | 38(74.5) | 159(78.7) | 197(77.9) | 0.417 | 0.518 |
| No | 13(25.5) | 43(21.3) | 56(22.1) | | |
| **TPOAb, N=113** | | | | | |
| Positive | 9(42.9) | 59(64.1) | 68(60.2) | 3.229 | 0.072 |
| Negative | 12(57.1) | 33(35.9) | 45(39.8) | | |
| **Eye signs, N=253** | | | | | |
| Yes | 27(52.9) | 90(44.6) | 117(46.2) | 1.152 | 0.283 |
| No | 24(47.1) | 112(55.4) | 136(53.8) | | |
| **Family history of GD, N=253** | | | | | |
| Yes | 3(5.9) | 22(10.9) | 25(9.9) | 1.147 | 0.284 |
| No | 48(94.1) | 180(89.1) | 228(90.1) | | |
| **Diabetes Mellitus, N=253** | | | | | |
| Yes | 3(5.9) | 13(6.4) | 16(6.3) | 0.021[F] | 1.000 |
| No | 48(94.1) | 189(93.6) | 237(93.7) | | |
| **Hypertension, N=253** | | | | | |
| Yes | 12(23.5) | 66(32.7) | 78(30.8) | 1.596 | 0.206 |
| No | 39(76.5) | 136(67.3) | 175(69.2) | | |
| | **Median(IQR)** | **Median(IQR)** | | **U** | ***p*-value** |
| **Pre-treatment TSH, N=253 (mIU/L)** | | | | | |
| Median (range) | 0.01 (0.01 – 6.86) | 0.01 (0.01 – 10.65) | | 4434.500 | **0.036*** |
| **Pre-treatment fT4, N=253 (pmol/L)** | 37.10(22.43-58.25) | 34.00(20.40-55.30) | | 4784.000 | 0.675 |
| **Pre-treatment TSHRAb, N=253 (U/L)** | 18.80(4.86-29.17) | 16.88(4.90-34.25) | | 532.000 | 0.939 |
| **Duration of ATD use, N=253 (months)** | 4.00(1.00-12.25) | 6.00(3.00-12.00) | | 1356.500 | **0.048*** |
| **Duration of GD symptoms prior to RAIT, N=253 (months)** | 6.50(4.00-19.00) | 8.50(4.00-23.00) | | 3842.500 | 0.403 |

**$\chi^2$**: Chi square test; **t**: Independent Samples T test; **U**: Mann-Whitney U test; *: *p* value <0.05; **HIV**: Human Immunodeficiency Virus; **IRIS-GD**: Immune Reconstitution Inflammatory Syndrome-associated Graves Disease; **ATD**: Anti-Thyroid medication; **TPOAb**: Thyroid Peroxidase Antibody; **TSH**: Thyroid Stimulating Hormone; **fT4**: free Thyroxine; **TSHRAb**: Thyroid Stimulating Hormone Receptor Antibody; **GD**: Graves Disease; **RAIT**: Radioactive Iodine Treatment

To correct for the effect of the differences between the two groups of patients, we matched in a 1:1 ratio all patients with IRIS-GD with a pretreatment serum TSH of ≤0.01 mIU/L with respect to age and gender (n=43) with HIV-uninfected patients with GD. We found no significant difference in the proportion of patients who achieved successful RAI treatment outcome at 3- or 6-month post-treatment. Table 6 shows the details of the matching and comparison between the two groups.

**Table 6**: Age- and gender-matched comparison of patients with IRIS-GD and HIV-uninfected patients with GD whose pre-treatment TSH level was ≤ 0.01 mIU/L

| Variable | IRIS-GD n (%) | HIV-uninfected GD n (%) | Total N (%) | $\chi^2$ | *p*-value |
|---|---|---|---|---|---|
| **Age, N=86** | | | | | |
| Mean ± SD | 38.95 ± 8.49 | 38.84 ± 8.56 | | 0.063[t] | 0.950 |
| **Gender, N=86** | | | | | |
| Male | 2 (4.7) | 2 (4.7) | 4 (4.7) | 0.000[F] | 1.000 |
| Female | 41 (95.3) | 41 (95.3) | 82 (95.3) | | |
| **Successful treatment outcome at 3 months, N=86** | | | | | |
| Yes | 17 (39.5) | 25 (58.1) | 42 (48.8) | 2.978 | 0.084 |
| No | 26 (60.5) | 18 (41.9) | 44 (51.2) | | |
| **Successful treatment outcome at 6 months, N=86** | | | | | |
| Yes | 30 (69.8) | 34 (79.1) | 64 (74.4) | 0.977 | 0.323 |
| No | 13 (30.2) | 9 (20.9) | 22 (25.6) | | |

**$\chi^2$**: Chi square test; **F**: Fisher's exact test; **t**: Independent T test; **IRIS-GD**: Immune Reconstitution Inflammatory Syndrome-associated Graves Disease; **HIV**: Human Immunodeficiency Virus

## Discussion

Graves disease is a known manifestation of IRIS in people living with HIV infection who commenced treatment with effective ART at a very advanced disease stage. GD is one of the autoimmune disorders described to result from the immune dysregulation that characterizes the restoration of the immune function in response to ART [22]. Compare to infectious and neoplastic diseases, autoimmune disorders are less frequently seen during HIV IRIS. In a retrospective analysis of a United Kingdom cohort of HIV-infected people, the estimated prevalence of IRIS-GD was 3% for women and 0.2% for men [18]. There was a disproportional racial distribution of the disease in affected patients in the study, with black Africans having above four-fold higher risk of IRIS-GD than expected [18]. In this study, we reviewed the patients referred to our department for GD treatment with RAI. In a cohort of 253 patients who met the criteria for inclusion in this study, 51 patients (20.2%) met the diagnostic criteria for IRIS-GD as previously defined by Chen and colleagues [18]. This represents the largest cohort of IRIS-GD in people living with HIV infection ever published in the literature. South Africa carries the largest burden of HIV infection globally. One in every five HIV-infected persons receiving ART world-wide resides in South Africa [23]. This large population of HIV-infected persons and the magnitude of our country's ART program means that we may have a larger prevalence of IRIS-GD in our population.

In the cohort of patients with IRIS-GD, HIV diagnosis and commencement of ART occurred at a very advanced disease stage, evident by a low median CD4 cell count of 66 cells/μL. The median interval between HIV diagnosis and the diagnosis of IRIS-GD was 63 months. This time interval for IRIS-GD is comparable what has been previously reported by others in earlier published studies. In the review of IRIS-GD cases published up till 2005 performed by Crum et al., the time interval between HIV diagnosis and IRIS-GD diagnosis ranged from 9 to 48 months [20]. In the most recent study and the largest series of IRIS-GD published to date that included 24 subjects, the median interval median HIV diagnosis and the diagnosis of GD was 87 months [21]. At the time of GD diagnosis, all patients in our cohort had achieved complete suppression of plasma HIV viremia, and CD4 cell count had improved to a median of 729 cells/μL, consistent with findings from published series [20,21]. These findings make IRIS the plausible cause of GD in these patients. Several factors influence the rate of CD4 cell recovery and may affect the time of occurrence of IRIS-GD. These factors include the age of the patients, CD4 cell count at the time of ART commencement, and the effectiveness of the ART regimen. Patients starting ART at a very low CD4 cell count usually take longer to achieve immune recovery [24]. Also, treatment with a less potent ART regimen may delay the attainment of immune recovery. These factors may influence the time of occurrence of IRIS-GD since published data consistently support the occurrence of IRIS-GD at a high CD4 cell count level.

We compared epidemiological, clinical, and biochemical characteristics of patients with IRIS-GD versus HIV-uninfected patients with GD. There was no significant age difference between the two groups, but we found fewer males among patients with IRIS-GD. Patients with IRIS-GD had a significantly shorter ATD use before referral for RAI treatment and, consequently, a lower pretreatment serum TSH level. These two findings suggest that while the proportion of patients with IRIS-GD treated with ATD was not significantly different from the cohort of HIV-uninfected patients with GD, a shorter duration of ATD use resulted in a poorer level of control of thyroid function. Poor thyroid function control (low serum TSH level) increases the rate of thyroid hormone production with a consequent decrease in the residence time of the RAI within the thyroid gland. This decrease in residence time may negatively impact treatment outcome.

In our study, GD responded well to RAI treatment, with 57.7% of all patients achieving a euthyroid or hypothyroid state at 3-month post-treatment and increasing to 76.3% at 6-month post-treatment. IRIS-GD had a poorer response to RAI treatment assessed at 3- and 6-month follow-up. RAI has a protracted duration of action with time to achieving euthyroid or hypothyroid state ranging from 6 to 18 weeks [25]. The poorer response of IRIS-GD to RAI treatment may be related to the prevailing condition within the thyroid at the time of RAI – a relatively poorer thyroid function control. This finding is supported by our results of a significant impact of pretreatment TSH and fT4 on RAI treatment outcome assessed at 3-month. After correcting for differences in TSH level, age, and gender, we found no significant difference in IRIS-GD response to RAI treatment compared with GD in HIV-uninfected patients. The presence of thyroid eye signs had a significant impact on the 6-months treatment outcome but not the 3-months outcome. This finding may suggest a more severe disease resistant to RAI in patients with GD who have associated thyroid ophthalmopathy than patients who did not. A consistently significant factor (at 3- and 6-months post-RAI treatment follow-ups) was the duration of ATD use prior to RAI treatment. A shorter pretreatment interval in a patient with a highly hyperactive gland may mean thyroid function is still poorly controlled at the time of RAI with an attendant decrease in the residence time of RAI given for treatment.

The putative pathophysiologic basis of IRIS-GD includes an increase in memory CD4 cells release from sites of sequestration in inflamed lymphoid tissues; increase thymic production in naïve CD4 cells; an altered cytokine milieu that favors a Th1 and Th17 profile, leading to a pro-inflammatory

immune response and a subsequent increase in the Th2 response that may allow autoantibody formation; and dysregulation of CD4, CD25 and FoxP3 T regulatory cells [26]. The level of similarity, if any, in the pathophysiologic characteristics of IRIS-GD and GD occurring in the HIV-uninfected population is not currently known. RAI treatment continues to play a frontline role in the treatment of hyperthyroidism. The success of the RAI treatment of hyperthyroidism is greatly influenced by the dose of radioactive iodine I-131 administered for therapy. There are two ways to determine the dose of I-131 to administer for treatment, dosimetry method and empirical dosing. Dosimetry method is a more precise method whose utilization is hampered by cost, patient inconvenience, and the requirement for a specialized skill for its application. The empirical method is simple and easy to apply. Using the empirical dosing technique, all patients with specific clinical profiles are treated with the same dose. In our practice, we applied 15mCi for empirical treatment of patients receiving RAI for the first time and who have small- to medium-sized thyroid gland determined on clinical examination. Before now, the impact of HIV infection on RAI treatment outcome is unknown. Our study provided data supporting the empirical treatment of IRIS-GD with a similar dose as used for GD patients without HIV infection.

Our study reports the characteristics of IRIS-GD in the largest cohort of patients so far published. We made a comprehensive comparison of the clinical and biochemical characteristics of IRIS-GD versus GD in the HIV-uninfected population. For the first time, we report the response of IRIS-GD to RAI treatment compared to GD in the HIV-uninfected population. At the same time, our study has some important limitations that must be borne in mind when applying our results in routine clinical practice. We studied patients referred for RAI at a specialized center. Our data may not reflect the community-level patient characteristics. At present, there are no biomarkers that distinctly differentiate IRIS-GD from GD seen in HIV-uninfected patients. Therefore, our definition of IRIS-GD was based on the temporal sequence of events as utilized in previous studies [16,20,21]. Additional limitations of our study relate to its retrospective design and the modest number of patients with IRIS-GD, even if this represents the largest series so far published. Our study did not include patients with HIV and GV who did not fulfil the case definition of IRIS-GD. Inclusion of this group of patients could have further our understanding regarding the characteristics of GD in HIV-infected patients and the impact of HIV infection and its treatment on RAI outcome in this group of patients.

## Conclusions

IRIS-GD occurs after immune reconstitution in HIV-infected patients starting ART at an advanced disease stage. In our cohort, patients with IRIS-GD were more likely to have been treated for a shorter duration and to have poorer control of thyroid function at the time of RAI therapy. This may result in a suboptimal response to RAI. After correcting for possible confounders, the response of IRIS-GD to RAI treatment is similar to GD response among HIV-uninfected patients.

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## Conflicts of Interest

No conflict of interest declared.

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No external funding was received for the conduct of this study.

## Author contributions

Conception and design of the study: IOL, ATO, MMS

Data acquisition: IOL, KMGM, TL

Data analysis: IOL, GOP

Manuscript draft: IOL

Intellectual contribution to manuscript: IOL, ATO, GOP, KMGM, TL, MMS

All authors read and approved the final version of the manuscript.

## Data availability statement

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

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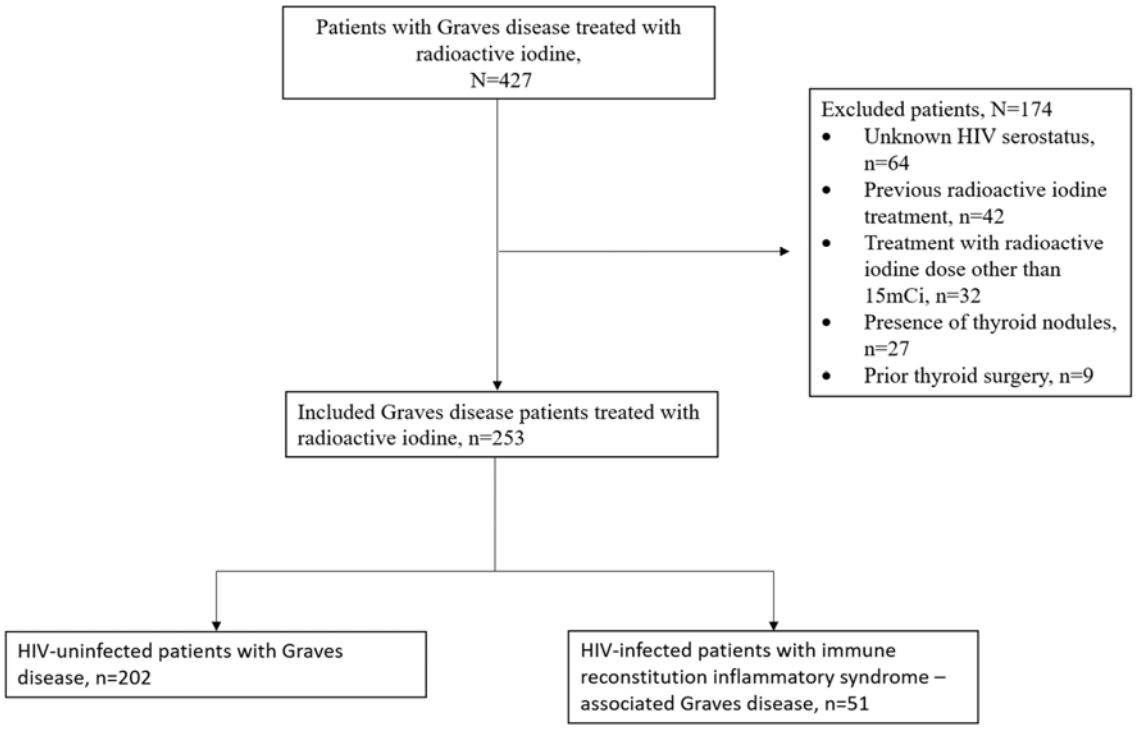


**Figure S1:** Proportion of patients with and without HIV infection with successful treatment outcomes at 3 and 6 months post radioactive iodine treatment. Successful treatment is defined as patients who achieved euthyroid or hypothyroid state after radioactive iodine treatment.