TUBERCULIN SKIN TEST AND SERUM ALBUMIN AMONG SPUTUM SMEAR POSITIVE PULMONARY TUBERCULOSIS PATIENTS IN MAIDUGURI, NIGERIA

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ABSTRACT

Background: Delayed-type hypersensitivity reaction, the hallmark of the Mantoux response is markedly depressed in the setting of malnutrition. **Objectives:** To determine the relationship between the intensity of Mantoux responses and serum albumin in patients with sputum smear-positive non-HIV associated pulmonary tuberculosis. **Methods:** This was an observational cross-sectional study of consecutive newly diagnosed sputum smear positive Pulmonary Tuberculosis (PTB) patients that were HIV-negative. Five tuberculin units of Purified Protein Derivative (PPD) was injected intradermally and response determined after 72 hours. Serum albumin was measured using Colorimetric Bromocresol green method. **Results:** Seventy three patients, 44 (60.3%) males and 29 (39.7%) females with a mean age of 31.2 ± 11.8 years were enrolled. The mean Mantoux response and serum albumin were 12.5 ± 7.3 mm and 3.5 ± 0.5 g/dl, respectively. Thirty three (45.3%) patients had serum albumin level < 3.5 g/dl and forty (54.8%) patients had serum albumin level of ≥ 3.5 g/dl. Eight (10.96%) patients with serum albumin < 3.5 g/dl had Mantoux response of 0 mm, whereas 1 (1.37%) had Mantoux of 8 mm. Twenty four with serum albumin of <3.5g/dl had Mantoux of ≥ 10 mm. Of those with serum albumin ≥ 3.5 g/dl, 7 (9.59%) had Mantoux response of 0 mm and 33 (45.21%) had response of ≥ 10 mm. There were no significant difference between Mantoux responses of 0 mm and ≥ 10 mm between patients with serum albumin of < 3.5 g/dl (p = 0.68) and those of ≥ 3.5 g/dl (p = 0.33). **Conclusion:** The study has shown that the magnitude of Mantoux response is independent of the levels of serum albumin among patients studied.

KEYWORDS : Serum albumin, Mantoux test, Sputum smear-positive PTB and HIV negative.

INTRODUCTION

Tuberculosis is caused by an acid fast and alcohol fast bacterium called *Mycobacterium tuberculosis* (MTB) and transmission occurs almost exclusively from human to human.[1] About a third of the world's population is infected with MTB. [1] Among communicable diseases, tuberculosis (TB) is the second leading cause of death worldwide after HIV/AIDS, killing nearly two million people each year. [1] Cell- mediated immunity (CMI) and Delayed-type hypersensitivity (DTH) play key role in pathogenesis of TB. [2] Delayed-type hypersensitivity is immunologically the same process as CMI, involving T cells and cytokines. CD4 T helper (Th) 1 cells differentiate from naïve Th-cells by Interleukin (IL)-12 and IL-18 produced from macrophages, play a regulatory role in the expression of DTH. [3] The sequence of cellular events in DTH can be exemplified by Tuberculin reaction. [4] Tuberculin reaction is induced by injecting an antigen of MTB intradermally. If the host has been previously exposed to the bacterium, swelling and induration will result. [5] The commonly used test employs the Mantoux technique, [6] wherein a small amount of

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tuberculin, a Purified Protein Derivative (PPD), is introduced into intradermal tissues using a small-gauge needle. The amount of induration is measured in mm after 48-72 hours. [7]

In the setting of malnutrition, DTH responses are markedly depressed. [8] The significance of these effects depends on previous nutritional status of the individual, and the nature and duration of infection. [9] Malnutrition is observed frequently in patients with PTB [10] and it has a profound effect on cellular immune function. It is an important risk factor for TB, given that CMI is the key host defence against TB. [11]

The aim of the study was to determine the relationship between the intensity of Mantoux responses and serum albumin in HIV-negative sputum smear positive PTB patients in Maiduguri, Borno State, Nigeria.

MATERIALS AND METHODS

This was a cross-sectional study in which 73 consecutive newly diagnosed HIV-negative, sputum smear positive PTB patients were enrolled. The study was conducted between April and August, 2007 at the University of Maiduguri Teaching Hospital, State Specialist Hospital Maiduguri, Chest Hospital and Chest Clinic, all located in Maiduguri, Borno State, Nigeria. Inclusion was based on PTB status as determined by Ziehl-Neelsen's (ZN) staining of sputum smear and a negative result on screening for HIV infection. Newly diagnosed sputum smear positive HIV negative adult patients between the ages of 18 and 75 years and consenting patients were studied.

Exclusion criteria included refusal of consent, HIV-positive patients, diabetics, patients diagnosed with connective tissue diseases, concurrent treatment with corticosteroids and cytotoxic drugs or use of anti-tuberculosis drugs for more than two weeks, past treatment for TB, recent Mantoux test within the preceding week, underlying malignancies, nephrotic syndrome, acute and chronic renal failure, chronic liver disease and diarrhoea. Patients with history of chronic alcohol ingestion, recent surgical therapy, haemorrhages and pregnant women were also excluded.

All patients gave informed written consent. Review and authorisation of all aspects of the study was done by the Ethical Committee of University of Maiduguri Teaching Hospital and permissions were obtained from the Ethical Committees of the other study centres.

Detailed history was obtained from each subject using a study questionnaire. The height and weight of the study subjects were recorded and the Body Mass Index (BMI) calculated. A questionnaire was administered to each subject giving details regarding demographic data. The Mantoux test technique was used for the TST. Tuberculin PPD (Mammalian, batch No 4240-06) containing Tween 80 (0.005%) and Phenol ($\leq$ 0.25%) was used. The reagent was refrigerated at an optimal temperature of 2-8 ^{0}C and reconstituted such that 0.1ml contained 5TU of PPD prior to use. Five tuberculin units (5TU) equivalent to 0.1ml of PPD [7] was injected intra-dermally on the volar aspect of the left forearm four finger breadths from the antecubital fossa using a 26G disposable tuberculin syringe and needle. A mark was applied to encircle the area of injection and patients were instructed not to tamper with it. Reading was done after 72 hours using plastic meter ruler employing the "ball point pen" technique of Sokal [12]. The Mantoux responses were categorised into: 0 mm that is no response (Anergy), 1-5 mm , 6-9 mm and $\geq$ 10 mm responses.

Colorimetric Bromocresol Green Method (BRCG-M) [13] was used for serum albumin estimation. Bromocresol Green kit (Randox Laboratory limited, United Kingdom) with reference and lot number AB362 and 050454, respectively stored at an optimal temperature of 15 ^{0}C was used. Three millilitres of blood was drawn from the Right ante-cubital fossa

vein of each subject with a 5ml syringe and needle after cleansing the site with methylated spirit. The Blood sample was placed in a labelled plain bottle and analysed using BRCG-M in the Chemical pathology laboratory of the University of Maiduguri Teaching Hospital (UMTH). Serum samples that were not immediately used were frozen and stored at -20 ^{0}C. The data obtained were analysed using SPSS version 13.0 (SPSS, Ill., Chicago, USA). Patients were categorised into two: those with serum albumin levels < 3.5 g/dl and those with serum albumin levels of ≥ 3.5 g/dl and their corresponding Mantoux responses in mm were compared. Tables and figure were used for illustration as appropriate. Values were expressed as Mean ± Standard deviation (SD). Comparison of means was done with the Student's t-test and p value of < 0.05 was considered significant.

RESULTS

Clinical and demographic details of the subjects are illustrated in table 1. The mean age of the subjects was 31.2 ± 11.8 years. Mean ages of the males and the females did not differ significantly, whilst their mean BMI was 17.8 ± 2.7 kg/m^2. The mean serum albumin (± SD) was 3.5 ± 0.5 g/dl. The mean serum albumin (± SD) of males was 3.4 ± 0.6 g/dl and that of females was 3.6 ± 0.5 g/dl. There was no significant difference between the mean serum albumin of males and females (p = 0.14). The mean Mantoux response (± SD) was 12.5 ± 7.3 mm. Fifteen (20.5%) patients had Mantoux response of 0-5 mm, 1 (1.4%) patient had Mantoux response of 8 mm and 57 (78.1%) patients had Mantoux response of ≥10 mm. Anergy occurred independent of the serum levels of albumin.

The relationship between serum albumin and Mantoux responses is illustrated in figure 1. Eight (10.96%) patients with serum albumin < 3.5 g/dl, had Mantoux response of 0 mm, 1 (1.37%), 8 mm while 24 (32.99%) had response of ≥10 mm. Of those that had serum albumin ≥3.5 g/dl, 7 (9.59%) had Mantoux response of 0 mm and 33 (45.21%) had response of ≥10 mm.

Table 1: Clinical and demographic characteristics of patients studied (n=73)

Parameter	Males (n=44)	Females (n=29)	P value
Age (yrs)	30.1 ± 9.8	32.9 ± 14.1	0.31
BMI (kg/m^2)	17.7 ± 2.5	17.9 ±2.9	0.75
Mean serum albumin (g/dl)	3.4 ±0.6	3.6 ± 0.5	0.14
Levels of serum albumin (g/dl)			
< 3.5	22	11	
≥3.5	22	18	
Mean Mantoux response (mm)	12.7 ± 6.9	12.3 ± 8.0	0.82
Magnitude of Mantoux responses (mm)			
0	8	7	
1-5	0	0	
6-9	1	0	
≥10	35	22	

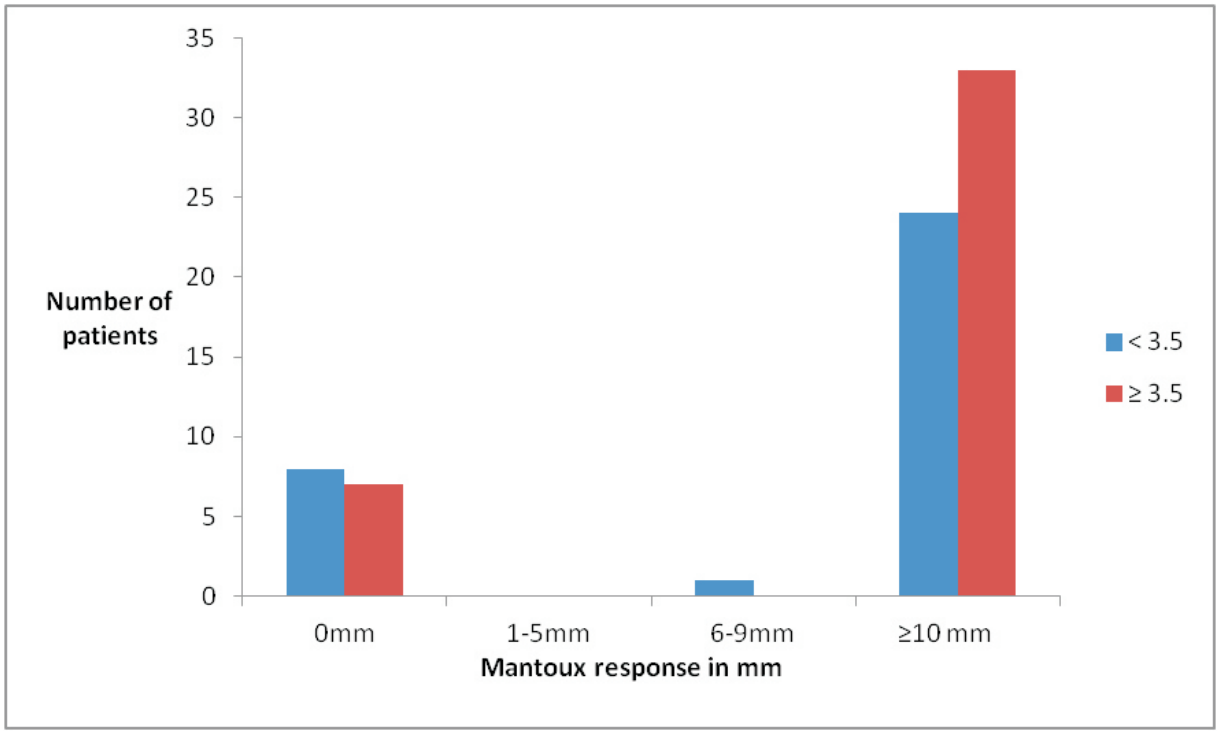

Figure 1: Magnitude of Mantoux response stratified based on levels of serum albumin

DISCUSSION

The age distribution of the patients is similar to that reported by Wokoma [14] in Nigerian PTB patients in south-south and that reported at Ile-Ife by Erhabor et al [15] in south-western region of Nigeria. The involvement of relatively younger age may among others, be due to exposure to predisposing risk factors for PTB such as sustained contact with people having PTB early in life as reported in other studies in sub-Saharan Africa [16].

The mean serum albumin of 3.5 ± 0.5 g/dl and mean BMI of 17.8 ± 2.7 kg/m2 in this study were similar to that reported by Kuppamuthu et al [17] of 3.6± 0.7g/dl and 18.5 ± 0.6 kg/m2, respectively. It has been known that serum albumin level may be low in PTB patients and the possible causes are nutritional factors, enteropathy and acute phase reactant proteins [18]. The hepatic synthesis of acute phase reactant proteins induced by cytokines such as interleukins and tumour necrosis factor-α inhibit production of serum albumin [19]. The low BMI may be due to poor dietary intake, anorexia, impaired absorption of nutrients or increased catabolism. [20]

The mean Mantoux response of 12.5 ± 7.3 mm in our study is similar to that reported by Odelowo. 21 The Mantoux responses of 0mm and ≥ 10mm of patients with serum albumin level of < 3.5 g/dl were not significantly different as compared to those patients with serum albumin level of ≥ 3.5 g/dl. This finding is different to that reported by Yoneda [22] where he showed that patients with lower serum albumin had lower Mantoux response compared to those with normal serum albumin level.

Fifteen (20.5%) of the studied patients had skin anergy, occurring to the same degree across all levels of serum albumin. A plausible explanation for this is that anergy could occur in persons infected with PTB due to compartmentalization [23]. The cavitary lesions may collect most of the antigen-specific circulating T-cells, so that few are available to participate in the dermal tuberculin reaction.

This concept is further proven by the fact that lymphocytes from diseased tissues obtained through bronchoalveolar lavage or from pleural exudates contain a greater proportion of antigen-specific T-cells. These antigen-specific T-cells secrete greater quantities of lymphokines and show a greater tendency to proliferate in the presence of specific antigens than do the T-lymphocytes in the peripheral blood [23, 24, 25]. Tuberculin-negative patients with active TB also have a greater number of suppressor monocytes and lymphocytes in their peripheral blood [24, 26]. However, this does not prove that T-cells within tuberculous lesions are suppressed. In the peripheral blood of such patients, mononuclear cells produce transforming growth factor-beta (TGF-β) and IL-10, which are, at least in part, responsible for the immunosuppressive effects on tuberculin sensitivity as are reactive mitogen intermediates, free fatty acids and phospholipids [27].

In conclusion, our study has shown that the magnitude of Mantoux response is independent on the levels of serum albumin among patients studied. ▬

REFERENCES

1. Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C. Tuberculosis. Lancet 2003; 362: 997-899
2. Dannenberg AM Jr. Delayed-type hypersensitivity and cell-mediated immunity in the pathogenesis of tuberculosis. Immunol. Today 1991; 12: 228-233
3. Kazuo k, Kenji K, Tsuyash K. Immunopathogenesis of Delayed-type hypersensitivity. Microsc. Res. Tech. 2001; 53: 241-245
4. Kaufmann SHE. Immunity to intracellular microbial pathogens. Immunol. Today 1995; 16: 338-343
5. Black CA. Delayed type hypersensitivity: current theories with a Historic perspective. Dermatology Online Journal 1999; 5(1): 7-8
6. Bernstein IL, Storm WW. Diagnostic tests of cell mediated immune reactions. Principal parameters for allergy diagnostic testing. Ann Allergy 1995; 75: 543-625
7. Paul AJ, Laurent AL, Michael FT, Renee R. Guidelines for preventing the transmission of mycobacterium tuberculosis in the Health-care setting. Morbidity and Mortality weekly Report 2005; 54(17): 1-141.
8. Chandra RK. Nutrition and the immune system: an introduction. Am J Clin Nutr 1997; 66(2): 460s-463s
9. Nevin SS, John PS. Synergism of nutrition, infection and immunity: an overview. Am J clin Nutr 1997; 66: 464s-477s
10. Elvina K, Werner S, Ronald H, Nelwan H, Rainer G, Zulkifli A. Poor micronutrient status of active pulmonary tuberculosis patients in Indonesia. J. Nutr 2000; 130: 2953-2958
11. Cegielski JP, McMurray DN. The relationship between malnutrition and tuberculosis: evidence from studies in human and experimental animals. Intern J Tuberc Lung Dis 2004; 8(3): 286-298
12. Sokal JC. Measurement of delayed skin responses. N Engl J Med 1975; 293: 501-502
13. Brackeen GL, Dover Js, Long Cl. Serum albumin. Differences in assay specificity. Nutr Clin Practice 1989; 4: 203-205
14. Wokoma FS. Human Immuno-Deficiency Virus (HIV) status of adult Nigerian patients suffering from pulmonary tuberculosis. Nigerian Med.Pract. 1997; 34:22-24
15. Erhabor GE, Aghanwa HS, Yusuph M, Adebayo RA, Arogundade FA, Omidiora A. Factors influencing compliance in patients with tuberculosis on directly observed

therapy at Ile-Ife, Nigeria. East Afri Med J 2000; 75: 325-329
16. Lienhardt C, Fielding K, Sillah J, et al. Risk factors for tuberculosis infection in sub-Saharan Africa: a contact study in the Gambia. Am J respire Crit Care Med 2003; 15; 168 (4): 448-455
17. Kuppamuthu R, Rajaiah S, Karuppusamy K et al. Serum Zinc and Albumin levels in Pulmonary Tuberculosis patients with and without HIV. Japan J Infect Dis 2008; 61: 202-204
18. Karyadi E, Schultink W, Nelwan RHH et al. Poor micronutrient status of active pulmonary tuberculosis patients in Indonesia. J Nutri 2000; 130: 2953-2958
19. Xing Z, Gauldie J, Cox G et al. IL-6 is an anti-inflammatory cytokine required for controlling local and systemic inflammatory responses. J Clin Invest 1998; 101: 311-320
20. Hopewell PC. Overview of clinical tuberculosis. In Bloom B.R (1st edition). Tuberculosis, pathogenesis, protection and control. Text book. 1stedition. Washington DC, USA. ASM Press 1994: Pp 25-36
21. Odelowo EO. Mantoux test tuberculin reaction in Nigerians. Afr J Med Med Sci 1994; 23(1): 91-98
22. Yoneda T. Relationship between malnutrition and cell-mediated immunity in pulmonary tuberculosis. Kekkaku 1989; 64(10): 633-640
23. Barnes PF, Fong Sj, Brennan PJ et al. Local production of tumour necrosis factor and IFN-gamma in tuberculous pleuritis. J Immunol 1990; 145: 149-154
24. Tsuyuguchi I. Regulation of human immune response in tuberculosis. Infect Agents Dis 1996; 5: 82-97
25. Rohrbach MS, Williams DE. T-lymphocytes and pleural tuberculosis. Chest 1986; 89: 473-474
26. Hirsch CS, Hussain R, Toosi Z, Dawood G, Shahid F, Ellner JJ. Cross-modulation by transforming growth factor beta in human tuberculosis: suppression of antigen-driven blastogenesis and interferon gamma production. Proc Natl Acad Sci USA 1996; 93: 3193-3198
27. Hirsch CS, Toossi Z, Othieno C, et al. Depressed T-cell interferon gamma responses in pulmonary tuberculosis: analysis of underlying mechanism and modulation with therapy. J Infect Dis 1999; 180: 2069-2073

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