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Biochemical, Hematological and Immunological Indices Among MDR-TB on Second Line Anti TB and MDR-TB-HIV Co-Infected Patients on Second Line Anti TB and ARV Drugs for a Six -Month Treatment

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Abstract

Background
Tuberculosis (TB) is a major global health problem, and anti-tuberculosis drugs can impose a hazardous effect on the patient's liver, leading to hepatotoxicity, and are a major problem in tuberculosis patients. This study aimed to determine hematological, biochemical and Immunological indices in patients with Multi Drug Resistant Tuberculosis TB (MDR-TB) with or without HIV infection, undergoing treatment with second-line Anti TB plus ARV's or second-line anti TB.

Objective
To assess the hematological, biochemical and immunological indices of patients with or without HIV-MDR-TB at the Kibong'oto Infectious Disease Hospital over a six-month treatment.

Methodology
This was a descriptive study. A total of 198 MDR-TB patients aged between 18 and 50 years or above, were collected from Kibong'oto Infectious Disease Hospital from April 2017 to October 2019 involving patients who were on treatment for MDR-TB and MDR-TB/HIV. Selected hematological, biochemical and immunological indices were determined at the onset of treatment from both groups of patients, and then same parameters determined after six months of treatment with second line anti-TB and second line anti-TB plus ARVS for both groups, respectively. Data were analyzed using paired samples t-test by Stata version 13.

Results
There was an elevated level of AST and ALT in MDR-TB with HIV infected patients compared to the MDR-TB group. Liver enzyme activities (ALT, AST, and ALP) were increased significantly after six months of treatment in MDR-TB/HIV co-infection compared to MDR-TB ($p<0.01$). While Hb decreased significantly over the six months in the MDR-TB-HIV co-infected patients, WBC increased significantly during the same group and treatment period ($p<0.01$).

Conclusion
Our findings imply that a combination of second-line anti TB with ARVs confers more toxicity to the liver and induce more hematological effects than anti-TB use alone. Our findings underscore the need for close monitoring during MDR-TB especially in co-infection with HIV for optimal management.

Keywords: *Tuberculosis, MDR-TB, HIV, Hematology, Biochemical.*

Introduction

Multidrug-resistant tuberculosis (MDR-TB), defined as resistance to both isoniazid and rifampicin, is a growing public health problem in resource-poor regions where adequate diagnosis and treatment are often unavailable. Treatment of MDR-TB is more complicated compared to susceptible TB since it requires multiple second anti TB drugs which are administered over a period of up to two years (1). An important concern in MDR-TB regimens is their potential for causing undesirable effects (2). One of the main frequently reported undesirable effects associated with first and second-line anti-TB treatment is the development of hepatotoxicity due to the toxic nature of these drugs (3). Substantial morbidity and mortality are caused by anti-tuberculous drug-induced hepatotoxicity (ATDH) due to its effect to diminish cure, thus hepatotoxicity can be deadly if not recognized early and when therapy is not interrupted in time (4). This scenario is worse in settings where high co-infection with the human immunodeficiency virus (HIV) are reported, whereby there is a high potential of overlap and additive drug toxicity of anti-retroviral treatment (ART) (5). Adverse drug reactions (ADRs) have been reported in many studies that involved MDR-TB patients with varying profiles between different settings, populations and individuals (6). However, many of these studies provide limited insight into the comparative evolution of hematological, biochemical and immunological parameters among MDR-TB and MDR-TB-HIV co-infected patients undergoing treatment.

Hematological, biochemical, and immunological parameters have many causative factors including medications, infections, and malnutrition. Zidovudine (AZT) and Stavudine (d4T) for example, can be toxic to the bone marrow. As a result, AZT use is known to cause anemia (lowered red blood cell levels) and neutropenia (lowered neutrophil or white blood cell counts), some side effects of AZT can be serious (7). *Cryptosporidium parvum, Isospora belli, Cyclospora cyatinis*, and *Microsporidium* species are among the known opportunistic protozoa associated with diarrhea in HIV/AIDS patients with consequent nutritional anemia (8,9).

Despite the known causes of changes in hematological, biochemical and immunological parameters among MDR-TB patients, the extent to which MDR-TB medications induce these changes in comparison to MDR-TB-HIV medications is largely unstudied. Because nutritional factors, concurrent infections, and other environmental factors affect MDR-TB and MDR-TB-HIV co infected patients more or less equally in a given community, a comparative

analysis of these parameters undergoing different treatment regimens may provide insights into the differential effects of medications administered to MDR-TB and MDR-TB-HIV patients. This study, therefore, was carried out to determine hematological, biochemical, and immunological (CD4+ counts) changes in MDR-TB and MDR-TB/HIV co-infected patients who were undergoing treatment with the standard protocol of anti TB and anti-TB plus ARVs.

Materials and Methods

Study design

This was a descriptive cross-sectional study, conducted from April 2017 to October 2019.

Study Area

This study was conducted at Kibong'oto Infectious Diseases Hospital (KIDH) in Northern Tanzania, equipped with a 340-bed capacity in addition to separate facilities for treatment of drug-susceptible TB. The hospital provides long-term hospitalization and provides all drugs required for the treatment of MDR-TB. KIDH receives all MDR-TB patients for treatment from all parts of Tanzania as referred cases, it also houses a major HIV care and treatment support Centre (CTC).

Sample size and Sampling Technique

A time delimit consecutive sampling technique was employed to recruit the study subjects. All confirmed MDR-TB cases by Gene Xpert and line probe assay that were referred to KIDH during the study period of April 2017 to October 2019 were enrolled in this study consecutively. Patients fulfilling the inclusion criteria were serially enrolled until the sample size was reached. A total of 198 confirmed MDR-TB patients were enrolled. Tanzania boasts of a rich cultural diversity, with over 120 indigenous ethnic groups and over 100 different dialects spoken across the country. Each of these ethnic groups has their unique language, social structure, and culture, with notable similarities between them. Ethnicity in Tanzania is also a product of the geographic area, as each tribe is concentrated on various parts of the country (10). It is exceedingly likely that this group of people have diverse levels of exposure to the disease and consequently inconsistency in occurrence.

Inclusion Criteria

Patients included in the study were those confirmed to be MDR-TB and MDR-TB/HIV co infected cases, and who have consented to participate in the study and aged 18 years and above.

Exclusion Criteria

Patients with the following conditions were excluded from the study; Inadequate medical records to allow complete analysis, patients with any type of hepatitis complications at the time of PTB diagnosis, hemoglobinopathy, patients with a clinical history of chronic renal failure, pregnancy at the time of PTB diagnosis, neoplastic disease and collagen vascular disease, sputum sample taken after the initiation of treatment and Malaria and worm-infested PTB diagnosis. Patients who met all the inclusion criteria but not Tanzanian residents were excluded from the study.

Criteria for the Confirmation of Drug-induced Hepatotoxicity among Tuberculosis Patients

The verification of drug-induced hepatotoxicity among MDR-TB and MDR-TB/HIV co-infected patients was based on biochemical, hematological and immunological criteria of the laboratory results. The biochemical criteria included the increased level of liver enzymes in response to anti-TB medication and included the rise in 3-5 fold times the upper limit of ALT, and AST. (11)

Data Collection Procedures and Tools

Demographic Data

Social demographic characteristics such as age, sex, marital status, residence, occupation, and clinical data were gathered using a pre-tested and structured questionnaire. A clinician, trained clinical nurse, and laboratory technologist working at the KIDH clinic collected the demographic characteristics of the patients.

Analysis of Samples

Determination of Hematological, Biochemical and Immunological parameters

Hematological, biochemical and Immunological indices were analyzed on admission before starting second-line anti TB for both, MDR-TB and MDR-TB/HIV co-infected participants and after six months being on anti-TB second-line treatment for MDR-TB and anti-TB plus ARVs

for MDR-TB/HIV co-infected individuals. For hematological markers, a 10 ml volume of venous blood was obtained from each participant confirmed to be MDR-TB patient; 2ml was dispensed into EDTA containers for Hemoglobin (Hb), white blood cell count (WBC) and platelet (Plt) count. Hematological counts were performed using an automated blood analyzer Sysmex Kx-21 S/N A8893 hematology analyzer (Sysmex Corporation, Kobe, Japan). Four ml of the remaining blood was dispensed into a plastic bottle containing sodium citrate (0.11 molar solution) to give a final blood/citrate ratio of 9:1. Immunological parameter (CD4+ cell count) was performed using Partec® Cytoflow Counter (PCC) (Munster, Germany), as described in the PCC manual (12). Briefly, to a 20 µL of CD4+ monoclonal antibody (MAb), already pipette into a Partec test tube, 20 µL of well-mixed whole EDTA blood collected within 6 hours was added, properly mixed by gentle tapping and incubated in the dark for 15 minutes. The mixture was agitated during incubation every 5 minutes. Afterward, 800 µL of CD4+ buffer was added and mixed thoroughly before CD4+ cell counting by an automated counter.

For biochemical indices, the remaining 3 ml of blood was allowed to clot in a plain vacutainer tube and serum was separated by using a Pasteur pipette immediately and used for Aspartate Aminotransferase Aspartate (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), total bilirubin and direct bilirubin, random blood sugar (RBS). The analysis was carried out using Biochemistry auto analyser-Model, Hitachi - Cobas C311 luems S/N0 46-02 (Cobas Roche GmbH-D68298 Mannheim, Germany) and reagent kits from Roche GmbH-D68298 (Mannheim).

Quality Control

Serum/Plasma

For quality control, biochemical analyzer and reagents were checked with quality control kits of known values for various constituents for each test. Each run and each assay on the Cobas ® C311 System was validated through our trusted control concept. Before using the Cobas instrument, the machine was calibrated using the manufacturer's calibration procedure.

HIV Testing

HIV testing was performed using the current National algorithm for HIV serodiagnosis. This involved the use of three rapid diagnostic kits, according to their manufacturer's instructions.

Briefly, each candidate's serum was screened for the presence of HIV antibodies using Determine (Abbott Laboratories, Tokyo, Japan) and Unigold HIV (Trinity Biotech Plc Bray, Co. Wicklow, Ireland). When both kits showed positivity, the patient was regarded as HIV positive. One ml of blood was dispensed into sterile EDTA tubes. Then plasma samples from each of the participants were tested using Determine and Unigold rapid test kits. The tests used have different antigenic compositions.

Statistical Analysis

The analysis was performed using STATA version 13 (Stata Corp, College Station, TX, USA). Descriptive data were presented as frequencies and respective percentages. Comparison of mean biochemical, haematological and immunological values between two groups, one on second-line anti-MDR-TB-HIV and the other on second-line anti-MDR-TB medications. Statistical analysis was done by using the paired student's t-test to determine differences in haematological, biochemical and immunological values at the time of admission and six months after treatment. Statistical significance was decided using a p-value of ≤ 0.05.

Ethical approval and consent to participate

The current investigation followed the tenets of the Declaration of Helsinki and Ethical clearance was obtained from KCMUCo's College Research and Ethical Review Committee (CRERC). Permission from KIDH authority to conduct the study was sought. Patients' privacy and confidentiality were strictly observed throughout the study according to Good clinical practice (GCP) requirements. Before the investigations were performed, written informed consent was obtained from all study participants after the explanation was given regarding the study aims, procedures, risks and benefits.

Results

Baseline Characteristics of Study Patients

This study included 198 patients who were confirmed as MDR-TB cases and were enrolled to evaluate different hematological, some biochemical and immunological markers. Of 198 MDR-TB patients enrolled in this study, 92 (46.5%) were identified as MDR-TB/HIV co-infected and 106 (53.5%) were MDR-TB without HIV infection. The mean age of the patients was 37.5±12.5 years with the age categories of 20–50^{+} years. Most of the patients had

primary school education 158 (79.8%) as their highest level of education. Among 198 patients 122 (61.6%) were male and 105 (53.03%) were single. Of the 198 enrolled patients, 194(97.98%) were aged between 20-50 years. The majority of patients had primary school education.

Table 1: Hematological, Biochemical and Immunological Indices among MDR-TB Patients on a Six Months Treatment Period with Second Line Anti TB (n=106)

Variable	Mean ± SD	95% CI (LL, UL)	p-value
Hb (g/dl)	0.5±1.2	0.3 - 0.7	0.0
WBC Count (×10^9L)	-0.7±1.5	-0.9 – (-0.4)	0.0
Plt Count (×10 ^{9}L	5.9±99.8	-13.3 - 25.2	0.5
CD4+ count cells /µL	-83.5±97.4	-102.3 – (-64.8)	0.0
RBS mmol/L	-0.6±1.6	-0.9 – (-0.3)	0.0
AST(IU/L)	-4.8±6.0	-6.0 – (-3.6)	0.0
ALT(IU/L)	-4.0±7.7	-5.4 – (-2.4)	0.0
ALP(IU/L)	-2.8.2	-4.2 – (-1.1)	0.0
TB mmol/ L	-1.1±1.9	-1.4 – (-0.7)	0.0
DB	-0.3±0.8	-0.4 – (-0.1)	0.0
TC mmol/ L	0.8±1.1	0.6 - 1.0	0.0

Legend: LL: *Lower Limit;* ***UL:*** *Upper Limit;* ***WBC****: White Blood Cell,* ***Cd4+****: Cluster of differentiation 4;* ***Hb****: Hemoglobin;* ***Plt****: Platelet Count;* ***AST****: Aspartate Aminotransferase;* ***ALT:*** *Alanine Aminotransferase;* ***ALP:*** *Alkaline Phosphatase;* ***TB****: Total Bilirubin;* ***DB:*** *Direct Bilirubin;* ***RBS:*** *Random Blood Sugar and* ***TC****: Total Cholesterol*

Hematological, Biochemical and Immunological Indices among MDR-TB Patients on a Six Months Treatment Period with Second Line Anti TB

The WBC, Hb, PLT counts, CD4+, RBS, ALT, AST, DB and TB, ALP, and TC of MDR-TB patients were investigated before and after using drugs. After using the second line anti TB drugs, CD4+ count mean was higher by 83.6 after six months of treatment, which shows a significant increase (p=0.0) (Table 1). The present study showed that there was a significant increase (p=0.0) of liver enzymes six months after being treated by anti TB and it was higher by 4.764 in AST and ALT was higher by 3.9 compared to the reading at admission. ALP was higher (2.7), compared to the reading at admission (p 0.0) (Table 1). In the MDR-TB group, the mean for Plt count was not significant (p= 0.5) even after six months of treatment.

Table 2: Hematological, Biochemical and Immunological Indices among MDR-TB/HIV Co-Infected Patients on a Six-Month Treatment Period with Second Line Anti TB and ARVs (n=92)

Variable	Mean ± SD	95% CI (LL, UL)	p-value
Hb(g/dl)	1.0±1.2	0.8 - 1.3	0.0
WBC Count (×10 ^{9}L	-2.0±2.1	-2.4 – (-1.5)	0.0
Plt Count (×10 ^{9}L	-81.6±54.9	-92.9 – (-70.2)	0.0
CD4+ count cells /µL	-132.3±93.6	-151.7 – (-112.9)	0.0
RBS mmol/ L	-1.3±1.3	-1.6 – (-1.0)	0.0
AST (IU/L)	-28.0±54.7	-39.3 – (-16.7)	0.0
ALT (IU/L)	-30.8±55.2	-42.3 – (-19.4)	0.0
ALP (IU/L)	-40.8±85.1	-58.4 – (-23.2)	0.0
TB mmol/ L	-17.9±42.1	-26.6 – (-9.1)	0.0
DB mmol/ L	-3.7±9.0	-5.6 – (-1.9)	0.0
TC mmol/ L	0.3±0.9	0.1-0.5	0.0

Legend: LL: *Lower Limit;* ***UL:*** *Upper Limit;* ***WBC****: White Blood Cell;* ***CD4+****: Cluster of differentiation 4;* ***Hb****: Hemoglobin;* ***PLT****: Platelet Count;* ***AST****: Aspartate Aminotransferase;* ***ALT:*** *Alanine Aminotransferase;* ***ALP:*** *Alkaline Phosphatase;* ***TB****: Total Bilirubin;* ***DB:*** *Direct Bilirubin;* ***RBS:*** *Random Blood Sugar and* ***TC****: Total Cholesterol*

Hematological, Biochemical and Immunological Indices among MDR-TB/HIV Co-infected Patients on a Six-Month Treatment Period with Second Line Anti TB and ARVs

There are significant changes in biochemical, hematological and immunological parameters of tuberculosis patients before and after the treatment course (Table 2). The mean Hb was lower by (1.038) after six months treatment using second-line anti TB drugs, while the WBC count was higher (-2.0) compared with that on admission and biochemical parameters (ALAT, ASAT, ALP, TB, and DB) in the study groups were significantly increased in patients after using the drugs, in comparison to before using the drugs ($p<0.0$) (Table 2). Table 2 shows that in the MDR-TB/HIV co-infected group of patients, the mean of Plt count, CD4+ count, AST, ALT, and ALP were significantly increased ($p=0.0$) after using anti TB drugs plus ARVs drugs. There readings were higher; 81.6, 132.3, 28.0, 30.8, and 40.8, respectively after six months compared with the reading at admission before using drugs (Table 2). The

mean of Hb, WBC, RBS and TC though it seems were significant were not so much higher compared to the rest, they were slightly different to the mean during admission.

Discussion

In this study, age groups of (20–40 years) with a mean age of 37.5 ± 12.5 years were commonly affected compared to other age groups in the study. Our study findings were similar to findings reported from different studies (13,14). TB is primarily a disease of the adults who make the productive group. Thus, TB affects the economically productive population, which bears a direct impact on individuals, families, communities, and the country at large. Due to this, it is urgent that public policy response with special reference to education and emergency care of patients are deployed.

The risk of acquiring TB infection increases with age from infancy to early adult life. Although a clear explanation is lacking, it is thought that probably it is due to the increased number and higher frequency of contacts in this group. Our study showed that MDR-TB and MDR-TB/HIV co-infection were more frequent in male patients older than 20 years and that coincided with the observed increase in the male to female ratio. In this study, HIV infections were seen in middle-aged men. On a gender basis, MDR-TB and MDR-TB/HIV co-infected patients' hepatotoxicity in this study were mainly recorded more in males than the female population. Our results reflect that the incidence is higher in males which correlates to findings from other studies (15). Male predominance in this study is due to their habit of traveling more frequently; have more social contacts; spend more time in settings that may be conducive to transmission, such as bars; and engage in professions associated with a higher risk for tuberculosis, such as mining (16). Also As a general rule, females exhibit more-robust immune responses to antigenic challenges, such as infection and vaccination, than males (17).

This study revealed that, MDR-TB/HIV co-infected patients had a significant elevation of mean values of platelets and CD4+ count compared to the MDR-TB group of patients. Platelets are effector cells that play an important role in the inflammatory and immunological response and have the capacity to release cytokines and chemokines, thus acting as an immune regulator. (19). Therefore, the direct relationship between platelet and CD4+ count similar trend between Plt and CD4+ is logical because when there is an immune response at PTB and HIV infection, platelets and CD4+ tend to increase in response to treatment,

indicating immune response action. Also, the literature shows that the hematopoietic system is responding to treatment. Both myeloid and lymphoid cell lines and plasma components are the cause of these outcomes in our study (20).

In our study, all enrolled patients were tested for liver enzymes levels - the ALT, AST, and ALP. It was found that the level of the liver enzymes was elevated from its normal range, consequently, cause damage to the liver. By increasing all of the three enzymes, ALT, AST, and ALP indicate that Hematological, biochemical and immunological indices in MDR-TB and MDR-TB/HIV co-infection are common and may be valuables to detect disease. This finding compares to findings from another study which reported that elevation in serum enzyme ALT and AST levels indicate cellular leakage and liver cell membrane functional integrity loss, hence causing toxicity or failure of the human liver during treatment (20). Hematological, biochemical, and immunological indices were generally more significantly elevated after 6 months of treatment with second-line anti-TB plus ARVs in the MDR-TB/HIV infected patients compared to MDR-TB patients without HIV infection who used second line anti TB only. This implies and signifies that a combination of second-line anti TB with ARVs confers more toxicity to the liver and affects hematological parameters. Our findings underscore the need for close monitoring during MDR-TB and MDR-TB-HIV management.
Our study had a small sample size, prospective studies with larger populations and over longer periods is necessary to confirm the differential effects of the two regimens among MDR-TB and MDR-TB/HIV co-infected patients

Conclusion

There was an elevated level of AST and ALT in MDR-TB with HIV infected patients compared to the MDR-TB patients' group. Liver enzyme activities (ALT, AST, and ALP) were increased significantly after six months of treatment in MDR-TB/HIV co infection compared to MDR-TB without HIV infection. This is due to the use of anti TB and ARVs.

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Competing Interest

The authors declare that they have no competing interests.

Author Contributions

JCM, OSM conceived and designed the experiments. JCM performed the experiments. JCM, OSM analyzed the data. JCM contributed reagents/materials/analysis tools. JCM, JOC, BMN, HHS. wrote the paper.

Abbreviations

ATDH	Anti-Tuberculous Drug-Induced Hepatotoxicity
ADRs	Adverse Drug Reactions
ALT	Alanine Aminotransferase
ART	Anti-Retroviral Treatment
AST	Aspartate Aminotransferase
d4T	Stavudine (Dideoxy-4-thymidine)
AZT	Zidovudine (Azidothymidine)
EDTA	Ethylenediamine Tetraacetic Acid
SD	Standard Deviation
MDR-TB	Multi Drug Resistant Tuberculosis

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