Male gender and duration of anti-tuberculosis treatment are associated with hypocholesterolemia in adult pulmonary tuberculosis patients in Kampala, Uganda

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Abstract

Background: Patients with Pulmonary tuberculosis (PTB) and hypocholesterolemia have an altered immune function, delayed sputum conversion at two months and increased mortality. However, the assessment for dyslipidemias is not often done in our setting.

Methods: A cross-sectional study was conducted among adults at an urban TB clinic in Kampala, Uganda. We included different participants at diagnosis (0), 2, 5, 6 and 8 months of anti-TB treatment. Data was collected from a complete physical examination, a pre-tested structured questionnaire, six-hour fasting lipid profiles and random blood glucose levels.

Results: Of the 323 included participants, 63.5% (205/323) were males and the median age was 30 years, IQR (23-39). The prevalence of hypocholesterolemia was 43.65% (95% CI 38.3-49.2). The participants at diagnosis had the highest hypocholesterolemia prevalence, 57.3%, 95% CI (46.7-67.2); and lowest amongst those completing treatment at 6/8 months, 32.2%, 95% CI (21.6-45.2). Significant factors associated with hypocholesterolemia were: male gender (PR 1.52, 95% CI: 1.13-2.03), and duration of anti-TB treatment (0.88, 95% CI: 0.80-0.98).

Conclusion: Hypocholesterolemia is common among patients with PTB. The risk of hypocholesterolemia increases with being male and reduces with increased duration of treatment. There is a need for further research in lipid abnormalities in TB patients.

Keywords: Hypocholesterolemia, pulmonary tuberculosis, duration of anti-tuberculosis treatment, gender.

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Introduction

Pulmonary Tuberculosis (PTB) is still a major global health problem estimated to cause 10.4 million new cases and 1.8 million related deaths annually. Africa bears about 25% of the world's PTB cases and yet it has the lowest number of health professionals, with the majority of sub-Saharan Africa countries like Uganda having less than 5 physicians per 1000 population. The incidence of PTB in Africa is estimated at 280 per 100,000 compared to the global incidence of 126 per 100000. In comparison, Uganda has an estimated incidence of TB at 202/100000 population. PTB patients often have deranged serum lipid levels, especially low cholesterol levels (hypocholesterolemia) which are an important cause of morbidity. The hypo-
cholesterolemia in PTB patients has been shown to alter the body immunity, and could possibly be associated with delayed sputum conversion and increased mortality especially in circumstances of drug resistance.

Lipid abnormalities in PTB patients are associated with Anti-retroviral therapy (ART) especially Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs), HIV, malnutrition, cavitary disease on the X-ray and older age. However, the characterization of lipid abnormalities in sub-Saharan Africa which has a high prevalence of HIV and malnutrition is not well documented. In Uganda, the WHO and National TB and Leprosy Programme (NTLP) recommend clinical, bacteriological, nutritional assessment and monitoring of TB patients at diagnosis and throughout the course of treatment but this is rarely done and if performed only stops at anthropometric measurements. This paper, therefore, presents the prevalence and factors associated with hypocholesterolemia among adult PTB patients either at diagnosis, 2, 5 and 6 /8 months of anti-TB treatment at an urban out-patient clinic located in Kampala, Uganda.

Methods
Design and setting
This was a cross-sectional study conducted at the Uganda National TB and Leprosy Programme (NTLP) Clinic, Mulago National Referral and Teaching Hospital Complex between February and April 2016. Mulago hospital serves patients from all over Uganda and residents of the surrounding areas in Kampala district. The NTLP clinic serves about 60-80 patients per month of whom a quarter are new patients. The number of patients bacteriologically confirmed each month varies depending on the test used, with about 60-65, 20-28 and 6-12 patients confirmed using Gene Xpert, sputum smear, and solid culture respectively. The clinic provides out-patient and in-patient care to PTB patients of all categories including new, relapse, and continuation phase. Patients are followed up regularly as per national treatment guidelines that is to say at diagnosis (0), 2, 5, 6, 8 months after starting treatment and wherever it is necessary. Patient follow-up clinic visits entail drug refills, consultation, and management of any arising complications including adverse effects according to the national TB program guidelines.

Eligibility criteria
We included all adults (≥18 years) with PTB at either: diagnosis (0) or at 2, 5, 6 /8 months of treatment. Mycobacterium tuberculosis was bacteriologically confirmed either with Gene Xpert, Sputum smear, or culture. For participants at diagnosis, their current diagnostic result was considered. Among those at 2, 5, 6/ 8 months of treatment, their patient charts were retrieved and initial diagnostic result before starting treatment was considered before enrollment into the study. All participants provided signed informed consent before enrolment into the study. We excluded participants taking any lipid-lowering drugs for example statins and fibrates, and those who were very ill with the kanorffsky score less than 20% at the time of enrollment.

Sample size calculation
We estimated a sample size of 427 adults using the Kish Leslie formula, assuming a 50% prevalence rate, 0.05 precision level, and factoring for 10% non-response.

Sampling procedure
The participants were consecutively enrolled with no stratification according to the duration of treatment.

Data collection
Study procedures included a complete physical examination, blood draws for laboratory tests (lipid profile, random blood sugar) and administration of a pre-tested structured questionnaire. Information collected on the questionnaire included socio-demographic characteristics, nutritional status, co-morbid illnesses, socio-economic characteristics, and lifestyle and clinical factors. We drew 4mls of venous blood samples from the antecubital fossa for each participant, 6 hours after the last meal to assess the lipid profile and fasting blood sugar. The samples for lipid profiles were analyzed using COBAS 6000 (Roche Diagnostics Ltd) at Mulago Hospital clinical chemistry laboratory. A Fasting blood sugar was measured on-site using a SOFTSTYLE® glucometer in accordance with standard operating procedures. All participants included in the study had undergone a test for HIV infection following the national HIV serial testing algorithm.

We also took anthropometric measurements such as height and weight. Weight was taken using an adult SECA
digital electronic scales to the nearest 100g. Height was measured to the nearest cm of standing height using a stadiometer. All anthropometric measurement values were means of duplicates. Body Mass Index (BMI) was calculated as weight divided by height in meters squared. The participants recruited at diagnosis had a posteroanterior chest X-ray taken as per national guidelines. For patients enrolled at 2, 5, 6 or 8 months of treatment, their initial chest X-rays at diagnosis were retrieved from the repository and read.

**Data management and analysis plan**

Data were double entered into Epidata version 3.1 and analyzed using STATA version 12.0 (Stata Corp College Station TX, USA). Categorical variables were summarized using proportions while means, median and inter-quartile range were used to summarize continuous variables. The prevalence of hypocholesterolemia was defined as the proportion of individuals with total serum cholesterol levels less than 3.7 mmol/l amongst the total number of participants included in the study. A Diagnosis of diabetes mellitus was made if a participant had a six-hour fasting blood sugar ≥126 mg/dl. We estimated prevalence ratios in bivariate and multivariate analyses using a generalized linear model with a log identity and binomial link. Participants with hypercholesterolemia (total serum cholesterol levels more than 5.7 mmol/l), were excluded from further analysis in assessing for factors associated. Clinical significance and a cut-off P value of ≤ 0.25 during bivariate analyses were used to select variables for multivariate analysis. Two-way product terms were formed among the variables in the model to assess for interaction using the chunk test. We also assessed for confounding by checking for a 10% change in the effect measure.

**Ethical consideration**

Ethical approval was obtained from the Makerere University School of Medicine Research and Ethics Committee (#REC REF 2016-017). All patients provided written informed consent. All study data were kept under lock and key to maintain privacy and confidentiality. We also referred all participants with hypocholesterolemia to clinicians for nutritional counseling.

**Results**

**Description of the study population**

Of the 371 participants screened, 323 were included in the study (Figure 1). Most of the participants were male (63.2%), and Catholics (35.9%). The median age of the participants was 30 years, Inter Quartile Range (23-39), (Table 1).

**Figure 1: The profile of TB patients enrolled in the study at Mulago Hospital TB ward, February –April 2016**

- Total number screened - 371
- Excluded
  - 32 had extra pulmonary TB
  - 2 declined to consent
  - 2 were too ill to complete the interview
- Enrolled 335 participants
- Excluded
  - 12 had missing laboratory results for lipids
- 323 participants were included in the analysis
Table 1. Socio-demographic characteristics of the study participants with PTB at Mulago Hospital, Kampala February –April 2016 (N=323)

| Characteristic       | Number | Percentage (%) |
|----------------------|--------|----------------|
| **Gender**           |        |                |
| Male                 | 205    | 63.5           |
| Female               | 118    | 36.5           |
| **Religion**         |        |                |
| Protestant           | 93     | 28.8           |
| Catholic             | 115    | 35.6           |
| Moslem               | 66     | 20.4           |
| Pentecostal          | 40     | 12.4           |
| Seventh Day Adventist| 7      | 2.2            |
| Others*              | 2      | 0.6            |
| **Distance from clinic** |    |                |
| < 5km                | 144    | 44.6           |
| >=5 km               | 179    | 55.4           |
| **Marital status**   |        |                |
| Married              | 117    | 36.2           |
| /Divorced            | 72     | 22.3           |
| Single               | 134    | 41.5           |
| **Occupation**       |        |                |
| Employed**           | 210    | 65.0           |
| None                 | 113    | 35.0           |
| **Level of education** |      |                |
| None                 | 27     | 8.4            |
| Primary              | 106    | 32.8           |
| Secondary            | 138    | 42.7           |
| Tertiary level       | 52     | 16.1           |

*included those who had no religion, traditionalist
**includes self-employed, salaried employee and peasant farmers
About 64.1% of participants had normal BMI (18.5-24.5), 31.9% were HIV positive and 27.9% had cavities on chest X-ray (Table 2). About 95.2% of the HIV positive participants were currently taking anti-retroviral drugs.

Table 2. Clinical characteristics of the study participants with bacteriologically confirmed TB at diagnosis and during Anti TB treatment at Mulago Hospital Kampala, February – April 2016. (N=323)

| Characteristic                          | Frequency | Percentage |
|----------------------------------------|-----------|------------|
| **Duration of Anti-TB treatment**      |           |            |
| Diagnosis                              | 89        | 27.6       |
| 2 months                               | 93        | 28.8       |
| 5 months                               | 82        | 25.4       |
| 6/8 months                             | 59        | 18.2       |
| **HIV infection status**               |           |            |
| Positive                               | 103       | 31.9       |
| Negative                               | 220       | 68.1       |
| **Confirmation of TB disease**         |           |            |
| Sputum Smear grade                     |           |            |
| AFB 1+                                 | 58        | 17.9       |
| AFB 2+                                 | 52        | 16.1       |
| AFB 3+                                 | 46        | 14.2       |
| Gene Xpert                             | 157       | 48.7       |
| Sputum culture*                        | 10        | 3.1        |
| **Body mass index(BMI)**               |           |            |
| <18.5                                  | 82        | 25.4       |
| 18.5-24.5                              | 207       | 64.1       |
| >24.5                                  | 34        | 10.5       |
| **Chest X-ray**                        |           |            |
| Cavities                               | 89        | 27.9       |
| No cavities**                          | 230       | 72.1       |
| **Diabetes Mellitus**                  |           |            |
| No                                     | 283       | 87.6       |
| Yes                                    | 40        | 12.38      |
| **ART use(n=103)**                     |           |            |
| Currently on ARVs                      | 98        | 95.2       |
| Not on ARVs                            | 5         | 4.8        |
| **Type of ARVs(n=98)**                 |           |            |
| Protease Inhibitors                    | 3         | 3.0        |
| NNRTIs                                 | 95        | 97.0       |
| **Smoking status**                     |           |            |
| ever smoked                            | 54        | 16.7       |
| Never smoked                           | 269       | 83.3       |
| **Alcohol intake**                     |           |            |
| Currently drink                        | 52        | 16.1       |
| Do not drink                           | 271       | 83.9       |
| **Hypertension**                       |           |            |
| No                                     | 287       | 88.9       |
| Yes                                    | 36        | 11.1       |

* had only culture results at entry into the study
** Includes infiltrates, hilar lymphadenopathy, pleural effusions, normal findings, and 4 participants had missing chest x-rays.
Prevalence of hypocholesterolemia and associated factors.

The prevalence of hypocholesterolemia among the study participants was 43.6 % (95 % CI: 38.3-49.2). This was highest amongst those at diagnosis (57.3 %) and lowest (32.2 %) among those at 6/8 months that is to say the end of treatment (Table 3).

Table 3. Proportions of participants with cholesterol abnormalities at diagnosis and during anti-TB treatment among PTB adults attending Mulago Hospital, February-April, 2016

| Total serum cholesterol | Diagnosis (n=89) | 2 months (n=93) | 5 months (n=82) | 6/8 months (n=59) | Total (N=323) |
|-------------------------|-----------------|-----------------|-----------------|------------------|---------------|
| Low levels (<3.7mmol/l), n (proportion, 95 % CI) | 51 (57.3, 46.76-67.22) | 40 (43.0, 33.28-53.32) | 31 (37.8, 27.92-48.8) | 19 (32.2, 21.46-45.23) | 141 (43.65, 38.31-49.15) |
| Normal (3.7-5.7mmol/l), n (proportion, 95 % CI) | 35 (39.33, 29.67-49.89) | 47 (50.5, 40.40-60.63) | 48 (58.5, 47.53-68.75) | 34 (57.6, 44.64-69.64) | 164 (50.8, 45.31-56.22) |
| High (>5.7 mmol/l), n (proportion, 95 % CI) | 3 (3.3,1.07-10.03) | 6 (6.6, 2.91-1.37) | 3 (3.7, 1.17-10.85) | 6 (10.2,4.59-21.02) | 18 (5.6, 5.53-8.69) |

The Bivariate analysis showed that having diabetes mellitus was associated with a 44% increased risk of hypocholesterolemia (PR: 1.44, 95 % CI (1.09, 1.90) compared to those without diabetes mellitus. Being greater than 30 years old increased the risk of having hypocholesterolemia by 9 % (PR: 1.09, 95 % CI (0.86, 1.40) when compared to those less than 30 years. The participants who were HIV positive were 0.86 times less likely (PR: 0.86, 95 % CI (0.65-1.13) to have hypocholesterolemia when compared to their HIV sero-negative counterparts (Table 4). HIV status, gender, diabetes, duration of anti-TB treatment and the presence of cavities were considered for a series of multivariate models based on previous studies, clinical significance and P-value less than 0.25. HIV status, diabetes, and presence of cavities were also considered for assessment of interaction and confounding based on biologic plausibility and previous studies. None of these variables had any significant interaction terms nor meaningful confounding effects. The final model had two covariates (Table 4). Being male was associated with a 52% statistically significant increase in the prevalence of hypocholesterolemia compared to females (PR=1.52 95% CI: (1.13-2.03), P=0.005). Participants who had had 5 months of treatment were 30% less likely to have hypocholesterolemia (PR=0.71 95% CI: (0.51-0.97), P=0.033) when compared to those who were at diagnosis (not yet started treatment). Similarly, participants who were at 6/8 months of treatment were 35% (PR 0.65, 95% CI: (0.44-0.97), P=0.033) less likely to have hypocholesterolemia when compared to those who were at diagnosis.
Table 4. Regression analysis of factors associated with hypocholesterolemia among adults with PTB at Mulago Hospital, February –April 2016. (N=305)

| Characteristic               | Hypcholesterolemia N (%) | Normal levels N (%) | Unadjusted, PR* (95% CI) | Adjusted, PR (95% CI) | P value |
|-----------------------------|--------------------------|---------------------|--------------------------|-----------------------|---------|
| HIV infection status        |                          |                     |                          |                       |         |
| Negative                    | 102 (48.3)               | 109 (51.6)          | 1                        |                       |         |
| Positive                    | 39 (41.5)                | 55 (58.5)           | 0.86 (0.65, 1.13)        |                       |         |
| Gender                      |                          |                     |                          |                       |         |
| Female                      | 37 (33.6)                | 73 (66.4)           | 1                        | 1                     |         |
| Male                        | 104 (53.3)               | 91 (46.7)           | 1.59 (1.18, 2.13)        | 1.52 (1.13, 2.03)     | 0.005   |
| Diabetes mellitus           |                          |                     |                          |                       |         |
| No                          | 117 (43.8)               | 150 (56.2)          | 1                        |                       |         |
| Yes                         | 24 (63.2)                | 14 (36.8)           | 1.44 (1.09, 1.90)        |                       |         |
| Age                         |                          |                     |                          |                       |         |
| <30                         | 66 (44.0)                | 84 (56.0)           | 1                        |                       |         |
| >=30                        | 75 (48.4)                | 80 (51.6)           | 1.09 (0.86, 1.40)        |                       |         |
| Alcohol status              |                          |                     |                          |                       |         |
| No                          | 119 (46.1)               | 139 (53.9)          | 1                        |                       |         |
| Yes                         | 22 (46.8)                | 25 (53.2)           | 0.98 (0.71, 1.37)        |                       |         |
| Smoking status              |                          |                     |                          |                       |         |
| No                          | 117 (45.7)               | 139 (54.3)          | 1                        |                       |         |
| Yes                         | 24 (49.0)                | 25 (51.0)           | 0.93 (0.68, 1.28)        |                       |         |
| Duration of TB treatment    |                          |                     |                          |                       |         |
| Diagnosis                   | 51 (59.3)                | 35 (40.7)           | 1                        | 1                     |         |
|                            |                          |                     |                          |                       |         |
|                            | 2 months                 | 40 (46.0)           | 47 (54.0)                | 0.77 (0.58, 1.033)    | 0.124   |
|                            |                          |                     |                          |                       |         |
|                            | 5 months                 | 31 (39.2)           | 48 (60.8)                | 0.66 (0.48, 0.92)     | 0.033   |
|                            |                          |                     |                          |                       |         |
|                            | 6/ 8 months              | 19 (35.8)           | 34 (64.2)                | 0.60 (0.41, 0.90)     | 0.033   |
| Hypertension                |                          |                     |                          |                       |         |
| No                          | 124 (45.9)               | 146 (54.1)          | 1                        |                       |         |
| Yes                         | 17 (48.6)                | 18 (51.4)           | 1.05 (0.73, 1.52)        |                       |         |
| Chest X-ray                 |                          |                     |                          |                       |         |
| No cavities                 | 102 (46.6)               | 117 (53.4)          | 1                        |                       |         |
| Cavities                    | 36 (43.9)                | 46 (56.1)           | 0.94 (0.71, 1.25)        |                       |         |

*PR: Prevalence Ratio
Discussion

Nearly half of the participants in the study population had hypocholesterolemia. This is among the first studies to document the prevalence of hypocholesterolemia in TB patients in Uganda.

We found a high prevalence of hypocholesterolemia among the study participants with the highest proportion being detected among those at diagnosis and lowest among those who had received 6/8 months of treatment. The high proportions of hypocholesterolemia at diagnosis may be due to inflammation caused by PTB which may worsen as the duration of symptoms and severity of the disease increases\textsuperscript{19,20}. Also, at diagnosis, hypocholesterolemia proportions may be a consequence of the TB disease although it cannot be elucidated whether it is a factor contributing to the development of the active disease\textsuperscript{4}. In our study, participants who were at 5 months of anti-TB treatment had slightly higher proportions of hypocholesterolemia when compared to those who were at 6/8 months of treatment (37.8 % versus 32.2 %) respectively. This is contrary to the expected close proportions of hypocholesterolemia in the two groups. This may be due to the fact that those who were at 6/8 months of treatment received the anti-TB drugs for a longer duration and may have received different types of drug regimens. The lowered hypocholesterolemia proportions among participants at 6/8 months (end of TB treatment) may possibly be explained by better response to treatment with improved appetite and increased nutrient intake especially of cholesterol-rich foods like eggs and fish.

The study results demonstrated that duration of anti TB treatment was associated with hypocholesterolemia. This may be due to the fact that the longer an individual is adherent to TB medications, the more their immunity is improved leading to reduced metabolic disturbances and improved appetite\textsuperscript{21}. This improvement leads to nutritional recovery during the course of anti-TB treatment.

Males were 52% more likely to develop hypocholesterolemia as compared to females. This finding may possibly be attributed to the hormonal differences, reduced immunity and reduced nutrient intake especially fats which may occur differentially among men and women with PTB\textsuperscript{21}. The varying poor nutrient uptake in male PTB patients may probably be mediated by inflammatory markers released during TB disease (especially tumor necrosis factor-alpha) which interacts with human metabolic pathways that lead to anorexia\textsuperscript{22}. Furthermore, males in our study presented with more severe disease (cavities on chest X-ray) possibly leading to a higher predisposition to hypocholesterolemia\textsuperscript{23}.

The strength of this study hinges on the fact that we considered participants at different time points of treatment which gives a broad spectrum of dyslipidemias among TB patients although a cohort study with larger sample size and a homogenous group of study participants followed through time would be the ideal.

The interpretation of findings from this study should be made with caution as it had some limitations. First, we studied different sub-populations at varying time points of TB treatment. There could be underlying low levels of total serum cholesterol and thus the observed increase in mean total serum cholesterol concentrations as the duration of anti TB treatment may differ if the same individuals are followed up over time during care. Further misclassification bias was minimized by training of the research assistants, pre-testing the questionnaire before data collection, calibration of the weighing scale and stadiometer daily.

It is also important to note that this being a cross-sectional study, causality could not be assessed. However, we recruited participants that had taken treatment at varying time points to mimic a cohort study in temporality. Selection bias which may have arisen from referral bias since Mulago hospital is a tertiary referral center was minimized by recruiting participants from the catchment area of the population. The findings from this study are generalizable to adults with PTB in African settings seeking care at tertiary level health centers.

Conclusion

The overall prevalence of hypocholesterolemia among adult PTB patients presenting at Mulago NTLP clinic in Kampala was high. Being male and duration of anti-Tuberculosis treatment were significantly associated with hypocholesterolemia. The findings from our study may need further evaluation in longitudinal studies employing
larger sample sizes to determine the effect of low total serum cholesterol levels on the parameters used to assess response to anti-TB treatment like sputum conversion, cure, and mortality.

Conflict of interest
All authors declare no conflict of interest.

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