Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2012, 2 (6):2010-2014

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Plasma IgE level and Eosinophil count in smear positive tuberculosis patients with and without helminthic infections at Gondar University Hospital, Northwest Ethiopia.

Aschalew Gelaw[1], Ebba Abate[1], Jonna Idh[2], Andargachew Mulu[1], Belay Anagaw[1], Yeshanbel Belyhun[1], Thomas Schon[3]

[1]University of Gondar, College of Medicine and Health Sciences, School of Biomedical and Laboratory Sciences, Department of Medical Microbiology
[2]Department of Medical Microbiology, Faculty of Health Sciences, Linköping University, Linköping, Sweden
[3]Department of Clinical Microbiology, Kalmar County Hospital, Kalmar, Sweden

---

ABSTRACT

In Sub-Sahara Africa and developing countries, where the prevalence of parasitic infection is very high a dominant Th2 immune response has been reported and suggested to increase susceptibility to mycobacterium tuberculosis infection. Such an imbalance in an increase is Th2 cells favor IgE production. Peripheral eosinophilia is widely recognized as a useful indicator of parasitic diseases mainly of helminthes. the aim of the study was to determine plasma IgE level and blood eosinophil count in smear positive tuberculosis patients with and without helminthic infection. A crossectonal study was conducted. One hundred and twelve smear positive tuberculosis patients were included. Plasma IgE level, and eosinophils count was analyzed and the stool sample was processed for intestinal parasites. The mean concentration of plasma total IgE (828 U/mL) study subjects with helmenthic infection were significantly higher than those without helmenthic infection (668 U/ml) P= 0.045. The peripheral eosinophil count in smear positive TB patients with intestinal helmenthic infection (352/mm^3) was significantly higher than those without helmenthic infection (112/mm^3) P= 0.033. Ascaris lumbricoids, Hookworm, Strongloides stercoralis, Trichuris trichiura, and S. mansoni were more prevalent. The prevalence of worm infection in HIV-negative TB patients 31.0%; 18/58) was similar to that of HIV positive patients (25.9%, 14 /54; P=0.39). Intestinal parasitic coinfection in tuberculosis patients up-regulates the Th2 immune response and supports the hypothesis that, there is an increase in the concentration of plasma IgE and peripheral eosinophils.

Key words: Plasma IgE, Helminths, eosinophils, tuberculosis

INTRODUCTION

Tuberculosis (TB) is the classic human mycobacterial disease acquired through inhalation of aerosolized infectious particles. The disease has high prevalence in countries like south East Asia and Sub-Saharan Africa [1]. Mycobacterium tuberculosis can elicited the immune system for the production of both cellular and humeral

responses. Immunoglobulin E is one of the five classes of immunoglobulin that serve as a receptor for allergens, mast c ells, parasitic antigens. It is important for protection against parasitic diseases [2]. Like tuberculosis, parasite diseases are more common in developing and resource scarce countries of the world mainly of the Sub-Saharan Africa [3].

Peripheral eosinophilia is widely recognized as a useful indicator of parasitic diseases mainly of helminthes. Eosinophils are heavily granulated cells with a bilobed nucleus that stain with the acid dye eosin and their basic proteins are toxic to parasitic infection [4]. It has been well established that cytokines released during specific immune response are characteristic of $CD4^+$ T-helper (TH) cells whose polarized form are Th 1 and Th2 [5]. The Th1 response is a local response which usually occurs early in an infection and amplifies local inflammatory reactions by activating macrophages, natural killer cells and $CD8^+$ Cytotoxic T-cells. Thus, it is important in the control of mycobacterial and other intracellular infections [2, 5]. Whereas Th_2 response results latter and acts systemically to activate B-cells and it is important in the control of helmenthic infections [2, 5]. In Sub-Sahara Africa and developing countries, where the prevalence of parasitic infection is very high a dominant Th2 immune response has been reported and suggested to increase susceptibility to mycobacterium tuberculosis infection [6]. Such an imbalance in an increase is Th2 cells favor IgE production [7]. Th_1 and Th_2 cells have been reported to negatively cross regulate each other in vivo and in experimental animals [8].

There have been few or no reports on immunological interaction between tuberculosis and intestinal parasites in Ethiopia, where the prevalence of these infections is very high [1, 3, 6]. Therefore, the aim of this study is to investigate whether co-infection by mycobacterium and intestinal parasites alter plasma IgE level and Eosinophil count of these specific groups of subjects.

MATERIALS AND METHODS

Study design, area and period

This cross sectional study was conducted at the University of Gondar Teaching Hospital, Northwest Ethiopia. It is a tertiary level teaching and referral hospital rendering services for inhabitants of the Amhara regional state. The study was conducted from September 2010 to March 2012.

Study subjects

The study subjects were all smear positive TB patients who self reported for anti TB treatment at the DOTS clinic in Gondar university hospital. Diagnosis of pulmonary tuberculosis was made based on the clinical presentations of the patient, positive sputum smear and radiographic examination consistent with TB.

Sample size determination and sampling

Time delimited convenient sampling technique was used. All the 112 consecutive smear positive tuberculosis patients who self reported for health service were included in the study.

Sample collection and processing

Socio-demographic and clinical data were collected using questionnaire. Blood samples were collected before the patients started antituberculosis chemotherapy (week 0). About 10 ml venous blood was collected in vacontainer tubes containing EDTA and divided into 2 aliquots. One of the aliquots was poured in test tubes and used for eosinophil count. The second aliquot was centrifuged to separate the plasma as per the standard procedure. Then, the plasma was stored at -20^0c until the assay for HIV and total plasma IgE were done.

Eosinophil count

Total white blood cell count was done for each study subjects using automethod hematology analyzer (Sysmex-kx-21 model) with the anti-coagulated blood as per the manufacturer's protocol. The percentage of eosinophil was obtained by analyzing the thin blood film stained with Wright stain microscopically. Then, by using the total WBC count and the percentage, the absolute count of eosinophils per millimeter cube of blood was calculated.

Serum IgE determination

The plasma IgE level was quantified by total IgE ELISA kit (IBL Immunobiological Laboratories, Hamburg, Germany) following the manufacturer's instructions. Ten micro liters (10ul) of plasma was poured in duplicates into wells of micro titration plates percolated with monoclonal mouse antihuman IgE antibody together with peroxidase-

conjugated antihuman IgE. After incubation for 30 minutes at room temperature the plates was rinsed with diluted wash buffer to remove unbound material. Then, a substrate solution (tetra methyl Benzedrine) was poured and incubated for 15 minutes to induce development of color. The reaction was terminated by the addition of stop solution and the resulting color intensity was measured in a spectrophotometer at 450 nm against the substrate blank. The IgE concentration of the sample was read from a standard curve. Mean values of two separate determinations from each sample was used as serum IgE level of a particular study subject.

HIV testing and stool examination

The HIV status of TB patients was determined in the routine provider initiated HIV counseling and testing (PIHCT) program using rapid HIV kits, Determine (Determine® HIV-1/2 Ag/Ab Combo, US) Capillus (Trinity Biotech, US) and Unigold (Trinity Biotech, US). Following collection stool sample was examined for the intestinal parasites using direct saline preparation and Kato Kat thick methods for three consecutive days.

Data analysis

The data was analyzed using SPSS version 16 statistical package. The results of the study were explained in words and tables. Proportions for categorical variables were compared using chi-square test. In all cases P-value, less than 0.05 was taken as statistically significant.

Ethical consideration

The study was reviewed and approved by ethical review committee of the University of Gondar. Ethical clearance was obtained from the Research and publication office (RPO). Informed consent was obtained from each subject during data collection.

RESULTS

One hundred twelve Tuberculosis patients were included to take part in the study. More than half, (52.7%) of the patients were females. The mean age of the participants was 29.27 years. Ninety one (81.3%) of the patients was younger than 40 years. Fifty-four (48.2%) of the TB patients were seropositive for Human Immunodeficiency Virus. Intestinal helminthic parasite was detected in 32(28.6%) of the smear positive TB patients. Thirteen (11.6%) of the patients had HIV and intestinal parasite co-infection (Table 1).

Table 1. Socio-demographic and clinical data of smear positive TB patients by worm status in the study from 2009-2011

Parameters		Worm status		chi-square	p-value
		Positive(n=32)	Negative(n=80)		
Demographic data					
Age range	< 40 years	27(84.4)	64(80)	0.287	0.592
	>40years	5(15.6)	16(20)		
Sex	Male	15(46.9)	38(48)	0.004	0.952
	Female	17(53.1)	42(52)		
Clinical characteristics					
HIV status	Positive	13(59.4)	19(59.4)	1.03	0.31
	Negative	18(40.6)	39(30.6)		
Eosinophil count	< 300/mm^3	12(37.5)	64(80)	18.93	0.000
	>300/mm^3	20(52.5)	16(20)		
IgE level	< 100IU/mL	3(11.5)	30(46.1)	9.63	0.002
	>100IU/mL	23(88.5)	35(53.8)		

Absolute eosinophil and total plasma IgE level was determined for 100% and 79% of the TB patients, respectively. The mean concentration of plasma total IgE (828 U/mL) of smear positive TB patients with helmenthic infection were significantly higher than those without helmenthic infection (668 U/ml) P= 0.045 .The peripheral eosinophil count in smear positive TB patients with intestinal helmenthic infection (352/mm^3) was significantly higher than those without helmenthic infection (112/mm^3) P= 0.033. Peripheral eosinophilia (>300/mm^3) and elevated level of total IgE (>100U/mL) had statistically significant association with intestinal helmenthic infection in patients with smear positive tuberculosis (X^2= 18.93 p=0.000; X^2= 9.63, p=0.002), respectively.

According to our statistical analysis and the calculated p-values total IgE level (p=0.54) and peripheral eosinophilia (p = 0.34) of the smear positive tuberculosis patients don't have statistically significant association with their HIV status.

Table 2. Plasma IgE concentration and eosinophil count of smear positive tuberculosis patients by their worm status

Parameters	Worm positive patients		Worm negative patients	
	Eosinophil count	IgE concentration	Eosinophil count	IgE Concentration
Mean	352	828	112	668
Median	330	384	147	113

Of the total 112 smear positive tuberculosis patients, 32(28.6%) of them tested positive for Intestinal Helminthes. The parasites, which were prevalent in this specific population, include *Ascaris lumbricoids*, Hookworm, *Strongloides stercoralis, Trichuris trichiura, S. mansoni*. The prevalence of worm infection in HIV-negative TB patients 31.0%; 18/58) was similar to that of HIV positive patients (25.9%, 14 /54; P=0.39) (Table 3).

Table 3. Prevalence of intestinal helminthes in smear positive TB patients by HIV status

Parasite	HIV status of TB Patients		OR (95% CI)
	HIV positive N=54	HIV negative N=58	
Ascaris lumbricoids	5(9.3)	5(8.6)	0.62 (0.14-2.74)
Hook worm	2(3.7)	6(10.3)	0.33 (0.07-1.69)
Strongiloids stercoralis	3(5.6)	0(0)	0.94 (0.88-1.01)
Schistosoma mansoni	0(0)	4(6.9)	1.075 (1.01-1.15)
Tricuris tricuria	4(7.4)	3(5.2)	1.47 (0.313-6.88)

DISCUSSION

Tuberculosis, intestinal parasites and their coinfection rate is highly prevalent in countries like Sub-Saharan Africa and south East Asia [1]. Because of the chronic nature of tuberculosis, it is difficult to determine whether helminth infection preceded the development of active TB or not. It is possible that having active TB will predispose to helminth infections. However, the immune modulation induced by helminth infection may affect immunity to TB. The presence of intestinal worms may shift the immune background towards a Th2 profile [9], which may favour the establishment of mycobacterial infection and the development of active disease. Consistent with this explanation, other reports have shown that development of active TB is associated with enhanced Th2 type immune responses [10]. This would argue against the explanation that having active TB favoured infection with intestinal helminths.

In the present study plasma IgE levels in smear positive tuberculosis patients have been shown to be associated with eosinophilia (p=0.035) and intestinal parasitoses (p. 0.002). The elevated IgE profile in smear positive TB patients with intestinal helminths coinfection might be due to the fact that, intestinal parasitic infections are potent stimulators of IL-4 dependent synthesis of both parasite specific IgE, which is important in the host immune response to parasites, and polyclonal IgE [11]. Parasite specific IgE attaches to high affinity Fc receptors on mast cells, and neighbouring specific immunoglobulin cross-link and trigger mast cell degranulation. This process mediates an antiparasite response via the release of proinflammatory cytokines [12]. This mechanism has been suggested as the major cause of the elevated serum levels of IgE in tropical populations where the prevalence of parasites is very high [13].

In our study, only 32 positive cases of helmenthic infections were detected by the examination of at least three-consquetive stool sample. The finding of *Ascaris lumbricoids*, Hookworm, *Strongloides stercoralis*, *Trichuris trichiura, and S. mansoni* is in agreement with most of the previous studies [14]. In the current study we showed that, prevalence of worm infection in HIV-negative TB patients (31.0%; 18/58) was similar to that of their HIV positive counter parts (25.9%, 14 /54; P=0.39). Logistic Regression analysis to assess the association between individual helminth species and HIV status found no significant association between HIV status and any of the helminth species observed. These findings are similar with a study done in Gondar to ascertain the effect of intestinal parasites for the development of active tuberculosis [10].

CONCLUSION

The present study confirms that intestinal parasitic coinfection in tuberculosis patients up-regulate a Th2 immune response and supports the hypothesis that, there is an increase in the concentration of plasma IgE and peripheral eosinophils. Further longitudinal studies, following the patients until completion of anti-TB chemotherapy is recommended to fully understand the role of therapy on the concentration of plasma IgE and eosinophils. .

Acknowledgments
The University of Gondar research and community services core process supported this study. This work was also partly supported by the Deworming project. We would like to express our heartfelt gratitude to staff of Gondar University Hospital laboratory and Deworming project.

REFERENCES

[1] World Health Organization report: Global tuberculosis control. WHO **2011**.
[2] Murrey PR, Rossental KS, Kobayashi GS, Pfaller MA. Medical microbiology: Basic concepts in immunology. **2009**.
[3] Kloss H, Zein AZ in: Tesfayohans TM (Ed.), Intestinal parasites in Ethiopia (Oxford, west view press, **1992**) 223.
[4] Fiscbach FT; A manual of laboratory and diagnostic tests, **1992**.
[5] Mosmann TR, Charueinski H, Bons MW, Giedlin MA, Coffman RL, *J Immunel* **1986**, 136, 2348.
[6] Bentwith Z, Kalinkovich A, Weisman Z, *Immunol today*, **1995**, 16,187.
[7] Costat SL, Botomyl K, *Ann Reu Immunol*, **1997**, 15,297.
[8] Abbas AK, Murphy KM, Shar A; Functional diversity of helper T-lymphocytes *nature*, **1996**.
[9] Van den Biggelaar AH, van Ree R, Rodrigues LC, *Lancet*, **2000**,356, 1723.
[10] Elias D, Mengistu G, Akuffo H and Sven B. Tropical Medicine and International Health, **2006**, 11, 551.
[11] Lynch NR In: Pawlowiski Z, (ed.) Bailliere's Clinical Tropical Medicine and Communicable Diseases (London, Bailliere Tindall,) **1987**, 573.
[12] Else KJ, Finkelman FD, Int J Parasitol, **1998**,28,1145.
[13] Bentwich A, Weisman Z, Moroz C, Bar-Yehuda S, Kalinkovich A., *Clin Exp Immunol* **1996,** 103,239
[14] Nagaraj S, Raghavan R, Macaden R, Kurpad AV, *Indian journal of medical microbiology*. **2004**, 22, 54