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Effects of Pulmonary Tuberculosis Treatment for 2 Months, 4 Months and 6 Months on Total Protein, Albumin, Globulin, 1gG & 1gM in Pulmonary Tuberculosis Infected Participants

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Abstract
This present study was designed to determine the effect of pulmonary tuberculosis treatment for 2 months, 4 months and 6 months on total protein, albumin, globulin, IgG and IgM in pulmonary tuberculosis infected participants in Anambra State, Nigeria. A total of 220 pulmonary tuberculosis infected subjects aged 18 – 60 were recruited for the study. After informed consent and ethical approval, blood samples were collected for analysis. Serum proteins were assayed using biuret method. IgG and IgM were assayed using turbidimetric method. Tuberculosis diagnosis was also carried out using Ziehl Neelsen technique. The result showed that the mean ±SD concentration of total protein (g/dl), globulin (g/dl), IgG (mg/dl) and IgM (mg/dl) were significantly higher in tuberculosis infected participants compared (in each case) with the control subjects, 2 months, 4 months and 6 months treatment subjects ($P<0.05$). The mean ±SD albumin concentration was significantly lower in tuberculosis infected participants compared in each case with the control subjects ($P<0.05$). The present study showed increase total protein, globulin, 1gG and 1gM in pulmonary tuberculosis infection. The serum concentration of these parameters returned to normal as patients undergo treatment. Therefore we recommend that total protein, albumin, globulin, 1gG and 1gM should be considered as one of the baseline test for tuberculosis infection.
Keywords: Pulmonary tuberculosis, total protein, albumin, globulin, 1gG, 1gM.

1. Introduction

Pulmonary tuberculosis (PTB) is a common disease in developing countries. Efforts have been made to diagnose this group of patients presenting with complex immunological and biochemical abnormalities (Bradley *et al*, 2003). Pulmonary tuberculosis spread through the air when people who have an active *Mycobacterium tuberculosis* infection cough, sneezes or spit (Bradley *et al*, 2003, Konstantinous 2010). It can also be gotten through eating meat infected with tuberculosis and drinking unpasteurized milk (Kumar *et al*, 2007). Most infection in humans result in an asymptomatic latent infection and about 1:10 latent infections eventually progresses to active diseases especially in immune compromised states like HIV (Kumar *et al*, 2007).

Pulmonary tuberculosis infection is common in Nigeria at an incidence rate of 27 per 100,000 populations. Tuberculosis accounts for 1.8 million deaths and is the world's greatest infectious killer of man and the leading cause of death among people with HIV/AIDS (WHO 2009). The emergence of multiple drug resistant strains (MDR-TB) has also contributed to this new epidemic with from 2000 to 2004, 20% of tuberculosis cases being resistant to standard treatment (WHO 2009).

Few studies have been reported on the effects of pulmonary tuberculosis on serum proteins and immunoglobulins especially in Nigerian literature. Biochemical abnormalities such as hypoalbuminaemia, hyperproteinaemia and hyperglobulinaemia have been reported in patients with pulmonary tuberculosis (Ferrara 2006). It has been shown in previous studies that a high proportion of patients with tuberculosis has significantly increased levels of antibodies to *Mycobacterium tuberculosis* (Al- Omar *et al*, 2009). Increased levels of IgG

with low titers of IgM in patients with pulmonary tuberculosis have been reported (Geisberger *et al*, 2006).
The objectives of this study are therefore to explore avenues to aid in the improved understanding of the disease entity. This will enhance the diagnosis and treatment of PTB infected persons by determining the effects of pulmonary tuberculosis treatment on some biochemical parameters in pulmonary tuberculosis infected participants.

2. Materials and Methods

2.1 subjects

A total of two hundred and twenty pulmonary tuberculosis infected patients aged 18 – 60 years were recruited for the study from the Direct Observation and Treatment (DOTS) centre of NAUTH, Nnewi based on sputum smear acid fast bacilli positive by Ziehl Neelsen stain. One hundred (100) subjects were in their pretreatment stage and 40 subjects each in their 2 months, 4 months and 6 month's post treatment stages respectively. Eight (80) age and sex matched apparently healthy subjects who visits the hospital for medical check- up served as control. Ethical approval was obtained from the ethics committee of Nnamdi Aziikiwe University Teaching Hospital, Nnewi before embarking on the study. Informed consent was also obtained from the subjects.

2.2 methods

The TB positive subjects were identified based on sputum smear acid fast bacilli positive by Ziehl Neelsen stain technique which relies on the principle that *M. tuberculosis* is acid fast and stains red due to mycolic acids (fatty acids) in the cell wall which form a complex with carbol fucsin (an arylmethane dye) and cannot be removed by the acid in the decolorizing reagent (Cheesbrough 2000). A piece of clean stick was used to transfer and spread sputum materials evenly covering an area of about 15-20mm diameter on a slide. The smear was air dried and labeled.

The slide with the smear uppermost was rapidly passed three times through the flame of a Bunsen burner and allowed to cool. The slide containing the smear was placed on a slide rack and the smear covered with carbol fuchsin stain. The stain was heated until vapour just begins to rise. The heated stain was allowed to remain on the slide for 5 minutes. The stain was washed off with clean water and then covered with 3% v/v acid alcohol for 5 minutes or until the smear is sufficiently decolorized, i.e. pale pink. The slide was washed off with clean water. The smear was covered with methylene blue stain for 1-2 minutes and then washed off with clean water. The back of the slide was wiped clean and placed in a draining rack for the smear to air-dry. The smear was examined microscopically using the 100 X oil immersion objective. Scanning of the smear was done systematically and when any definite red bacilli was seen, it was reported as AFB positive. Two mililiters of blood was collected from all the participants for the analysis of the parameters. Serum proteins were assayed using biuret method as described by Gornall et al, (1949). 1gG and 1gM were assayed by turbidimetric method as described by the manufacturer of the kit linear chemicals, Barcelona, Spain which relies on the principle that Anti-human IgG or IgM antibodies form insoluble complexes when mixed with samples containing IgG or IgM. The scattering light of the immuno-complexes depends on the IgG or IgM concentration in the patient sample and can be quantified by comparison from a calibrator of known IgG or IgM concentration. The reagent for the assay was pre-warmed to 37^0C. Distilled water was used to zero the photometer at 540nm. Seven microlitres of the serum was mixed with 1.0ml of the reagent (R_1) provided in the kit in a cuvette. The absorbance was recorded after 2 minutes. This procedure was performed on the different dilutions of the calibrator as described in the kit's manual. The different absorbance value is plotted against the IgG or IgM concentration of each calibrator dilution. IgG or IgM concentration in the sample was calculated by interpolation of its absorbance value in the calibration curve.

2.3 statistical methods

Results generated in this study were tabulated using excel, statistical analysis was done using SPSS package. The variables were expressed in mean and standard deviation. The Student's t-test and ANOVA were used. A p-value of less than 0.05 ($P<0.05$) was considered statistically significant.

3. Results

The in between comparison in the subjects showed that the pretreatment subjects presented significantly higher mean total protein (g/dl), Globulin (g/dl), IgG (g/dl), IgM (g/dl) levels. However, the Albumin (g/dl) mean ±SD values was significantly lower in pretreatment compared with control subjects ($P < 0.05$). In the comparison between the groups; control and 2 months, 4 months, 6 months treatment (in each case), there were no significant difference observed [table 1]. The comparison between 2 months and 4 months, 6 months treatment (in each case) was significantly similar in mean parameters. Also 4 months treatment and 6 months treatment compared showed no significant different in mean parameters. The mean ±SD comparison between variables in the pretreatment and 2 months, 4 months, and 6 months treatment (in each case) showed statistically higher mean

levels at pretreatment in total protein(g/dl), Globulin(g/dl), IgG(g/dl), and IgM(g/dl) than the post treatment stages. However, the pretreatment subjects presented lower significant mean level of Albumin (g/dl) compared with the mean levels in 2 months , 4 months and 6 months (in each case) post treatment ($P<0.05$). The comparison of the parameters between the sex in the control subjects, pretreatment and all the post treatment stages shows no significant difference in all except IgG. IgG showed significantly higher value in female when pretreatment PTB only was compared.

Table 1**:** Mean ± SD of Total Protein , Albumin , globulin , IgG and 1gM compared among control non pulmonary tuberculosis subjects (group A), pretreatment pulmonary tuberculosis patients (group B) and pulmonary tuberculosis patients in their 2 – month (group C), 4-month (group D) and 6-month (group E) treatment.

Groups	**Total Protein (g/dl)**	**Albumin (g/dl)**	**Globulin (g/dl)**	**IgG (g/dl)**	**IgM (g/dl)**
A – Control (n=80)	**7.16 ±0.68**	**3.96 ±0.22**	**3.21 ±0.63**	**989.10 ±241.72**	**134.21 ±48.05**
B – Pretreatment (n=100)	**9.26 ±0.77**	**3.25 ±0.45**	**5.83 ±1.05**	**1841.80 ±265.24**	**260.61 ±203.45**
C – 2-month treatment (n = 40)	**7.70 ±0.41**	**3.95 ±0.21**	**3.73 ±0.44**	**1092.30 ±186.94**	**144.59 ±45.65**
D – 4-month treatment (n = 40)	**7.47 ±0.50**	**4.01 ±0.17**	**3.42 ±0.62**	**1053.10 ±192.86**	**154.94 ±40.89**
E – 6-month treatment (n = 40)	**7.45 ±0.59**	**4.02 ±0.20**	**3.43 ±0.58**	**1099.50 ±226.14**	**152.36 ±47.39**
F(p)value	**142.65 (0.00)**	**94.01 (0.00)**	**168.11 (0.00)**	**192.72 (0.00)**	**15.05 (0.00)**
A vs B p value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
A vs C p value	**0.92**	**1.00**	**0.75**	**0.08**	**0.78**
A vs D p value	**0.05**	**0.67**	**0.45**	**0.52**	**0.11**
A vs E p value	**0.13**	**0.66**	**0.31**	**0.11**	**0.29**
B vs C p value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
B vs D p value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
B vs E p value	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
C vs D p value	**0.17**	**0.55**	**0.09**	**0.89**	**0.82**
C vs E p value	**0.21**	**0.54**	**0.09**	**1.00**	**0.95**
D vs E p value	**1.00**	**1.00**	**1.00**	**0.86**	**1.0**

Key = ($p<0.05$)

4. Discussion

In this present study, increased levels of total protein, globulin, 1gG and 1gM observed in pulmonary tuberculosis participants were corrected after 2 months of treatment with anti-tuberculosis drugs (Isoniazid, Ethambutol and Rifampcin). The present study showed also that reduced level of albumin observed in pretreatment pulmonary tuberculosis patients was corrected after 2 months of treatment with anti tuberculosis drugs. This report however contradicts earlier reports of decreased concentrations of 1gM in pulmonary tuberculosis patients (Grange *et al*, 2007). The present study correlates with earlier report of increased levels of total protein, globulin and decreased levels of albumin in PTB patients (Ferrara 2006, Daniel & Baum 2008). The increase in total protein in pre-treatment PTB patients correlates with the works of Ferrara (2006). The return of total protein level to normal is a good indication of disease control in that they correlate with sputum conversion to acid-fast bacilli negative. The increase in globulin level seen in pretreatment PTB patients correlates with ealier works by Gornall *et al,* (1949). Serum proteins abnormalities are related to factor such as fever with respect to Albumin and Globulin (Ferrara 2006). Immunoglobulin G was significantly higher in pre-treatment PTB patients when compared with control non PTB subjects, 2 months treatment PTB patients, 4 months treatment PTB patients and 6 months treatment PTB patients. This report correlates with the earlier

reports (Bradley *et al*, 2003). Immunoglobulum M was also significantly higher in pre-treatment PTB patients when compared with control non PTB subjects, 2 months treatment PTB patients, 4 months treatment PTB patients and 6 months treatment PTB patients. This report however contradicts the earlier reports of decreased titres of IgM in pre-treatment PTB patients (Grange *et al*, 2007). It has been shown in previous studies that a high proportion of patients with PTB has significantly increased levels of antibody to *Mycobacterium tuberculosis* (Geisberger *et al*, 2006, Alarcon-Sergovia & Fishbein 2003). Increase in IgG and IgM are therefore due to humoral response to mycobacterial antigen (Daniel & Baum 2008). Increase in IgM may probably also be due to secondary superadded bacterial and fungal infections that these patients are so prone to develop.

5. Conclusion

In conclusion, the present study showed increase total protein, globulin, 1gG and 1gM in pulmonary tuberculosis infected patients. The study also indicates that serum concentration of these parameters returned to normal as patients undergo treatment. Based on these findings it is recommended that baseline levels of these parameters are obtained prior to treatment in order to monitor the progress of treatment in pulmonary tuberculosis patients.

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