

# Combination of Reduced Levels of Serum Albumin and Alpha-2-Macroglobulin Differentiates Newly Diagnosed Pulmonary Tuberculosis Patients from Patients on Chemotherapy

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**ABSTRACT:** Pulmonary tuberculosis (PTB) is global disease affecting about one third of the world's population with its attendant mortality and morbidity. Acute phase proteins have been used in monitoring the progression of infections but not in relation to PTB in this environment. The levels of total protein, albumin,  $\alpha$ -2-macroglobulin, transferrin, and haptoglobulin were determined in 23 patients with PTB and 17- age / sex matched PTB-free controls using spectrophotometric and immunodiffusion methods respectively. The result showed that  $\alpha$ -2-macroglobulin was significantly raised in PTB patients compared with controls ( $p < 0.001$ ), while the levels of transferrin and albumin were significantly reduced in PTB patients compared with the controls ( $p < 0.001, 0.000$  respectively). The levels of  $\alpha$ -2-macroglobulin and albumin were significantly raised in PTB patients on treatment compared with newly diagnosed PTB patients ( $p = 0.05, p = 0.01$  respectively). The combination of reduced levels of albumin and  $\alpha$ -2-macroglobulin may be used to differentiate newly diagnosed PTB and those on chemotherapy

**Keywords:** Tuberculosis, chemotherapy, acute phase proteins, albumin, Nigeria.

## INTRODUCTION

Pulmonary tuberculosis (PTB) is communicable disease of global importance. It is caused by *Mycobacterium tuberculosis*, a fastidious slow growing bacterium. In PTB, the primary infection is usually sub-pleural often confined to mid- to upper-zones. The infection grows when the organism replicates and spreads from the primary focus to secondary sites such as the mediastinum and the other lymph nodes. The disease can also assume a systemic spread that is also referred to as miliary. Today, one-third of the world's population is infected with tubercle bacilli, nine million of these develop the disease and almost 2 million die out of this curable

disease (WHO, 2001). Inflammatory response that is usually determined by acute phase reactants among other parameters was reported in tuberculosis (Grange et al., 1984).

Acute phase reaction is a collective designation for changes in serum protein profile and cellular immune response encompassing symptoms like fever, tiredness and general malaise induced by infection, inflammation or trauma. The group of acute phase proteins includes C-reactive protein (CRP), haptoglobin, alpha-1-acid glycoprotein (AAGP), caeruloplasmin, fibrinogen and alpha-1- antitrypsin (AAT) (Grange et al., 1984).

Immunity in tuberculosis is complex involving T lymphocytes (Mackness G.B., 1971), macrophages, neutrophils (Appleberg and Silva., 1989) and antibodies (Chaparas D.S., 1982). Several studies (Grange et al., 1984, Immanuel et al., 1990, Wong and Saha., 1999, Wong and Saha., 1989) have been carried

out on acute phase proteins in pulmonary tuberculosis with varying results. Grange et al (1984) found that certain acute phase proteins: AAT,  $\alpha$ -2-macroglobulin, AAGP, CRP, caeruloplasmin and haptoglobin were significantly increased in tuberculosis except transferrin. Others found that CRP, caeruloplasmin, haptoglobin, and alpha-1-acid glycoprotein were reduced in response to treatment in PTB patients (Onadeko and Sofowora, 1978, Ige et al., 2000). This study determined the levels of  $\alpha$ -2-macroglobulin (A2MG), transferrin (TRF), and haptoglobin (HPT)), total protein (TP) and albumin (ALB) in newly diagnosed PTB patients and those on chemotherapy. This study aimed at providing additional indices (index) to monitor the effectiveness of chemotherapy in PTB.

## MATERIALS AND METHODS

**Participants:** Twenty-five pulmonary tuberculosis patients (7 newly diagnosed and 18 on PTB treatment) were selected based on positivity for acid-fast bacilli using Ziehl – Nielson staining method (Crime I.M., 1987) and radiological findings (Fahey and Mackelvey., 1965). The patients ( $36 \pm 19$  yrs of age) were recruited from the both chest clinic of the University College Hospital and Chest Clinic Jericho, Ibadan. Informed consents were obtained from all the subjects before the commencement of the study. The controls (mean age  $35.0 \pm 10.1$  yrs) were 17- sex and age matched apparently healthy subjects. They included members of staff and students of University College Hospital, Ibadan, Nigeria who had been screened to be free from PTB.

**Determination of serum concentrations of acute phase proteins:** Five (5) ml of whole blood was collected from each subject by venepuncture into a clean plain sample bottle and allowed to clot. The serum was separated after the clot had retracted at room temperature. The acute phase protein levels were measured by single radial immunodiffusion technique of Fahey and Mckelvey (12). A volume of monospecific antiserum was mixed with noble agar and poured on glass plate. Wells of equal diameter were cut in the antibody agar mixture and filled with test or standard serum. The plates for the different acute phase proteins (alpha-2 macroglobulin, transferrin, and haptoglobin) were incubated at room temperature for 18 hours. After incubations, the diameters of the precipitin rings were measured using a Hyland precision viewer with a micrometer eyepiece.

**Determination of serum concentrations of total protein and albumin:** Serum total proteins were assayed colorimetrically using Biuret reagent while serum albumin were determined by bromocresol green method.

**Statistical Methods:** - The data generated in this study was analyzed for mean, standard deviation and Students t – test.

## RESULTS

In Table 1, transferrin and albumin were significantly reduced while alpha-2 macroglobulin was significantly higher in PTB patients compared with the control. Alpha-2 macroglobulin and albumin were significantly lower in newly diagnosed PTB compared with those on chemotherapy (Table 2).

**Table 1:** Levels of acute phase proteins, total protein and albumin in PTB patients compared with controls.

N	A2MG	TRF	HPT	TP(g/L)	Alb(g/L)
Controls 17	128.8 $\pm 41.4$	270.4 $\pm 22.8$	71 $\pm 11$	8.38 $\pm 1.1$	4.99 $\pm 0.5$
TB patients 23	215.8 $\pm 76.6$	220.7 $\pm 53.1$	84 $\pm 46$	7.89 $\pm 1.0$	3.6 $\pm 0.5$
t, p	4.24, 0.001	4.00, 0.001	1.5, 0.31	1.33, 0.66	8.18, 0.000

KEY:  $p < 0.05$  is significant

**Table 2:** The levels of acute phase protein in newly diagnosed PTB patients compared with PTB patients on drug treatment.

Parameter	Newly Diagnosed (n = 7)	On treatment (n =18)	t- values	p- values
A2MG	233.9 $\pm 70.4$	182.10 $\pm 42.5$	2.02	0.05
TRF	218.18 $\pm 54.7$	244.6 $\pm 61.1$	1.02	0.32
HPT	75.5 $\pm 9$	79.8 $\pm 11$	0.83	0.60
Age(years)	34.7 $\pm 12.8$	35.4 $\pm 13.9$	0.13	0.89
Weight(Kg)	48.3 $\pm 9.4$	51.3 $\pm 7.8$	0.90	0.62
Alb(g/L)	3.6 $\pm 0.7$	4.3 $\pm 0.6$	2.33	0.01
TP(g/L)	8.0 $\pm 4.9$	8.4 $\pm 5.0$	0.18	0.96

KEY:  $p < 0.05$  is significant.

## DISCUSSION

This work compares the levels of A2MG, transferrin, haptoglobin, albumin and total protein in Nigerians with PTB and controls. PTB is a chronic inflammatory disease that elicits T cell reaction, with the

development of cellular immunity that can be demonstrated 3-8 weeks after the initial infection (Begin et al., 1974). Cellular immune responses have been extensively studied in PTB and levels of immunoglobulin classes were also determined in Nigerians with PTB (Appleberg and Silva., 1989., Arinola and Igbi., 1998). The present study assessed the levels of acute phase reactants in Nigerian PTB patients.

In this study, the levels of A2MG and HPT were significantly raised in PTB subjects compared with controls. This could be accounted for by the acute haemodynamic changes that occur in response to tissue damage in patients with PTB. This acute phase reaction in PTB patients may also explain decreases in the concentrations of many plasma proteins like albumin and transferrin, since both albumin and transferrin are negative acute phase reactants. Similar inflammatory response in PTB patients was previously confirmed by increase in the concentration of CRP (Gabay and Kushner., 1999). Three important nutritional indices (transferrin, total protein and albumin) are reduced in PTB patients considered for this study., thus raising the possibility of *Mycobacterium tuberculosis*-induced malnutrition.

Immanuel et al., (1990) found a decrease in concentration of transferrin with severity of PTB. In our study, the levels of transferrin, haptoglobin, total protein, and albumin were found to be reduced in newly diagnosed PTB patients compared with those on treatments. This is suggestive of reduced synthesis of certain proteins by liver in PTB patients with increase in severity, also supporting induction of malnutrition and loss of weight by *Mycobacterium tuberculosis* infection.

This study observed that only A2MG showed a significant increase in PTB patients on treatment compared with the newly diagnosed patients. A2MG binds endopeptidases such as trypsin and chymotrypsin; resulting in reduced tissue damage and encouragement of tissue repair. This observation could therefore account for the increased A2MG in PTB patients on treatment.

Based on the fact that both albumin and A2MG were reduced in newly diagnosed PTB patients compared PTB patients on chemotherapy; both albumin and A2MG may therefore be used as an indices of differentiating these categories of PTB. This could be in addition to fibrinogen, ESR and leucocytes count previously suggested by Hansson et al., (1995) to differentiate mild, moderate and severe PTB.

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