ABSTRACT

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SERUM APOLIPOPROTEIN B INCREASED AMONG TUBERCULOSIS PATIENTS COMPARED TO HEALTHY SUBJECTS

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BACKGROUND
The burden of tuberculosis (TB) and cardiovascular disease (CVD) is enormous worldwide. Public health programs have been challenged with the overlapping of TB and CVD epidemics. Mycobacterium tuberculosis (MTB) is also a main driver of atherogenesis, suggesting a potential pathogenic role of tuberculosis in cardiovascular disease. The objective of this study was to compare the serum levels of apolipoprotein B (apo B), apolipoprotein B48 (apo B48) and apolipoprotein B100 (apo B100) between patients with tuberculosis and healthy subjects.

METHODS
A cross-sectional study was conducted involving 251 subjects consisting of 120 treatment naïve active TB patients [26 HIV co-infected (TB+HIV+), and 82 TB+], 12 malaria parasite co-infected (TB+MP+), 26 latent TB infected (LTB) and 105 healthy controls. Their body mass index (BMI) was calculated. Mycobacterium tuberculosis infection was determined by Ziehl-Nelsen (ZN) sputum smear microscopy and confirmed positive using GeneXpert. Latent TB was determined by Mantoux test, MP was evaluated by microscopy while HIV by immunochromatographic techniques using serial algorithm. Apolipoproteins were determined using spectrophotometry. A one-way ANOVA test and LSD’s post hoc multiple comparisons were used for statistical analyses.

RESULTS
Significantly lower mean levels of BMI were observed in LTB, TB+, TB+HIV+ and TB+MP+ compared with the controls (p<0.005). The mean serum levels of apo B, apo B48 and B100 were significantly higher in LTB, TB+, HIV+TB+ and TB+MP+ compared with apparently healthy controls (p<0.05).

CONCLUSION
Elevated levels of apolipoproteins among infected TB individuals might predispose them to cardiovascular disease.

Keywords: Apolipoprotein, atherosclerosis, tuberculosis patient, healthy subjects
INTRODUCTION

Tuberculosis and cardiovascular disease (CVD) pose great public health challenges globally. The World Health Organization has been challenged with the increasing burden of tuberculosis and CVD epidemics. There is increasing evidence that many infections contribute to the pathogenesis of CVD including Mycobacterium tuberculosis. Some authors also revealed the association of latent tuberculosis with acute myocardial infarction (AMI). The potential mechanism for this association relied on a study that showed continuous activation of the immune system in latent and active tuberculosis. Antibodies to mycobacterial HSP65 cross-reacting with self-antigens in human blood vessels leading to autoimmunity may also have an effect on CVD risk. Monocytes/macrophages, lymphocytes and cytokines engaged in cell-mediated immune responses against Mycobacterium tuberculosis are also main drivers of atherogenesis, suggesting a potential pathogenic role of tuberculosis in CVD via mechanisms that have been described for other pathogens that establish chronic infection and latency.

Consequently, apolipoprotein-B (apo B) containing lipoproteins are a suspected cause of atherosclerosis which is the main underlying cause of CVD. Apolipoprotein-B enters the wall of the arteries and can transport cholesterol into the arterial wall; if present in increased numbers, may be the main initiating factor in atherosclerosis. Apolipoprotein B100 (apo B100) is the largest of the apoB group of proteins, consisting of 4563 amino acids. Apolipoprotein B100 is the apolipoprotein found in lipoproteins synthesized by the liver and is found in chylomicrons, very low-density lipoprotein cholesterol (VLDL-C), intermediate-density lipoprotein cholesterol (IDL-C), low-density lipoprotein cholesterol (LDL-C) and lipoprotein(a) [LP(a)] particles. All these particles are atherogenic. Each of these particles contains a single apo B molecule.

Several studies have shown that apo B may be a better predictor of cardiovascular disease risk than LDL-C. Furthermore, it has been shown that apo B may be raised despite normal or low levels of LDL-C. Apolipoprotein-B also appears to predict on-treatment risk, when LDL-C has been lowered by statin therapy. Similarly, a study by Ihim et al., on free fatty acid and lipid profile in active tuberculosis, latent tuberculosis, active TB and HIV, active TB and malaria parasite in subjects in Anambra concluded that low level of free fatty acid and hypolipidaemia in TB subjects could be attributed to tuberculosis. This observation is in line with the findings by Oyedeji et al. in their study on oxidative stress and lipid profile status in pulmonary tuberculosis patients in South Western Nigeria. The evaluation of apo B represents the total burden of the main lipoprotein particles involved in the atherosclerotic process. But another study showed different results, in that there was no significant difference in Apo B between patients with TB and healthy subjects.

It has been shown that Mycobacterium tuberculosis obtains nutrients and metabolizes host derived lipids mainly fatty acids and cholesterol and uses them to cause and maintain disease. Despite evidence of hypolipidaemia among individuals with TB infection, CVD is still on the increase among them. Consequently, this research objective was to compare the serum levels of apo B, apolipoprotein B48 (apo B48) and apo B100 between patients with tuberculosis and healthy subjects.

METHODS

Research design

This cross-sectional 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 apparently 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
Consecutive non-random sampling techniques was used to select individuals from the TB DOT centres before the initiation of therapy. Participants were individuals who met the inclusion criteria and consented after the purpose was explained to them and were recruited as they became available until the sample size was attained.

Study subjects
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 0.89 and a medium effect size. Based on these, the calculated total sample size of 245 has 89% power to detect a difference of 0.25 at a significance level of 0.05. A total of 251 study subjects composed of 120 treatment naïve active TB patients [26 HIV co infected (TB+HIV+) and 82 TB+], 12 malaria parasite co-infected (TB+MP+), 26 latent TB infected (LTB) and 105 healthy controls (TB-HIV”TST”) were recruited. At recruitment, all study subjects were interviewed using a standard questionnaire and demographic data were collected. The weight and height of all the subjects were measured and used to determine their body mass index (BMI). Mycobacterium tuberculosis infection was determined by Ziehl-Neelsen (ZN) sputum smear microscopy and confirmed positive using GeneXpert®.

Newly diagnosed TB positive individuals with or without MP, and or HIV co -infections were recruited. The individuals above were those who did not receive any tuberculosis treatment before they were recruited (Category one, first line TB positive individuals), were between 15-66 years old, and attending the TB DOTS Clinic, NAUTH, Nnewi, State Anambra.

Individuals infected with TB and on antiretroviral therapy were excluded. Patients diagnosed with pulmonary tuberculosis but having diabetes mellitus were also excluded from the study. Tobacco smokers, alcohol drinkers and participants who had other clinical problems such as diabetes and 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) under no. NAUTH/CS/66/VOL.7/79, Nnewi, Anambra State, Nigeria.

Informed consent
The aim, benefits and purpose of the study was explained to the individuals. Participation was voluntary and informed consent was obtained from all of 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 positive using the GeneXpert® by Cepheid.

Blood sample collection
Blood samples were collected once from individuals with active Mycobacterium tuberculosis infection. 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). Blood samples were collected once from individuals with latent TB and from apparently healthy individuals (controls). Eight milliliters (8mls) of blood was collected from each individual at each period of blood collection, thick and thin blood films were made for microscopic detection of *P. falciparum* on recruitment and malaria *Plasmodium falciparum/pan* rapid test (Carestart™, Access Bio, USA) which is a chromatographic immunoassay for the qualitative detection of circulating *P. falciparum* antigen in whole blood was also used. Two milliliters (2ml) of blood was dispensed in ethylene diamine tetra acetic acid (EDTA) bottles and 6ml of blood was dispensed in plain tubes to separate serum for various biochemical assays. The blood in the plain tubes 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 the clot with a micropipette into a sterile plain tube for the measurement of biochemical parameters. Each individual’s blood sample was stored frozen at -20°C in aliquots, in three cryovials to avoid repeated thawing and storing that would affect the result of the analysis.

**Diagnostic assessments**

The HIV status of the study subjects was determined using the Determine HIV-1/2 (Abbott Laboratories, Japan) as the screening test, the Capillus HIV-1/2 (Trinity Biotech, Ireland) as the confirmatory test and Uni-Gold HIV-1/2 recombinant (Trinity Biotech, Ireland) as a tie breaker test.

**Diagnosis of malaria**

*P. falciparum* malaria was detected using thick and thin blood smears for microscopic detection (employing Giemsa staining technique) and malaria plasmodium falciparum rapid test device (CARESTART™Malaria HRP2 (Pf) by ACCESS BIO, INC. USA).

**Mantoux test**

Tuberculin purified protein derivative (PPD) was utilized for the Mantoux test to assist in clinical diagnosis of tuberculosis for diagnosis and differential diagnosis of tuberculosis, early detection of tuberculosis and screening for infection by *M. tuberculosis* (latent tuberculosis infection). This test is known as the tuberculin skin test. The PPD used was obtained from BB – NCIPD Ltd, Sofia, Bulgaria. Each vial contained 1ml (10 doses) containing 50TU of PPD = 5TU/0.1ml per dose.

**Estimation of Apo B, Apo B48, and Apo B100**

Apo B, Apo B48, and Apo B100 were estimated by sandwich enzyme immunoassay technique as described by Brodsky and Edward.

**Statistical analysis**

The IBM Statistical Package for Social Sciences (SPSS) version 21 was used for the statistical analysis. ANOVA was used to determine if there were any statistical differences between the means of different groups of subjects. LSD’s post hoc multiple comparisons were run to confirm where the differences occurred in the groups. The results were presented as mean ± standard deviation. Significant levels were considered at p<0.05.

**RESULTS**

Significantly lower mean levels of BMI were observed in individuals with LTB (25.57 ± 6.53 kg/m²), active TB (24.11 ± 3.84 kg/m²), human immunodeficiency and tuberculosis co-infection (22.48 ± 2.38 kg/m²) and tuberculosis and malaria parasite co-infection (TBMP) (26.69 ± 4.01 kg/m²) compared with the controls (29.19 ± 7.22 kg/m²) (p<0.05).

The mean serum level of apolipoprotein B was significantly higher in individuals with active TB infection, TBHIV and TBMP co-infections (2879.3 ± 1.03 µg/mL, 2759.6 ± 0.37 µg/mL, and
2656 ± 0.462 µg/mL) respectively compared with LTBI (2493.7 ± 0.83 µg/mL) and apparently healthy controls (2033.2 ± 1.10 µg/mL) (p<0.05). Furthermore, the mean serum level of apolipoprotein B 48 in individuals with active TB infection, TBHIV and TBMP co-infections (364.4 ± 8.5 µg/mL, 358.7 ± 1.98 µg/mL and 386.4 ± 2.36 µg/mL, respectively) is significantly higher than in individuals with LTBI (106.3 ± 1.10 µg/mL) and apparently healthy controls (100.2 ± 5.50 µg/mL)(p<0.05). The mean serum level of apolipoprotein B 100 (µg/mL) was significantly higher in individuals with LTB, active TB infection, TBHIV and TBMP (2489.4 ± 0.84, 2879.5 ± 1.03, 3448.3 ± 0.22 and 2880.8 ± 0.46, respectively) than in apparently healthy controls (2032.3 ± 1.11)(p<0.05).

DISCUSSION

BMI has been used as an indicator of malnutrition or total adiposity, although with many limitations. However, the mean BMI values of the entire study group were within the normal reference range. The lower mean levels of BMI observed in the test groups might be the result of depletion in body lipids and free fatty acids associated with Mycobacterium tuberculosis infection. These lower mean levels of BMI observed in the test groups are in agreement with the studies by Yen et al. and Casha and Scarci. The significantly higher mean serum levels of apo B, B48 and B100 in Mycobacterium tuberculosis infected individuals in this study could be attributed to Mycobacterium tuberculosis infection. Several studies have shown that apo B may be a better predictor of cardiovascular disease risk than LDL-C. Apolipoprotein B is an important component of most atherogenic lipoprotein particles. The apolipoproteins were higher in the active TB and co-morbidity groups than in the controls. Apolipoprotein B containing lipoproteins are the ones that are most likely to enter the wall of the arteries. They are capable of trafficking cholesterol into the artery

Table 1. Sociodemographic characteristics parameters of individuals with LTB, active TB infections, HIV&TB, TB & MP and apparently healthy controls (AHC)

<table>
<thead>
<tr>
<th></th>
<th>AHC (n=105)</th>
<th>LTB (n=26)</th>
<th>TB (n=82)</th>
<th>TB HIV (n=26)</th>
<th>TB MP (n=12)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>35.61 ± 9.60</td>
<td>38.31 ± 10.83</td>
<td>36.44 ± 14.20</td>
<td>34.61 ± 11.12</td>
<td>39.33 ± 7.43</td>
<td>0.315</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.19 ± 7.22</td>
<td>25.57 ± 6.53</td>
<td>24.11 ± 3.84</td>
<td>22.48 ± 2.38b</td>
<td>24.21 ± 3.85b</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Means in a row without a common superscript letter differ (p<0.05), as analyzed by one-way ANOVA.

AHC: apparently healthy controls; LTB: latent tuberculosis; TBHIV: tuberculosis and HIV co-infection, TBMP: tuberculosis and malaria parasite co-infection. Results are expressed as mean ± SD and are statistically significant at p<0.05

Table 2. Serum levels of apolipoprotein B, apolipoprotein B48 and B100 among healthy controls, LTB, active TB, TBHIV and TBMP individuals

<table>
<thead>
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<th>TB MP (n=12)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Apo B (µg/mL)</td>
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<td>2493.7 ± 0.83b</td>
<td>2879.3 ± 1.03c</td>
<td>2759.6 ± 0.37c</td>
<td>2656 ± 0.462a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apo B 48 (µg/mL)</td>
<td>100.2 ± 5.50a</td>
<td>106.3 ± 1.10a</td>
<td>364.4 ± 8.5b</td>
<td>358.7 ± 1.98b</td>
<td>386.4 ± 2.36b</td>
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AHC: apparently healthy controls; LTB: latent tuberculosis; TB+HIV+: tuberculosis and HIV co-infection, TB+MP+: tuberculosis and malaria parasite co-infection. Results are expressed as mean ± SD and are statistically significant at p<0.05
wall, and if present in increased numbers they may be the main initiating factor in atherosclerosis. Retention of apo B containing lipoprotein particles within the arterial wall is an essential part of the process. The measurements of apo B represent the total burden of the main lipoprotein particles involved in the atherosclerotic process. Apolipoprotein B occurs in two main forms, apoB48 and apoB100. ApoB48 is synthesized mainly by the small intestine and is primarily found in chylomicrons. Apo B100 is the largest of the apo B group of proteins, consisting of 4563 amino acids. Apolipoprotein B100 is the apo-lipoprotein found in lipoproteins synthesized by the liver and it is found in chylomicrons, VLDL, IDL, LDL and LP (a) particles. All these particles are atherogenic. Each of these particles contains a single apo B molecule. Therefore, from the viewpoint of atherosclerosis and cardiovascular risk, apoB100 is the important one. Furthermore, the mean serum levels of apo lipoprotein B, apo lipoprotein B48 and B100 were significantly higher in individuals with TBHIV and TBMP co-infections. Hence, they might be at a higher risk of predisposition to cardiovascular disease. This finding is in line with previous studies and confirmed that high levels of apo B are indicative of a higher risk to cardiovascular disease in tuberculosis and concluded that concentrations of apo B are superior indicators of vascular/heart disease and CVD risk prediction than standard lipid profile. Lack of prior awareness of the participants, on the importance of research was a major constraint in carrying out this study. Monitoring serum apolipoprotein B levels may help reduce the occurrence of cardiovascular disease in patients with tuberculosis. Further research is necessary using a longitudinal follow up design to assess the effect of Mycobacterium tuberculosis (MTB) infection on serum levels of apo B, apo B48, apo B100, atherogenic index and coronary risk index in individuals with MTB infection before, during and after treatment.

CONCLUSION

It was observed in this research that higher mean serum levels of apo B, apo B48 and apo B100 in tuberculosis patients might predispose them to cardiovascular disease.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTORS

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

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