Apo Lipoproteins A1 and B in Multidrug-Resistant Tuberculosis (MDR-TB)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The medical management of multidrug-resistant tuberculosis (MDR-TB) has become a major public health issue. Apolipoproteins play a key role in lipoprotein metabolism such as the recognition of receptors involved in lipoprotein metabolism. Thus, the study of the inter- relationships between apolipoproteins (A1 and B) and MDR-TB could represent an important approach to the biological management of MDR-TB patients.

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Methodology: This is an experimental study carried out on eighty-two (82) patients including thirty-eight (38) MDR-TB patients which age ranged from 18 to 60 years old recruited from three tuberculosis centers (CAT) in the city of Abidjan and forty-four (44) non-tuberculosis patients used as control aged 18 to 60 years old recruited at the National Blood Transfusion Center (CNTS) in Treichville (Abidjan, Côte d'Ivoire). Total cholesterol, HDL-cholesterol and triglycerides were measured by the colorimetric-enzymatic method. Apolipoproteins A1 and B were measured using the immunoturbidimetric method.

Results and Conclusion: Showed a dyslipidemia concerning cholesterol and its HDL fraction, triglycerides and apolipoproteins A1 and B suggest an atherogenic profile in multidrug-resistant TB patients.

ABBREVIATIONS

ATC : Anti Tuberculosis Centers
NBTC : National Blood Transfusion Center

1. INTRODUCTION

Multidrug-resistant tuberculosis (MDR-TB) is a sickness caused by strains of Mycobacterium tuberculosis resistant to both isoniazid and rifampicin, the two major first-line anti-tuberculosis drugs. This resistance is due to a mutation in the targets of rifampicin and isoniazid (the β- subunit of RNA polymerase and mycolic acid synthesis respectively). The emergence of these multidrug-resistant bacilli has increased the complexity of TB control programmes [1]. The high rates of treatment failure with second generation anti-tuberculosis drugs leading to new resistance make it difficult to provide medical care for patients suffering from this form of tuberculosis. The pathophysiology of tuberculosis highlights the presence of a lipid-rich granuloma during infection through inhalation. These lipids contained in the center of the granuloma in the form of cytoplasmic lipid bodies originating from foamy macrophages are stored by Mycobacterium tuberculosis which uses them as a source of energy and carbon for its growth [2]. Also, the bacillus uses host lipids to protect itself from external process caused by the mycobacterial infection [3,4]. Aqueous insoluble lipids assemble with proteins called apolipoproteins to be transported in the plasma as lipoproteins. Cholesterol is transported by high-density lipoproteins (HDL) and low-density lipoproteins (LDL) whose specific proteins are apolipoproteins A1 and B respectively [5]. Apolipoproteins play an important role in lipoprotein metabolism, including recognition of receptors involved in lipoprotein metabolism [5,6] Thus a modification of apolipoproteins linked to oxidative stress induced by the Mycobacterial bacillus could alter the metabolism of lipoproteins and lead to the formation of atheroma and cardiovascular diseases [7]. Studies have identified apolipoproteins A1 and B as biomarkers for the diagnosis and treatment monitoring of TB and showed a disturbance of the lipid profile during TB [8,9]. However, the interrelation between apolipoproteins (A1 and B) and MDR-TB was not investigated.

The general objective of this study is to contribute to a better biological management of multidrug-resistant tuberculosis patients (MDR-TB).

And by specifically:

- Determine the variations in serum concentrations of apolipoproteins A1 and B during multidrug-resistant tuberculosis compared to control patients
- Determine the variations in serum concentrations of apolipoproteins A1 and B during the intensive treatment phase of multidrug-resistant tuberculosis
- To assess the apolipoprotein-related cardiovascular risk in patients with multidrug-resistant tuberculosis compared to control patients.

2. MATERIALS AND METHODS STUDY POPULATION

This is an experimental study carried out on eighty-two (82) patients including thirty-eight (38) MDR-TB patients which age ranged from 18 to 60 years old and recruited from Adjame, Koumassi and Yopougon antituberculosis centers (ATC), Three municipalities of Abidjan city and forty-four (44) non-tuberculosis patients

used as control aged between 18 and 60 years old recruited at the National Blood Transfusion Center (NBTC) in Treichville (Abidjan, Côte d'Ivoire).

2.1 Materials

The biological material was made of the sera of MDR-TB patients and non-tuberculosis control patients. Blood samples were collected from patients at different stages of their follow-up, such as, at the initial checkup, after confirmation of the multidrug resistance test and before starting the treatment (M_0) and at three and six months of second-generation anti-TB treatment respectively (M_3 and M_6). For control patients, sampling was carried out in a single blood collection.

The technical equipment consisted of a centrifuge (Horizon Drucker 755-24, USA) which was used for the separation of blood components and a biochemistry automate (Cobas C311 Hitachi type, Roche France) used to measure studied parameters.

2.1.1 Reagents

For the various biochemical parameter assays, ready to use kits for total cholesterol; HDL-cholesterol; triglycerides; APOAT and APOBT were obtained from the supplier.

2.2 Methods

The determination of total cholesterol, HDL-cholesterol and triglycerides was performed by thecolorimetric-enzymatic method [10 -12].

Apolipoproteins A1 and B were determined by immunoturbidimetry [13] and LDL cholesterol was estimated by the formula of Friedewald [14].

LDL-C (g/l) = TC (g/l) - [HDL-C (g/l) +TG/5] (g/l) with TG < 3.5 g/L (4 mmol/L)

Data were processed using Graph Pad Prism 5.0 (Microsoft, USA). The results were presented as mean ± standard deviation. The comparison of means was done by Student's t-test for the different parameters. The differences were considered: *: significant at $P < 0.05$; **: highly significant at $P < 0.01$ and ***: very highly significant at $P < 0.001$.

3. RESULTS AND DISCUSSION

3.1 Results

The results showed a disturbance of the lipid profile in MDR-TB patients compared to non-tuberculosis controls.

Indeed, the triglyceridemia of MDR-TB was significantly higher than that of controls ($P = 0.018$). However, a significant decrease was observed in serum concentrations of total cholesterol, HDL cholesterol ($P < 0.0001$), Apo A1 ($P < 0.0001$) and Apo B ($P = 0.0004$) were in TB-MDRs than that of controls. There was no significant change in LDL cholesterol ($P = 0.88$).

The decrease in HDL-C and Apo A1 resulted in a significant increase in the TC/HDL ($P < 0.0001$) and ApoB/Apo A1 ($P = 0.0007$) atherogenicity indices in MDR-TB compared to controls (Table 1).

**Table 1. Variation in mean serum values of lipoprotein constituents in mute-resistant TB patients and controls**

| Parameters	Controls (n=44)	MDR-TB (n= 38)	P-value
CT (g/l)	1.61 ± 0.059	1.37 ± 0.10	0.011**
TG (g/l)	0.74 ± 0.051	0.96 ± 0.08	0.018*
HDL-C (g/l)	0.49 ± 0.024	0.15 ± 0.01	<0.0001***
LDL-C (g/l)	1.02 ± 0.049	1.03 ± 0.10	0.88
APO A1 (g/l)	1.20 ± 0.042	0.62 ± 0.07	<0.0001***
APO B (g/l)	0.86 ± 0.036	0.60 ± 0.04	0.0004***
CT/HDL (g/l)	3.54 ± 0.16	13.04 ± 1.57	<0.0001***
APOB/APOA1 (g/l)	0.79 ± 0.077	2.65 ± 0.56	0.0007***

Apo A1: Apolipoprotein A1; Apo B: Apolipoprotein B; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; TC: Total Cholesterol; TG: Triglycerides

The results showed, at (M3), a significant decrease in total cholesterol (P = 0.05) and its LDL- cholesterol fraction (P = 0.03) compared to the initial assessment (M0), with no change in HDL- cholesterol (P > 0.05) and Apo A1 (P > 0.05). There was also a non-significant decrease in triglyceridaemia, Apo B (P > 0.05) and Apo B/ApoA1 ratio (P > 0.05) with a significant decrease in TC/HDL atherogenicity index (P = 0.004) at M3 compared to M0. Comparison of the results between M0 and M6 showed that triglyceridaemia, total cholesterol, LDL-cholesterol, Apo A1, Apo B, HDL-cholesterol, TC/HDL atherogenicity index and ApoB/ApoA1 did not vary significantly (*P* > 0.05).

However, a significant increase in total cholesterol (*P* = 0.009) and its LDL fraction (*P* = 0.026) with no change in triglyceridaemia, HDL cholesterol, Apo A1 and Apo B were observed at M6 compared to M3. A non-significant (*P* > 0.05) increase in TC/HDL and Apo B/Apo A1 atherogenicity index was also observed at M6 compared to M3.

After analysis of the results, it was found that Apo A1, HDL-cholesterol and Apo B did not vary from M0 to M6. hus these parameters were not influenced by the treatment.

A

B

C

D
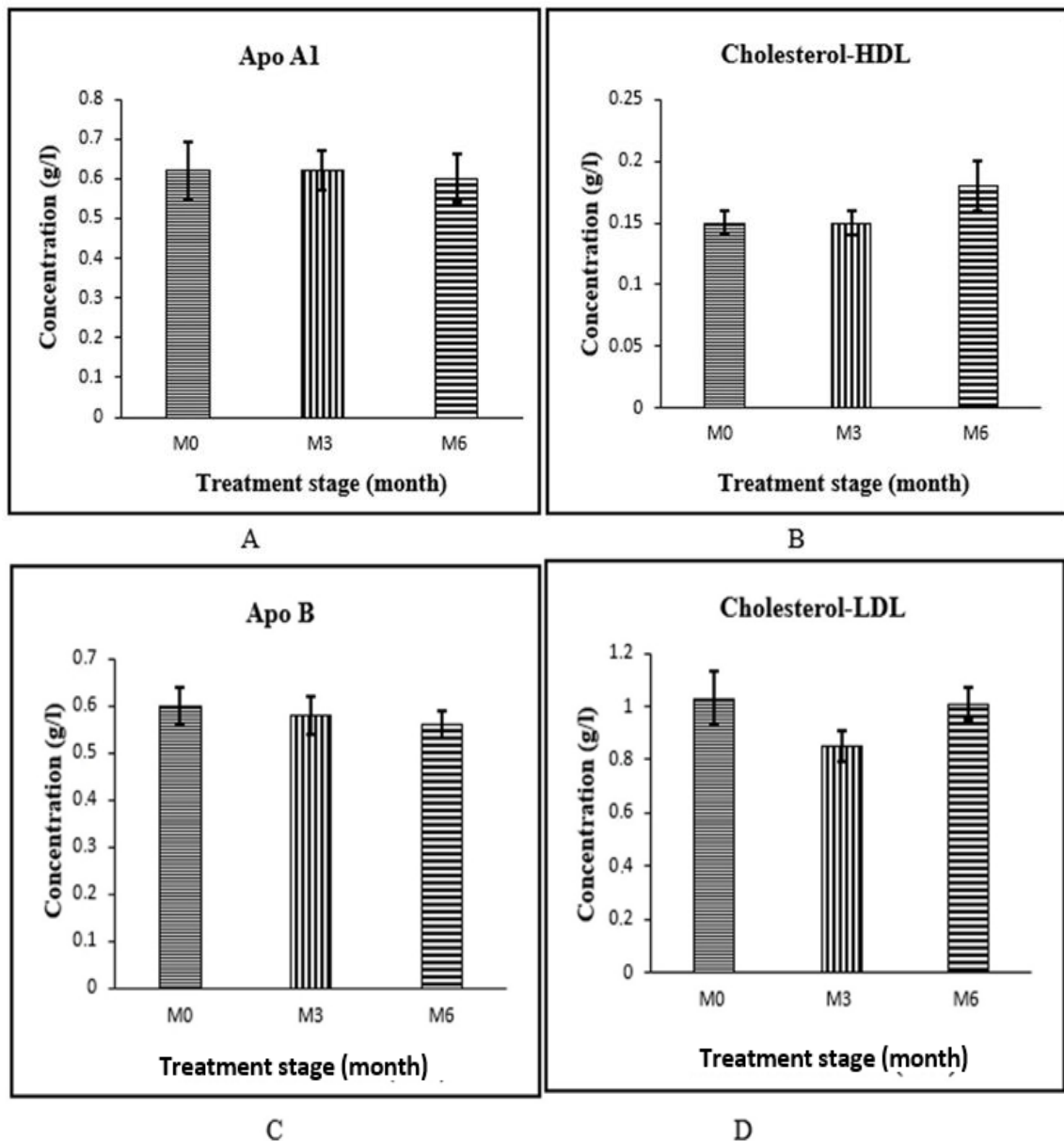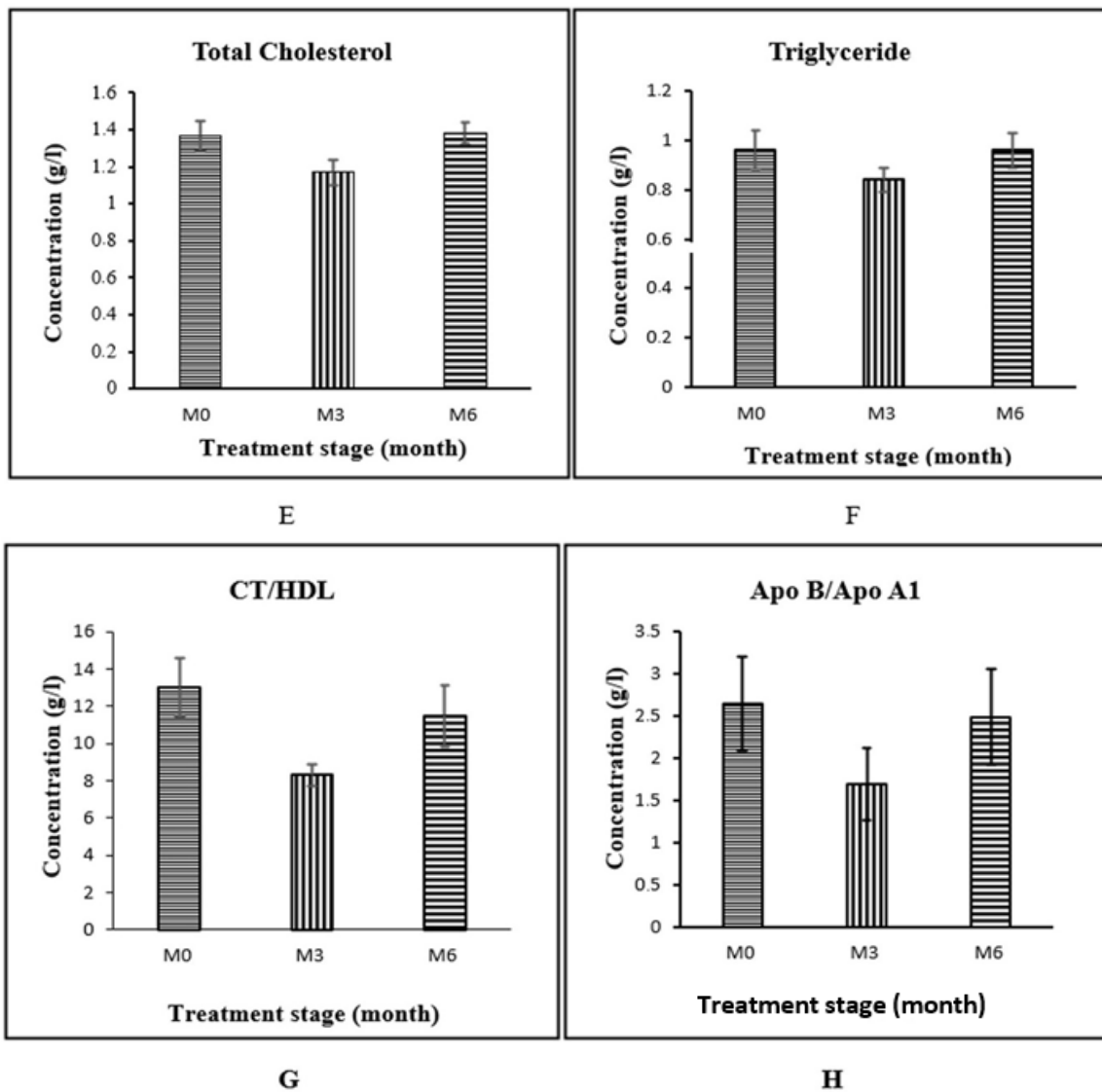
Fig. 1. Variation in lipoprotein contents of MDR-TB patients according to treatment stage
Legend: A: Apolipoprotein A1, B: HDL-Cholesterol, C: Apolipoprotein B, D: LDL-Cholesterol, E: Total Cholesterol
F: Triglycerides, G and H: Atherogenicity Indices

3.2 Discussion

The overall objective of this study was to contribute to the biological management of patients with multidrug-resistant tuberculosis by measuring Apolipoproteins A1 and B. Analysis of results showed a disturbance of the lipid and lipoprotein profile in MDR-TB patients. While control patients showed a normal lipid profile, results showed dyslipidaemia in MDR-TB patients. Indeed, compared to control patients, an increase in triglyceride concentrations, a decrease in HDL-cholesterol, Apo A1, Apo B, total cholesterol without variation in LDL-cholesterol and an increase in atherogenicity

indices were observed compared to control patients exposing MDR-TB patients to a cardiovascular risk. For the lipid profile of MDR-TB patients, it was noticed that the rise of triglyceride concentrations in MDR-TB patients compared to controls was in contrast with Wang et al. [15] who found a decrease in triglyceride concentration in MDR-TB patients. These results could be explained by an inflammation-induced disorder of triglyceride metabolism during MDR-TB. Several studies link hypertriglyceridaemia to inhibition of lipoprotein lipase messenger RNA transcription by proinflammatory cytokines such as tumor necrosis factor-α (TNFα), interferonγ (INFγ) and

Interleukin-1 [16]. Lipoprotein lipase is the enzyme that catabolises the hydrolysis of triglycerides from chylomicrons and VLDL. This inhibition could lead to the accumulation of triglyceride-rich lipoproteins, particularly chylomicrons and VLDL, by reducing their catabolism. In addition, a significant decrease in total cholesterol concentration was observed in patients with MDR-TB compared to control patients. Similar results have been reported by other authors [17,18]. Hypocholesterolemia could be due to the inflammation caused by tuberculosis (TB). An adequate concentration of cholesterol is necessary for the proper functioning of the immune system. Perez-Guzman et al. [17] showed that a high cholesterol diet accelerates bacteriological sterilisation in tuberculosis patients. Furthermore, the significant decrease in total cholesterol and HDL-cholesterol concentrations in MDR-TB patients observed in this study is consistent with the work of Akpovi et al. [19] and Wang et al. [15] who reported a reduction in HDL-C concentration in MDR-TB patients compared to control. The decrease in HDL-Cholesterol concentration could be explained by a deficiency in transporter protein, like ApoA1 and an increase in HDL catabolism due to oxidative stress during inflammation caused by the mycobacterial bacillus [20]. Indeed, the response to inflammation during the acute phase of tuberculosis is characterised by an over expression of proteins such as phospholipase A2 and circulating amyloid A, stimulating the catabolism of HDL-cholesterol [18] In addition, HDL-cholesterol and Apo A1 are known to be protective of the arterial wall of the circulatory system. Therefore, the decrease in HDL-cholesterol and ApoA1 concentrations in MDR-TB patients puts them at risk for cardiovascular disease [21]. A significant decrease in apolipoprotein A1 and B concentrations in MDR-TB patients was observed compared to control patients. These results are contrasting that of Wang et al. [15] who pointed out a constant concentration of Apo B and a decrease of Apo A1 in MDR-TBs. This decrease in Apo A1 and B could be explained by the activation of the peroxisome proliferator receptor (PPAR) caused by the inflammatory response. Indeed, the hepatic cells producing Apo A1 and B carry on their surface PPARα receptors which have a role in regulating the expression of some genes. The activation of these receptors during inflammation represses the transcription of apolipoprotein genes, thereby lowering their concentrations [22]. The significant decrease in HDL-cholesterol and

Apo A1 in patients with MDR-TB compared to controls resulted in a significant rise of the atherogenicity index TC/HDL and ApoB/ApoA1 above cardiovascular risk, placing patients with MDR-TB at risk for cardiovascular disease. These results are in accordance with those of McQueen et al. [23] but differ from those obtained by Akpovi et al. [19]. As for the influence of treatment on lipid and lipoprotein concentrations from M_0 to M_6. Thus, from M_0 to M_3, there was a significant decrease in total cholesterol and its LDL fraction without any variation in HDL cholesterol. These results were reflected in a decrease in the CT/ c-HDL atherogenicity index at M_3 compared to M_0. Triglyceride, Apo A1, Apo B concentrations did not change significantly during treatment. The decrease observed in total cholesterol is similar with the study of Albana et al. [8]. These results could be explained by the treatment of MDR-TB. Indeed, these patients are treated according to a therapeutic protocol using isoniazid whose hepatotoxic effect is well documented. The synthesis of cholesterol takes place in the liver. In addition, studies have shown that a low total cholesterol concentration is associated with the presence of isoniazid in the treatment of tuberculosis in experimental animals. Furthermore, these animal experiments showed that before the development of a tuberculosis lesion, the concentration of cholesterol in the lungs was increased and inhibited by isoniazid treatment. Thus isoniazid has a lipid- lowering effect [24]. The decrease in LDL-C could therefore be explained by a deficiency of Apo B related to isoniazid treatment, Apo B is the main structural protein of LDL cholesterol [25]. From M_3 to M_6, there was a significant increase in serum concentrations of total cholesterol and its LDL fraction. The elevated concentrations of total cholesterol and its LDL fraction at M_6 compared to M_3 do not agree with those of Wang et al. [15] who found a decrease in total and LDL cholesterol at M_6 compared to M_2. The rise of cholesterol and its LDL fraction could be justified by the withdrawal of some molecules in the fourth month of MDR-TB treatment such as isoniazid which has a lipid-lowering role.

4. CONCLUSION

This study showed dyslipidaemia in MDR-TB. Indeed, compared to control patients, an increase in serum triglyceride concentrations, a decrease in total cholesterol, HDL-cholesterol, Apo A1, Apo B and an increase in atherogenicity

indices were observed, exposing MDR-TB subjects to a risk of cardiovascular disease. The results also showed that second-generation anti-TB treatment led to a disturbance of the parameters measured, such as total cholesterol and its LDL fraction. In contrast, HDL-cholesterol and triglyceride concentrations, Apo A1 and Apo B did not change during treatment.

We can conclude that dyslipidaemia of cholesterol and its HDL fraction, triglycerides and apolipoproteins A1 and B suggest an atherogenic profile in adult patients with multiresistant TB. These results show that lipids and lipoproteins could represent biological markers for a better medical management of patients suffering from multidrug-resistant tuberculosis and also in the monitoring of the treatment.

CONSENT AND ETHICAL APPROVAL

This study was approved by the National Committee on Ethics and Research of Côte d'Ivoire (NCER/N/Réf: 0115/MSLS/CNER-dk). The approval and informed consent were obtained from MDR-TB patients and control participants for the use of their blood for research purpose.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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