Journal of Infectious Diseases and Immunity Vol. 4(2), pp. 16-19, February 2012
Available online at http://www.academicjournals.org/JIDI
DOI: 10.5897/JIDI11.055
ISSN 2141-2375 ©2012 Academic Journals

Full Length Research Paper

Serum proteins in health and in patients with pulmonary tuberculosis in Nigeria

Damburam A.[1], Garbati M. A.[2]* and Yusuph H.[3]

[1]Department of Medicine, Federal Medical Centre, Adamawa State, Yola, Nigeria.
[2]Division of Infectious Diseases, Department of Medicine, King Fahad Medical City, Riyadh, 11525, Saudi Arabia.
[3]Department of Medicine, University of Maiduguri, P. M. B. 1069, Maiduguri, Nigeria.

Accepted 10 February, 2012

Tuberculosis (TB) remains an important communicable disease all over the world. We therefore undertook this study to evaluate the relationship between TB and serum protein levels. 210 patients with pulmonary TB (PTB) aged 18 years and above from Lagos metropolis and age- and sex-matched controls were studied. Both cases and controls had serum proteins levels evaluated. Serum total globulin was estimated by subtracting the albumin from the total serum protein. Screening for HIV antibodies was done using ELISA (Welcome Diagnostics, pasteur, Marnes-la-cognetee, France); and confirmed with Western Blot test (Dupont de Nemours, USA). Hypoalbuminaemia was detected in 184 (87.60%) of the cases versus 26 (12.38%) of the controls (P=0.001). Hypergammglobulinaemia was higher among cases compared to controls, 189 (90%) versus 90 (42.86%) (P=0.001). The protein sub-fraction, gammaglobulin was also higher in the cases compared with controls [mean 41.45 ± 11.21 vs. 29.42 ± 6.76% (p=0.005)]. Alpha-1 and alpha-2 globulin sub-fractions were however normal in both study groups. HIV antibody test was positive in 11/153 (7.19%) of those who accepted testing. Patients with PTB had lower serum total proteins and serum albumin but higher plasma gammaglobulin levels than controls.

Keywords: Serum proteins, TB, HIV, Nigeria.

INTRODUCTION

Tuberculosis (TB) remains a major public health problem throughout the world, especially in developing countries. The reasons for the unacceptably high prevalence may be related to the socioeconomic status of the patients, mostly being in depressed economies of the world. Other contributory factors are the high prevalence of malnutrition, poor health education and increasing threat posed by infection with the Human Immunodeficiency Virus (HIV).

Cure rates for pulmonary tuberculosis (PTB) cases of up to 90% can be achieved with the use of the directly observed treatment short course (DOTS) strategy (Rieder, 2002). However, the success of the treatment does not only depend on the use of appropriate anti-tubercular drugs. Other factors like adherence of the patient to treatment, the sensitivity of the mycobacteria to anti-tubercular drugs, and the control of associated diseases also contribute to the success or otherwise of the treatment program. An additional factor that could negatively affect the efficacy of the anti-tubercular treatment is a deficiency in cellular immunity, which in turn can be influenced by nutritional status (Redinger, 2003).

Nutritional deficiencies and excesses influence various components of the immune system. Early studies investigating the association between nutrition and immunity focused on generalized protein-energy malnutrition, particularly in developing countries. The extent of immunological impairment depends not only on the severity of malnutrition but also on the presence of infection, among other factors. Deficiencies of protein and some amino acids, as well as vitamins are associated with reduced immune-competence. Dysproteinemic syndromes are found frequently in clinical practice and the determination of the different protein fractions found

*Corresponding author. E-mail: musagarbati@yahoo.com. Tel: +966-55-203-7801.

in the plasma may be made by either of these methods: neutral salts, electrophoresis, and ultracentrifugation. However, in this study the electrophoretic approach was used.

In the study of the relationship between TB and nutrition, four protein fractions can be isolated from the serum, i.e. albumin, alpha-, beta- and gamma-globulins, serum albumin being one of the common parameters used to evaluate nutritional status. Among these fractions, the gamma-globulin region has attracted much of the clinical interest because of its relationship with immunoglobulins. Tuberculosis being one of the causes of polyclonal gammopathy is also associated with a rise in C - reactive protein (CRP) levels, an acute phase reactant located in the area between the beta and gamma components (Jacoby and Cole, 2000).

This paper provides a brief review of the link between tuberculosis and serum protein levels. Often, TB is viewed merely as a problem of a small and special fragment of the population such as the homeless, and its wide and diffuse connections with nutrition are likely to be overlooked.

MATERIALS AND METHODS

This was a case-control study in 210 consecutive adults aged 18 years and above with newly diagnosed sputum smear positive PTB attending the medical clinics of the Lagos University Teaching Hospital (LUTH); the Infectious Diseases Hospital, Yaba; the General Hospital, Lagos; and the General Hospital, Ikeja between April 1996 and April 1997. Ethical committees from all four enrollment centers (LUTH, GH Lagos, IDH Yaba, and GH Ikeja) gave approval for the conduct of the study and also signed informed consent for participating in the study. The control group was made up of medical students and relations of patients (matched for age and sex).

Whole blood was collected in to plain bottles and centrifuged at 3000 rpm for about 10 minutes and the serum was harvested and stored at 4°C until analyzed. Serum total protein was measured colorimetrically using the Biuret method (Kingsley, 1942; Gornall et al., 1949) while serum albumin was determined by bromocresol green method. For the assay, 0.1 ml of serum was mixed with 2.4 ml of normal saline and 2.5ml of Biuret reagent, and incubated at 37°C for 10 min. The absorbance was measured with SP- 500 spectrophotometer at 540 nm in 1 cm cuvette against a reagent blank. A protein standard (std) was similarly treated. The total protein was expressed in g/L, calculated from the formula:

$$\text{Total protein} = \frac{\text{Absorbance of test} \times \text{Concentration of std. (g/L)}}{\text{Absorbance of std.}}$$

Normal total serum protein by this method in the LUTH laboratory ranged between 52 and 82g/L.

Serum albumin was measured by direct spectrophotometric determination of albumin in human serum by dye binding method, using bromocresol green dye (Doumas et al., 1971; Corcoran and Durna, 1977). After mixing; the solution was left at room temperature for 10 minutes. This absorbance was read at 630nm against a reagent blank, using SP-500 spectrophotometer. Albumin standard was similarly treated. The albumin concentration was expressed in g/L, using the formula:

$$\text{Albumin} = \frac{\text{Absorbance of test} \times \text{Concentration of std. (g/L)}}{\text{Absorbance of std.}}$$

Serum total globulin was estimated by subtracting albumin from the total serum protein. For serum protein electrophoresis, 4µL of serum was deposited gently on agarose gel, using a template as a guide. The gel was placed in an electrophoresis tank, containing barbital buffer at a pH of 8.6. Electrical current (100v) was passed for 25 min. The gel was then removed and subsequently fixed in acid alcohol and dried in an oven. Staining was done using coomasie brilliant blue. Gel was finally passed through a densitometer, with the different bands quantitated.

Screening for HIV-1 and HIV-2 antibodies was done using ELISA (Welcome Diagnostics, pasteur, Marnes-la-cognetee, France). Sera found to be positive by screening were subjected to Western Blot test (Dupont de Nemours, USA) for confirmation.

Epi-Info (6.1) statistical software was used for data entry and analysis; while Chi Square and student's t-tests were used to test for association in contingency tables and for comparison of proportions. P values of ≤ 0.05 were considered significant. The method of Galen and Gambino was used to determine the measures of validity (Galen and Gambino, 1975) that is, sensitivity, specificity and positive and negative predictive values.

RESULTS

The mean ages of cases and controls (Table 1) were comparable as shown (29.33 ± 11.08 vs. 29.55±11.38 years) (p>0.05) respectively. The proportion of people in the low socioeconomic class was much higher in the cases than in the controls. The difference was however not significant (p=0.06).

Although the pre-morbid weights of the study subjects were not known, it was observed that the cases had significantly lower BMIs compared to the control group (18.82 ± 2.75 vs. 22.65± 3.10 Kg/m^2), (p=0.001). Of the 210 cases, only 159 returned to have their Mantoux reaction read. The default rate was 24.28%. All the 42 healthy individuals in the control group for TST tested negative to the Mantoux reagent, with zero percent default rate. Among the 159 cases that came back for review, 13 (8.2%), 2 (1.30%), and 144 (90.50%) were categorized to have 0 to 5, 6 to 9 and ≥10 mm in duration respectively. In contrast, 38 (90.50%) of the control group had 0 to 5mm in duration with only 4 (9.50%) in the >5 mm category. The cases had more significant reactions (X^2 = 117.68) and p<0.001).

The mean total serum protein was significantly lower in the cases than in the controls; 73.25 ± 10.51 vs. 82.09 ± 63.57 g/L (p=0.04), as shown in Table 2 below. Similarly, the mean serum albumin was also significantly lower in the cases compared to the controls; 29.37 ± 8.71 vs. 36.09 ± 6.34g/L (p=0.001). The prevalence of hypoalbuminaemia in the cases was higher compared with controls; 184 (87.60%) vs. 26 (12.38%) (P=0.001). Similarly, the prevalence of hypergammglobulinaemia was significantly higher among cases compared to controls, 189 (90%) vs. 90 (42.86%) (P=0.001).

Table 3 shows the relative percentages of protein sub-fractions in randomly selected sub-populations of cases

Table 1. Socio-demographic characteristics of cases and controls.

Characteristics	Cases	Controls	P value
Age (years) ± SD	29.33±11.08	29.55±11.38	0.84
Sex			
M	129 (61.40%)	126 (60.00%)	0.76
F	81 (38.60%)	84 (40.00%)	0.76
Socioeconomic status			
Low	171 (81.40%)	154 (73.40%)	0.06
High	39 (18.60%)	56 (26.70%)	0.06
Alcohol (Yes)	26 (12.40%)	6 (2.99%)	0.003
Cigarette Smoking (Yes)	23 (10.90%)	5 (2.38%)	0.003

Table 2. Serum protein pattern in the study population.

Protein fraction (g/L)	Cases	Controls	P value
Total Protein (Mean ±SD)	73.25 ± 10.51	82.09 ± 63.57	0.04
Albumin (Mean ±SD)	29.37 ± 9.22	40.18 ± 6.23	0.001
Globulin (Mean ±SD)	45.63 ± 8.71	36.09 ± 6.43	0.001
Hypoalbuminaemia	184 (87.62%)	26 (12.38%)	0.001
Hypergammglobulinaemia	198 (90.00%)	90 (42.86%)	0.001

Hypoalbuminaemia: Albumin < 35 g/L; Hypergammglobulinaemia: Globulin > 35g/L.

as well as the controls. The relative percentage of albumin was much lower in the cases compared with the controls (mean 28.41 ± 10.88 vs. 42.55 ± 5.13%), p =0.001). The relative percentage of gammaglobulin sub-fraction was much higher in the cases than in the controls [mean 41.45 ± 11.21 vs. 29.42 ± 6.76% (p=0.005)]. However, there was no difference in the proportion of other globulin sub-fractions between cases and controls as in Table 3 .

Among the 210 cases, 153 consented to have HIV screening test. Out of these 11 (7.19%) tested positive for HIV antibodies. All controls were negative for HIV antibodies.

DISCUSSION

The study population consisted of adult Nigerians with newly diagnosed PTB (aged 18-78 years) and a control group which comprised of healthy individuals matched for age and sex. The mean ages of both cases and controls were comparable (29.33 ± 11.81 versus 29.55 ± 11.38 years) (p= 0.08). Predominantly those aged 20 to 39 years were mainly affected. This agrees with previous reports from developing countries where about 80% of affected individuals were aged below 50 years (Highman 1970; Ekweani, 1989; Ali-Gombe, 1991; Idigbe et al., 1995) as compared with reports from developed countries where the majority of the infected population were more than 50 years of age. The finding of low serum proteins among those in the low social class agrees with the WHO declaration in 1982 that the prevalence of TB is inversely related to socio-economic conditions (WHO, 1982).

The high false negative rate for the Mantoux reaction of 9.40% among the cases might not be unconnected to the fact that majority of them presented with either severe disease or malnutrition or both. This agrees with previous reports (Nash and Douglas, 1980). Energy rate among patients was 20% and it was recorded among those co-infected with HIV. The occurrence of hypoalbuminemia, and hypergammaglobulinemia, was significantly higher in patients with PTB. These serum protein changes (elevated gammaglobulins with a concomitant decrease in albumin) have been reported to be associated with PTB (Bovornkiti, 1962; Gilliand et al, 1956; Leggart 1957; Volk et al., 1953; Adedapo et al., 2006). The hypoalbuminemia may be due to poor nutritional status of the patients. The hypergammaglobulinemia, on the other hand, is most probably due to immunologic response to the tubercle bacilli.

The alpha-1 and alpha -2 sub-fractions were, however, similar for both cases and controls on the serum electrophoresis. The elevated pattern of gammaglobulin sub-fraction in is agreement with that of earlier workers who found that gammaglobulin, (with or without alpha- 1

Table 3. The Relative sub-fractional composition of serum proteins by electrophoresis in some sample of cases.

Protein Sub-fraction	Cases (Mean ±SD)	Controls (Mean ±SD)	P value
Albumin	28.41 ± 10.88	42.55 ± 5.13	0.013
Alpha-1 globulin	8.03 ± 5.04	11.19 ± 2.84	0.098
Alpha-2 globulin	11.18 ± 3.15	11.46 ± 2.03	0.810
Beta globulin	10.26 ± 6.61	10.54 ± 5.41	0.810
Gamma globulin	43.45 ± 11.21	29.42 ± 6.67	0.005

and alpha -2 sub-fractions), is usually elevated in PTB (Baldwin and Hand, 1953; Bhave et al., 1989). They observed that elevation of Alpha-1 and Alpha-2 globulins was seen in severely ill patients.

In this study, both alpha-1 and -2 globulins were found to be normal. A probable explanation for the apparently normal levels of the alpha-1 and alpha -2 globulins sub-fractions in the study subjects may be the fact that most of the cases were not severely ill.

Conclusion

Dysproteinemic syndromes occur with increasing frequency in patients with PTB, especially among those with severe disease. This study therefore highlighted the complex relationship that exists between poverty and disease. Significant reductions occurred in serum total protein and serum albumin; while increased levels of gamma globulin was observed among the patients. The earlier reports of elevation of alpha globulins was however not observed in these patients, probably because of less sever disease.

REFERENCES

Rieder HL (2002). Interventions for tuberculosis control and elimination . International Union Against Tuberculosis and Lung Diseases. Paris, France, pp. 15-93.

Redinger RN (2003). Nuclear receptors in cholesterol catabolism: molecular biology of the enterohepatic circulation of bile salts and its role in cholesterol homeostasis. J. Lab. Clin. Med., 142: 7-20.

Jacoby RF, Cole CE (2000). Molecular diagnostic methods in cancer genetics. In: Abeloff MD, et al., eds. Clinical oncology. 2d ed. New York: Churchill Livingstone, pp. 119-121.

Kingsley GR (1942). The direct biuret method for the determination of serum proteins as applied to photoelectric na d visual colorimetry. J. Lab. Clin. Med., 27: 840-845.

Gornall AG, Bardawill CJ, David MM (1949). Determination of serum proteins by means of the biuret reagent. J. Clin. Med., 177: 751-766.

Doumas BT, Watson WA, Biggs HC (1971). Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta, 31: 87-96.

Corcoran RM, Durna SM (1977). Albumin determination by a modified bromocresol green method. Clin. Chem., 23: 765-766.

Galen A, Gambino SR (1975). Beyond normality. The predictive value and and efficiency of medical diagnosis. John Wiley and sons New York.

Highman DT (1970). Pulmonary Tuberculosis - Radiographic presentation. Clin. Rad., p. 21.

Ekweani CN (1989). Streptomycin and thiazina in the treatment of tuberculosis in Kaduna. A preliminary report. FNMC Dissertation.

Ali-Gombe A (1991). Factors affecting sputum conversion in patients with active pulmonary tuberculosis using the modified short-course therapy and the conventional short-course therapy. FWACP Dissertation.

Idigbe EO, Sofola TO, John EKO, Okoye R, Onugbogu C, Begg O, Giwa-Amu J (1995). Trend of pulmonary tuberculosis in Lagos, Nigeria 1982-1992. Biomed. Lett., 51: 99-109.

World Health Organization. Tuberculosis Control (1982). Report of the IUATLD/WHO study group. WHO Tech. Ser., 671: 1-26.

Nash DR, Douglas JE (1980). Anergy in active pulmonary tuberculosis. Chest, 77: 32.

Bovornkiti S (1962). Serum protein changes in tuberculosis in humans, with particular reference to alpha-2 globulin. Am. Rev. Res. Dis., 85: 58-65.

Gilliand IC, Johnson RN, Stradling P, Abdel-Wahab EM (1956). Serum proteins in pulmonary tuberculosis. Brit. Med. J., 1: 1460-1464.

Leggart PO (1957). Serum protein changes in pulmonary tuberculosis. Brit. J. Tuberc. Dis. Chest, 51: 130-145.

Volk BW, Saifer A, Johnson LE, Oreskes I (1953). Electrophoretic and chemical serum protein fractions in pulmonary tuberculosis. Am. Rev. Tuberc., 67: 299-321.

Adedapo KS, Arinola OG, Ige OM, Adedapo ADA, Salimonu LS (2006). Combination of Reduced Levels of Serum Albumin and Alpha-2-Macroglobulin Differentiates Newly Diagnosed Pulmonary Tuberculosis Patients from Patients on Chemotherapy. Afr. J. Biomed. Res., 9: 169-172.

Baldwin RW, Hand CN (1953). Electrophoretic studies of the serum proteins in tuberculosis. Am. Tuberc. 68: 372-381.

Bhave GG, Pathare AV, Dagha CC, Chabria LL, Dalvi SG (1989). Immunoprofile of pulmonary tuberculosis-comparison with normal healthy controls. J. Postgrad. Med., 35: 24-29.