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Original Research Article

Evaluation of Interleukin 10 Levels among Pulmonary Tuberculosis Patients Attending Aminu Kano Teaching Hospital, Kano

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Abstract

Despite medical advances, tuberculosis is responsible for over one billion deaths in the last 200 years. This study was carried out to determine interleukin 10 levels in serum of tuberculosis patients attending Aminu Kano Specialist Hospital, Kano (AKTH). A total of 330 subjects were enlisted for this study. The subjects were grouped into: Group A (30 control) and Group B (300 subjects). DNA sequences specific for *Mycobacterium tuberculosis* and rifampicin resistance were detected using Gene Xpert MTB/RIF assay. 5mls of blood were aseptically collected from subjects using standard venipuncture procedure into plain tubes for HIV and Interleukin 10 assay. TB patients co-infected with HIV were excluded from this study. The Interleukin 10 level was measured using commercial Sandwich ELISA kits. The result revealed that there was significant difference ($p<0.05$) between the interleukin 10 levels of the test (11.30±3.79) and control group (9.38±4.39). Furthermore, the result showed that there was no significant difference ($p>0.05$) between the interleukin-10 levels of rifampicin resistant (11.80±3.87) and non-rifampicin resistant patients (11.65±3.55). Interleukin 10 was also found to be higher among patients aged 30-39 (12.33±3.31) and female patients (11.95±3.50) than in male patients (11.45±3.65). Furthermore, Interleukin 10 level was found to be higher in TB patients before anti TB treatment (12.54±3.82) than in patients after treatment (9.86±3.59). The findings of the study showed that interleukin 10 is a viable biomarker in the pathogenesis of TB infection and can be used as prognostic marker for management of TB patients.

Keywords: Tuberculosis, Interleukin 10, Rifampicin Resistance, ELISA, HIV, Anti TB Treatment.

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is responsible for over one billion deaths in the last 200 years, more than any other single pathogen (Walzl G *et al.*, 2015). Despite the increased global attention, the expansion of therapeutic drug regimens, and the widespread use of existing vaccine, approximately 1.8 million people still die every year as a result of this devastating disease (Abdalla A *et al.*, 2015). Furthermore, increasing outbreaks of drug-resistant TB pose a significant threat to treating and preventing further transmission (Bhembe N *et al.*, 2014). Available anti-TB drugs have a profound effect on drug-susceptible TB with greater than 90% cure rates. But resistance to almost all of the available drugs is rapidly emerging in the form of multi, extremely and totally drug-resistant TB (MDR, XDR, and TDR-TB), and the development of new anti-TB drugs severely lags behind (Evans T *et al.*, 2013). The current vaccine, Bacilli Calmette–Guérin (BCG), has been available for almost 70 years, but it is not very effective and provides only partial and inconsistent protection (0–70%) (Ouyang W *et al.*, 2011).

In the last 20 years, a concerted worldwide effort has been prompted to develop a new preventive and/or therapeutic TB vaccine. Unfortunately, none of them showed sufficient efficacy through clinical trials (Abulalgasim E *et al.*, 2016). Clearly, something is missing. Development of a new effective vaccine against TB remains challenging due to a poor understanding of immune-correlates of protection and disease pathogenesis (Sabat R, 2010).

Interleukin 10 is one of the most important anti-inflammatory cytokines reported to affect multiple cell types including macrophages, monocytes, dendritic cells, CD4 and CD8 T cells (Schmitt E *et al.*, 2012. The dominant function of interleukin 10 is to down regulate the immune response and limit tissue injury (Zdanov A, 2010). However, the excessive production of this cytokine directly inhibits CD4 T cells responses which may result in failure to control infections.

Increased levels of IL-10 have been associated with increased activity of programmed cell death, protein and cytotoxic T-cell lymphocyte antigen 4 (CTL4-A) co-receptors on T-cell which function as negative regulators of T-cells, while also being associated with decreased CD28 co-receptor activity on T-cells, all of which are associated with reduced effector T-cell function (Cai G, 2012). Additionally, IL-10 is effective at decreasing levels of pro-inflammatory cytokines: IL-2, IL-6, IL-1β, IL-12, GM-CSF, TNF-α and IFN-γ, in both stimulatory and naive situations related to the presence or absence of sufficient antigen (Tang-Feldman Y *et al*., 2011). The reduction of these cytokines minimizes leukocyte maturation and recruitment.

Most research targeting host immunity has so far focused on generating and maintaining antigen-specific adaptive immune responses against Mtb as an effective way to prevent and/or treat Mtb infections. Despite significant effort and resources, they have not been very successful yet. There is a serious need to more comprehensively understand the network of immunological mechanisms underlying protection and/or clearance of TB infection, which allow a precise balance between host protective responses and immune pathogenesis. The aim of this research was to determine the Interleukin 10 levels in serum of tuberculosis patients attending AKTH, Kano.

MATERIALS AND METHODS

Study Area

This study was carried out at Aminu Kano Teaching Hospital (AKTH), Kano, situated in Kano and a referral center for both private and public health institution in and around the neighboring states.

Study Population

This study consisted of Consented Tuberculosis patients of both sexes and different age groups attending Aminu Kano Teaching Hospital, Kano. 30 apparently healthy individuals were used as control group for this study. TB patients co-infected with HIV and those who declined consent were excluded from the study.

Ethical Consideration

Ethical clearance/approval was obtained from the Research and Ethics Committee of Aminu Kano Teaching Hospital before the commencement of the study. A consent form containing the research topic, researchers name and the purpose of the study was administered to the subject for their consent. Informed consent of the subjects was sought before the commencement of the study and the subjects had rights to accept or reject enrolment into the study. The data for this study was collected using a structured questionnaire to assess subjects' bio data and socio-demographic characteristics (Appendix III). Information on medical history of the patients was obtained from their hospital medical records.

Sample Size Determination

Determination of sample size:

The Sample size was determined by the following formula (Lwanga and Lemeshaw, 1991):

$$n = \frac{Z^2 Pq}{d^2}$$

P = The prevalence rate for tuberculosis in Minna and Suleja, Niger State, Nigeria was 25.5% (Sani T, 2015).

$n \cong 292$

Thus, to improve precision and to account for missing or incomplete data, the number of respondents that were recruited for this study was 300.

Study Sampling Technique

Subject for the study were obtained by random selection of patients among the study population. The sampling interval was obtained using the formula: N/n=K

Data and Sample Collection

Socio demographic data for this study was collected using structured questionnaire. Sputum samples were collected from the consented subjects to detect for DNA sequences specific for *Mycobacterium tuberculosis* as well as most of the clinically relevant Rifampicin resistance inducing mutations in the RNA polymerase beta (rpoβ) gene in the *Mycobacterium tuberculosis* genome. About 5mls of whole blood was collected aseptically from each subject using standard venipuncture procedure. Samples were dispensed into appropriately labeled screw-capped containers and left at room temperature for about an hour, after which it was spinned at 3,000 rpm for 10minutes to separate serum from whole blood clot. Serum was dispensed into corresponding labeled plain containers and stored at -20°Cuntil needed for HIV and Interleukin 10 elisa assays.

Analysis of samples

Gene Xpert Assay

The Gene Xpert Assay detects DNA sequences specific for *Mycobacterium tuberculosis* and rifampicin resistance by polymerase chain reaction. It is based on

the Cepheid GeneXpert system, a rapid, simple to use nucleic acid amplification tests (NAAT). The Xpert® MTB/RIF purifies and concentrates *Mycobacterium tuberculosis bacilli* from sputum samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR. The process identifies most of the clinically relevant Rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the *Mycobacterium tuberculosis* genome in a real time format using fluorescent probes called molecular beacons. Results are obtained from unprocessed sputum samples in 90 minutes, with minimal biohazard and very little technical training required to operate (Van Rie A *et al.*, 2010).

Working area was disinfected and each cartridge was labeled with laboratory serial number. Sample reagent and sample were mixed in 2:1 proportion respectively and mixed vigorously 20 times. The mixture was incubated at room temperature for 10 minutes. The mixture was mixed again vigorously 20 times and observed until it liquefies with no clumps or sputum visible.

The liquefied sample was aspirated into transfer pipette until the meniscus is above the minimum mark of 2mls. Sample was transferred into the open port of Xpert MTB/RIF cartridge making sure no aerosols were created. The pipette was transferred into tubercoidal disinfectant and cartridge was closed making sure the lid snaps firmly into place. The cartridge was loaded into the instrument module door which displays green light and closed firmly, the test starts and the green light stopped blinking. Once the run is completed, results were printed automatically in less than 2 hours (Van Rie A *et al.*, 2010).

HIV Testing

Tuberculosis positive subjects for this study were tested for HIV in accordance with National Serial Algorithm for HIV testing.

Determination of Interleukin 10 levels

Determination of interleukin 10 levels was done using a commercial Sandwich Elisa detection method (SinoGeneClon Biotech Co., Ltd, China).

All reagents working standards, blank and samples were prepared according to the manufacturer's instruction, the number of the samples was determined using Assay layout sheet, and the unused wells were stored at 4°C.

Interpretation of Result

Standard concentration is taken as the horizontal, the OD value for the vertical, a standard curve was drawn on graph paper. The corresponding concentration is determined according to the sample OD value by the sample curve and multiplied by the dilution multiple. The sample actual concentration is determined by multiplying the sample concentration with the dilution factor.

Data Analysis

All generated data were analyzed using SPSS software version 20.0 (2011), IBM California, USA). The results obtained were expressed in simple proportion and percentages for the study groups. Chi square contingency table was used to compare socio demographic factors in TB infections. Student T test was used to compare the Interleukin 10 levels between the study subjects and control group. A degree of freedom of 95%, *p* value of less than 0.05% was considered as statistically significant.

RESULTS

The result of this study revealed that male participants that aged between 20 and 29 years had the highest population distribution compared to other age ranges. Similarly, female participants that aged between 20 and 29 years were observed to have the highest population distribution. The result further indicated that majority of the male (73.37%) and female (63.36%) participants completed secondary education, and were observed to majorly reside in urban areas (Table 1).

Table 1: Demographic Characteristics of the Study Participants

Characteristics	Test Group		χ^2	*P*-value
	Male (*n* = 169)	Female (*n* = 131)		
Age (years)				
10 – 19	27	14	54.11	< 0.0001
20 – 29	45	30		
30 – 39	40	27		
40 – 49	27	19		
50 – 59	10	23		
60 – 69	16	16		
> 70	4	2		
Educational Level n (%) n (%)				
Primary	14 (8.28)	8 (6.11)	182.85	< 0.0001
Secondary	124 (73.37)	82 (63.36)		

Tertiary	21 (12.43)	18 (12.98)		
None	10 (5.92)	23 (5.92)		
Address				
Rural	49 (28.99)	39 (29.77)	42.43	< 0.0001
Urban	120 (71.01)	92 (70.23)		
Marital Status				
Single	58 (34.32)	22 (16.79)	64.57	< 0.0001
Married	111 (65.68)	109 (83.21)		

The result of the distribution of the possible risk factors among the study participants revealed that 276 (92%) of the 300 study participants had contact with TB patients, while 218 (73%) had knowledge on TB infection. The result further showed that only 16 (5%) out of the 300 study participants were health care workers, whereas 20 (7%) of the population had other medical conditions. Of the 300 study participants, 97(32%) were exposed to indoor and outdoor pollution, whereas 71(24%) of the respondents were involved in smoking and/or alcohol or/and drug consumption. In addition, the findings of the current study showed that only 60 (20%) of the study participants were hospitalized whereas only 36 (12%) of the study participants had family history of TB other than index case (Table 2).

Table 2: Distribution of the Possible Risk Factors among the Study Participants

Risk Factors	Frequency	Percentage
Contact with TB Patients		
Yes	24	8
No	276	92
Knowledge on TB		
Yes	218	73
No	82	27
Medical condition (HIV and diabetes)		
Yes	20	7
No	280	93
Hospitalization		
Yes	60	20
No	240	80
Family history of TB other than index case		
Yes	36	12
No	264	88
Smoking/Alcohol and drugs consumption		
Yes	71	24
No	229	76
Occupational risk		
Yes	16	5
No	284	95
Indoor pollution		
Yes	97	32
No	203	68

Table 3 shows the result of the comparison of IL-10 level between test and control groups. The result revealed that the test group had higher IL-10 level (11.30±3.79) compared to the control group (9.38±4.39). The difference between the test and control groups was statistically significant at $P< 0.05$. The IL-10 levels obtained in the present study were within the normal range of (undetectable to 13.68 pg/mL).

Table 3: Comparison of Interleukin (IL) 10 level between test and control group

Groups	No. Examined	Mean±SD	*t-value*	*p-value*
Test Group	300	11.30±3.79	2.687	0.011
Control Group	30	9.38±4.39		

Figure 1 shows the result of the changes in IL-10 level between the different stages of anti-tuberculosis treatment. The result revealed that the mean IL-10 level in TB patient before anti-tuberculosis

therapy (ATT) was higher (12.54±3.14) compared to the TB patients on ATT for 3 months (11.29±3.82) and 6 months (9.86±3.59). However, the difference between the treatment groups was not statistically significant (*P*> 0.05).

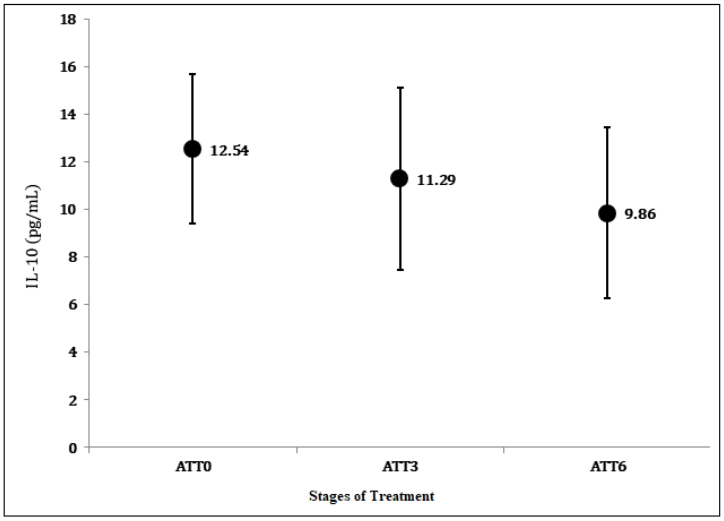


Figure 1: Changes of IL-10 levels in plasma of TB patients (n=300) before anti-tuberculosis therapy (ATT0) (*n*=19), after three months (ATT3) (*n* =173) and six months treatment (ATT6) (*n*=108), Changes are non-significant (NS) at *P* = 0.852

The result of the IL-10 variation between rifampicin resistance and non-rifampicin resistance showed that participants that presented rifampicin resistance (11.80±3.87 pg/mL) were slightly higher than the non-rifampicin participants (11.65±3.55 pg/mL). However, the difference between the two groups was statistically in-significant (*P*> 0.05) (Table 4).

Table 4.4: Interleukin (IL) 10 between Rifampicin Resistance and Non-Rifampicin Resistance

Groups	No. Examined	Mean±SD	*t-value*	*p-value*
Rifampicin Resistance	45	11.80±3.87	-0.172	0.864
Non-Rifampicin Resistance	255	11.65±3.55		

The result of the demographic and IL-10 characteristics of the study participants showed that study participants that aged between 30 and 39 years had the highest mean IL-10 level of 12.33±3.31 pg/mL. However, those that aged 70 and above had the least IL-10 level of 10.43±3.63 pg/mL (Figure 2).

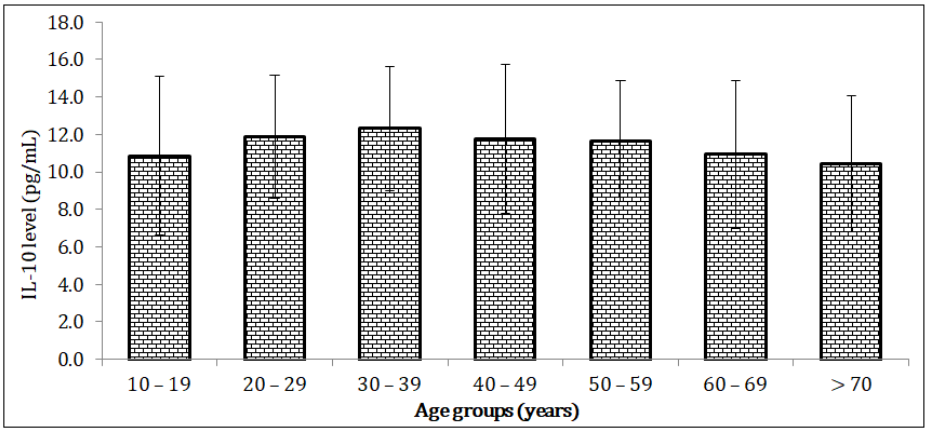


Figure 2: Multi-variance among demographic characters (age groups) and interleukin (IL) 10

In addition, the female study participants were observed to have slightly higher mean IL-10 value of 11.95±3.50 pg/mL than the male participants (11.45±3.65 pg/mL) (Figure 3).

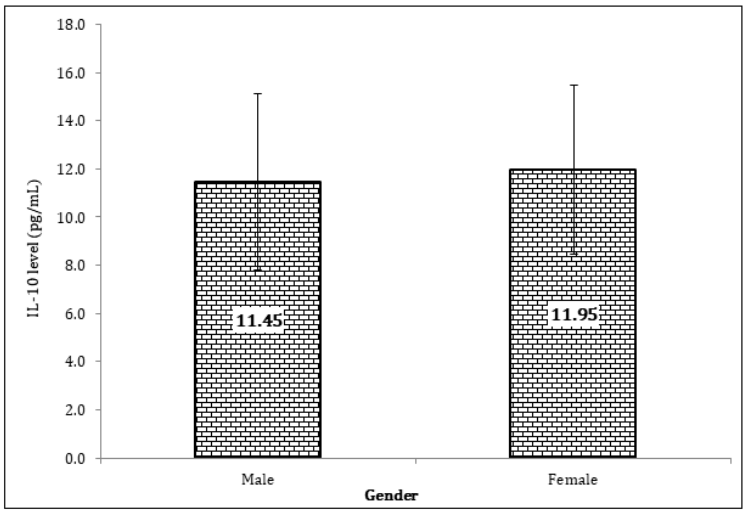


Figure 3: Multi-variance among demographic characters (gender) and interleukin (IL) 10

DISCUSSION

Tuberculosis (TB) remains one of the world's deadliest communicable diseases (Lonnroth K *et al.*, 2010). The result of the current study showed that there was higher prevalence of TB patients in male population. The finding that the majority of TB patients were males is in accordance with other studies which revealed that there are generally more males than females suffering from TB worldwide (Chiang C *et al.*, 2006). Studies have found that TB is gender-sensitive. The burden of the disease is greater on women due to stigma and societal settings than on men, even though more men suffer from TB worldwide than women (Eftekhar M *et al.*, 2015). In a study by Rao S (2009) that looked at TB and patient gender, it was observed that the male-to-female ratio in pulmonary tuberculosis patients was 2:1, and that this was in agreement with other reports (Horton C *et al.*, 2016).

The finding of this study confirms that healthcare workers are at increased risk of exposure to TB. Joshi L *et al.*, (2015) summarized evidence on the incidence and prevalence of latent TB infection (LTBI) and disease among healthcare workers in low and middle-income countries. In their review of 51 studies the authors found that the prevalence of LTBI among healthcare workers was on 55% (CI = 33–79), the estimates of the annual risk of LTBI ranged from 0.5 to 14.3%, and the annual incidence of TB disease ranged from 69 to 5780 per 100 000.

Similarly, indoor air pollution is a very possible risk factor to exposure to TB as observed in the present study. In developing countries; the percentage usage of solid fuels for cooking is more than 80% (Kolappan C & Subramanai R, 2009). Biomass combustion is shown to release large particulate matter (PM) such as carbon monoxide (CO), nitrogen oxide, formaldehyde, and poly-aromatic hydrocarbons which can deposit deep into the alveoli and can cause considerable damage (Silva D *et al.*, 2018).

In the present study, the proportion of smokers/or drug or/and alcohol consumers among TB patients were 24%. Smoking increases TB morbidity risk and mortality rates (Lienhardt C *et al.*, 2012), as it leaves a negative impact on lungs and immunity. in the same way, consumption of alcohol at least once a week increases TB morbidity risk by 2.0 times compared with people who drink less often and may cause changes in the immune system and leadto immunodeficiency, resulting in susceptibility to pneumonia, TB and other infectious disease (Anthony B *et al.*, 2019).

Poverty and illiteracy has been found to be strongly associated with TB (Bennadi D, 2013). According to a review by (Bhatias *et al.*, 2002), poverty, illiteracy and the tubercle bacillus create a second vicious circle. Poor people living in rural areas, suffering from hunger and crowded into close non-hygienic places are easily victimized in an environment where TB is easily spread (Amelio *et al.*, 2017). Meanwhile, in the current study, TB was found more in individuals living in the city, urban and suburban areas. The present findings also concurred with the result observed in Ghana by Anthony B *et al*, 2019 who observed higher number of Multidrug Resistant (MDR) Tuberculosis and Drug Responsive Tuberculosis Patients in Ghana lived in cities and other urban areas. Due to the abundance of drug stores in these areas compared to the rural areas, there is the greater tendency for such people to self-medicate. Additionally, the busy lifestyle of people living in the city and other urban areas could result in TB patients not completing their treatment courses. These situations encourage the emergence of drug-resistant TB strains (Anthony B *et al.*, 2019) and could explain the link between residency and TB observed in this study. Similar to findings from the current study, Bhatias S *et al.*, 2002 reported on the

link between illiteracy, unemployment, non-adherence of TB patients to TB medication and the development of drug resistance.

Chronic immune activation, which might be because of exposure to a high load of environmental antigens, has mostly characterized the immune profile of people living in sub-Saharan Africa. Such exposure has been observed to impair the host's immune response to *M. tuberculosis* and HIV, which are widespread in sub-Saharan Africa (lago P *et al.*, 2012). Infection with intracellular parasites such as *Mycobacterium tuberculosis* is known to induce Th1 immune response (Sahiratmadja E *et al.*, 2007). The protective immunity against the TB pathogen is said to be mediated by cytokines such as IFN-γ, TNF-α, IL-12, IL-6 and IL-18 during the initial stage of infection (Ndishimye P *et al.*, 2015). Interleukin-10 (IL-10) is one of the most important anti-inflammatory cytokine reported to inhibit CD4+ T cell responses by inhibiting APC function of cells infected with mycobacteria. IL-10 has been identified to play a significant role in suppressing macrophage and dendritic cell (DC) function, which helps control and initiate the immune responses (Boussiotis *et al.*, 2000). Therefore, it was not surprising that in the present study, significant (P = 0.011) elevated plasma levels of IL-10 were found in TB participants compared to the control participants. However, the IL-10 levels obtained in the present study were within the normal range of IL-10 (undetectable to 13.68 pg/mL) as described by Fayad *et al.*, (2001). This correlated with a study that showed elevated levels of cytokines in TB patients compared to controls (Tang-feldman Y *et al.*, 2011). Several studies have reported increased levels of cytokines in the serum of TB patients (Amelio *et al.*, 2017). Cytokines are factors both in the protection against tuberculosis and in immunopathology. The high levels of IL-10 in TB patients in the current study may be due to a marked tissue necrosis during the disease occurrence that led to progressive TB and eventually rifampicin resistance. This might have resulted in the release of IL-10 into circulation and eventually contributed to systemic indicators of TB, such as fever and cachexia.

To evaluate the association of IL-10 cytokine with various disease stages of TB, plasma levels of IL-10 were measured in active TB patients before treatment, after three months of treatment and at the end of treatment. In the present study, the possible effect of treatment on plasma IL-10 levels in TB patients was evaluated. All the patients that received anti-tuberculosis treatment were given isoniazid, rifampin, pyrazinamide and ethambutol (2HRZE/4HR) according to the national TB program. IL-10 levels were determined in 19 patients before treatment, 173 patients after three months of treatment and 108 patients after six months TB treatment. The finding showed that IL-10 level decreased during treatment. In agreement with the present study, Lago *et al.*, (2012) observed correspondence a consistent decrease in IL-10 levels in TB patients during therapy. Sahiratmadja *et al.*, (2007) also observed reduced IL-10 production during TB therapy, suggesting that this cytokine may be a useful biomarker signature to assess the disease progression. Collectively, these studies concluded that IL-10 was functioning to limit the immune response to Mtb and may contribute to TB pathogenesis.

Interleukin (IL)-10, which is a suppressor T cell or T-regulatory cytokine, is known to play a critical role during chronic and latent stages of pulmonary TB. The IL-10 production is said to be elevated during the infection, promoting reactivation of TB (Figen D *et al.*, 2005). The excessive production of this cytokine usually results in failure to control the infection, and this could account for why IL-10 was elevated in the MDR-TB participants. Increased production of IL-10 in patients with active disease including MDR-TB has been reported in Turkey (Figen D *et al.*, 2005). This high IL-10 production in MDR-TB might also indicate suppression of the immune response, leading to an inadequate balance of pro- and anti-inflammatory cytokines. Interleukin-10 functions to limit the immune response to TB and may contribute to TB pathogenesis.

CONCLUSION

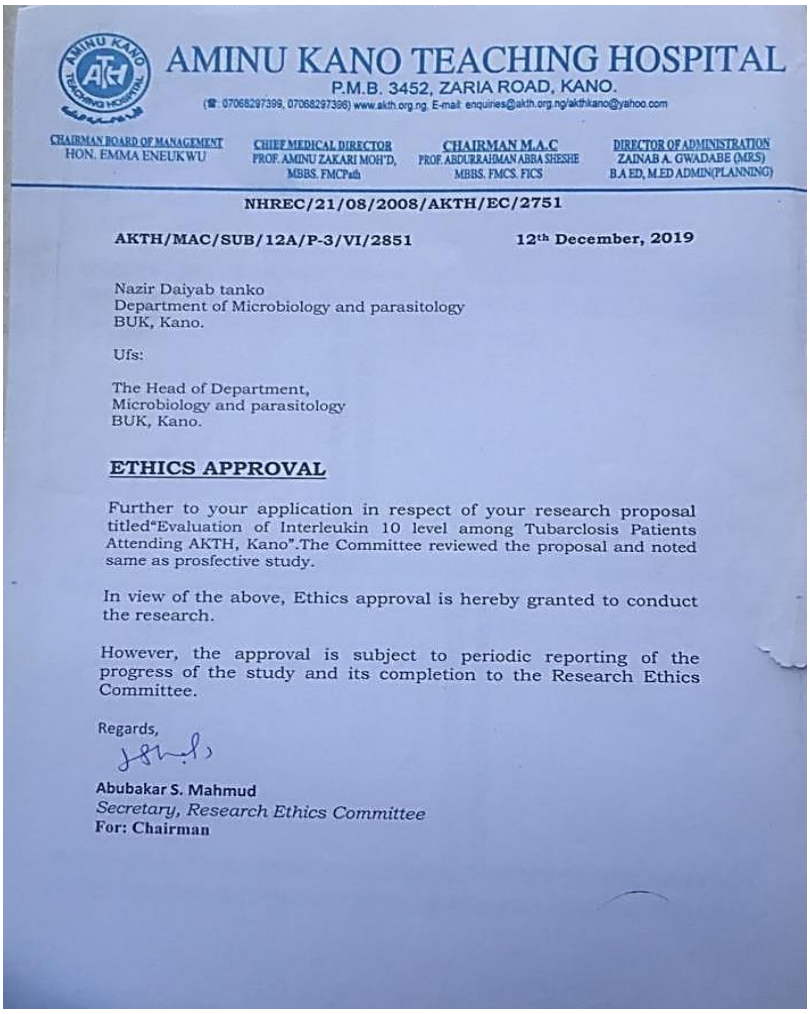
Tuberculosis (TB) is a chronic bacterial disease caused by *Mycobacterium tuberculosis.* The study showed that there was higher prevalence of TB infection in male than female in the sample population. The findings of the current study further showed that majority of the study population only acquired the secondary level of education and lived in the urban areas of the state. The levels of anti-inflammatory (IL-10) cytokine were observed to be significantly higher in TB patients compared to the healthy subjects. However, the result revealed no significant difference between the interleukin-10 levels of rifampicin resistant and non-rifampicin resistant patients. Furthermore, the results of this study showed there was higher Interleukin 10 levels before anti TB treatment. Interleukin-10 can be used as a prognostic marker in management of TB patients.

RECOMMENDATIONS

Interleukin 10 should be used in monitoring and treatment of tuberculosis.

This study was carried out on the role of interleukin-10 in the management of TB infection, however, further studies should investigate the roles of other cytokines such as IFN-γ, TNF-α, IL-12, IL-6 and IL-18 in the protective immunity against the TB pathogen.

Ethical Clearance



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NHREC/21/08/2008/AKTH/EC/2751

AKTH/MAC/SUB/12A/P-3/VI/2851 12th December, 2019

Nazir Daiyab tanko
Department of Microbiology and parasitology
BUK, Kano.

Ufs:

The Head of Department,
Microbiology and parasitology
BUK, Kano.

ETHICS APPROVAL

Further to your application in respect of your research proposal titled"Evaluation of Interleukin 10 level among Tubarclosis Patients Attending AKTH, Kano".The Committee reviewed the proposal and noted same as prosfective study.

In view of the above, Ethics approval is hereby granted to conduct the research.

However, the approval is subject to periodic reporting of the progress of the study and its completion to the Research Ethics Committee.

Regards,

Abubakar S. Mahmud
Secretary, Research Ethics Committee
For: Chairman

CONSENT FORM FOR RESEARCH STUDY

I am NazirDaiyabTanko, a student from the department of medical microbiology and parasitology, Bayero University Kano. I am conducting a research titled, EVALUATION OF INTERLEUKIN 10 LEVELS AMONG PULMONARY TUBERCULOSIS PATIENTS ATTENDING AMINU KANO TEACHING HOSPITAL, KANO, which is a part of the requirement for award of Master of Science Degree in Medical Microbiology.

You have fulfilled the criteria to serve as a volunteer for this study which is to be carried out solely for research purpose. Data obtained shall be kept confidential and may be significant for other patients.

You may wish to participate in this study and you may withdraw freely at any point. If you are willing to participate in this study, please sign or thumbprint below. Thank you for your cooperation.

Study Number:……..
Name of patient:……....
Signature:……..

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