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Levels of Selected Lipids, Apolipoproteins and Malondialdehyde In *Mycobacterium Tuberculosis* Infected Individuals Before, During And After Treatment

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Abstract

Cardiovascular risk prediction is of high importance for clinicians and patients to assess the risk of developing cardiovascular disease (CVD), thereby allowing for preventive interventions to be instituted in those patients. Lipids and malondialdehyde (MDA) status of individuals with active *Mycobacterium tuberculosis* (MTB) infection were determined before, after two, and six months' treatment. This prospective follow-up study recruited 159 tuberculosis (TB) treatment-naive individuals. They were followed up on a six-month course of anti--tuberculosis therapy (ATT).120 individuals completed the study. Lipids and malondialdehyde were measured before ATT, at two and six months post-treatment. MTB was detected by microscopy and Genexpert methods. Lipids and malondialdehyde levels were determined spectrophotometrically. The mean levels of FFA, MDA, Apolipoprotein B, and B 48 were significantly lower in individuals with active TB at 2 months and 6 months on ATT compared with the baseline ($p<0.05$). The mean levels of Apolipoprotein B100 were significantly higher in individuals with active TB at 2 months and 6 months following ATT compared with the baseline ($p<0.05$). These findings showed reduced levels of MDA, Apo B, and B 48 with increased levels of Apo B100 in individuals with active MTB infection following treatment. The observed significantly raised level of Apo B100, even with treatment, indicates a higher risk of cardiovascular disease. Lipid profile and apo B100 levels significantly increased while malondialdehyde, apolipoproteins B, and B 48 significantly decreased after treatment indicating a good therapeutic response.

Keywords: *Cardiovascular Disease, Mycobacterium tuberculosis, Lipid Profile, Free Fatty Acid, Apolipoprotein B, Apolipoprotein B 48, Apolipoprotein B 100, Malondialdehyde.*

Abstrait

La prédiction du risque cardiovasculaire est d'une grande importance pour les cliniciens et les patients afin d'évaluer le risque de développer une maladie cardiovasculaire (MCV), permettant ainsi d'instituer des interventions préventives chez ces patients. Le statut des lipides et du malondialdéhyde (MDA) des personnes atteintes d'une infection active à Mycobacterium tuberculosis (MTB) a été déterminé avant, après deux et six mois de traitement. Cette étude de suivi prospective a recruté 159 personnes n'ayant jamais reçu de traitement contre la tuberculose (TB). Ils ont été suivis pendant un traitement antituberculeux (ATT) de six mois. 120 personnes ont terminé l'étude. Les lipides et le malondialdéhyde ont été mesurés avant ATT, à deux et six mois après le traitement. MTB a été détecté par microscopie et méthodes Genexpert. Les taux de lipides et de malondialdéhyde ont été déterminés par spectrophotométrie. Les niveaux moyens de FFA, MDA, Apolipoprotéine B et B 48 étaient significativement inférieurs chez les personnes atteintes de TB active à 2 mois et 6 mois sous ATT par rapport à la valeur initiale ($p<0,05$). Les niveaux moyens d'apolipoprotéine B100 étaient significativement plus élevés chez les personnes atteintes de tuberculose active à 2 mois et 6 mois après l'ATT par rapport à la ligne de base ($p<0,05$). Ces résultats ont montré des niveaux réduits de MDA, d'Apo B et de B 48 avec des niveaux accrus d'Apo B100 chez les personnes atteintes d'une infection MTB active après le traitement. Le niveau significativement élevé d'Apo B100 observé, même avec un traitement, indique un risque plus élevé de maladie cardiovasculaire. Le profil lipidique et les niveaux d'apo B100 ont augmenté de manière significative tandis que le malondialdéhyde, les apolipoprotéines B et B 48 ont significativement diminué après le traitement, indiquant une bonne réponse thérapeutique.

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Introduction

Cardiovascular disease (CVD) has been known as the major cause of death globally and may continue to rise if unchecked in the preceding years. [1,2]. According to the American Heart Association (AHA), one in three people will be affected by some form of CVD during their lifetime [3]. The two most common clinical manifestations of CVD are coronary artery disease (CAD) and ischemic stroke [4]. Complications of atherosclerosis, including myocardial infarction, chronic kidney disease, and stroke, are major contributors to the financial burden of healthcare costs globally [5,6]. The overall approach to reducing CVD morbidity and mortality is focused on primary and secondary prevention and control of modifiable risk factors [7,8]. Despite all efforts, substantial residual risk remains and new lines of attack against atherosclerotic CVD are needed [9]. One avenue that may be explored is apolipoprotein B (apo B) and its prominent position as a causal factor in atherosclerosis [10].Atherosclerosis is a progressive disease of large- and medium-sized muscular arteries, characterized by elevated lesions called fibrous plaques that encroach upon the vessel lumen and disturb blood flow. Atherosclerosis is the major cause of CVD. A hallmark of atherosclerosis is the retention of cholesterol-rich low-density lipoprotein (LDL-C) and other apoB-containing lipoproteins within the arterial wall [11]. The development of the fatty streak and subsequent transition to the fibrous plaque is primarily dependent upon the absorption of modified forms of cholesterol by subendothelial macrophages in an inflammatory setting. Thus, elevated levels of cholesterol in the circulation promote atherosclerosis and CVD [12,13]. Measurement of serum apoB reflects total LDL-C, intermediate-density lipoproteins (IDL-C), VLDL-C, and lipoprotein a) (Lp(a)) particle concentrations because each particle contains exactly one molecule of apoB100. Thus, apoB can be considered a powerful tool for the assessment of atherogenic lipid status. The hypothesis that tuberculosis leads to CVD comes from case studies of tuberculosis causing cardiovascular death as well as from population-based studies that show an increased risk of cardiovascular events. Tuberculous granuloma formation affecting the coronary arteries has been described as a rare cause of myocardial infarction in young patients [14,15]. It has been reported that during *M. tuberculosis* infection, there is increased production of reactive oxygen species (ROS) also known as free radicals as a result of phagocyte respiratory burst [16], and they play a major role in the etiology of a wide variety of diseases including CVD by causing significant damage to cell structures, enhanced ROS and free radical production may lead to an imbalance in the host antioxidant capacity. [17] This may cause oxidative stress and lipid peroxidation [18].Expectantly, a good therapeutic response to anti-tuberculosis therapy should show decreased production of reactive oxygen species and a reversal of oxidative stress and lipid peroxidation. Free radicals generate the lipid peroxidation process in an organism. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde is commonly known as a marker of oxidative stress [19].Serum level of Malondialdehyde has been known to be raised in inflammation, and infectious diseases including tuberculosis[20]. It is possible that tuberculosis could be contributing to premature cardiovascular death in areas of high tuberculosis prevalence. *M. tuberculosis* may not only affect the coronary vessels, but also the myocardium. Liu et al. summarized available clinical cases of tuberculous myocarditis and related sudden cardiac death (SCD) [21]. The proposed underlying mechanism leading to SCD was ventricular tachyarrhythmia, likely a consequence of aberrant conduction from extensive tuberculous septal involvement or ventricular wall necrosis [14, 22]. Pulmonary tuberculosis without obvious direct mycobacterial involvement of the myocardium or coronary arteries has also been linked to SCD, but the exact mechanisms of death remain obscure [23]. A large population-based retrospective cohort study conducted in Taiwan goes beyond these individual clinical observations to look at baseline CVD risk elevation in patients who had tuberculosis disease. The researchers looked at 10,168 patients with a history of tuberculosis disease and 40,672 control patients without a history of tuberculosis disease. After adjusting for important co-morbidities, those with a history of tuberculosis had a 40 % increased risk of the composite endpoint of unstable angina and AMI compared to the control. This elevated risk persisted for the entire study period of up to 14 years [24]. The potential effects of tuberculosis disease do not appear to be limited to coronary heart disease (CHD) but extend to other atherosclerosis-mediated vascular diseases such as stroke. A study in Taiwan followed patients with a history of non-meningeal tuberculosis disease and no history of stroke, as well as control patients. The authors found a 50 % increased risk of ischemic stroke in the tuberculosis group after 3 years of follow-up. This study also showed an increased risk of CHD in those with tuberculosis [25]. The mechanism of cardiovascular disease in tuberculous myocarditis or coronary arteritis, and the relationship between infection and CVD is direct and apparent. Hence, Levels of Selected Lipids, Apolipoproteins,

and Malondialdehyde in *Mycobacterium Tuberculosis* Infected Individuals Before, During, and After Treatment was Investigated in this study.

Materials and Methods

Research design: This prospective follow up research was conducted at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria, between May 2015 and January 2018 to compare the serum levels of apo B, apo B48 and apo B100 among individuals with MTB and healthy controls.

Study population: The study population consisted of 5518 suspected individuals with cardinal symptoms of tuberculosis who presented at Tuberculosis Directly Observed Therapy (TBDOT) clinics of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, between May, 2015 and January, 2018.

Sampling technique: The blood sample was collected from each individual before initiation of antituberculosis therapy, two months into ATT, six months into ATT, grouped accordingly and involved, laboratory investigations

Study participants: Sample size was calculated using G*Power software version 3.0.10 (Universität Düsseldorf, Germany). Power analysis for one way ANOVA was conducted in G*Power to determine a sufficient sample size using an alpha of 0.05, a power of 0.95 and a large effect size. Based on these, the calculated total sample size of 102 has 95% power to detect a difference of 0.25 at a significance level of 0.05.

A total of 120 study individuals composed of 120 treatment naïve active TB patients were recruited. *Mycobacterium tuberculosis* infection was determined by Ziehl-Neelsen (ZN) sputum smear microscopy and confirmed positive using GeneXpert®.

Inclusion criteria: Newly diagnosed TB positive individuals with or without MP, and or HIV co – infections aged (15-66) years (Category one, first line TB positive individuals), and attended the TB DOTS Clinic, NAUTH, Nnewi, Anambra State were recruited.

Exclusion criteria: Individuals infected with TB and on antiretroviral therapy, patients diagnosed with pulmonary tuberculosis but having diabetes mellitus, tobacco smokers, alcohol drinkers and participants who had cardiovascular diseases were excluded from the study.

Ethical consideration: Ethical approval for the study was obtained from Nnamdi Azikiwe University Teaching Hospital Ethics Committee (NAUTHEC) NAUTH/CS/66/VOL.7/79, Nnewi, Anambra State, Nigeria.

Informed consent: The aim, benefits and purpose of the study were explained to the individuals. Participation was voluntary and informed consent was obtained from the individuals. The individuals were allowed at any time they so desired to discontinue and that would not in any way affect their care. The information obtained from the individuals was kept highly confidential in observance of the privacy act.

Sample collection: Sputum collection and processing

Sputum samples were collected using the Directly Observed treatment short Course (DOTs) strategy specification and were processed using the Ziehl-Neelsen staining method and confirmed using the GeneXpert® by Cepheid.

Blood sample collection: Blood samples were collected three times from the participants. Firstly, immediately the individual was confirmed to be positive for pulmonary tuberculosis by Ziehl Neelsen's staining technique and GeneXpert MTB/RIF assay, before the initiation of anti tuberculosis treatment (ATT).Secondly on successful completion of two months course ATT. Thirdly on successful completion of six months course ATT. Eight milliliters (8mls) of blood was collected from each individual at each period of blood collection, and dispensed in plain tube to separate serum for various biochemical assays .The blood in the plain tube was allowed to stand for 30 minutes to clot and further centrifuged at 3500 rpm for five minutes using Wisperfuge model 1384 centrifuge (Samson, Holland). Serum was separated from clot with micropipette into sterile serum sample bottle for the measurement of biochemical parameters. Each individual's blood sample was stored frozen at -20^{0}C in aliquot, in three vials to avoid repeated thawing and storing that would affect the result of the analysis.

Diagnostic assessments

Free Fatty Acid was determined by colorimetric technique as described by [26].

Principle

Fatty Acids are converted to their CoA derivatives, which are subsequently oxidized with the concomitant generation of fluorescence. C-8 (octanoate) and longer fatty acids can then be easily quantified by either colorimetric (spectrophotometry at 570 nm

wavelenght) or fluorometric (at Ex/Em = 535/587 nm) methods with detection limit 2 ìM free fatty acid in variety samples.

Procedure

All reagents, standard solutions, and samples were prepared as instructed and brought to room temperature before use.

Standard Curve Preparation:

For the colorimetric assay, 0, 2, 4, 6, 8, 10 ìl Palmitic Acid Standard was added into 96-well plate individually and volume was adjusted to 50 ìl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Fatty Acid Standard.

Sample Preparation: different volume of samples was added to each well in a 96-well plate and the volume was brought up to 50 ìl/well with Assay Buffer.

Acyl-CoA Synthesis: 2 ìl ACS Reagent was added into all the standard and sample wells. Mixed well; the reaction was incubated at 37°C for 30 min. 50 ìl of the Reaction Mix was added to each well containing the Standard or test samples. The reaction was incubated for 30 min at 37°C, avoiding exposure to light. O.D. was measured at 570 nm for colorimetric assay in a micro-plate reader. The Fatty Acid amount in the sample wells were calculated from the standard curve. Fatty Acid Concentration = Fa/Sv (nmol/ìl or mM) Fa is the Fatty Acid amount (nmol) in the well obtained from standard curve. Sv is the sample volume (ìl) added to the sample well.

Total cholesterol, triglycerides, High Density Lipoproteins-cholesterol (HDL-C), and Low Density Lipoprotein Cholesterol (LDL-C) were determined by colorimetric method as described by [27]

Total Cholesterol was estimated by Cholesterol enzymatic End-point Method *[27]*

Principle

Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4- amino antipyrine in the presence of phenol and peroxidase.

Cholesterol ester + H_2O $^{\text{cholesterolesterase}}$ Cholesterol + fatty acids

Cholesterol + O_2 $^{\text{Cholesterol oxidase}}$ Cholestene-3-one + H_2O_2

$2H_2O$ + phenol + 4-Aminoantipyrine $^{\text{peroxidase}}$ quinoneimine + $4H_2O$

Procedure

10(ul) of serum, distilled water, control and standard cholesterol was collected and transferred into four test tubes labelled sample ,reagent blank,control and standard respectively.1000(ul) of reagent(R1) was added into all the test tubes, mixed and incubated for 5 minutes at 37^{O}C. The absorbance of the sample (A_{sample}) against the reagent blank was read spectrophotometrically within 60 minutes at 500nm.

Triglycerides was estimated by colometric method [27]

Principle

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide,4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.(Tietz,1990 and Trinder,1969).

Triglycerides + H_20 $^{\text{lipases}}$ glycerol + fatty acids

Glycerol +ATP ^{GK} glycerol-3-phosphate +ADP

Glycerol-3-phosphate + O_2 ^{GPO} dihydroxyacetone + phosphate + H_2O_2

$2H_2O_2$ +4-aminophenazone +4 chlorophenol ^{POD} quinoneimine +HCl +$4H_2O$

Procedure

10(ul) of serum, distilled water, control and standard cholesterol was collected and transferred into four test tubes labelled sample ,reagent blank, control and standard respectively.1000(ul) of reagent(R1=reagent mixture R1a +R1b) was added into all the test tubes, mixed and incubated for 5 min at 37^{O}C. The absorbance of the sample (A_{sample}) against the reagent blank was read spectophotometrically within 60 minutes at 500nm.

High Density Lipoproteins-cholesterol [27]

Principle

Low Density Lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitatively from serum by the addition of phosphotungstic acid in the presence of magnessium ion. After centrifugation, the cholesterol concentration in the HDL (High Density Lipoprotein) fraction which remains in the supernantant is determined.

Procedure

Precipitation reaction, 500(ul) of sample and standard was transferred into two test tubes, each containing 1000(ul) of precipitant (R1), and labelled, sample and standard respectively. The tubes were mixed and allowed sitting for 10minutes at room temperature. Then centrifuged for 10 minutes at 4000 rpm, or 2 minutes at 12000rpm. The cleared supernatant was separated off within two hours and was used for determining the cholesterol content as follows.100 (ul) of supernatant, distilled water and standard supernatant was collected and transferred into three test tubes labelled sample, reagent blank and standard respectively.1000 (ul) of reagent (R1) was added into

all the test tubes, mixed and incubated for 5 min at 37^{O}C. The absorbance of the sample (A_{sample}) against the reagent blank was read spectophotometrically within 60 minutes at 500nm.

Low Density Lipoprotein Cholesterol (LDL-C) in mmol/l was estimated by a clearance method for the direct measurement of LDL cholesterol using Randox Diagnostic LTD kit.
Principle

The assay consists of two distinct reaction steps
Elimination of chylomicrons, VLDL-Cholesterol and HDL-Cholesterol by cholesterol esterase, cholesterol oxidase and subsequently catalase. Specific measurement of LDL-Cholesterol after release of LDL-Cholesterol by *Cholesterol enzymatic End-point Method.*

Determination of Apolipoprotein B (APOB)
APOB was estimated by sandwich enzyme immunoassay technique as described by Brodsky [28].

Principle
This assay employs a quantitative sandwich enzyme immunoassay technique that measures human ApoB in less than 4 hours. A polyclonal antibody specific for human ApoB has been pre-coated onto a 96-well microplate with removable strips. ApoB in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for ApoB, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Procedure
All reagents, standard solutions, and samples were prepared as instructed and brought to room temperature before use. The assay was performed at room temperature (27-30°C).Excess microplate strips were removed from the plate frame and returned immediately to the foil pouch with desiccants inside. The pouch was resealed securely to minimize exposure to water vapor and stored in a vacuum desiccator.50 µl of Human ApoB Standard or sample was added per well. Wells were covered with a sealing tape and incubated for 2 hours. The timer was started after the last addition to time for 2 hours.Wells were washed five times with 200 µl of Wash Buffer manually. The plate was inverted each time and the contents decanted; hit 4-5 times on absorbent material to completely remove the liquid.50 µl of Biotinylated Human ApoB Antibody was added to each well and incubated for 1 hour. The microplate was washed again as described above. 50 µl of Streptavidin-Peroxidase Conjugate was added to each well and incubated for 30 minutes. The Microplate reader was turned on and set up for the program in advance.The microplate was washed again as described above.

50 µl of Chromogen Substrate was added per well and incubated for 10 minutes or till the optimal blue color density developed. The Microplate was gently tapped to ensure thorough mixing and bubbles in the well were broken with pipette tip. 50 µl of Stop Solution was added to each well. The color changed from blue to yellow.Absorbance was read on a microplate reader at a wavelength of 450 nm immediately and results obtained were recorded.

Evaluation of Apolipoprotein B48.
Apolipoprotein B48 was evaluated by sandwich enzyme immunoassay technique as described by [28]
Principle
The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to ApoB48. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for ApoB48 and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain ApoB48, biotinylated detection antibody and Avidin-HRP conjugate appeared blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of ApoB48.Concentrations of ApoB48 in the samples were calculated by comparing the OD of the samples to the standard curve.

Procedure
All reagents, standard solutions, and samples were prepared as instructed and brought to room temperature before use. The assay was performed at room temperature (27-30°C). The samples were centrifuged again after thawing before the assay. All the reagents were mixed thoroughly by gently swirling before pipetting and foaming was avoided. Add Sample: 100iL of Standard, Blank, or Sample was added per well. The blank well was added with Reference Standard and Sample diluent. Solutions were added to the bottom of micro ELISA plate well, foaming and inside wall touching were avoided. The solution was mixed gently. The plates were covered with sealer provided with the kit and incubated for

90 minutes at 37°C. Biotinylated Detection Ab: The liquid of each well were removed by aspiration without washing. Immediately, 100ìL of Biotinylated Detection Ab working solution was added to each well. Covered with the Plate sealer. The plate was gently tapped to ensure thorough mixing and incubated for 1 hour at 37°C.

Wash: Each well was aspirated and washed; the process was repeated three times. Washing was done by filling each well with Wash Buffer (approximately 350iL). Complete removal of liquid at each step was essential. After the last wash, the remaining Wash Buffer was removed by decanting. The plate was inverted and patted against thick clean absorbent paper.

HRP Conjugate: 100ìL of HRP Conjugate working solution was added to each well and the plate was covered with the sealer and incubated for 30 minutes at 37°C. The wash process was repeated for five times as in above. Substrate: 90iL of Substrate Solution was added to each well. Covered with a new Plate sealer. Incubated for 15 minutes at 37°C and was protected from light. (The reaction time could be shortened or extended according to the actual color change, but not more than 30minutes). When apparent gradient appeared in standard wells, user should terminate the reaction.

Stop: 50iL of Stop Solution was added to each well. Then, the color turned to yellow immediately.

OD Measurement: The micro-plate reader was opened in advance, preheated and the testing parameters were set. The optical density (OD value) of each well was determined at once, using a micro-plate reader set to 450 nm. The concentrations of the samples were calculated from the standard curve.

Evaluation of apolipoprotein B100 was evaluated by sandwich enzyme immunoassay technique as described by [28]

Principle

This assay employs an antibody specific for human ApoB100 coated on a 96-well plate. Standards and samples are pipetted into the wells and ApoB100 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human ApoB100 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of ApoB100 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Procedure

All reagents, standard solutions, and samples were prepared as instructed and brought to room temperature before use. Removable 8-well strips were labeled appropriately for the experiment.100 ìl of each standard and sample wash added into appropriate wells. The plates were covered well and incubated for 2.5 hours at room temperature. The solution was discarded by decanting and washed 4 times with Wash Buffer. The plate was inverted and blotted against clean paper towels.100 ìl of 1X prepared biotinylated antibody was added to each well and incubated for 1 hour at room temperature with gentle shaking. The solution was discarded and washing was repeated as in above.100 ìl of prepared Streptavidin solution was added to each well. The plate was incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded by decanting and washed 4 times with Wash Buffer. 100 ìl of TMB One-Step Substrate Reagent was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50ìl of Stop Solution was added to each well. Absorbance was read at 450 nm immediately.

Malondialdehyde was estimated by the method of [20]

Principle:

Malondialdehyde (MDA) is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline condition, it forms a pink coloured product, which has absorption maximum at 540nm.

Procedure

Three test tubes were set up and labeled test, blank and serum control. 50ìl of serum, distilled water (d/w), and control were added to the test tubes respectively. 1ml of 1% TBA in 20% NaOH was added to all the test tubes. And 1ml of glacial acetic acid was added to all the test tubes respectively. The reagents were mixed and incubated in boiling water for 15 minutes. Allowed to Cool and absorbance of the tests were read at 532 nm against the reagent blank. Results were calculated using MDA (nmol/ml) = (OD X 1000000)/156000

Where E_{532} = Molar extinction coefficient for MDA ($1.56x10^5$ $M^{-1}cm^{-1}$)

Statistical analysis

The IBM Statistical Package for Social Sciences (SPSS) version 21 ANOVA and LSD's post hoc were used for statistical analyses. The results were presented as mean ± standard deviation. Significant levels were considered at $p< 0.05$

Results

A significant increase was observed in the mean serum levels of TC (mmol/l) at 6months (2.90±1.89) and at 2months (2.75±1.39) on ATT compared with baseline (2.55±1.55) ($p<0.05$). Also a significant increase existed in the mean serum levels of LDL-C (mmol/l) at 6months (0.73±0.30) compared with 2months (0.56±0.52) and baseline (0.52±0.49) on ATT ($p<0.05$) while no significant increase was observed in the mean serum level of LDL-C(mmol/l) at 2months (0.56±0.52) compared with baseline(0.52±0.49) on ATT($p>0.05$). Further the mean serum level of VLDL-C (mmol/l) at 6months (0.49±0.22) and 2months (0.48±0.22) on ATT were significantly increased compared with baseline (0.40±0.21) ($p<0.05$) while no significant increase existed in the mean serum level of VLDL-C (mmol/l) at 6months (0.49±0.22) compared with 2months (0.48±0.22) on ATT ($p>0.05$). Furthermore, the mean serum levels of TG (mmol/l) following treatment for 6months (1.742±0.30) was significantly increased compared with 2months (1.01±0.48) and baseline (0.52±0.27) ($p<0.05$) on ATT while the mean serum levels of FFA (nmol/il) following treatment for 6months (0.11±0.07) was significantly decreased compared with 2months (0.15±0.09) and baseline (0.20±0.08) ($p<0.05$).

There were significantly decreased mean serum levels of Apo B (µg/ml), Apo B48 (ng/ml) and MDA (nmol/ml) after 6months treatment. (1179.2±85.5; 158.6±60.3; 1.39±0.65) compared with 2months (1263.4±72.3; 162.8±67.4; 1.49±0.86) and baseline levels (1366.1±39.3; 359.7±81.2; 1.74±0.77) ($p<0.05$). A significantly increased mean serum level of Apo B100 (ng/ml) was observed in individuals with active TB at 2months (3439.5±9.16) and 6months (3442±9.04) following treatment compared with baseline (3003.4±9.35) ($p<0.05$). Furthermore, the mean serum levels of Apo B (µg/ml), Apo B48 (ng/ml) and MDA (nmol/ml) were significantly increased at 2months (1263.4±72.3; 162.8±67.4; 1.49±0.86) following treatment compared with baseline (1366.1±39.3; 359.7±81.2; 1.74±0.77) ($p<0.05$) respectively, while the mean serum levels of MDA (nmol/ml) was significantly decreased at 2months (1.49±0.86) following treatment compared with baseline (1.74±0.77) ($p<0.05$).

Table 1: Serum levels of lipid profile and FFA at baseline, two months and six months following treatment (mean±SD)

Group	TC (mmol/l)	LDL-C (mmol/l)	VLDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)	FFA (nmol/il)
Baseline1	2.55±1.55	0.52±0.49	0.40±0.21	1.65±1.38	0.52±0.27	0.20±0.08
2months2	2.75±1.39	0.56±0.52	0.48±0.22	1.67±1.37	1.01±0.48	0.15±0.09
6months3	2.90±1.89	0.73±0.30	0.49±0.22	1.70±1.35	1.742±0.30	0.11±0.07
1 vs 2	<0.021	0.342	<0.001	<0.021	<0.001	<0.001
1 vs 3	<0.021	<0.001	<0.001	<0.021	<0.001	<0.001
2 vs 3	<0.020	<0.001	0.574	<0.021	<0.001	<0.001

n=120

Table 2: Serum levels of apolipoprotein B, apolipoprotein B48, apolipoprotein B100 and MDA at baseline, two months and six months following treatment (mean±SD)

Group	APOB (µg/ml)	APOB48 (ng/ml)	APOB100 (ng/ml)	MDA (nmol/ml)
Baseline1n=120	1366.1±39.3	359.7±81.2	3003.4±9.35	1.74±0.77
2months2 n=120	1263.4±72.3	162.8±67.4	3439.5±9.16	1.49±0.86
6months3n=120	1179.2±85.5	158.6±60.3	3442.9±9.04	1.39±0.65
1 vs 2	<0.01	<0.01	<0.01	<0.01
1 vs 3	<0.01	<0.01	<0.01	<0.01
2 vs 3	<0.01	<0.01	<0.01	<0.01

Discussion

Cardiovascular risk prediction is of high importance for clinicians and patients to assess the risk of developing cardiovascular disease (CVD), thereby allowing for preventive interventions to be instituted in those patients.[29] It has been suggested that high concentrations of FFAs are associated with insulin resistance, fatty liver disease, atherosclerosis, and myocardial dysfunction[30]. In this study, free fatty acid concentration was significantly lower at 6 months and 2 months following anti-tuberculosis therapy. Hence reduction in the FFAs level could be likened to a good therapeutic response. An increase in FFAs is followed by the production of ROS and activation of endogenous radical oxygen scavengers [31] hence in this study, malondialdehyde a lipid peroxidation marker was also significantly decreased at 6 months and 2 months following treatment than at baseline. The reduction in MDA and FFAs levels at 6 months and 2 months indicates absolute or diminished assault on the polyunsaturated fatty acids by oxidants, enhanced tubercle bacilli clearance, and deactivation of endogenous radical oxygen scavengers with treatment duration which clinically signifies an indication of good therapeutic response. A similar observation of low serum MDA concentrations was made in the study [20] under the title "Evaluation of Some Antioxidants in tuberculosis Patients" where serum MDA concentrations were lower in healthy participants and antioxidants were higher compared with participants infected with tuberculosis. This finding is in agreement with the work of [32] which reported that levels of lipid peroxidation products malondialdehyde (MDA) were increased significantly in individuals with TB before treatment and decreases with treatment. Furthermore, the observed significantly increased mean serum levels of TC, LDL-C, VLDL–C, HDL-C, and TG, following treatment at6 months and 2 months shows improvement due to response to TB treatment. Malnutrition and tuberculosis are synergistically associated with each other. The combination of malnutrition leading to decreased "supplementation" of lipids and reduction of immune parameters might be indicating the pathogenesis of tuberculosis. *Mycobacterium tuberculosis* activates invaded macrophages resulting in free radical bursts. High serum levels of free radicals and high concentrations of lipid peroxidation products are characteristics of patients with advanced tuberculosis [33]. Lipid peroxidation could cause reduced concentration of serum lipids and tissue inflammation. Similarly,[34] has stated that low serum cholesterol in the new cases may be due to high oxidative stress. Hence this study observed progressive reduction of MDA and restoration of serum levels of lipid parameters which are dependent on treatment duration. The rise in lipid profile concentration after treatment in TB patients might be due to the nutritional status and immune function improving and the cleaning of circulating bacilli in the blood. The finding that TB patients on ATT had significantly raised lipid profile concentration compared to baseline is consistent with the previous findings [35,33]. Likewise, significantly lower mean serum levels of Apo B and Apo B48 following treatment in individuals with TB at 2 months and 6 months were observed. The progressive reduction in ApoB and ApoB48 with ATT observed in this study corroborated the work of [36], whose research indicated that isoniazid (H) was associated with a significant decrease in total cholesterol and apo B levels. In line with their study, isoniazid (H) was used with rifampicin (R) throughout the six months treatment period for the TB individuals recruited in this study. Further, there was a significantly decreased mean serum level of Apo B100 in TB individuals at baseline. The previous study has shown that ApoB 100 is the apolipoprotein found in lipoproteins synthesized by the liver and it is found in chylomicrons, VLDL, IDL, LDL, and LP (a) particles [37]. All these particles are atherogenic [38] The observed increase in ApoB 100 at 2 months and 6 months on ATT could be the predisposing factor for the risk of cardiovascular disease in tuberculosis patients even on treatment [38], have shown in their work that the viewpoint of atherosclerosis and cardiovascular risk, apoB100 is the important one. Further, there was a significantly increased mean serum level of MDA in individuals with TB at baseline compared with individuals with TB at 2 months and 6 months on ATT. This finding is in agreement with the work of[34,35,36], which reported that levels of lipid peroxidation products malondialdehyde (MDA) were increased significantly in individuals with TB before treatment and decreased with treatment, hence, oxidative stress was observed in all the TB patients irrespective of treatment status.

Conclusion

Lipid profile level was restored with antituberculosis therapy while serum levels of FFA, apolipoproteins B, and B 48 progressively reduced with treatment, however, the serum level of atherogenic apolipoprotein B100 increased progressively with the duration of treatment, suggesting the persistent risk of cardiovascular events in individuals with active *Mycobacterium tuberculosis* infection even on treatment.

Recommendation

Measurement of the levels of apo B 100 should be considered routine in the prognostic cardiac evaluation

of individuals with active *Mycobacterium tuberculosis* infection. Further research in this area with a larger sample size is additionally recommended.

Contributors

ACI, OCC, and SCM conceived and designed the research proposal. POM, AEA, and ACI performed sample collection, experiments and data analysis. CAN, ACI and SCM contributed to the final version of the manuscript. All authors have read and approved the final manuscript.

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Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of interest:

None declared.

Ethical approval:

Ethical approval for the study was obtained from Nnamdi Azikiwe University Teaching Hospital Ethics Committee (NAUTHEC) under no. NAUTH/CS/66/VOL.7/79, Nnewi, Anambra State, Nigeria.

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