

Original Research

Study the role of serum protein fractions as Early Index of Clinical Recovery in Pulmonary Tuberculosis patients

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ABSTRACT:

Aim: To study the role of serum protein fractions as Early Index of Clinical Recovery in Pulmonary Tuberculosis patients. **Methods:** This cross-sectional study was done the Department of chest and T.B. A total of 80 subjects aged between 19 and 49 years were conveniently recruited for this study. 40 were clinically confirmed PTB subject with no HIV nor malaria co-infections. They were further sub-divided into TB subjects on ATT 20 and drug naive TB subjects 20. The remaining were 40 aged matched apparently healthy controls. **Results:** The results showed that BMI (kg/m^2) in drug naive TB subjects (19.36 ± 2.76) and in the TB subjects on ATT (20.41 ± 2.91) was significantly lower when compared to control subjects (24.69 ± 3.16) ($p < 0.000$). Similarly, waist and hip circumferences (cm) of the drug naive TB subjects (50.23 ± 1.45 , 70.69 ± 2.11) and the TB subjects on ATT (51.15 ± 2.99 , 70.57 ± 2.76) were significantly lower when compared to control subjects (52.35 ± 4.57 , 72.34 ± 3.55) ($p < 0.04$, 0.03). However, the mean value of WHR in drug naive TB subjects (0.73 ± 0.05) and in TB subjects on ATT (0.75 ± 0.06) were not statistically significant when compared to control subjects (0.74 ± 0.07) ($p < 0.27$). Also, the mean weight (kg) of TB subjects on ATT (58.28 ± 4.71) when compared with that of the drug naive TB subjects (51.58 ± 10.61) was not statistically significant ($p < 0.12$). The results showed that the mean serum total protein levels (g/dl) in TB subjects on ATT (8.93 ± 1.75) and in the drug naive TB subjects (9.13 ± 1.88) were significantly higher when compared with the control subjects (7.89 ± 0.98) ($p < 0.003$). Mean serum globulin (g/dl) level in TB subjects on ATT (3.52 ± 1.63) was significantly lower when compared to their counterparts not on ATT (6.34 ± 1.97) ($p < 0.000$). **Conclusion:** We concluded that the BMI was found to be significantly lower in both drug naive PTB subjects and in PTB subjects on ATT when compared with the control subjects.

Key words: Serum protein fractions, Pulmonary Tuberculosis, BMI

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INTRODUCTION

Tuberculosis (TB) is a common and often deadly infectious disease caused by various strains of mycobacterium usually Mycobacterium Tuberculosis in humans.¹ It was originally referred to as Koch's bacillus after Robert Koch who first identified it in 1882 in Berlin, Germany.² Mycobacterium tuberculosis is found in infected human and is transmitted to primates, dogs and other animals that are in close association with man. M. bovis is the causative agent of tuberculosis in cattle and is transmitted to man, primates, pigs and domestic animals.² TB usually attacks the lungs but can affect other parts of the body such as the lymph nodes, kidneys, bones, central nervous system, peritoneum,

eye as well as the skin and spread through air when people who have the disease sneeze or spit.³ When people with active pulmonary TB cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets 0.5 to 5.0 μm in diameter. A single sneeze can release up to 40,000 droplets.⁴ Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very low (the inhalation of fewer than 10 bacteria may cause an infection).⁵ People with prolonged, frequent, or close contact with people with TB are at particularly high risk of becoming infected, with an estimated 22% infection rate.⁶

Report has shown that there are changes in levels of serum proteins fractions in response to both acute and

chronic infections including tuberculosis.⁷⁻¹⁰ Shingdang and colleagues reported that the change shows the overall production and breakdown of proteins due to microbial interactions.⁸ In chronic infectious TB disease, the albumin shows a decrease while globulin content shows an increase leading to low albumin to globulin (A/G) ratio.⁷ This shows that there are significant alterations in proteins fractions in tuberculosis infections.

The present study therefore, is designed to assess the prognostic value of albumin and albumin/globulin ratio as index of treatment recovery in TB patients especially in resource limited setting.

MATERIAL AND METHODS

This cross-sectional study was done the Department of chest and T.B., after taking the approval of the protocol review committee and institutional ethics committee.

A total of 80 subjects aged between 19 and 49 years were conveniently recruited for this study. 40 (20 males and 20 females) were clinically confirmed PTB subject with no HIV nor malaria co-infections. They were diagnosed based on sputum smear microscopy and radiography, (class 3 TB) according to WHO guidelines.¹¹ They were further sub-divided into TB subjects on ATT 20 (8 females and 12 males) and drug naive TB subjects 20(13 females and 7 males). The TB subjects on ATT had been on the DOTS (regimen of Rifampicin (R), Isoniazid (H), Pyrazinamide (Z), and Ethambutol (E) or Streptomycin (S) for eight (8) weeks. The remaining were 40 (20 females and 20 males) aged matched apparently healthy controls.

METHODOLOGY

Anthropometric measurements included weight, which was measured with light clothes and without shoes and approximated to the nearest 0.1kg on a mobile lever scale, while height was measured to the nearest 0.1cm using a stadiometer. BMI was calculated as weight (in kilogrammes) divided by the square of height (in meters). The waist circumference was measured by using a flexible, non-stretch measuring tape to measure the waist circumference midway between the lower rib margin and iliac crest with the tape all-round the body in a horizontal position. The hip circumference was taken as the measurement around the widest portion of the buttocks, with the tape parallel to the floor. Measurements were recorded to the nearest centimetre. The WHR was calculated as waist circumference divided by hip circumference. 5ml of whole blood was collected from each subject using aseptic venipuncture from the cubital fossa into labelled plain test tubes. The samples were allowed to clot, and then centrifuged at 3500 rpm for 5 minutes. The sera was then transferred into properly labelled plain tubes in aliquots and stored at -20°C until assayed for total protein and albumin.

Serum total protein was determined using Biuret method as described by Dale.¹² The Biuret test (Piotrowski's test) is a chemical test used to detect the presence of peptide bonds. In the presence of peptides, a copper (II) ion forms violet coloured coordination complexes in an alkaline solution. Serum Albumin was determined using BromoCresol Green (BCG) method as described by Savoury *et al*¹³ The measurement of serum albumin is based on its quantitative binding to the indicator BCG. Albumin at pH 4.2 is sufficiently cationic to bind the anionic dye bromocresol green (BCG) to form a blue-green coloured complex. The intensity of the blue green colour is directly proportional to the albumin concentration in the specimen when the absorbance is read at 620-630nm Serum globulin concentration was obtained by subtracting values of serum albumin from that of total serum protein. The value for albumin-globulin ratio was obtained by dividing albumin value by globulin value.

STATISTICAL ANALYSIS

The Statistical Package for Social Science, SPSS version 24.0 for windows was used, groups mean \pm SD was calculated for each parameter and significant difference between means evaluated using analysis of variance (ANOVA). T-test was used to assess variation between groups, while $p \leq 0.05$ was considered as statistically significant.

RESULTS

The results showed that BMI (kg/m^2) in drug naive TB subjects (19.36 ± 2.76) and in the TB subjects on ATT (20.41 ± 2.91) was significantly lower when compared to control subjects (24.69 ± 3.16) ($p \frac{1}{4}$ 0.000). Similarly, waist and hip circumferences (cm) of the drug naive TB subjects (50.23 ± 1.45 , 70.69 ± 2.11) and the TB subjects on ATT (51.15 ± 2.99 , 70.57 ± 2.76) were significantly lower when compared to control subjects (52.35 ± 4.57 , 72.34 ± 3.55) ($p \frac{1}{4}$ 0.04, 0.03). However, the mean value of WHR in drug naive TB subjects (0.73 ± 0.05) and in TB subjects on ATT (0.75 ± 0.06) were not statistically significant when compared to control subjects (0.74 ± 0.07) ($p \frac{1}{4}$ 0.27). Also, the mean weight (kg) of TB subjects on ATT (58.28 ± 4.71) when compared with that of the drug naive TB subjects (51.58 ± 10.61) was not statistically significant ($p \frac{1}{4}$ 0.12) (Table 1).

The results showed that the mean serum total protein levels (g/dl) in TB subjects on ATT (8.93 ± 1.75) and in the drug naive TB subjects (9.13 ± 1.88) were significantly higher when compared with the control subjects (7.89 ± 0.98) ($p \frac{1}{4}$ 0.003).

In TB subjects on ATT, the mean (\pm SD) serum albumin (g/ dl) was significantly higher (5.52 ± 1.58) when compared with control subjects (3.84 ± 1.16) ($p \frac{1}{4}$ 0.000). In contrast, the drug naive TB subjects had a significantly lower mean serum albumin ($2.89 \pm$

0.88) when compared with the control (3.84 ± 1.16) ($p \leq 0.000$).

Mean serum globulin (g/dl) level in TB subjects on ATT (3.52 ± 1.63) was significantly lower when compared to their counterparts not on ATT (6.34 ± 1.97) ($p \leq 0.000$). However, both groups of TB subjects had significantly higher mean serum globulin

levels when compared with control subjects (3.96 ± 1.51) ($p \leq 0.000$). Similarly, the mean level of albumin-globulin ratio was significantly higher in TB subjects on ATT (2.44 ± 1.88) but decreased significantly in drug naive TB subjects (0.54 ± 0.33) when compared with control subjects (1.18 ± 0.67) ($p \leq 0.000$ respectively). (Table 2).

Table 1: Anthropometric parameters in TB subjects on ATT, drug naive TB subjects and control subjects

Groups	Height (m)	Weight (kg)	BMI (kg/m ²)	WHR
TB subjects on ATT (A) n = 20	1.78 ± 0.26	58.28 ± 4.73	20.41 ± 2.91	0.75 ± 0.06
Drug naive TB subjects (B) n = 20	1.75 ± 0.25	51.58 ± 10.61	19.36 ± 2.76	0.73 ± 0.05
50 Control (C) n = 20	1.83 ± 0.25	73.44 ± 12.97	24.69 ± 3.16	0.74 ± 0.07
F- value	2.75	23.08	29.67	1.32
P- value	0.08	0.00	0.00	0.27
A vs B	0.35	0.12	0.34	0.41
A vs C	0.66	0.00	0.00	0.98
B vs C	0.07	0.00	0.00	0.28

P < 0.05 Significant. Data was expressed as mean \pm SD.

Table 2 Levels of Total protein, Albumin, Globulin and Albumin-Globulin ratio in TB subjects on ATT, drug naive TB subjects and control subjects.

Groups	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)	AGR
TB subjects on ATT (A) n = 20	8.93 ± 1.75	5.52 ± 1.58	3.52 ± 1.63	2.44 ± 1.88
Drug naive TB subjects (B) n = 20	9.13 ± 1.88	2.89 ± 0.88	6.34 ± 1.97	0.54 ± 0.33
40 Control (C) n = 20	7.89 ± 0.98	3.84 ± 1.16	3.96 ± 1.51	1.08 ± 0.58
F- value	6.03	40.49	24.68	14.43
P- value	0.005	0.00	0.00	0.00
A vs B	0.87	0.00	0.00	0.00
A vs C	0.03	0.00	0.48	0.003
B vs C	0.005	0.001	0.00	0.17

P < 0.05 Significant. Data was expressed as mean \pm SD.

DISCUSSION

In the present study, there was no significant difference between the weights of the TB subjects on ATT and their drug naive counterparts. This finding is in line with earlier reports by PrayGod *et al*¹⁴ and Umo *et al*¹⁵. Previous study has attributed it to differences in the contribution of weight gain in different body compartments despite strong anabolic response.¹⁶ Significant decrease in body weight has been a common finding in patients with active tuberculosis, and this has been attributed to the likely combination of associated tissue inflammations and immune responses. The authors noted that weight gain during anti-tuberculosis therapy is unreliable indicator of overall treatment response.^{14,15} Bekker *et al*¹⁷ had also earlier reported that clinical and functional recovery often lags behind microbiological cure. Even though weight gain is frequently used as a measure of treatment response in tuberculosis,¹⁸ the results of this study also showed significantly lower albumin and albumin globulin ratio in drug naive TB subjects when compared with control subjects. Depleted serum albumin in drug naive TB subjects is suggestive of a chronic infectious process and may be attributed to oedema or malnutrition. The decrease may also be attributed to several factors such as acute and chronic inflammatory responses, nephrotic

syndrome, malnutrition and decreased immunity. This is in consistent with other findings.^{7,8} Poor nutritional status in patients with active pulmonary tuberculosis compared with healthy controls has also been reported elsewhere.¹⁹ Other studies have reported reduced plasma albumin and total protein concentration in chronic TB infection.^{9,10}

The findings from this present study also showed significantly increased albumin and albumin-globulin ratio among the TB subjects on ATT when compared to their drug naive counterparts. This may be due to some level of improvement occasioned by the ATT regimen. This is in concordance with the findings of Egah *et al*²⁰. The author noted that increased albumin-globulin ratio is possibly due to the role of albumin as an antioxidant to prevent cellular damage and tissue wasting in TB individuals. The increases observed in serum albumin of the TB subjects on ATT may also be attributed to increased albumin synthesis by the liver which may be directly or indirectly linked to the effect of the anti-tuberculosis therapy (ATT).

The increase in serum total proteins and albumin observed in pulmonary tuberculosis subjects in this study may be attributed to the anti-tuberculosis drugs Isoniazid and Rifampicin suggesting significant improvement with the treatment regimen. This was in line with previous report,²⁰ and contrary to report by

Damburam *et al*²¹The author reported decreased serum levels of total protein and albumin in tuberculosis. This discordance may be due to sample size and differences in patient distribution in different geographical locations. The variations in the level and pattern of serum proteins in TB subjects have been reported to be due to parasitic infestations, culture and socio-economic status.²²

Increased serum globulin levels in tuberculosis patients were also noted when compared with the apparently healthy controls. This could be attributed to the fact that in infectious diseases such as tuberculosis, serum globulin formation increases significantly as a result of increased immune response where antibodies are produced. Elevated serum globulin in the drug naive TB subjects may be attributed to the host immune response to the TB infection. This was consistent with the findings by Edozien.²³

The elevated albumin-globulin ratio observed in the present study is a direct consequence of depleted serum albumin (hypoalbuminaemia) and elevated globulin. This is in agreement with some previous findings.^{19,21,24} Damburam *et al*²¹ attributed the increased serum globulin levels to the host immunologic response to the tubercle bacilli which elicits the production of gamma globulins. Arinola and Igbi²⁵ also reported high levels of serum IgG and IgM in pulmonary tuberculosis. This observation was in discordance with other reports.²⁰

CONCLUSION

We concluded that the BMI was found to be significantly lower in both drug naive PTB subjects and in PTB subjects on ATT when compared with the control subjects. The depletion of serum albumin and significant reduction in the albumin-globulin ratio observed in the drug naive TB subjects is suggestive of chronic infectious process and may be attributed to oedema or malnutrition and may be due to delayed diagnosis.

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