ORIGINAL ARTICLE

Multidrug Resistant Tuberculosis in HIV Positive Patients and its Effect on IL-10 and IL-12

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ABSTRACT

Background: *Multidrug resistant tuberculosis and HIV have profound effects on the immune system, which may negatively affect viral replication and activate it, control how T cell activation is done. Dysregulation of the cytokine production required to combat HIV and MDR-TB could ultimately have a significant impact on the treatment's outcomes and in the progression of MDR-TB and HIV infection.* ***Objective****: is evaluation of plasma level of IL-10 and IL-12 in multidrug resistant tuberculosis (MDR-TB) co infected with HIV and compare with MDR-TB monoinfected patients.* ***Methodology:*** *Using a case-control design, sought to find plasma concentration of anti-inflammatory cytokine (IL-10) and pro-inflammatory (IL-12) cytokine in MDR-TB/HIV co-infected patients and MDR-TB monoinfected patients. This study determined the differences in the quantity of IL-10 and IL-12 cytokines in MDR-TB/HIV co-infected patients and MDR-TB monoinfected patients. IL-10 and IL-12 plasma levels were assessed in 130 participants (comprising MDRTB/ HIV co-infected treatment naïve patients, MDR-TB/HIV co-infected treatment experienced patients, MDR-TB monoinfected treatment naïve patients, MDR-TB experienced monoinfected treatment patients, DS-TB/HIV patients who have had co-infection treatment and control groups using ELISA.* ***Results:*** *MDR-TB/HIV co-infected individuals did not differ significantly from MDR-TB patients in any way, both individuals who have received treatment before and those who have not ($P > 0.05$) in IL-10 and IL-12 concentrations. MDR TB/HIV co-infected patients and MDR-TB-co-infected showed comparable concentration of IL-10 and IL-12 cytokine patterns. Antiretroviral therapy and anti-TB therapy, however, result in a non-significant reduction in concentrations of IL-10 and IL-12. These cytokines can serve as a signal for early MDR-TB and HIV co-infection detection.* ***Conclusion:*** *Comparing apparently healthy controls to MDR-TB/HIV co-infected treatment-naïve patients, MDR-TB monoinfected treatment-naïve patients, MDR-TB/HIV co-infected treatment-experienced patients and MDR-TB monoinfected treatment-experienced patients, apparently healthy controls had considerably increased amount of IL-10 and IL-12. MDR-TB/HIV co-infected patients and MDR-TB monoinfected patients display similar plasma cytokine pattern.*

INTRODUCTION

Multidrug resistant tuberculosis (MDR-TB) is TB that is resistant to the two first line effective anti-TB drugs; Rifampicin and Isoniazid [1]. The MDR-TB is mismanagement of TB treatment and person to person transmission[2]. The host protective defense against intracellular antigens is induced by cell mediated immunity[3]. The immune response is negatively impacted by both HIV and TB, a disruption of the normal balance of cytokines is characterized by the cytokine network's ability to function. The imbalance of cytokines generated by macrophages and T cells plays an important role in determining the potency of the defense system's reaction to certain pathogens [4-6]. When cytokine secretion is too high and low than others, HIV infection has an impact on how well the immune system works and how the illness, boosting or reducing viral replication[4,5]. The deliberate prevention of persistent Mycobacterium can also worsen the immunological reaction brought on by HIV-1 infection with TB. Cytokines are soluble protein or glycoprotein molecules that are released by variety of cells and act as a bridge for immune system cell communication[7]. Cytokines act as intracellular-signaling proteins, they regulate local and systematic immune and inflammatory response as well as hematopoiesis and many other biological activity [7]. The proinflammatory cytokines are produced

by T cells [8]. To control and eliminate intracellular pathogens, including viruses, pro-inflammatory cytokines typically govern immune cell proliferation, activation, differentiation, and homing to the sites of infection [8]. Immunoregulatory molecules that regulate the pro-inflammatory cytokine response are involved in the anti-inflammatory cytokines, the interleukin (IL)-1 receptor antagonist, IL-4, IL-10, IL-11, and IL-13 are important anti-inflammatory cytokines [9, 10]. It prevents Th-1 cells from producing cytokines, which causes immune responses to switch to a Th-2 type 10. 6 IL-12 is produced by T cells very early during infections or immune response, and exerts important proinflammatory functions and enhancement of innate resistance by activating natural killer cells and, through IFN-γ induction, phagocytic cells [11]. Cytokines are regulatory peptides that are synthesized by different cell types in the body, and often have pleiotropic regulatory effects on hemopoietic, lymphoid, and inflammatory cells [12, 13]. As a result, it may be crucial to evaluate the cytokines produced during MDR-TB and HIV co-infection during antiretroviral therapy in order to forecast how the disease will progress and how well the treatment will work. Clinicians can use the measurement of cytokine concentrations as prognostic indicators to track immune system remission or suppression [13]. The generation of different cytokines at the plasma level in MDR-TB/HIV co-infection is now still not properly understood. Almost minimal research has been done on cytokine status in MDR-TB/HIV patients co-infection before and during the antiretroviral and ant-itubercular medications therapy [12, 13]. However, it is critical to precisely establish how severely perturbed the manufacturing of a variety of substances is in order to understand the etiology of co-infection and develop appropriate treatments cytokines in co-infected individuals. The objective of this work was to evaluate the expression of IL-10 and IL-12 in individuals with MDR-TB and HIV co-infection who had just received a diagnosis, both before and during antiretroviral/antituberculosis therapy to evaluate the disease's progression and the effectiveness of treatment.

METHODOLOGY

Study Area

This study was carried out at Infectious Diseases Hospital and Aminu Kano Teaching Hospital Kano, Nigeria.

Study Subjects

- **Group 1:** a total of 30MDR-TB/HIVco-infected patients were divided into group1a (treatment naive) and group1b (treatment experienced) 15patientseach.
- **Group 2:** a total of 40MDR-TB monoinfected patients were divided into group 2a (treatment naive) and group 2b (treatment experienced) 20 patients each.
- **Group 3:**a total of 20 drug susceptible tuberculosis (DS-TB) co-infected with HIV treatment-experienced patients.
- **Group c:** 40 individuals

Ethical Approval

The ethical approval for this research work was obtained from the Ethics and Research committee of AKTH and from the Kano state ministry of health.

Informed Consent

Written informed consent was obtained from all study participants before enrolment.

Sputum Samples Collection and Processing

The participants were counseled about sputum production at the directly observed treatment, short-course center and given wide mouthed sputum containers to produce sputum for Xpert MTB/RIF assay. The sputum collection device's cover was the two volumes of Gene Xpert sample reagent were unscrewed and added to the one volume of container for the sputum sample (2:1 v/v), vigorously blended and incubated for 15 minutes at room temperature. The samples were liquefied completely and no clumps of sputum were visible [14, 15].

Blood Samples Collection and Processing

Three milliliters of blood specimen were collected into a sterile EDTA vacuntainer blood specimen bottle, and centrifuged at 2000-3000 A sterile EDTA vacuntainer blood specimen vial was used to collect blood, which was then centrifuged at 2000–3000 r.p.m. for 20 minutes to obtain clear unhaemolyzed plasma. The plasma was harvested into sterile plasma separation tubes and rapidly stored at -200C until assayed in batches; for IL-10 and IL-12. Two milliliter of the blood specimen weretrasfered into a sterile EDTA blood specimen bottle, and used to re-determined and confirmed HIV-status.

LABORATORY ANALYSIS

Detection of MTB and Rifampicin Resistance:

Detection of MTB and drug resistance was determined by Gene XpertMTB/RIF technique according to standard operating procedure [14, 15].

HIV Screening Test:

The HIV screening test was carried out using the WHO screening criteria for developing countries which entails the use of a parallel testing algorithm for serological testing of HIV antibodies in the patient's sera using a combination of three (3) different screening methods, in a stepwise order for the detection of HIV-1 and HIV-2 in the blood [16].

HIV screening one was conducted using rapid test, Determine HIV-1 and 2 kits (Abbot Japan Co ltd. 2 Tokyo, Japan). Fifty ml of participant plasma samples were put to sample pads with the proper labels. The findings were read after 15 minutes of sample

application. The kit's built-in quality control verifies the outcomes. Two clearly visible red lines appearing in the test and control regions indicate an HIV seropositive reaction, while one red line in the control region validates the test kit. Two clearly visible red lines occurring in the patient and control regions indicate an HIV seropositive reaction, while one red color in the control window indicate an HIV seronegative reaction.

The HIV screening two was carried out using Uni-Gold HIV-1 and H-2 test kit (manufactured by Trinity Biotech Plc Co Wicklow Ireland). The plasma sample was carefully put over the sample pad in two drops (60µl). The wash reagent was then added in two drops (60µl) to the sample port after that. Ten minutes after adding the wash reagent, the result was represented by the emergence of one or two pink/red bars was read.

The Tie breaker or HIV screening 3 test was performed when the results of the screening I and II were indeterminate (discordant). STAT- PAK HIV 1and 2 assay test kit (made in New York, USA, by Chembio Diagnostic System INC). This technique makes use of immobilized antigen to find HIV-1 and HIV-2 antibodies in human plasma. Following the dispensing of 50µl of plasma sample into properly labeled sample wells, three drops of running buffer were added dropwise to the sample wells. Ten minutes after the running buffer was added, the test results were read. The outcomes of this procedure were validated by an internal quality control system. A single pink line at the control region indicates an HIV seronegative reaction, while the appearance of two pink lines in the test sample and control regions suggests an HIV seropositive reaction. Participants who initially presented with HIV infection were confirmed using HIV seropositive results from these two techniques.

Cytokines Quantitation:

The cytokines, IL-10 and IL-12 was assessed using the ELISA human IL-10 and IL-12 immunoassay kits (Nanjing Pars Biochem CO., Ltd, China). In accordance with recommended practices and the manufacturer's guidelines and standard procedure.

Statistical Analysis

Frequencies and percentages were used to express the variables. Statistical analysis was carried out using statistical package for social sciences (SPSS) software (SPSS Inc. Chicago, IL, USA, 2020). ANOVA and the student t test were applied. P-values of less than or equal to 0.05 was considered statistically significant.

RESULTS

Socio-demographic Characteristics

The majority of the study population were male (66.92%) with only (33.08%) female, most of them were married (56.92%) followed by single (29.23%). One-third of the study group's patients were younger than the age range of 18 to 29 (46.67%). Most of them attained secondary school level (46.92%) of education and self-employed (50.00%) (Table 1).

Table 1: Socio Demographic MDR-TB/HIV Co-infected Group

Characteristics	n	Gender		Percentage (%)
		Male	Female	
Age (years)	**130**			
18-29		40	17	43.8
30-49		38	19	43.8
50≤		9	7	12.3
Marital Status	**130**			
Married		34	40	56.9
Single		15	23	29.2
Widowed		3	4	5.4
Divorced		4	7	8.5
Educational Level	**130**			
No formal		7	12	14.6
Primary		17	10	20.8
Secondary		40	21	46.9
Tertiary		12	11	17.7
Employment Status	**130**			
Civil Service		9	5	10.8
Self employment		45	20	50.0
Student		10	8	13.8
Unemployed		12	21	25.4

Effects of HIV on IL-10 and IL-12 in MDR-TB

Table 2 showed the mean of IL-10 and IL-12 in MDR-TB/HIV co-infected treatment-naïve (group 1a), MDR-TB monoinfected treatment-naïve patients (group 2a), MDR-TB/HIV co-infected treatment-experienced patients (group 1b) and MDR-TB monoinfected treatment experienced patients (group 2b) were significantly lower (P<0.05) compared with similar value in apparently healthy control (group c). No significant difference was found in IL-10 and IL-12 production between MDR-TB/HIV co-infected treatment nainve patients and MDR-TB monoinfected treatment naïve patients, but there was a marked reduction in IL-10 production in MDR-TB monoinfected treatment naïve patients (Table 2, 3).

Table 2: Plasma levels of IL-10 and IL-12 in Studied Groups

Group	n	IL-10 (pg/ml)	IL-12 (pg/ml)
Group 1a	15	57.68 ± 26.85^{b1}	17.30 ±4.10
Group 1b	15	49.74 ±17.55	14.94 ± 2.68^{c1}
Group 2a	20	47.59 ±40.50	17.71 ±3.17
Group 2b	20	44.08 ±26.98	16.75 ±5.36
Group 3	20	137.33 ±2.78	22.77 ±2.87
Group c	40	135.8 ±5.09	20.07 ±4.31
Total	130		

KEY: Values are mean ± standard deviation; n = number of Subjects; ; MDR-TB = multidrug resistant tuberculosis; DS-TB = drug susceptible tuberculosis; HIV = human immunodeficiency virus; ART= antiretroviral therapy; ATT=anti-tuberculosis therapy; IL-10 = interleukin 10; IL-12 = interleukin 12; Significant differences: **b**^{1} (P<0.001)= Group 1b versus Group c; **c**^{1} (P<0.011)= Group 1b versus Group 3 by Tukey-Kramer Multiple Comparisons Test.
GROUP 1a = MDR-TB co-infected with HIV ATT and ART treatment-naïve patients.
GROUP 1b = MDR-TB co-infected with HIV ATT and ART treatment-experienced patients.
GROUP 2a = MDR-TB ATT treatment naïve patients.
GROUP 2b = MDR-TB ATT treatment-experienced patients
GROUP 3 = DS -TB co-infected with HIV ATT and ART treatment-experienced patients
Group c = apparently healthy control.

Table 3: Effect of HIV on Plasma Level of IL-10 and IL-12 on MDR-TB

Group	n	IL-10 (pg/ml)	IL-12 (pg/ml)	p-value
MDR-TB/HIV Treatment-Naïve	15	57.69±26.85	17.30±4.10	0.122
MDR-TB Treatment-Naive	20	47.59±40.50	17.71±3.17	0.294
Total	35			

KEY: Values are mean ± standard deviation; n = number of Subjects; IL-10 = interleukin 10; IL-12 = interleukin 12; ART = antiretroviral therapy; ATT = anti-tuberculosis therapy; SD = standard deviation; MDR-TB = multidrug resistant tuberculosis; HIV = human immunodeficiency virus; p-values of less than or equal to 0.05 was considered statistically significant.
MDR-TB/HIV = MDR-TB co-infected with HIV ATT and ART treatment-naïve patients.
MDR-TB = HIV negative MDR-TB ATT and ART treatment-naive patients.

Impact of antiretroviral and antituberculous therapy on IL-10 and IL-12

We discovered that the concentration of IL-10 and IL-12 cytokines was not statistically different from one another between MDR-TB/HIV co-infected treatment naïve-patients (group 1a) and MDR-TB/HIV co-infected treatment-experienced patients (group1b) (Table 4). The mean 3 plasma levels of IL-10 and IL-12 were decreased in MDR-TB/HIV co-infected treatment-experienced patients than in MDR-TB/HIV co-infected treatment-naïve patients but not statistically difference (Table 4, 5).

Table 4: Effect of Antiretroviral Therapy and Anti-TB Therapy on IL-10 and IL-12

Group	n	IL-10 (pg/ml)	IL-12 (pg/ml)
MDR-TB/HIV Treatment-Naive	15	57.69±26.85	17.30±4.09
MDR-TB/HIV Treatment-Experienced	15	49.74±17.56	14.94±2.69
p-value		0.124	0.129
Total	30		

KEY: Values are mean ± standard deviation; n = number of Subjects; SD = standard deviation; IL-10 = interleukin 10; IL-12 = interleukin 12; ART=antiretroviral therapy; ATT=anti-tuberculosis therapy; MDR-TB = multidrug resistant tuberculosis; HIV = human immunodeficiency virus; p-values of less than or equal to 0.05 was considered statistically significant.

Table 5: Effect of Anti-TB Therapy on IL-10 and IL-12

Group	n	IL-10 (pg/ml)	IL-12 (pg/ml)
MDR-TB Treatment-Naive	20	47.59±40.50	17.71±3.17
MDR-TB Treatment-Experienced	20	44.08±26.98	16.75±5.36
Total	30		
p-value		0.085	0.057

KEY: Values are mean ± standard deviation; n = number of Subjects; IL-10 = interleukin 10; IL-12 = interleukin 12; ATT=anti-tuberculosis therapy; MDR-TB = multidrug resistant tuberculosis; p-values of less than or equal to 0.05 was considered statistically significant.

Effect of Anti-tuberculosis Treatment on IL-10 and IL-12 in MDR-TB Monoinfected Patients

We also measured IL-10 and IL-12 if these cytokines displayed a difference as a result of anti-TB treatment, between MDR-TB monoinfected treatment-experienced patients (group 2b) and MDR-TB monoinfected treatment-naive patients (group 2a). Statistically significant differences (P>0.05) were not observed between MDR-TB monoinfected treatment-naïve patients (group 2a) and MDR-TB monoinfected treatment-experienced patients (group 2b). (Table 6).

Table 6: Comparison between MDR -TB/HIV and DS -TB/HIV Treatment-experienced

Group	n	IL-10 (pg/ml)	IL-12 (pg/ml)
MDR-TB/HIV Treatment-Experienced	20	49.74±17.55	14.94±2.69
DS-TB/HIV Treatment-Experienced	20	137.33±2.78	22.77±2.87
Total	40		
p-value		0.001	0.001

KEY: Values are mean ± standard deviation; n = number of Subjects; SD = standard deviation; IL-10 = interleukin 10; IL-12 = interleukin 12; ART = antiretroviral therapy; ATT = anti-tuberculosis therapy; MDR-TB = multidrug resistant tuberculosis; DS-TB = drug susceptible tuberculosis; HIV = human immunodeficiency virus; p-values of less than or equal to 0.05 was considered statistically significant

Comparism between MDR-TB/HIV co-infected and DS-TB/HIV co-infected Treatment-experienced Patients

Table 6 findings demonstrate that the mean plasma level of IL-10 (137.33±2.78 pg/ml) were significantly (p<0.001) higher in DS-TB/HIV co-infected treatment-experienced patients (group 3) than the MDR-TB/HIV co-infected treatment-experienced patients (group 1b) (49.74±17.55 pg/ml). The concentration of IL-12 (22.77±2.87 pg/ml) in DS-TB/HIV co-infected treatment-experienced patients (group 3) was significantly (p<0.001) higher than MDRTB/ HIV co-infected treatment-experienced patients (group 1b) (14.94±2.69 pg/ml).

DISCUSSION

In this study, the finding of 35.24±1.53 years as means age for MDR-TB/HIV co infected patients are consistent with reports of previous studies Cherono et al.[17] who indicated that TB/HIV is known to affect reproductive age group of between twenty five to forty five years because these are individuals who are sexually active hence the transmission of HIV is very high. Marital status is a critical risk factor when exploring the patterns of MDR-TB/HIV co-infection. Married persons had higher proportion (56.92%) of co-infection than single (43.08%) counterpart. These findings are concurred with the finding of Cherono et al.[17].

Married couple is 38 at high risk of infection especially if one partner is unfaithful. Although patients in all education levels were predisposed to MDRTB/ HIV co-infection. Secondary education had higher proportion (46.92%). It was in contrast to the findings conducted in Zambia by Muyunda et al. [18] who reported higher percent among primary school level. It was also in disagreement with the findings of another researcher's study, which revealed that patients with high educational levels has high chances of being employed compared to those with unskilled occupations having attained low educational level [18].

Male had higher co-infection (66.92%) than females (33.08%). It was contrast with the finding of Cherono et al. [17] in Kenya who found higher prevalence of tuberculosis TB/HIV co-infection in females compared to males. Most of them were self-employed (50.00%) followed by unemployed (25.38%). Individuals with low income earning are more predisposed to the multidrug resistant tuberculosis co-infected with HIV than those with high income generating and stable jobs.

The study showed IL-10 and IL-12 in MDR-TB/HIV co-infected treatment-naïve patients, MDR-TB monoinfected treatment-naïve patients, MDR-TB/HIV co-infected treatment-experienced patients and MDR-TB monoinfected treatment experienced patients were significantly lower than those in the apparently healthy control group. Furthermore, the plasma levels of IL-10 in the DS-TB/HIV co-infected treatment-experienced patients were higher than those in MDR-TB/HIV co-infected treatment-experienced patients and apparently healthy control group ($p>0.05$). There was higher production of IL-10 in patients with MDR-TB/HIV co-infected treatment naïve patients compared to MDRTB monoinfected treatment-naïve patients but not statistically different (Table 3).

The study showed IL-10 and IL-12 in MDR-TB/HIV co-infected treatmentnaïve patients, MDR-TB monoinfected treatment-naïve patients, MDR-TB/HIV co-infected treatment-experienced patients and MDR-TB monoinfected treatment experienced. Although not significantly different, the level of IL-10 production after therapy dropped slightly in MDR-TB/HIV coinfected patients who had previously received treatment compared to MDR-TB/HIV co-infected patients who had not. Therefore, DS-TB/HIV co-infected patients had greater levels of IL-10 than MDR-TB/HIV coinfected patients who had received treatment, as well as the seemingly healthy control group ($p< 0.05$). The role of macrophage is suppressed by IL-10, which helps control and initiates the immune responses [19].

The IL-10 production was increased during the infection, inducing reactivation of TB [20, 21]. Increased plasma level secretion of IL-10 in patients with MDRTB has been reported in Turkey [22-24]. This elevated IL-10 in MDR-TB/HIV co-infection may also be a sign of immune system suppression, which results in an unbalanced production of pro-inflammatory and anti-inflammatory cytokines. Between MDR-TB monoinfected treatment-nave patients and MDR-TB/HIV co-infected treatment-nave patients, there was no discernible difference in IL-12 expression, but there was a marked decrease in MDR-TB monoinfected treatment naïve patients compared to MDR-TB/HIV co-infected treatment-naïve patients. It is clear that both HIV infection and TB affect the immune system in a way that interferes with cytokine release.

CONCLUSION

The syndemic interaction between the HIV and MDR-TB epidemics has had deadly consequences in Nigeria and yet, to the best of our knowledge, this study is the first to directly compare the concentration of cytokines (IL-10 and IL-12) in MDR-TB/HIV coinfected patients and MDR-TB monoinfected patients in the country. The concentration of IL-10 and IL-12 were significantly higher in apparently healthy control compared with MDRTB/ HIV co infected treatment-naïve patients, MDR-TB monoinfected treatment-naïve patients, MDR-TB/HIV co-infected treatment-experienced patients and MDR-TB monoinfected treatment-experienced patients. MDR-TB/HIV co-infected patients and MDRTB monoinfected patients display similar plasma cytokine pattern. Patients with MDRTB/HIV co-infection and MDR-TB monoinfection have lower levels of the anti-inflammatory cytokine IL-10 and the pro-inflammatory cytokine IL-12, which suggests a protracted dysregulation of the immune system and higher disease severity. We found that when evaluating individual with MDRTB/HIV co-infection, the concentration of IL-10 and IL-12 is crucial. The concentration of IL-10 and IL-12 in DSTB/HIV co-infected treatment-experienced patients demonstrated significant higher values than MDR-TB/HIV co-infected treatment-experienced patients.

Recommendation

According to the results of this study, it is advised that: Our study was a case control; further studies should be conducted to include larger sample size. Recombinant human interferon gamma interleukin 2 (IL-2) should be applied to boost immunity among MDRTB/ HIV co-infected patients.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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