Acute Phase Proteins and Stress Hormone Responses in Patients with Newly-Diagnosed Active Pulmonary Tuberculosis

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Abstract

*Introduction***:** Despite the high burden of disease, there have been surprisingly few studies of the acute phase and plasma catecholamine/cortisol stress hormone responses in patients with active pulmonary tuberculosis. We wished to document acute phase reactant and stress hormone responses in patients with newly-diagnosed, active pulmonary tuberculosis and to compare these responses to those of a group of surgical/medical cases with conditions other than tuberculosis.

Methods: This was a prospective study of consecutive patients with newly-diagnosed pulmonary tuberculosis, admitted to a tertiary hospital in Johannesburg, South Africa, documenting demographic, clinical, routine laboratory, acute phase protein and stress hormone responses relative to those of the control group.

Results: TB patients had a higher body temperature and pulse rate, as well as a platelet counts, ferritin, CRP and dopamine levels, with a tendency to higher cortisol levels compared to the control group. Conversely, they had a lower BMI, haemoglobin, leucocyte count, MCV, and epinephrine levels than the control group.

Conclusions: Patients with active pulmonary tuberculosis were documented to mount an acute stress response which was more intense than that of a control group of patients with surgical/medical conditions other than tuberculosis.

Keywords: Acute phase response, catecholamines, cortisol, stress hormones, tuberculosis

Introduction

It is now over 20 years since the World Health Organisation declared tuberculosis (TB) a global health emergency, and yet it currently continues to remain a major public health threat globally.[1-4] While the epidemiology of TB varies in different regions of the world, it is recognised that the highest rates occur in sub-Saharan Africa.[4] The human immunodeficiency virus (HIV) pandemic, affecting both the developing and developed countries, is one of the reasons for the enormous increase in TB infections worldwide, with the brunt of these co-infections occurring in developing countries, such that HIV/TB co-infection prevalence rates exceed 5% in some African countries.[1,2,5] South Africa is estimated to have the highest incidence rates and the highest prevalence of TB, the second highest number of cases of multi-drug resistant TB and also the highest number of TB-HIV co-infected cases.[6]

In response to an infection, such as TB, the human host mounts an acute phase response, which encompasses a diverse range of systemic effects that accompany inflammation.[7,8] These include, among others, changes in lipid profiles, release of cytokines, activation of complement and the clotting cascade, increases in adrenocorticotrophic hormone (ACTH) and glucocorticoid levels, and changes (increases or decreases) in a number of plasma proteins, collectively referred to as

the acute phase proteins.[7] However, despite such a high burden of TB, there have been surprisingly few studies describing the acute phase and stress hormone responses in the global context in patients with newly-diagnosed, active TB.[9-13]

The aim of the current study was to document the acute phase response, and associated catecholamine/cortisol release, in patients with newly- diagnosed active pulmonary TB. We have compared these responses in the patients with TB to a control group of patients with an acute phase response elicited by conditions other than TB.

Patients and Methods

This was a prospective study of consecutive patients with newly-diagnosed smear-positive TB, treated at the Helen Joseph Hospital in Johannesburg, South Africa. The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (Protocol number M01-11-14) and all patients gave written, informed consent before being enrolled into the study. The inclusion criteria were as follows; adult patients $\geq$ 18 years, history, physical examination, and radiological abnormalities consistent with a diagnosis of pulmonary TB, at least one positive sputum Ziehl Nielsen smear for TB, written informed consent. Exclusion criteria were as follows: previous episode of TB infection with treatment, concomitant immunosuppression other than HIV infection, known HIV infection with other AIDS-defining illness or patients on highly-active antiretroviral therapy, and the presence of any additional comorbid conditions.

For purposes of comparison a control group of patients requiring admission to hospital for mainly surgical conditions was also studied. The rationale for inclusion of this control group, rather than healthy controls, was based on the requirement for comparison with a group of patients with a hyperacute/acute stress response due to conditions other than TB. The group consisted of consecutive patients without TB, who were age, gender, race, and suburb of residence matched (to correct for socioeconomic circumstances) to the group of patients with TB, and who had surgical/medical conditions other than TB requiring their admission to hospital. Exclusion criteria for this group were the same as those described above.

A range of demographic (age, gender, race), clinical [blood pressure, pulse rate, temperature, body mass index (BMI), presence/absence of weight loss], routine laboratory data [haemoglobin, white cell count (WCC), mean corpuscular volume (MCV), platelet count, urea, electrolytes, albumin], were recorded in both groups, as well as plasma C-reactive protein (CRP), ferritin, cortisol and catecholamine stress hormone levels. The normal ranges for the acute phase reactants are <5 micrograms (µg)/mL for CRP, and, in the case of ferritin, 10-150 and 12-300 nanograms (ng)/mL for females and males respectively. Blood specimens for the specialised investigations were drawn from the patients between 08h00 and 09h00 following an 8 hour overnight fast and were processed promptly and as required for the various assays.

Plasma cortisol levels were measured by radioimmunoassay (ADVIA Centaur cortisol assay, Bayer Diagnostic, USA) and the catecholamine levels by a high performance liquid chromatography (HPLC) method (BIO-RAD Laboratories Diagnostics Group, California, USA). The results for cortisol are expressed as nanomoles (nmol/L) and those of the other 3 analytes as picomoles (pmol)/L. The normal ranges for each of these are 119-618 nmol/L (cortisol), 0-366 pmol/L (epinephrine), 560-2636 pmol/L (norepinephrine), and 0-890 pmol/L (dopamine).

With regard to statistical analyses, the control and study groups were compared in relation to clinical and laboratory data using SPSS 7.5 and/or Graphpad Instat software. Continuous variables were analysed using student's unpaired (2-tailed) t test and categorical variables using the Fisher's exact (2-tailed) test. Correlations were determined using the Spearman rank correlation coefficient.

Results

One hundred and sixty patients were enrolled in the study, 71 of whom were cases of newly- diagnosed, active TB and 89 were control patients. The control group of patients had the following diagnoses; assault (n=39), cellulitis or septic injury (n=9), traumatic injury at work (n=8), acute abdomen (n=8), gunshot wounds (n=7), motor vehicle accident (n=3), pedestrian vehicle accident (n=3), suicide attempt (n=3), human bite (n=2), deep vein thrombosis (n=2), and burns, lipoma, priapism, perianal

abscess, and tonsillitis in one case each. In the control group, only one patient was tested for HIV infection and was found to be negative, whereas in the study group, 25 patients were known to be HIV-seropositive and the HIV status was unknown in the remainder of the cases.

The mean ages of the study and control groups were 35.3 ± 9.3 years (range 18-72 years) and 32.3 ± 10.0 years (range 18-69 years) respectively. There were 38 males and 33 females in the study group and 62 males and 27 females in the control group. Overall, the majority of patients in both the study and control groups were black (88.7% and 82%, respectively), and the remainder of the patients were of mixed-race.

The comparative clinical features of the study and control groups are shown in Table 1. Overall, the study patients had a higher body temperature (38.7^{o}C ± 0.7 ^{o}C versus 37.1^{o}C ± 0.4 ^{o}C; *P*<0.01), a higher pulse rate (101.9 ± 15.1 beats/min versus 83 ± 8.9 beats/min; *P* <0.01), and a lower BMI (18.29 ± 3.8 kg/m^2 versus 23.20 ± 5.35 kg/m^2; *P*<0.01) than the control group. Weight loss was noted in a total of 69 patients (97.2%) in the group with TB compared with one (1.1%) in the control group (*P* <0.0001). There was no difference in the median systolic blood pressure between

Table 1: Clinical features of the patients, comparing the study and control groups

Clinical Feature	CONTROL Group	STUDY Group	Significance (*P* value)
Blood Pressure mmHg (range)	100/60-140/90	90/40-140/90	<0.0001*
Pulse (b/min) mean ± SD (range)	83.0 ± 8.9 (62-121)	101.9± 15.1 (62-136)	< 0.01
Temperature (°C mean± SD (range))	37.1± 0.4 (36.5-38.5)	38.7 ± 0.7 (37.0-40.0)	< 0.01
Weight loss (no. (%))	1 (1.1%)	69 (97.2%)	<0.0001
BMI (kg/m^2) mean ± SD (range)	23.20 ± 5.35 (15.57-44.96)	18.29 ± 3.8 (11.72-32.05)	< 0.01

* there was no difference in the median systolic blood pressure between the two groups, but there was a significant difference in median diastolic blood pressure, which was lower in the study group compared with the control group (60 mmHg (range 40-90 mmHg) versus 80 mmHg (range 54-95 mmHg)).

the two groups, but there was a significant difference in median diastolic blood pressure, which was lower in the study group compared with the control group (60 mmHg (range 40-90 mmHg) versus 80 mmHg (range 54-95 mmHg); p<0.0001).

The comparative routine haematology data are shown in Table 2. Overall the study group had a lower haemoglobin (9.6 ± 1.9 g/dL versus 12.9 ± 2.3 g/dL; *P*< 0.01), lower MCV (82.7 ± 7.5 fL versus 87.7 ± 7.9 fL; *P* =0.001), lower white cell count (8.7 ± 5.4 x 10^9/L versus 11.0 ± 4.9 x10^9/L; *P*=0.018), and higher platelet count (369.2 ± 190.8 x 10^9/L versus 295.9 ± 94.6 x10^9/L; *P* = 0.01) than the control group. There was no significant correlation between the haemoglobin and CRP levels.

Table 2: Haematological parameters in the study and control groups

ASSAY	**CONTROL Group**		**STUDY Group**		**Significance** (*P* value)
	Mean ± SD	**(Range)**	**Mean ± SD**	**(Range)**	
Haemoglobin (g/dL)	12.9 ± 2.3 (n = 58)	4.8 – 16.9	9.6 ± 1.9 (n = 56)	4.8 – 13.1	< 0.01
MCV (fL)	87.7 ± 7.9 (n = 54)	60.0 – 100.0	82.7 ± 7.5 (n = 57)	62.7 – 98.0	0.001
White cell count (x10^9/L)	11.0 ± 4.9 (n = 58)	4.8 – 26.7	8.7 ± 5.4 (n = 57)	1.4 – 36.3	0.018
Platelet count (x10^9/L)	295.9 ± 94.6 (n = 58)	62.0 – 609.0	369.2 ± 190.8 (n = 57)	82.0 – 940.0	0.01

The urea, electrolyte and albumin measurements are shown in Table 3. The only differences were that the plasma sodium and albumin concentrations were lower in the study group (132.6 ± 4.8 mmol/L versus 138.8 ± 3.4 mmol/L; *P* <0.01) and (24.9 ± 5.7 g/L versus 37.3 ± 8.8 g/L; *P* =0.001) respectively. In the group of patients with TB, there was also a significant, positive correlation between the plasma sodium and albumin levels (r=.78; p =0.0002). However, there was no significant correlation at all between the plasma sodium levels and any of the stress hormone levels (cortisol,

epinephrine, norepinephrine and dopamine) in the group of patients with tuberculosis.

Table 3: Blood chemistry parameters in the study and control groups

ASSAY	CONTROL Group		STUDY Group		Significance (*P* value)
	Mean ± SD	**(Range)**	**Mean ± SD**	**(Range)**	
Sodium (mmol/L)	138.8 ± 3.4 (n = 55)	132.0 - 148.0	132.6 ± 4.8 (n = 57)	121.0 – 142.0	< 0.01
Potassium (mmol/L)	3.8 ± 0.5 (n = 55)	2.6 – 5.5	3.8 ± 0.7 (n = 57)	2.4 – 6.1	0.728
Urea (mmol/L)	4.7 ± 2.3 (n = 53)	1.8 – 14.4	5.5 ± 6.9 (n = 57)	1.2 - 44.0	0.285
Creatinine (µmol/L)	83.3 ± 17.8 (n = 53)	41.0 – 140.0	96.5 ± 89.1 (n = 57)	39.0 – 552.0	0.292
Serum albumin (g/L)	37.3 ± 8.8 (n = 6)	25.0 - 47.0	24.9 ± 5.7 (n=18)	14.0 – 35.0	0.001

The comparative values for the acute phase reactants and the catecholamine stress hormones are shown in Table 4. The mean CRP and ferritin levels were higher in the study group compared to the control group (125.9 ± 54.8 µg/mL versus 77.2 ± 81.2 µg/mL; *P* <0.01), and (3005.9 ± 5023.3 ng/mL versus 466.5 ± 1774.8 ng/mL; *P* r<0.01) respectively. Eleven cases in the control group had infections other than tuberculosis and in this group of patients the CRP level was 97.0 ± 89.7 µg/ml, which was not significantly different from that of the patients with tuberculosis. Furthermore, the ferritin level (162.2ng/ml) was lower in this group than in both the patients with tuberculosis, as well as the control group as a whole.

Table 4: Acute phase protein and catecholamine stress hormone responses in the study and control groups

ASSAY	CONTROL Group		STUDY Group		Significance (*P* value)
	Mean ± SD	**(Range)**	**Mean ± SD**	**(Range)**	
Catecholamine Stress Hormones					
Cortisol (nmol/L)	392.7 ± 191.3 (n = 88)	6.0 – 1012.0	448.1 ± 197.4 (n = 68)	41.0 – 917.0	0.079
Epinephrine (pmol/L)	680.6 ± 743.8 (n = 64)	68.0 – 3498.0	449.4 ± 380.0 (n = 58)	0.0 – 1954.0	0.035
Norepinephrine (pmol/L)	2179.0 ± 1719.0 (n = 64)	126.0 – 8016.0	2531.6 ± 2043.6 (n = 57)	141.0-9250.	0.305
Dopamine (pmol/L)	293.4 ± 355.8 (n = 64)	40.0 – 1703.0	468.4 ± 377.6 (n = 57)	5.0 – 1439.0	0.01
Acute Phase Proteins					
CRP (mg/L)	77.2 ± 81.2 (n = 89)	0.2 - 346.0	125.9 ± 54.8 (n = 71)	0.2 - 271.0	<0.01
Ferritin (μg/L)	466.5 ± 1774.8 (n=89)	2.7 – 16,600.0	3005.9 ± 5023.3 (n=71)	12.7 – 26,700.0	<0.01

With regard to the stress hormone levels in the current study, cortisol and dopamine levels were in the normal range for both groups, while those of epinephrine were elevated in both groups, and norepinephrine only in the TB group. There was a tendency for the cortisol level to be higher in the study versus the control group, but this did not reach statistical significance (*P*=0.079). Dopamine levels were higher in the study group compared to the control group (468.4 ± 377.6 pmol/L versus 293.4 ± 355.8 pmol/L; *P* =0.01), while epinephrine levels were lower (449.4 ± 380.0 pmol/L versus 680.6 ± 743.8 pmol/L; *P* =0.035).

Discussion

This study has demonstrated a significant number of clinical and laboratory changes in patients with newly-diagnosed, active TB, which are consistent with an effective acute phase response. When comparing patients with newly-diagnosed, active TB with patients with acute injury/illnesses other than TB, those with TB had a higher temperature and pulse rate, and lower BMI compared to the control group (all P <0.01). They also had a lower haemoglobin, WCC and MCV than the control group, but a higher platelet count, as well as ferritin and CRP levels (all P <0.05). Furthermore, the patients with TB had higher dopamine and lower epinephrine levels, with a tendency to higher cortisol levels.

The changes in the temperature and pulse rate in the TB group relative to the control group are almost certainly the result of comparing a group of patients with infection to a group predominantly without infection. There was a significant difference in BMI between the two groups, being significantly lower (P <0.01) in those with TB. This correlates with the history of weight loss observed in almost all the TB patients versus only 1 patient in the control group (P <0.0001). It has previously been noted that weight loss in patients with TB is part of the acute phase response occurring as a consequence, at least partly, of cytokine release, together with release of other infection agent-related products, causing a decrease in appetite.[12]

The haemoglobin level in the patients with TB was also low, and was significantly lower than that of the control group (P< 0.01), and was associated with a lower MCV. The finding of the lower haemoglobin in the TB group is not surprising and has been documented previously in a case control study comparing patients with TB to both those with pneumonia and healthy controls.[12] In a previous study, a significant negative correlation between CRP and haemoglobin levels was noted.[12] However, in the current study no significant correlation between these parameters was detected. Interestingly, the WCC was lower in the TB group than in the control group (P=0.018). The reasons for this are uncertain. In one study comparing patients with TB to those with pneumonia, as well as to healthy controls, the leucocyte counts were found to be significantly higher in the TB patients than controls, but significantly lower than those with pneumonia.[12]

The platelet counts of the TB patients, although within the “normal” range were higher than in the control group (*P* = 0.01). This is consistent with a previous study that also documented higher platelet counts in TB patients, in a similar range to those of the current study patients.[13]

Of the routine urea and electrolyte measurements, only the plasma sodium showed a difference when comparing the TB and study groups. In the TB group the plasma sodium level was significantly decreased (*P*<0.01), while it was normal in the control group. It is recognised that hyponatraemia may occur in patients with TB due to a number of possible causes.[14] These include the syndrome of inappropriate antidiuretic hormone secretion, and tuberculosis involvement of endocrine organs causing adrenal insufficiency or hypopituitarism.[14] However, there was no significant correlation at all between the plasma sodium levels and any of the stress hormone levels (cortisol, epinephrine, norepinephrine and dopamine) in the group of patients with tuberculosis.

Serum albumin is also an important acute phase protein. In the current study, serum albumin was significantly lower in the TB group than in the control group (*P* =0.001). A low albumin has also been documented in a previous study of patients with TB in comparison with both cases of pneumonia and healthy controls, demonstrating an apparent relationship with disease severity.[12] Serum albumin was found to correlate with the degree of hyponatraemia (r=0.48), which resolved by end of treatment in 96% of cases in whom the data was available.[14] In the group of patients with TB in the current study, there was also a significant correlation between the plasma sodium and albumin levels (r=.78; p =0.0002).

CRP is a non-specific marker of systemic inflammation and is a well-characterised acute phase protein that appears to be a good marker of severity of infection.[12] In the current study, CRP levels were raised in both groups, but significantly more so in the TB group (125.9 $\pm$ 54.8 mg/L versus 77.2 $\pm$ 81.2 mg/L; *P* <0.01). Similarly, increased levels of CRP were documented in a study of TB patients compared to healthy controls previously.[13]

Ferritin is also an acute phase protein, the levels of which are raised in both infectious and non-infectious causes.[10,15] One study from South Africa documented 19 patients with ferritin levels > 10,000 ng/mL, with TB appearing to be the most common cause, accounting for 42% of cases.[10] In the current study, the patients in both the TB and the control groups had raised ferritin levels, with those in the TB group being significantly higher than in the control group (P <0.01).

With regard to the stress hormone levels in the current study, there was a tendency for the cortisol level to be higher in the study group versus the control group, but this did not reach statistical significance (P=0.079). Among the catecholamines, differences included higher dopamine (P =0.01) and lower epinephrine (P=0.035) levels in the TB group. A previous study has documented raised epinephrine, norepinephrine, dopamine and cortisol levels in relation to severity of TB infection, with norepinephrine being the most reliable.[9] In addition, a further investigation of the immune-endocrine metabolic alterations in patients with pulmonary TB documented moderate elevations in patients with TB compared to controls, with increasing levels in cases with increasing disease severity.[11]

The study has some potential limitations. For example, there were a higher number of males than females in the control group and this may have skewed some of the laboratory results. Similarly the lower levels of sodium and lower diastolic blood pressure in the group of patients with tuberculosis may have also influenced the stress hormone results; however, there was no correlation at all between plasma sodium levels and any of the stress hormone levels.

In conclusion, the current study has documented a number of clinical and laboratory changes consistent with an intense acute phase response in patients with newly-diagnosed active TB, relative to that of a control group of patients with a stress response due to conditions other than TB.

Conflict of interest: The authors declare that they have no conflict of interest

Acknowledgment: Professor Charles Feldman is supported by the National Research Foundation of South Africa

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