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EVALUATION OF LIPIDS AND PROTEIN PROFILES IN TUBERCULOSIS (TB) PATIENTS ON ANTI-TUBERCULOSIS THERAPY IN GENERAL HOSPITAL UMUGUMA, OWERRI.

OFOR, I. B.*[1], OBEAGU, E. I.[2], OCHEI K.C.[3] AND ODO ,M.[4]

[1]Department of Medical Laboratory Science. Imo State University, Owerri, Nigeria
[2.]Diagnostic Laboratory Unit,Department of University Health Services,Michael Okpara University of Agriculture,Umudike,Abia State,Nigeria.
[3.] Department of Health system strengthening & Laboratory, FHI 360 Country Office, Area 3 Garki Abuja.
[4.] Department of Prevention Care & Treatment, FHI 360 Country Office, Area 3 Garki Abuja.

*Corresponding Author: talk2igri@yahoo.com

Abstract

A total of 38 subjects were used to study the levels of serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), total protein, albumin and globulin in pulmonary TB patient on antituberculosis therapy. The subjects comprised of 8 patients recently diagnosed of pulmonary TB, 10 patients who have been on anti-TB therapy for 3 months, 10 patients who have been on anti-TB therapy for 6 months, and 10 healthy subjects who served as control. Plasma samples were analyzed using enzymatic colorimetric methods. Student t-test was applied to compare the values obtained using statistical analysis software (SPSS version 17 for windows). Regarding the whole studied groups, TC and albumin were found to be significantly lower in patients with new cases of pulmonary TB (128.50±19.27mg/dl and 3.49±0.23g/dl respectively) than in control. TC and albumin values significantly increased with regular intake of anti-TB therapy to 153.10±13.99mg/dl and 3.76±0.11g/dl respectively after 3 months. The values further increased to 157.70±23.11mg/dl and 4.21±0.31g/dl respectively after 6 months of anti-TB therapy. Globulin level was found to be significantly higher in new TB patients than in any other group. According to the results, the changes observed in levels of the parameters assayed were not sex dependent. Hypocholesterolemia and hypoalbuminemia were found to be of diagnostic importance in pulmonary TB infection. The findings of the study could be used to assess the severity of the disease and progress of treatment.

Keywords: Lipids, Protein, Tuberculosis.

Introduction

Tuberculosis (TB) is a highly prevalent chronic infectious disease caused by *Mycobacterium tuberculosis*, an aerobic intracellular binding bacterium (Oliva *et al.*, *2008*). *Mycobacterium tuberculosis* is a bacillus, a member of the *Mycobacterium tuberculosis* complex. After inhaling the bacillus, transmitted by tiny droplets of saliva, the infected individual may develop the disease depending on his immunological state (Amin, 2006). After taking up resistance in the lung, *M. tuberculosis* can disseminate to any part of the body (Lima-Filho, 1993).

Globally, *M. tuberculosis* infection remains at epidemic levels (Raviglione *et al., 1995*). One third of the world's population is infected and approximately 3 million people die annually from pulmonary tuberculosis, overtaking the number of deaths due to acquired immune deficiency syndrome (AIDS), Malaria, Diarrhea, Leprosy, and other tropical diseases combined (Sundre *et al.,* 1992).

Despite the grave statistics, TB is a treatable disease. Response rate to effective therapy are excellent when patients are compliant with their medications. An effective control has been achieved by the use of antimicrobial agents such as Isoniazid,Rivampicin, Streptomycin, Ethambutol,Pyrizinamide,Ethionamide, and Aminosalicylic acid. Treatment usually involves combination of four of these antibiotic drugs, given for at

least 6 months, sometimes for as long as 12 months. Firstly combined agents with the greatest level of efficacy and with an acceptable degree of toxicity include: Isoniazid, Ethambutol, Rivampicin, Streptomycin and Pyrizinamide (Hardman *et al.,* 2001). However many *Mycobacterium tuberculosis* strains are resistant to one or more of the standard TB drugs, which complicates treatment greatly (National Institute of Allergy and Infectious Diseases, 2009). The success of the treatment depends on the use of appropriate antituberculous drugs, the adherence of the patient to treatment, the sensitivity of the mycobacteria to drugs, and the control of associated diseases (Friedman and Selwyn, 1994). With the currently available drugs, about 90% of TB cases can be cured (Rieder, 2002).

Tuberculosis is one of the oldest diseases afflicting the human race since ancient times (Mohamed and Hesham, 2012). Guzman *et al.,* (2002) and Perez-Guzman (2008), found that most patients with pulmonary TB had low total serum cholesterol levels, and that values of about 90mg/dL were strongly associated with mortality in those patients with miliary disease.

Serum proteins perform many functions in the body. Due to their various kinds of functions, metabolism and site of origin, serum albumin and globulin are subjected to different influences and hence their concentrations vary, which is independent of one another. Alternations have been described in hepatic, renal, acute, and chronic infectious diseases like tuberculosis (Aziz *et al.,* 1988).
Cholesterol constitutes up to 30% of the total lipid content in the cell membrane, and participates in the fluidity of this structure (Delvin, 1992). Consequently cholesterol is involved in the activity of membrane-bound enzymes and membrane functions such as phagocytosis and cell growth. Thomas *et al.,* (2011) and Drabowsky *et al*., (1980), demonstrated that cholesterol content in the cell membrane of human lymphocytes is important for their cytotoxic function. Gatfield and Pieters (2000), observed a clear derangement of the ability of the macrophage to phagocytize mycobacteria when they were depleted of cholesterol. It is well established that changes in levels of serum protein occur in response to both acute and chronic infections. In air infection like Mycobacterium tuberculosis, it is to be expected that changes in plasma protein levels will occur in patients (Zia and Warke, 2012). However, the change in level of each protein at any particular time should reflect the net effects of both the rate of synthesis and rate of catabolism as a result of host-microbe interactions (Chong and Nilmani, 1989). These clinical observations are of considerable interest in the present study.

This present study is aimed at determining, the levels of serum total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins, total protein, albumin, and globulin in TB patients who have been on anti-tuberculosis therapy for 3 months and compare it with TB patients not on drugs; changes in serum total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins, total protein, albumin, and globulin in TB patients who have been on anti-tuberculosis therapy for 6 months and compare it with TB patients not on drugs; and to compare the levels of serum total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins, total protein, albumin, and globulin in male and female, treated and untreated TB patients.

Materials and Methods

Study Area

The study was conducted at the General Hospital Umuguma, Owerri. General Hospital Umuguma, Owerri, is one of the government approved centres for TB treatment in Imo state.
Owerri is the capital of Imo state, South-East Nigeria. Owerri consists of 3 local government areas; Owerri West, Owerri North, and Owerri Municipal, which cover an area of approximately 40 square miles (100km^2). It provides home for a population of 127,213 people of mainly Ibo ethnic group and non-indigenes, made up of 62,990 males and 64,223 females (N.P.C, 2006). Owerri lies within latitudes 5^{o}25' and 5^{o}29' and longitudes 6^{o}59' and 7^{o}3'E.

Ethics, Advocacy And Pre-Survey Contact

With a letter of introduction from The Head, Department of Medical Laboratory Science, Imo State University (Appendix i), the Head of Clinical Services and chairman Ethical Committee General Hospital, Umuguma, Owerri was contacted. On request, the research proposal (Appendix ii) was submitted and approval obtained (Appendix iii). Informed consent was sought from patients, after which the date for sample collection was fixed.

Study Population

A total of 76 subjects were used for the study. These include; 16 patients (10 males and 6 females) who had new cases of pulmonary TB, who had not started anti-TB therapy, 20 TB patients (12 males and 8 females) who had been on anti-TB therapy for 3 months, 20 TB patients (14 males and 6 females) who had been on anti-TB therapy for 6 months, and 20 (10 males and 10 females) healthy volunteers as control. All the subjects were in the age group of 15-60 years.

Patient Selection Criteria

(A) Inclusion Criteria

(i) Pulmonary TB patients of either sex in the age group of 15-60 years.

(ii) Patients with new cases of pulmonary TB confirmed by detection of acid-fast bacilli (AFB) by Ziehl Nelson staining technique.

(B) Exclusion Criteria

(i) Patients suffering from active liver disease.
(ii) Patients suffering from other associated diseases.
(iii) Pregnant and lactating female patients.
(iv) HIV-positive TB patients.
(v) Patients who did not indicate interest in the study.

(C) Selection of Control

(i) Apparently healthy volunteers of both sexes in the age group of 15-60 years.

Sample Collection

With a sterile syringe, 5ml of blood was collected from each subject from the antecubital vein, using the standard venepuncture technique between 9am and 12pm. The blood sample was dispensed into lithium-heparin anticoagulant tube and mixed. The sample was centrifuged at 3000rpm for 5 minutes to separate the plasma. The plasma obtained was stored at -20^{o}C prior to use for estimation of lipid and protein profiles.

Laboratory Procedures

All reagents were commercially purchased and the manufacturer's SOP was strictly
Followed:

(A) Lipid Estimation

Cholesterol Estimation

Cholesterol reagent manufactured by Agappe Diagnostics, Switzerland, LOT number 51204002 was used.

Principle As Modified By Agappe Laboratories

The enzymatic colorimetric determination of total cholesterol is based on the following reaction:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol ester} + O_2 \xrightarrow{\text{Cholesterol esterase}} \text{4-Cholesten-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 4H_2O$$

Normal range: 150-200mg/dL

Triglyceride Estimation

Triglyceride reagent manufactured by Agappe diagnostics, Switzerland, LOT number 51410002 was used.

Principle

The enzymatic determination of triglyceride is based on the following reactions:

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[Mg^{++}]{\text{glycerol kinase}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{TOPS} \xrightarrow{\text{POD}} \text{Violet coloured complex.}$$

Normal range:
Male: 60-165 mg/dL
Female: 40-140 mg/dL

Estimation of HDL Cholesterol

Agappe Diagnostics reagent for HDL-cholesterol, LOT number 51010001 was used.

Principle

The chylomicrons, VLDL, and LDL of the plasma are precipitated by phosphotungstic acid and magnesium ions. After centrifugation, HDLs are in the supernatant. The HDL content of the supernatant is measured by an enzymatic method.
Normal range:
men: 35-55mg/dL
women: 45-65mg/dL

Estimation of LDL-Cholesterol

LDL-cholesterol was mathematically determined using Friedewald equation:
LDL-cholesterol (mg/dL) = Total cholesterol - HDL-(Triglyceride/5)

(B) Protein Estimation

Estimation of Total Protein

The Burette reaction method for total protein estimation was used.

Principle

The cupric ion in alkaline solution reacts with the peptide bonds in protein to produce a violet colour complex which is proportional to the concentration of protein present in the sample.
Normal range: 6-8g/dL

Estimation of Albumin

Albumin reagent, LOT number 51001002 manufactured by Agappe Diagnostics, Switzerland, was used.

Principle

This is based on the reaction between albumin in serum and the dye Bromocresol-Green which produces a

change in colour that is proportional to the concentration of albumin.

Reference range: 3.5-5.5g/dL

Globulin Estimation

Globulin was mathematically obtained using the relationship:

Globulin (g/dL) = Total protein (g/dL) - Albumin (g/dL)

Statistical Analysis

The statistical significance was set at $p<0.05$. The results were expressed as the mean ± SD.

Results

Table 1: Values of serum Total cholesterol, Triglyceride (TG), High density lipoprotein (HDL-C), Low density lipoprotein (LDL), Total protein, Albumin, and globulin in the control (Group I), New TB patients (Group II), 3 months post treatment patients (group III) and 6 months post treatment patients (Group IV).

Parameters	Control (Group I)	New TB patients (Group II)	3 months post treatment (Group III)	6 months post treatment (Group IV)
Total cholesterol (mg/dl)	154.90±19.27	128.50±12.18*	153.10±13.99**	157.70±23.11**
Triglyceride (mg/dl)	102.98±12.68	72.08±10.07*	84.55±12.77**	113.03±15.02**
HDL (mg/dl)	39.00±2.24	40.59±7.41	53.60±9.06**	59.20±2.94**
LDL (mg/dl)	95.30±18.52 75.89±22.62	73.51±17.03		82.59±15.32
Total protein (g/dl)	6.33±0.43 6.66±0.35**	8.30 ±0.40*		6.95±1.14**
Albumin (g/dl)	4.23±0.21 4.21±0.31**	3.49±0.23*		3.76±0.11
Globulin (g/dl)	2.10±0.36 2.45±0.53**	4.81±0.53*	3.58±0.45**	

Values are presented as Mean ± Standard deviation.
* indicates statistically significant values when compared to control (group I).
** indicates statistically significant values when compared to new TB patients (group II).

Total cholesterol value (128.50±12.18mg/dl) in group II (New TB patients), was significantly lower ($p<0.05$), when compared to that of group I (154.90±19.27mg/dl). Total cholesterol of groups III and IV (153.10± 13.99mg/dl and 157.70±23.11mg/dl) were significantly higher ($P<0.05$) than that of the group II (128.50±12.18mg/dl).

The triglyceride value in group II (72.08 ±10.07mg/dl) was significantly lower ($P<0.001$) than that of the control group (102.98±12.68mg/dl). The triglyceride values in groups III and IV were significantly higher (84.55±12.77mg/dl and 113.03±15.02mg/dl respectively) when compared to group II.

The HDL value in group II (40.59±7.4mg/dl) was higher than that of group I (39.00±2.2mg/dl) but not statistically significant. There was a statistically significant increase ($p<0.0001$) in HDL values in groups III (53.60±9.06mg/dl) and IV (59.20±2.94) when compared to group II (40.59±7.41mg/dl). When compared to the control group, no statistically significant increase was observed in LDL value of group II. Also, there was no statistically significant difference ($P<0.05$) in the LDL values in groups III and IV (82.59±15.32mg/dl and 75.89±22.62mg/dl respectively) when compared to that of group II (73.51±17.03mg/dl).

In group II (new TB patients), statistically significant increase in serum total protein (8.30±0.40g/dl) was observed, as compared to group I (6.33±0.43g/dl). Also, statistically significant increase was observed in total protein level in groups III and IV (6.95±1.14g/dl and 6.66±0.35g/dl respectively) when compared to group II (8.30 ±0.40g/dl).

There was a statistically significant decrease ($p<0.05$) in serum albumin level in group II (3.49±0.23g/dl) when compared to group I (4.23±0.21mg/dl). Also, a significant increase in albumin level was observed in group IV, compared to group II (3.49±0.23g/dl).

Serum globulin level in group II(4.81±0.53g/dl) was found to be significantly higher when compared to groups I (2.10±0.36g/dl), III (3.58±0.45g/dl), and IV (2.45±0.53g/dl) respectively.

Table 2: Comparison of the levels of serum Total cholesterol (TC), Triglyceride (TG), High density lipoprotein (HDL-C), Low density lipoprotein (LDL), Total protein, albumin, and globulin in New TB patients according to sex.

Parameters	Male	Female	p value	Significance
TC (mg/dl)	123.20±12.38	137.33±5.03	0.115	>0.05
TG (mg/dl)	73.10±5.17	70.33±17.10	0.736	>0.05
HDL(mg/dl)	42.84±7.69	36.83±6.30	0.301	>0.05
LDL(mg/dl)	65.75±16.61	86.43±7.86	0.950	>0.05
Total protein (g/dl)	8.20±0.34	8.50±0.51	0.403	>0.05
Albumin (g/dl)	3.42±0.26	3.60±0.11	0.298	>0.05
Globulin (g/dl)	4.78±0.54	4.86±0.62	0.851	>0.05

P < 0.05 = Significant
P >0.05 = Non significant

No statistically significant difference was observed in the levels of serum total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, total protein, albumin, and globulin in males and females with new cases of pulmonary TB.

Table 3: Comparison of the levels of serum Total cholesterol (TC), Triglyceride (TG), High density lipoprotein (HDL-C), Low density lipoprotein (LDL), Total protein, albumin, and globulin in male and female pulmonary TB patients after 3 months of antituberculosis therapy.

Parameters	Male	Female	p value	Significance
TC (mg/dl)	148.66±13.25	159.75±14.00	0.240	>0.05
TG (mg/dl)	83.73±13.37	35.78±13.72	0.821	>0.05
HDL(mg/dl)	53.33±10.88	54.00±6.98	0.917	>0.05
LDL(mg/dl)	78.59±15.19	88.59±15.48	0.341	>0.05
Total protein (g/dl)	7.40±0.50	7.27±0.33	0.673	>0.05
Albumin (g/dl)	3.77±0.51	3.75±0.17	0.826	>0.05
Globulin (g/dl)	3.63±0.48	3.53±0.48	0.108	>0.05

Legend:
P < 0.05 = Significant
P >0.05 = Non significant

No statistically significant difference was observed in the levels of serum total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, total protein, albumin, and globulin in males and females with pulmonary TB after 3 months of anti TB therapy.

Table 4: Comparison of the levels of serum Total cholesterol (TC), Triglyceride (TG), High density lipoprotein (HDL-C), Low density lipoprotein (LDL), Total protein, albumin, and globulin in male and female pulmonary TB patients after 6 months of antituberculosis therapy.

Parameters	Male	Female	p value	Significance
TC (mg/dl)	149.43±22.99	177.00±4.36	0.081	>0.05
TG (mg/dl)	113.16±16.21	112.73±15.06	0.970	>0.05
HDL(mg/dl)	58.43±3.05	61.00±2.00	0.223	>0.05
LDL(mg/dl)	68.37±23.31	93.45±3.51	0.111	>0.05
Total protein (g/dl)	6.79±0.16	6.37±0.55	0.084	>0.05
Albumin (g/dl)	4.14±0.36	4.37±0.58	0.330	>0.05
Globulin (g/dl)	2.64±0.44	2.00±0.50	0.760	>0.05

Legend:
P < 0.05 = Significant
P >0.05 = Non significant

No statistically significant difference was observed in the levels of serum total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, total protein, albumin, and globulin in males and females with pulmonary TB after 6 months of anti TB therapy.

Discussion

The result of this study showed that patients with new cases of pulmonary TB in Owerri have hypocholesterolemia. This is in agreement with the report of Perez-Guzman *et al.*, 2002. They suggested that hypocholesterolemia was a risk factor for the disease. The present study supports the assertion by Mohamed and Heshman (2012), that patients with pulmonary TB have hypocholesterolemia that proved to be a consequence of the disease itself rather than a risk factor. The present study found that serum cholesterol increased significantly to a comparable level to that of control after 6 months of regular intake of anti-TB therapy. If hypocholesterolemia is being corrected during anti-TB therapy, then the patient would have had normal serum cholesterol value before being diseased and consequently, hypocholesterolemia would be considered as consequence of the disease rather than a risk factor, as risk factors should be present before, during, and after treatment.

Malnutrition is known to be a major cause of hypoalbuminemia. As evident in the result of this study, a significant decrease in albumin level was observed in new cases of pulmonary TB when compared to that of patients who have been on anti-TB therapy for 6 months. This observation is similar to that of Abdur-Rehman, 1995 and Kailasam *et al.,* 2005. The decrease in serum albumin in new TB patients could be attributed to loss of appetite in TB patients due to exotoxins produced by bacteria, which results in low intake of proper diet, thus leading to malnutrition.

The result also revealed that the increase in total protein before the commencement of treatment was due to the increase in globulin level. In infectious disease such as TB, one possible mechanism which may be at work is increased globulin formation in the body as a result of increased immune response involving antibody production. The results also showed that the changes observed in the levels of the parameters assayed were not sex dependent.

Conclusion

As evident in the results of this study, patients with pulmonary TB have hypocholesterolemia and hypoalbuminemia, which proved to be a consequence of the disease itself. The hypocholesterolemia and hypoalbuminemia proved to be correctable with regular intake of anti-TB therapy and normal diet. The findings of the study are of diagnostic importance in pulmonary TB infection and could be used to assess the severity of the disease and progress of treatment

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