



Antimycobacterial Drug Concentration In Serum Protein Levels Of Pulmonary Tuberculosis Patients

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ABSTRACT

Undesirable drug interactions may lead to altered serum concentration of the drugs and therefore reduced effectiveness of the drug. Effective monitoring of the serum concentration of antituberculosis drugs in patients can be an indicator for expressing potential clinical outcome. This study was therefore aimed at examining whether serum protein level monitoring could be used for effective determination of treatment response in TB patients. In this study, the effect of antituberculosis drugs on the serum proteins of pulmonary tuberculosis patients attending Evangelical Reformed Church of Christ, Medical Center, Alushi was investigated between May and September, 2018. Total protein was determined Spectrophotometrically by Biuret method while albumin was determined by bromocresol green method. Seventy-three (73) patients were enrolled for the study; thirty-five (35) were males while thirty-eight (38) were females. The levels of serum total proteins were higher in test groups (81.07 ± 12.27) than the control group (77.66 ± 4.75). Similarly, the level of serum albumin was higher in the control group (43.98 ± 0.4) when compared to the test group (33.67 ± 4.24). For serum globulin, the outcome was higher in the test group (33.67 ± 4.54). The statistical analysis of the result showed that the level of serum albumin between the test and control group was significant different ($p < 0.05$) while total serum proteins and globulin were not significantly different ($p > 0.05$) between the test group and control. Serum concentration of antituberculosis drugs are known to vary considerably between individuals therefore monitoring their serum levels in standard treatment settings will promote clinical outcomes.

Keywords: antituberculosis drugs, serum protein, serum albumin, serum globulin

INTRODUCTION

Blood protein also termed plasma proteins or serum proteins, are proteins in the plasma. They serve many different functions, including transport of lipids, hormones, vitamins and minerals in the circulatory system and the regulation of acellular activity and functioning of the immune system (Anderson and Anderson, 2007; Adkins *et al.*, 2010). Serum total protein measures the total amount of protein in the serum. Protein in plasma is made up of albumin and globulins. Serum albumin accounts for 55% of blood proteins and a major contributor in maintaining the osmotic pressure of plasma to assist in the transport of lipids and steroid hormones, while globulin makes up 38% of blood proteins (Adkins *et al.*, 2010).

The traditional method for measuring total protein uses the biuret reagent but other methods such as Kjeldahl, dye-binding and refractory now exist (Migliori *et al.*, 2009).

Serum albumin (blood albumin) is a type of globular protein found in vertebrate blood. Human serum albumin is encoded by the ALB gene. It is produced by the liver, occurs dissolved in blood plasma and is the most abundant blood protein in mammals. It is essential for maintaining the osmotic pressure needed for proper distribution of body fluids between blood vessels and body tissue, without albumin, the high pressure in the blood vessel would move fluids out into the tissues. Too much or little circulating serum albumin may be harmful (Um *et al.*, 2007).

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Globulin is made up of different types of proteins called alpha, beta and gamma. Some globulins are made by the liver, while others are made by the immune system. Some globulins bind with haemoglobin while others transport metals such as iron in the blood and help fight infections. Globulin is tested to determine the chances of developing an infection and to check for blood diseases such as multiple myeloma or macroglobulinemia (Somoskou *et al.*, 2001).

Tuberculosis (TB) is a serious, persistent worldwide health problem due to its high risk of person to person transmission, morbidity and mortality (Pasipanodya, 2013). Therapeutic regimens of antituberculosis drugs are extremely effective although most TB patients respond well to standard treatments, some show recurrence, acquired drug resistance or adverse effects. Studies have shown that undesirable drug interactions among the anti-TB drugs or between the antituberculosis drugs and other drugs used by the patients can occur, as can adverse reaction to varying degrees of severity (Blumberg *et al.*, 2003). Drug interaction can alter the serum concentrations of the drugs involved, thereby reducing their effectiveness (Pai, 2006).

Most challenging treatment outcomes of the antituberculosis drugs are related to various factors, among which are the dose and time of the day at which the medication is administered, patients' age, nutritional status, presence of preexisting disease and dysfunctions such as alcoholism, impaired liver and kidney functions and HIV co-infection (Brasil *et al.*, 2002).

Serum concentrations of anti-tb drugs are known to vary considerably between individuals (Pasipanodya *et al.*, 2012; Tostman *et al.*, 2013). Low serum concentration of anti-tb drugs may therefore be associated with poor treatment outcome (Pasipanodya, 2013; Alsultan and Peloquin, 2014). It is important therefore to examine whether effective monitoring of the serum concentration of anti-tb drugs in patients can be an indicator for expressing potential clinical outcome. This study was therefore aimed at examining whether serum protein level monitoring could be used for effective determination of treatment response in TB patients.

METHODOLOGY

Study Location

The study was carried out at Evangelical Reformed Church of Christ, Medical Center, Alushi located at latitude 8°54N and longitude 8°34E of Akwanga, Nigeria (Akwa *et al.*, 2007).

Sample population and size

The sample size was determined based on WHO/World Bank, (2001) sample size formula. A total of 73 subjects made of 53% males and 30% females aged between 18-70 years on antituberculosis treatment were recruited. Ethical approval was obtained from the management of the Hospital while consent was granted by patients to carry out the study.

Sample Collection

5mls of venous blood was collected from the subjects using disposable syringe and dispensed into plain labeled tubes. The specimens were centrifuged at 300rpm for 6 minutes. The serum was separated into aliquots in sterile eppendorf tubes by using sterile Pasteur pipettes. The entire procedure was carried out under aseptic conditions. The separated serum was stored at -4°C until ready for use.

Methods

Total serum protein was determined spectrophotometrically using Biuret (Doumas *et al.*, 1981). Here copper ion reacts with alkaline solution with protein peptides bonds to give a purple coloured biuret complex, where the amount formed is directly proportional to the amount of total of protein in the specimen.

Total serum albumin was determined by Bromocresol green method (Doumas *et al.*, 1981). The measurement of serum albumin is based on quantitative binding to the indicator 3,3',5,5'-tetrabromocresol sulphonephthalein (bromocresol green BCG) the albumin BCG-complex absorbs maximally the 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.

The total serum globulin was determined by subtracting albumin values from total protein.

Statistical Analyses

Data obtained from the test results were subjected to statistical analysis using Regression and Correlation Analysis with the help of Statistical Package for Social Sciences (SPSS).

RESULTS

The study population consisted of adult tuberculosis patients who were on anti-tuberculosis drugs and a control group which comprised of healthy individuals. A total of 73 individuals, made up of 63 patients on TB treatment and 10 apparently healthy subjects who served as control. 48% of the sampled population was males while 52% were female patients respectively.

The effect of anti-tuberculosis drugs on level of serum protein in the patients and control is as shown in Table 1. The result showed total albumin was significantly ($p < 0.05$) higher in the control group (43.78 ± 0.40) compared to the test (38.20 ± 4.24). Although the test group had numerically higher level of total serum protein (81.07 ± 12.27) and globulins (42.87 ± 14.51) compared to the control (77.66 ± 4.75 ; 33.67 ± 4.54), they were not significantly different ($p > 0.05$).

Table 1: Effect of anti-tuberculosis drugs on levels of serum proteins in pulmonary tuberculosis patients and control

Group	Control	Test
Total protein	77.66 ± 4.75^a	81.07 ± 12.27^a
Albumin	43.98 ± 0.40^a	38.20 ± 4.24^b
Globulin	33.67 ± 4.54^a	42.87 ± 14.51^a

Mean \pm standard deviation

Values with the same superscripts across a row shows not significantly different ($p > 0.05$), while different superscript across a row shows significantly different ($p < 0.05$).

The effect of anti-tuberculosis drugs on level of serum protein in the patients and control based on gender is as presented in Table 2. The effect of anti-tuberculosis drugs on the mean level of total protein of TB patients was high in females (92.63 ± 13.27) than male (74.03 ± 10.70). While in the control groups it was high in the male (78.67 ± 5.51) than female (74.27 ± 0.46). The effect on gender was statistically insignificant ($p > 0.05$). Also the effect on the level of albumin in TB patients was high in males (39.20 ± 2.52) than females (35.70 ± 7.59) this was same for the control group. The effect was statistically significant in male ($p < 0.05$) but statistically insignificant in females ($p > 0.05$). The level of

serum globulin of TB patients was high in female test group (54.00 ± 17.80 and 34.83 ± 8.21) than in males. The effect on control group was high in males (34.67 ± 5.51) than in females (30.40 ± 0.69). The effect on both male and female patients was statistically insignificant ($p > 0.05$).

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Anti-tuberculosis drug on the levels of serum total proteins in pulmonary tuberculosis patients according to age groups is reported in Table 3. The total protein in the test patients was higher in the age group 8-29 years (79.37 ± 2.37) than the control groups (78.00 ± 6.08), $p = 0.7352$, while the test age group ≥ 45 years (75.90 ± 8.95) had lower serum protein than the control (80.60 ± 6.15 , $p = 0.4290$). In the age group $> 30-44$ years, the control had more total serum protein (75.27 ± 0.64) than the test group (70.70 ± 7.62). For the serum albumin, the control group had higher albumin content (43.97 ± 0.06 , 43.83 ± 0.21 , 42.83 ± 2.57) across the age groups when compared to the test groups (39.13 ± 2.63 , 36.60 ± 2.17 , 39.40 ± 1.49). The level of albumin in the age group 18-29 years (0.0335) and 30-44 years (0.0045) was statistically significant while the group > 45 years (0.8939) was statistically insignificant. The effect of anti-tb drugs on levels of serum globulin in patients based on age showed considerably high levels of serum globulin in the test group 18-29 years (40.23 ± 2.29) followed by ≥ 45 years

(35.70±7.47) and lastly age group >30-44 years (34.10 ± 7.64). When compared to the control, the age group ≥45 years (36.47 ± 5.61) had higher level of serum globulin, followed by the

age group 18-29 years (30.00 ± 6.08) then age group 30-44 years (31.40 ± 0.53). The level of serum globulin for all the age groups were statistically insignificant ($P>0.05$)

Table 2: Effect of anti-tuberculosis drugs on levels of serum proteins in patients based on gender

Treatment	Concentration of Serum Proteins					
	Total Proteins		Albumin		Globulin	
	Male	Female	Male	Female	Male	Female
Test	74.03±10.70 ^a	92.63±13.27 ^a	39.20±2.52 ^a	35.70±7.59 ^a	34.83±8.21 ^a	54.00±17.80 ^a
Control	78.67±5.51 ^a	74.27±0.46 ^a	43.97±0.06 ^b	43.87±0.2 ^a	34.67±5.5 ^a	30.40±0.69 ^a

Mean ± standard deviation

Values with the same superscripts within a column shows not significantly different ($p>0.05$), while different superscript within a column shows significantly different ($p<0.05$)

Table 3: Effect of anti-tuberculosis drugs on levels of serum total proteins in patients based on the ages

Age Range (years)	Concentration of Serum Proteins					
	Total Proteins		Albumin		Globulin	
	Test	Control	Test	Control	Test	Control
18-29 (n=12)	79.37±2.37 ^a	78.00±6.08 ^a	39.13±2.63 ^a	43.97±0.06 ^b	40.23±2.29 ^a	34.00±6.08 ^a
30-44 (n=32)	70.70±7.62 ^a	75.27±0.64 ^a	36.60±2.17 ^a	43.83±0.2 ^b	34.10±7.64 ^a	31.40±0.53 ^a
≥45 (n=19)	75.10±8.9 ^a	80.60±6.15 ^a	39.40±1.49 ^a	42.83±2.57 ^a	37.70±7.47 ^a	36.47±5.61 ^a

Mean ± standard deviation

n = number of subjects,

Values with the same superscripts across a row shows not significantly different ($p>0.05$), while different superscript across a row shows significantly different ($p<0.05$)

DISCUSSION

In many diseases conditions, the serum proteins are altered. The general direction is a decrease in albumin and an increase in globulin concentrations respectively. These changes are familiarly recognized as alteration in the albumin/globulin ratio, and the standards usually accepted are those derived from chemical precipitation techniques. This study therefore highlighted the complex relationship that exists between treatment outcome and

disease state from the serum protein perspective.

While most of the observed changes are not in themselves diagnostic, they are indicative of the severity or other wise of the clinical state. Thus, study of these changes is more likely to yield information of clinical progress than to be of diagnostic help. Among the changes which occur on the serum protein of the patients suffering from pulmonary tuberculosis as observed in this study is the lowering of

albumin level in the Tb. patients. Attention has earlier been drawn to this fact by Um *et al.*, (2007). This implies that our finding agrees with Um *et al.*, (2007)

Low level of serum albumin as recorded in this study has possible treatment implication. Serum albumin is said to account for 55% of the blood proteins and serves as a major contributor in maintaining the osmotic pressure of plasma and also assist in the transport of lipid and steroid hormones. Since albumin is essential for maintaining the osmotic pressure needed for proper distribution of the body fluids between blood vessels and body tissue, its absence or near absence in TB infected individuals may result in the movement of fluids out into the tissues and may consequently, result in poor treatment response.

From our results it is evident that albumin in healthy population is higher, as compared to tuberculosis patients while globulin is lower when compared to tuberculosis patients. Our result showed a decrease in albumin concentration, an increase in the levels of total protein and globulin in pulmonary tuberculosis patients. This agrees with the findings of other researchers (Tappero *et al.*, 2005; Um *et al.*, 2007; Holland *et al.*, 2009). High level of total globulin in TB patients as observed in our study is of beneficial effect to the infected patients. Globulins play vital role in the system. Globulin boosts individual immunity. Some bind with hemoglobin while others transport metals such as iron in the blood and help to fight infection.

Although there have been several reports that showed low anti-TB drug levels in patients with Tuberculosis, (Tappero *et al.*, 2005; Um *et al.*, 2007; Srivastava *et al.*, 2010). Few of such studies have examined whether low drug concentrations affect patients response to TB treatment (Weiner, 2005; Heysell *et al.*, 2010; Srivastava, 2011). In this study we could not also establish any correlation between treatment outcome and drug concentration in serum level, as most challenging treatment outcomes of antituberculosis drugs are associated with various factors as; dose, time of drug administration, patients' age, nutritional status, dysfunctions such as impaired liver and kidney function and HIV co-infection (Basil *et al.*, 2002) However, this line of studies is

necessary in order to continuously monitor treatment progress in TB patients

Conclusion

This study highlighted the complex relationship that exists between biochemical parameters and disease state. Serum concentration of anti-mycobacterium drugs are known to vary considerably between individuals, therefore monitoring serum levels in standard treatment settings will promote clinical outcomes. Subsequent studies should therefore focus attention on the relationship of individual genetic variability, drug metabolism and serum protein level in patients undergoing Medicare.

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Conflict of interest

The authors declared no conflict of interest.

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