

*Full Length Research Paper*

## Tuberculosis treatment raises total cholesterol level and restores high density lipoprotein cholesterol (HDL-C) in patients with pulmonary tuberculosis

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The aim of this study was to determine whether tuberculosis (TB) treatment normalizes the lipid profile strongly affected by pulmonary TB. Serum levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were determined in 83 patients with pulmonary TB before and after treatment, and compared to results obtained from 100 control subjects without TB. Before treatment, levels of TC ( $p < 0.005$ ), HDL-C ( $p < 0.005$ ) and LDL-C ( $p < 0.005$ ) were significantly lower in pulmonary TB patients than normal subjects. Unlike TC and LDL-C, HDL-C decrease was correlated ( $r = 0.96$ ,  $p < 0.05$ ) with smear positivity extent (SPE). At the end of TB treatment, which lasted six months, TC ( $p < 0.01$ ) and HDL-C ( $p < 0.005$ ) levels were significantly increased than before treatment while LDL-C stayed relatively unchanged. The treatment significantly reduced the atherogenic indices TC/HDL-C ( $p < 0.001$ ), LDL-C/HDL-C ( $p < 0.001$ ) and  $\log$  (TG/HDL-C) ( $p < 0.001$ ) levels. Our results show that tuberculosis treatment increases TC levels and normalizes HDL while reducing atherogenic indices to below levels of controls.

**Key words:** Pulmonary tuberculosis, lipid profile, treatment, atherogenic index.

### INTRODUCTION

Cholesterol has received much attention in recent years mainly because of its involvement in cardiovascular disease (Smith et al., 1992). Current recommendations for treatment are all geared towards reducing the serum cholesterol levels (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). However, increasing evidence indicates a link between low blood cholesterol levels and a number of human diseases including tuberculosis (TB) (Perez-Guzman et al., 2005; Deniz et al., 2007). Specifically, it is

reported that hypocholesterolemia promotes the development of TB whereas hypercholesterolemia confers some protection against infection with *Mycobacterium tuberculosis* (MTB) (Wilson et al., 2003; Perez-Guzman et al., 2005). Despite the existence of such links between cholesterol and TB, it is not known to which extent the treatment of the disease affects lipid indicators in patients with TB. We formulate the hypothesis that TB treatment is associated with restoration of normal levels of cholesterol in patients with pulmonary TB.

## MATERIALS AND METHODS

### Patients

This study was carried out as a pre-post test design study with a control group at *Centre National Hospitalier de Pneumo-Phyysiologie Cotonou* (CNHPP) which is a reference center for TB diagnosis and treatment in West Africa. From October 2011 to June 2012, a total of 187 patients (72 females and 115 males) suffering from TB were admitted to CNHPP. Sputum and blood samples were systematically collected from all patients with clinical signs of pulmonary TB. Patients co-infected with MTB and human immunodeficiency virus, and those who underwent an incomplete TB treatment were excluded from the study. Furthermore, patients who were on antibiotic therapy or using any cholesterol lowering medication at the time of admission were also not included in the study. Eighty-three (83) out of 147 patients have met the criteria listed above and have been selected to participate in the study. All selected patients completed the treatment. One hundred (100) age- and sex-matched healthy subjects with no complaints and no known diseases were recruited at *Hôpital Saint-Luc* (Cotonou, Bénin) and enrolled as control group. This control group was tested negative upon TB diagnostic testing. Informed written consent was voluntarily obtained from each participant before entering the study and the local ethics board approved the study protocol.

### Diagnostic criteria and bacteriological examination

On the basis of clinical signs, bacteriological diagnosis was requested by the physician for each patient. Two sputum samples were collected in two consecutive days in all patients. Sputum samples were examined by direct smear examination. Acid-fast mycobacteria (AFB) resist decolorization by acid-alcohol after primary staining owing to the high lipid (mycolic acid) content in their cell walls. The direct smear examination of sputum was carried out by auramine O stain. The technique consists of making a smear and drying it for 15-30 min, fixing it over the flame and staining for 15 min. The slides were decolorated with 0.5% acid-alcohol, rinsed, counterstained with acridine orange for 2 min and air dried. The smears were examined under oil immersion lens of microscope (objective 40X, total magnification, X400). To determine the smear positivity extent (SPE), the number of AFB were counted by an experienced technician. The results are expressed as the number of AFB per number of fields (F) observed as follows: +/-: 1-18/50F, +: 4-36/10F, ++: 4-36/F, +++: >36/F.

### Measurement of biochemical parameters

Blood from fasting patients was collected in a dry tube (without anticoagulant) before and after treatment in experimental subjects and once in control subjects. In the event that the dosage was postponed, the serum was aliquoted into 1.5 ml Eppendorf tubes and stored at -20°C until needed. Total cholesterol (TC) (Biolabo, France), high density lipoprotein cholesterol (HDL-C) (Biolabo, France) and triglycerides (TG) (ELITech Group, France) were assayed by enzymatic methods. Low density lipoprotein cholesterol (LDL-C) was determined using the Friedewald formula.

### Treatment

The treatment was based on a combination of isoniazid (H), rifampicin (R), pyrazinamide (Z), ethambutol (E) and streptomycin (S) for a period of 6 months. It comprises an initial phase with a combination of ERHZ for two months, followed by a four month treatment of HR. In case of recurrence, the initial treatment comprised a two month treatment with ERH2S, followed by 1 month of ERHZ and five months of ERH for a total treatment time of 8 months.

### Statistical analyses

Data were evaluated by Student's t-test using the SigmaPlot statistical analysis software (Systat Software, Inc. San Jose, CA, USA). Quantitative data are expressed as mean  $\pm$  SEM. The null hypothesis was rejected at the level of 0.05.

## RESULTS

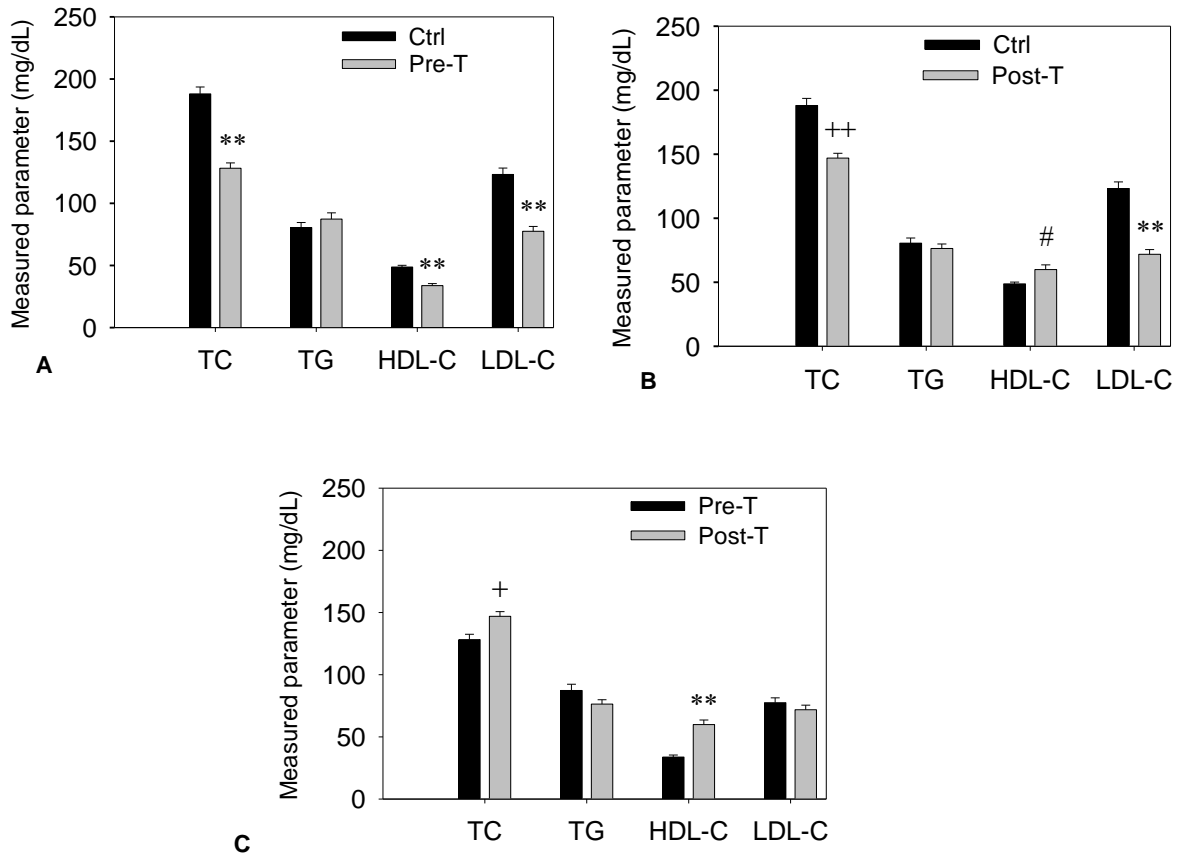
This study involved 83 TB patients as the experimental group and 100 normal subjects who had previously not been diagnosed or received treatment for TB as the control group. TB patients were aged between 12 and 62 years with an average age of  $32.37 \pm 12.87$  and included 57 men (68.67%) and 26 women (31.33%). The control group consisted of 35 women (35.00%) and 65 men (65.00%). The age of the control group was between 17 and 67 years with an average of  $35.70 \pm 12.48$ . There were no significant differences in age ( $p=0.078$ ) between the two groups.

Lipid parameters were measured in 83 experimental subjects suffering from TB and 100 control subjects. In TB patients, the same individuals were tested before treatment and at the end of treatment. Before treatment, TC ( $p<0.005$ ), HDL-C ( $p<0.005$ ) and LDL-C ( $p<0.005$ ) levels were significantly lower in TB patients as compared to normal subjects (Figure 1A). Triglycerides levels were unchanged between the two groups (Figure 1A). At the end of treatment, which lasted six months, the levels of TC remained significantly ( $p<0.01$ ) lower in TB treated patients when compared to controls (Figure 1B). However, compared with the levels before treatment, TC levels increased significantly ( $p<0.01$ ) (Figure 1C). TB treatment had not changed the levels of TG and LDL-C (Figures 1B and 1C). However, the levels of HDL-C were significantly increased after treatment compared to the control group ( $p<0.02$ ) (Figure 1B) and with respect to non-treated TB patients ( $p<0.005$ ) (Figure 1C).

In order to determine whether changes in the levels of lipid parameters correlated with the extent of bacterial load in TB patients, we compared the levels of TC, HDL-

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**Abbreviations:** TB, Tuberculosis; MTB, *Mycobacterium tuberculosis*; CNHPP, Centre National Hospitalier de Pneumo-Phyysiologie Cotonou; AFB, acid-fast mycobacteria; SPE, smear positivity extent; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; H, isoniazid, R, rifampicin; Z, pyrazinamide; E, ethambutol; S, streptomycin.



**Figure 1.** The mean levels of serum lipid parameters in treated and pre-treated tuberculosis patients. The mean levels of TC, TG, HDL-C and LDL-C of 83 tuberculosis patients prior to treatment (Pre-Treatment, Pre-T) (A) and at the end of treatment (Post-Treatment, Post-T) (B) were compared to that of 100 control patients. The values of lipid parameters in tuberculosis patients before (Pre-T) and after treatment (Post-T) were matched (C). The values shown are mean  $\pm$  SEM. (\*\* $p < 0.005$ ; + $p < 0.01$ ; ++ $p < 0.001$ ; # $p < 0.02$ ).

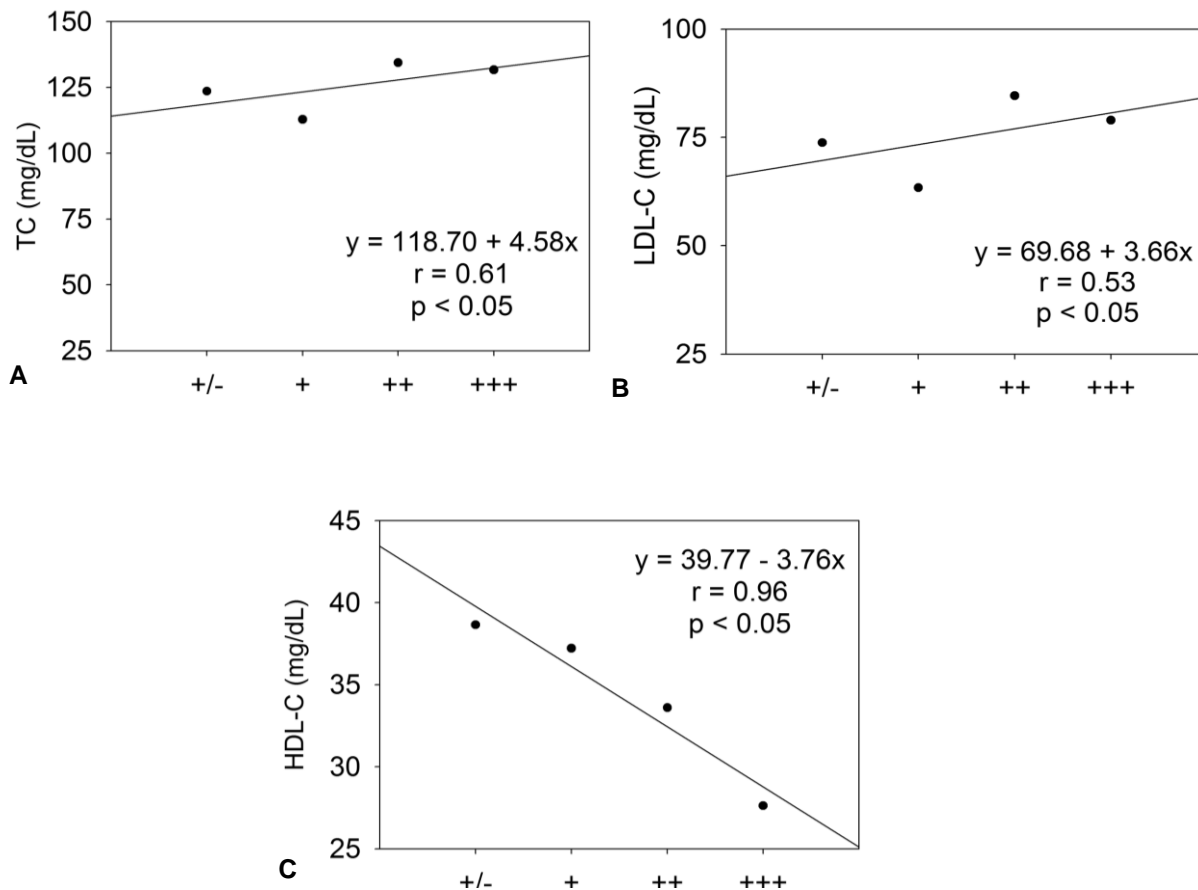
C and LDL-C to corresponding SPE before treatment. We did not observe any correlations between the levels of TC (Figure 2A) and LDL-C (Figure 2B) and SPE. However, we found a strong negative correlation ( $r = -0.96$ ,  $p < 0.05$ ) between HDL-C and SPE (Figure 2C).

We calculated atherogenic indices in order to determine whether TB treatment affects these predictors of disease associated with dyslipidemia in TB patients. The results are summarized in Table 1. TC/HDL-C and LDL-C/HDL-C in TB patients were not significantly different from those in normal subjects. However, indices TC/HDL-C ( $p < 0.001$ ) and LDL-C/HDL-C ( $p < 0.001$ ) declined significantly after six months of treatment compared to controls and to patients prior to treatment. Log (TG/HDL-C) is another index referred to as the atherogenic index of plasma and reflects the size of circulating LDL (Dobiasova and Frohlich, 2001). Log (TG/HDL-C) was significantly increased ( $p < 0.002$ ) in TB patients before treatment compared to controls and then fell significantly ( $p < 0.001$ ) after 6 months of treatment to levels lower than those in control subjects. We compared atherogenic indices with SPE in patients before treatment. TC/HDL-C

ratio increased significantly ( $p < 0.01$ ) in patients in whom SPE was the highest (Figure 3A). Log (TG/HDL-C) increased with the smear positivity to reach its maximum level ( $p < 0.05$ ) in patients with the highest SPE (Figure 3B). The index LDL-C/HDL-C did not vary significantly with increasing SPE (Figure 3C).

## DISCUSSION

This study has shown that the level of TC was significantly lower in patients with TB. Similar results were reported elsewhere (Deniz et al., 2006 and 2007; Perez-Guzman et al., 2005). An adequate level of cholesterol is necessary for the proper functioning of the immune system against infection (Heiniger and Marshall, 1982). Our results support the observation that the level of circulating TC decreases in severe infections (Gonzales and Sande, 2000). Perez-Guzman et al. (2005) have shown that a cholesterol-rich diet accelerates bacteriologic sterilization in patients with TB. We showed here that treatment of TB during six months



**Figure 2.** Correlations between smear positivity extent scores and lipid parameters. The values of SPE scores were correlated to the levels of TC (A), LDL-C (B) and HDL-C (C) in tuberculosis patients before the beginning of anti-tuberculosis treatment. (+/-, n = 15; +, n = 16; ++, n = 34; +++, n = 18).

**Table 1.** Atherogenic indices variation in TB patients.

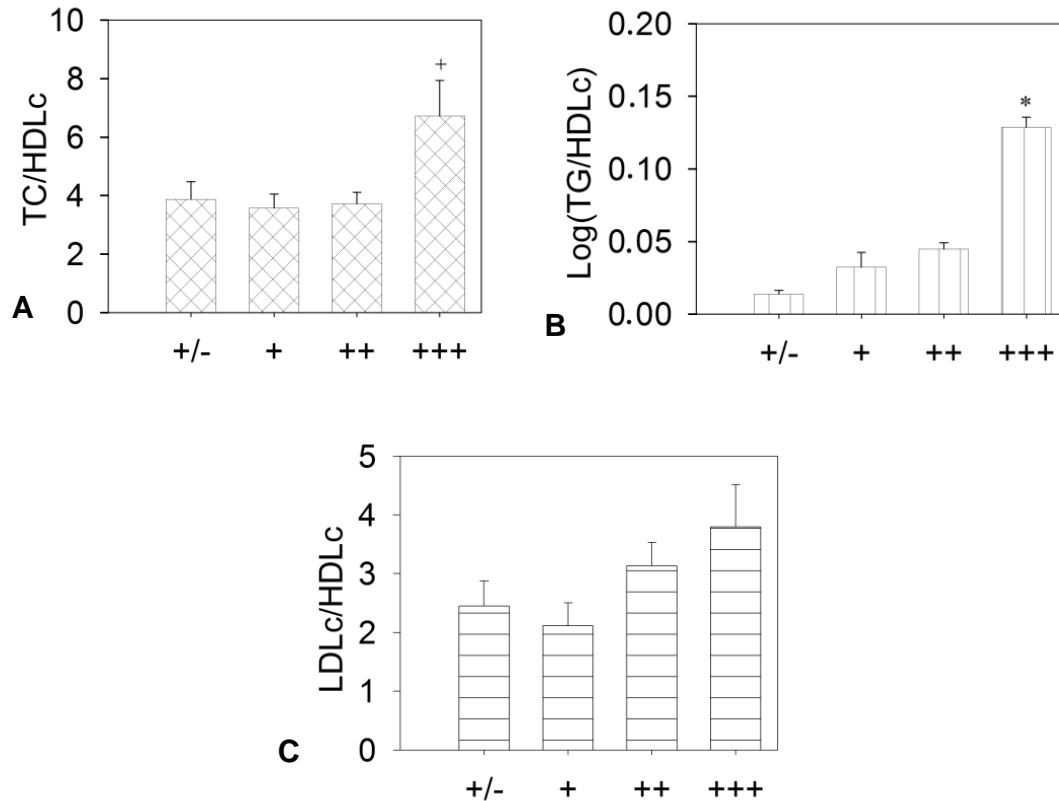
Atherogenic index	Controls (n=100)	Pre-T (n=83)	Post-T (n=83)
TC/HDL-C	4.16 ± 0.18	4.55 ± 0.28	2.78 ± 0.11 <sup>a,a</sup>
LDL-C/HDL-C	2.77 ± 0.16	2.89 ± 0.23	1.49 ± 0.10 <sup>a,a</sup>
Log (TG/HDL-C)	0.19 ± 0.03	0.41 ± 0.04 <sup>d</sup>	0.11 ± 0.03 <sup>b,a</sup>

Atherogenic indices were determined in TB patients pre- and post-treatment and in controls. The values are the mean ± SEM of individual index in 100 control participants and 83 TB patients prior to treatment (Pre-T) and post-treatment (Post-T). (<sup>a</sup>p<0.001 Post-T vs Control or Pre-T; <sup>b</sup>p<0.05 Post-T vs Control; <sup>d</sup>p<0.002 Pre-T vs control).

under a normal diet significantly raises the TC level. This indicates that hypocholesterolemia can be seen as a consequence of TB disease although it cannot be excluded as also being a factor contributing to the development of pulmonary TB (Pérez-Guzmán et al., 2005). We have shown that there is no correlation between the bacillary load and TC variations. This suggests that the use of host cholesterol by MTB (Van der Geize et al., 2007) contributes little to the lower TC level in TB patients.

We showed that HDL-C level decreased significantly in

non-treated TB patients compared to controls, and increased significantly after treatment above the control levels. Our results also showed that HDL-C decrease was strongly correlated with SPE. In general, HDL-C catabolism increases during inflammation. Response to inflammation during the acute phase of TB is characterized by an over expression of proteins such as phospholipase A2 and circulating amyloid A (Tietge et al., 2002) which stimulates HDL-C catabolism (Deniz et al., 2006). It is therefore expected that the levels of HDL-C increase after successful treatment in TB patients as



**Figure 3.** Smear positivity extent variation with atherogenic indices. Changes in atherogenic indices are represented according to the results of SPE scores in TB patients before treatment. The results are presented as mean  $\pm$  SEM of atherogenic indices value within SPE scores groups. (+ $p$ <0.01, \* $p$ <0.05), (+/-,  $n$  = 15; +,  $n$  = 16; ++,  $n$  = 34; +++,  $n$  = 18).

shown by our results. LDL-C level decreased significantly in non-treated TB patients, but was not affected by the treatment. This suggests that the mechanism underlying LDL-C implication in MTB infection is different from HDL-C. Oxidation followed by removal of LDL-C is reported during infection in Hamsters (Memon et al., 2000) but it is not known if this is the case in humans.

Atherosclerosis and cardiovascular disease are common in clinical situations where dyslipidemia is present. We estimated the atherogenic indices TC/HDL-C, LDL-C/HDL-C and log (TG/HDL-C) in order to determine whether TB and/or its treatment represent cardiovascular diseases risks. Our results showed that the atherogenic index of plasma log (TG/HDL-C) was significantly increased in TB patients without reaching the critical level synonymous of atherogenic risk (Maron, 2000). We demonstrated that the atherogenic indices TC/HDL, LDL/HDL and log (TG/HDL) were significantly increased in patients with the highest SPE. We also showed that TB treatment induced significant reduction in all three atherogenic indices despite elevated total cholesterol. Improvement (decrease) in atherogenic indices reflects the increase in HDL-C after treatment as earlier reported by Deniz et al. (2006).

## Conclusion

In this study, we showed that the recovery from TB is accompanied by normalization of lipid parameters such as cholesterol and HDL-C. Despite the rise of lipid parameter levels in TB treated patients, atherogenic indices were somewhat normal. Additional research is needed to more fully assess the link between TB treatment and levels of total cholesterol and its components in patients with pulmonary TB. It would be interesting to study the effectiveness of cholesterol supplement alone or in combination with antituberculosis therapy in TB patients care.

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