ApolipoproteinsA1andBinmultidrug-resistanttuberculosis(MDR-TB)

ABSTRACT:
Aims: Themedicalmanagementofmultidrug-resistanttuberculosis(MDR-TB)hasbecomeamajor public health issue. Apolipoproteins play a key role in lipoprotein metabolism such asthe 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 importantapproachto thebiological managementof MDR-TB patients.
Methodology: Thisisanexperimentalstudycarriedoutoneighty-two(82)patientsincludingthirty-eight (38) MDR-TB patients which age ranged from 18 to 60 years old recruited fromthree tuberculosis centers (CAT) in the city of Abidjan and forty-four (44) non-tuberculosispatients used as control aged 18 to 60 years old recruited at the National Blood TransfusionCentre(CNTS)inTreichville(Abidjan,Côted'Ivoire).Totalcholesterol,HDL-cholesterolandtriglycerides were measured by the colorimetric-enzymatic method. Apolipoproteins A_1 and Bweremeasured using theimmunoturbidimetricmethod.
Resultsandconclusion:showedadyslipidemiaconcerningcholesterolanditsHDLfraction,triglyceridesandapolipoproteinsA_1andBsuggestanatherogenicprofileinmultidrug-resistantTBpatients.

Keywords:MDR-TB;Apolipoproteins;Lipids;Abidjan;Côted'Ivoire.

Abbreviations:
ATC:AntiTuberculosisCenters
NBTC:NationalBloodTransfusionCentre

INTRODUCTION

Multidrug-resistant tuberculosis (MDR-TB) is a sickness caused by strains of Mycobacteriumtuberculosisresistanttobothisoniazidandrifampicin,thetwomajorfirst-lineanti-tuberculosisdrugs. 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 thesemultidrug-resistant bacilli has increased the complexity of TB control programmes [**1**]. Thehigh rates of treatment failure with second generation anti-tuberculosis drugs leading to newresistance make it difficult to provide medical care for patients suffering from this form oftuberculosis.Thepathophysiologyoftuberculosishighlightsthepresenceofalipid-richgranulomaduringinfectionthroughinhalation.Theselipidscontainedinthecenterofthe

granuloma in the form of cytoplasmic lipid bodies originating from foamy macrophages arestored by Mycobacterium tuberculosis which uses them as a source of energy and carbon forits growth [**2**]. Also, the bacillus uses host lipids to protect itself from external process causedby the mycobacterial infection [**3 ; 4**]. Aqueous insoluble lipids assemble with proteins calledapolipoproteins to be transported in the plasma as lipoproteins. Cholesterol is transported byhigh-density lipoproteins (HDL) and low-density lipoproteins (LDL) whose specific proteinsare apolipoproteins A1 and B respectively [**5**]. Apolipoproteins play an important role inlipoproteinmetabolism, including recognition ofreceptors involved in lipoprotein metabolism
[**5 ; 6**]. Thus a modification of apolipoproteins linked to oxidative stress induced by theMycobacterial bacillus could alter the metabolism of lipoproteins and lead to the formation ofatheroma and cardiovascular diseases [**7**]. Studies have identified apolipoproteins A1 and B asbiomarkers for the diagnosis and treatment monitoring of TB and showed a disturbance of thelipid profile during TB [**8 ; 9**]. However, the interrelation between apolipoproteins (A1 and B)andMDR-TB was not investigated.
The general objective of this study is to contribute to a better biological management ofmultidrug-resistanttuberculosis patients (MDR-TB).
Andbyspecifically:
- DeterminethevariationsinserumconcentrationsofapolipoproteinsA1andBduringmultidrug-resistanttuberculosis compared to controlpatients
- DeterminethevariationsinserumconcentrationsofapolipoproteinsA1andBduringtheintensivetreatmentphaseof multidrug-resistanttuberculosis
- Toassesstheapolipoprotein-relatedcardiovascularriskinpatientswithmultidrug-resistanttuberculosiscompared to controlpatients.

Material and methodsStudypopulation

Thisisanexperimentalstudycarriedoutoneighty-two(82)patientsincludingthirty-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 cityand forty-four (44) non-tuberculosispatients used as control aged between 18 and 60 years old recruited at the National BloodTransfusionCentre(NBTC) in Treichville(Abidjan, Côted'Ivoire).

Material

ThebiologicalmaterialwasmadeoftheseraofMDR-TBpatientsandnon-tuberculosiscontrolpatients.Bloodsampleswerecollectedfrompatientsatdifferentstagesoftheirfollow-up,suchas, at the initial checkup, after confirmation of the multidrug resistance test and before startingthetreatment(M_0)andatthreeandsixmonthsofsecond-generationanti-TBtreatmentrespectively ($M_3$and M_6).For control patients, sampling was carried out in a single bloodcollection.
The technical equipment consisted of a centrifuge (Horizon Drucker 755-24, USA) which wasusedfortheseparationofbloodcomponentsandabiochemistryautomate(CobasC311Hitachitype,Roche France) used tomeasure studied parameters.

Reagents

Forthevariousbiochemicalparameterassays,readytousekitsfortotalcholesterol;HDL-cholesterol;triglycerides;APOAT andAPOBT wereobtained fromthesupplier.

Methods

Thedeterminationoftotalcholesterol,HDL-

cholesterolandtriglycerideswasperformedbythecolorimetric-enzymaticmethod[**10 ; 11 ; 12**].

Apolipoproteins A1 andB were determined byimmunoturbidimetry[**13**].LDL cholesterolwas estimated by theformulaof [**14**]:

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

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

Results and discussionResults

Theresultsshowedadisturbanceofthelipidprofilein MDR-TBpatientscomparedtonon-tuberculosiscontrols.

Indeed, the triglyceridemia of MDR-TB was significantly higher than that of controls ($P = 0.018$).However,asignificantdecreasewasobservedinserumconcentrationsoftotalcholesterol,HDLcholesterol($P<0.0001$),ApoA1($P<0.0001$)andApoB($P=0.0004$)werein TB-MDRs than that of controls. There was no significant change in LDL cholesterol ($P = 0.88$).

The decrease in HDL-C and Apo A_1resulted in a significant increase in the TC/HDL ($P < 0.0001$) and ApoB/Apo A1 ($P = 0.0007$) atherogenicity indices in MDR-TB compared tocontrols(Table1).

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
APOA1(g/l)	1.20±0.042	0.62±0.07	<0.0001***
APOB(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***

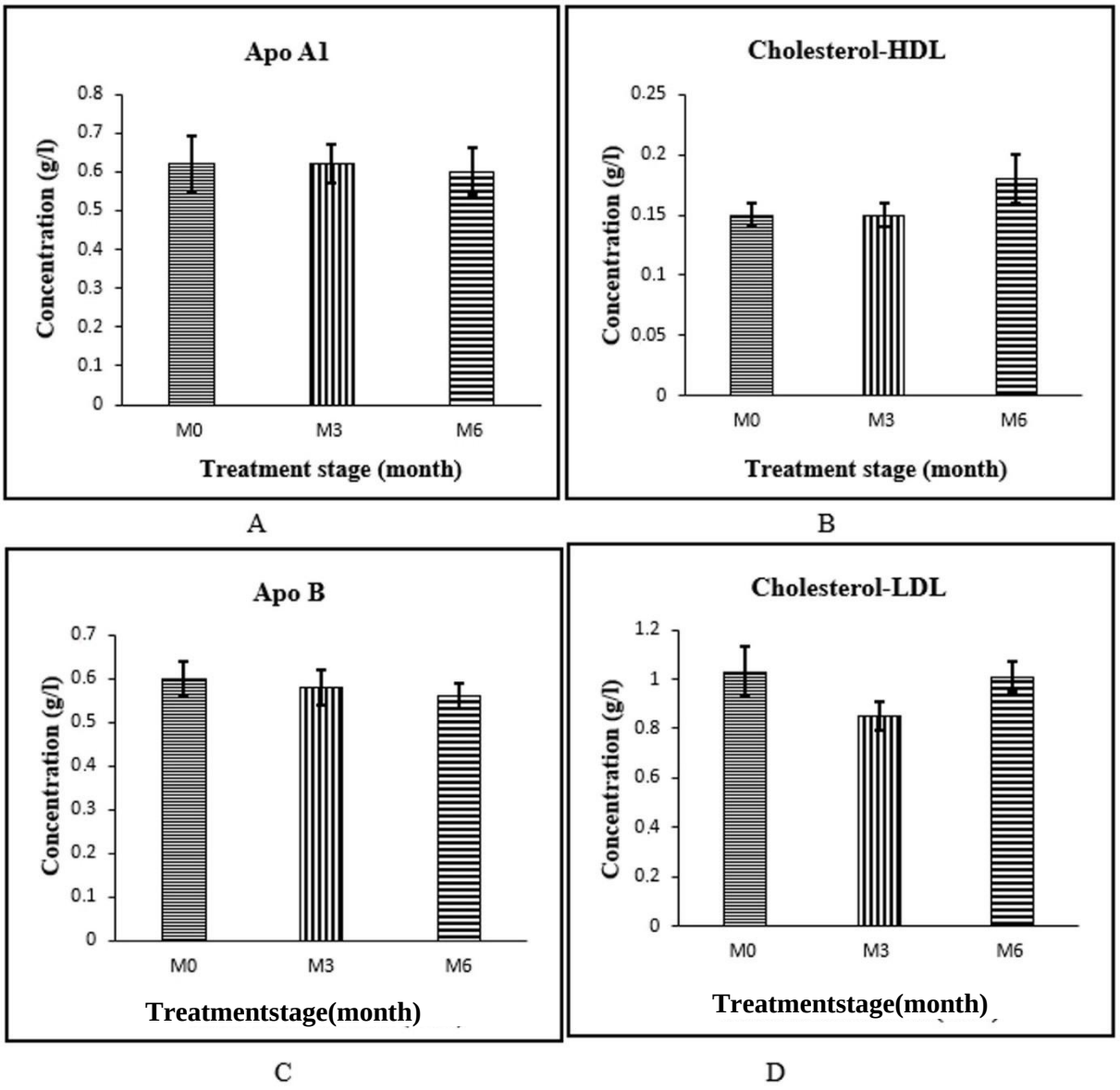
Apo A1: apolipoprotein A1; Apo B: apolipoprotein B; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; TC: total cholesterol; TG: triglycerides

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 M_0 and M_6 showed that triglyceridaemia, total cholesterol,LDL-cholesterol,ApoA_1,ApoB,HDL-cholesterol,TC/HDLatherogenicityindexandApoB/ApoA_1did notvary significantly ($P > 0.05$). However,asignificantincreaseintotalcholesterol(P=0.009)anditsLDLfraction(P=0.026)withnochangeintriglyceridaemia,HDLcholesterol,ApoA1andApoBwereobservedatM_6comparedtoM_3. Anon-significant(P>0.05)increaseinTC/HDLandApoB/ApoA_1atherogenicityindex was alsoobserved at M_6comparedto M_3.

AfteranalysisoftheresultsitwasfoundthatApoA_1,HDL-cholesterolandApoBdidnotvaryfromM_0toM_6. Thus theseparameters werenotinfluenced bythetreatment.



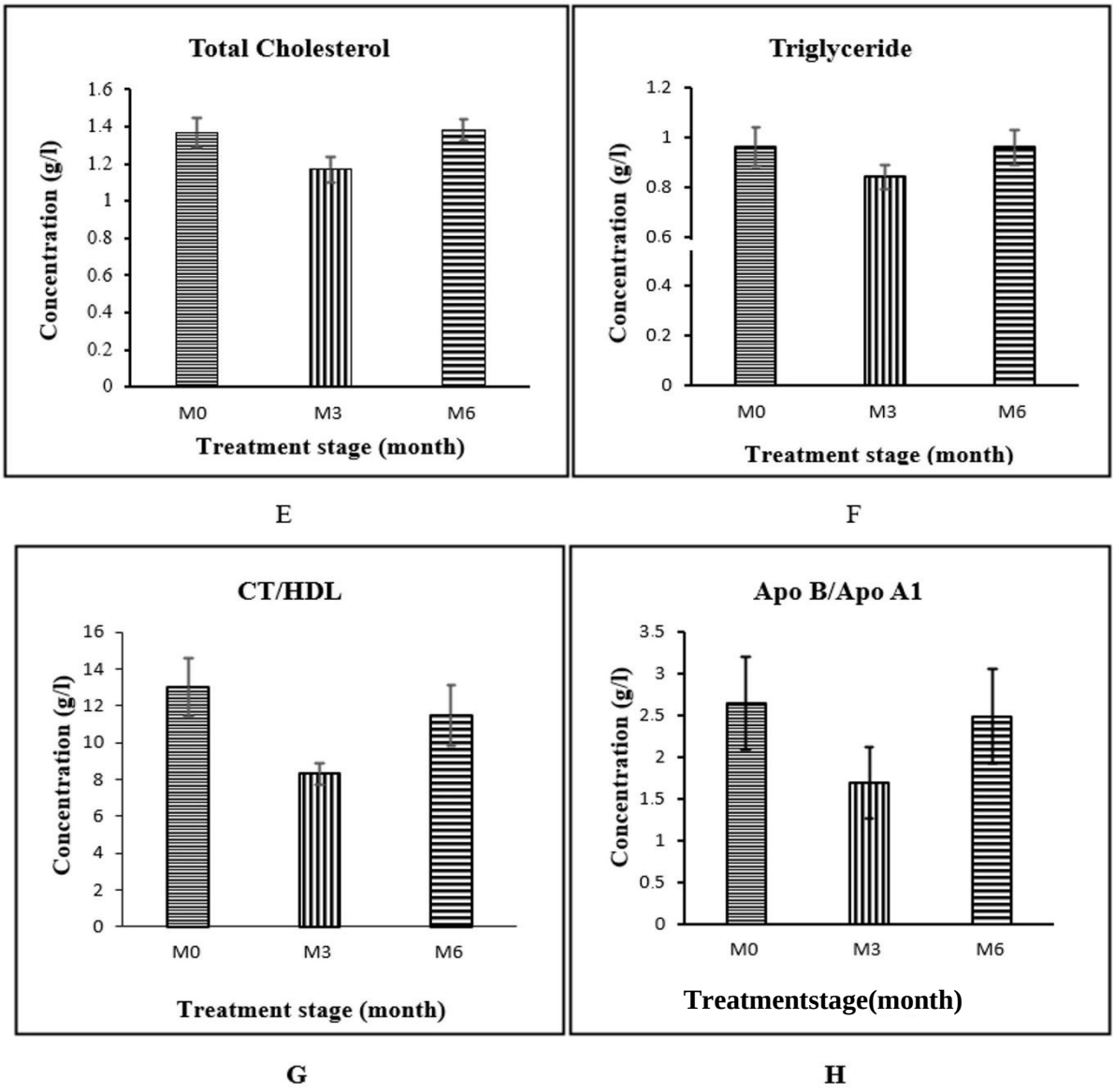


Figure 1: Variation in lipoprotein contents of MDR-TB patients according to treatmentstage

Legend: A:apolipoproteinA$_1$,B:HDL-cholesterol,C:apolipoproteinB,D:LDL-cholesterol,E:totalcholesterolF:triglycerides,GandH:atherogenicityindices

Discussion

The overall objective of this study was to contribute to the biological management of patientswith multidrug-resistant tuberculosis by measuring Apolipoproteins A$_1$and B. Analysis ofresults showed a disturbance of the lipid and lipoprotein profile in MDR-TB patients. Whilecontrol patients showed a normal lipid profile, results showed dyslipidaemia in MDR-TBpatients. Indeed, compared to control patients, an increase in triglyceride concentrations, adecrease in HDL-cholesterol, Apo A1, Apo B, total cholesterol without variation in LDL-cholesterol and an increase in atherogenicity indices were observed compared to controlpatients exposing MDR-TB patients to a cardiovascular risk. For the lipid profile of MDR-TBpatients,itwasnoticedthattheriseoftriglycerideconcentrationsinMDR-TBpatientscomparedtocontrolswasincontrastwith**Wanget*al.***[**15**]whofoundadecreaseintriglyceride concentrationinMDR-TBpatients.Theseresultscouldbeexplainedbyaninflammation-

induceddisorderoftriglyceridemetabolismduringMDR-TB.Severalstudieslinkhypertriglyceridaemia to inhibition of lipoprotein lipase messenger RNA transcription byproinflammatory cytokines such as tumor necrosis factor-α (TNFα), interferonγ (INFγ) andInterleukin-1[**16**].Lipoproteinlipaseistheenzymethatcatabolisesthehydrolysisoftriglycerides from chylomicrons and VLDL. This inhibition could lead to the accumulation oftriglyceride-richlipoproteins,particularlychylomicronsandVLDL,byreducingtheircatabolism.In addition, a significant decrease in total cholesterol concentration was observedin patients with MDR-TB compared to control patients. Similar results have been reported byother authors [**17 ; 18**]. Hypocholesterolemia could be due to the inflammation caused bytuberculosis(TB).Anadequateconcentrationofcholesterolisnecessaryfortheproperfunctioning of the immune system. **Perez-Guzman et *al*.** [**17**]showed that a high cholesteroldietacceleratesbacteriologicalsterilisationintuberculosispatients.Furthermore,thesignificantdecreaseintotalcholesterolandHDL-cholesterolconcentrationsinMDR-TBpatients 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 tocontrol. The decrease in HDL-Cholesterol concentration could be explained by a deficiency intransporter protein, like ApoA1 and an increase in HDL catabolism due to oxidative stressduringinflammationcausedbythemycobacterialbacillus[**20**].Indeed,theresponsetoinflammation during the acute phase of tuberculosis is characterised by an over expression ofproteins such as phospholipase A2 and circulating amyloid A, stimulating the catabolism ofHDL-cholesterol[**18**].Inaddition,HDL-cholesterolandApoA1areknowntobeprotectiveofthe arterial wall of the circulatory system. Therefore, the decrease in HDL-cholesterol andApoA1 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 wasobserved 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 peroxisomeproliferator receptor (PPAR) caused by the inflammatory response. Indeed, the hepatic cellsproducingApoA1andBcarryontheirsurfacePPARαreceptorswhichhavearoleinregulatingthe expression of some genes. The activation of these receptors during inflammation repressesthe transcription of apolipoprotein genes, thereby lowering their concentrations [**22**]. Thesignificant decrease in HDL-cholesterol and Apo A1 in patients with MDR-TB compared tocontrols resulted in a significant rise of the atherogenicity index TC/HDL and ApoB/ApoA1above 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 thoseobtained by **Akpovi et *al.*** [**19**].As for the influence of treatment on lipid and lipoproteinconcentrations from M_0 to M_6. Thus, from M_0 to M_3, there was a significant decrease in totalcholesterol and its LDL fraction without any variation in HDL cholesterol. These results werereflectedinadecreaseintheCT/c-HDLatherogenicityindexatM_3comparedtoM_0.Triglyceride,ApoA1,ApoBconcentrationsdidnotchangesignificantlyduringtreatment.Thedecrease observed in total cholesterol is similar with the study of **Albana et *al.*** [**8**]. Theseresults could be explained by the treatment of MDR-TB. Indeed, these patients are treatedaccordingtoatherapeuticprotocolusingisoniazidwhosehepatotoxiceffectiswelldocumented. The synthesis of cholesterol takes place in the liver.In addition, studies haveshown that a low total cholesterol concentration is associated with the presence of isoniazid inthe treatment of tuberculosis in experimental animals. Furthermore, these animal experimentsshowedthatbeforethedevelopmentofatuberculosislesion,theconcentrationofcholest

erolinthe lungs was increased and inhibited by isoniazid treatment. Thus isoniazid has a lipid-loweringeffect[**24**].ThedecreaseinLDL-Ccouldthereforebeexplainedbyadeficiencyof

ApoBrelatedtoisoniazidtreatment,ApoBisthemainstructuralproteinofLDLcholesterol [**25**].FromM_3toM_6,therewasasignificantincreaseinserumconcentrationsoftotalcholesterol and its LDL fraction. The elevated concentrations of total cholesterol and its LDLfractionatM_6comparedtoM_3donotagreewiththoseof**Wange*tal*.**[**15**]whofoundadecreaseintotalandLDLcholesterolatM_6comparedtoM_2.Theriseofcholesterolanditsfractioncould be justified by the withdrawal of some molecules in the fourth month of MDR-TBtreatmentsuch as isoniazid whichhas alipid-lowering role.

Conclusion

Thisstudyshoweddyslipidaemiain MDR-TB.Indeed,comparedtocontrolpatients,anincrease 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-TBsubjectstoariskofcardiovasculardisease.Theresultsalsoshowedthatsecond-generationanti-TB treatment led to a disturbance of the parameters measured, such as total cholesterol and itsLDL fraction. In contrast, HDL-cholesterol and triglyceride concentrations, Apo A1 and ApoBdid notchangeduring treatment.

We can conclude that dyslipidaemia of cholesterol and its HDL fraction, triglycerides andapolipoproteins A1 and B suggest an atherogenic profile in adult patients with multiresistantTB. These results show that lipids and lipoproteins could represent biological markers for abettermedicalmanagementofpatientssufferingfrommultidrug-resistanttuberculosisandalsointhemonitoring of thetreatment.

Ethicsapprovalandconsenttoparticipate

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 obtainedfromMDR-TBpatientsandcontrolparticipantsfortheuseoftheirbloodforresearchpurpose.

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