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Serum IgE Levels of Diarrheic Patients in Northwest Ethiopia with High Prevalence of HIV and Intestinal Parasitoses

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Abstract

Background: HIV/AIDS remains a major health problem in Sub-Saharan Africa. Co-infection with intestinal parasites has been suggested to worsen the outcome of infection by polarizing the immune response towards Th2. This study investigated the IgE profile in patients with diarrhea and with or without HIV and/or intestinal parasites co-infection at the time of diagnosis.

Methods and Materials: A cross-sectional study was conducted among diarrheic patients with and without HIV/AIDS attending at the outpatient Department of the University of Gondar teaching hospital, in Gondar, Ethiopia. Stool samples were examined using standard parasitological procedures. The presence of HIV antibodies was determined by an enzyme linked immunosorbent assay following the manufacturer's instruction.

Results: Among tested diarrheic patients, 109 (52.9%) of them were seropositive for HIV. Chronic and acute diarrheas were diagnosed in 114(55.3%) and 92(44.7%) of the patients, respectively. Intestinal parasites were detected in 30(27.5%) among HIV seropositive and 36(37.1%) of HIV seronegative diarrheic patients. Diarrhea and marked weight loss were found to be significantly associated with HIV infection ($P<0.05$). Median IgE concentration found in HIV positive diarrheic patients (618 IU/ml, IQR 107.25-971.25 IU/ml) was not significantly ($P>0.05$) higher than in HIV negative diarrheic patients (618 IU/ml, IQR 304.50-739 IU/ml). Significantly higher association of median total IgE level was obtained in diarrheic male patients (624 IU/ml, IQR 325.50-857.25 IU/ml) than diarrheic female patients (490 IU/ml, IQR 39-835 IU/ml), $P<0.001$. Adjusted estimates of the effects of HIV, age, and helminth infection on IgE concentration, estimated using linear regression did not showed significant associations.

Conclusion: There was a remarkably elevated IgE response in diarrheic patients irrespective of HIV and/or intestinal parasitic infection. The correlation of IgE levels with parasitic infection with or without HIV co-infection as well the immunological and molecular mechanisms of IgE overproduction and its role in HIV infection needs further investigation.

Keywords: Northwest Ethiopia; HIV/AIDS; Diarrhea; Intestinal parasitoses; Total immunoglobulin E

Introduction

Diarrheal diseases are one of the most important causes of morbidity and mortality in developing countries [1]. The situation is severe in sub-Saharan Africa, a region where an estimated 25.8 million adults and children are infected with HIV [2]. Diarrhea, the passage of loose or watery stools at least three times in 24 hours, is one of the clinical manifestations of HIV infection and usually tends to be chronic [1]. Chronic diarrhea, an episode that begins acutely and lasts for more than four weeks [1] in tropical countries is associated with weight loss and is often the presenting illness of HIV infected individuals. This diarrhea wasting syndrome in association with a positive HIV serology test is an AIDS defining illness in the World Health Organization's classification [3].

In addition, parasitic infection of the intestinal tract is a major source of disease in patients with HIV particularly in the tropics, where specific pathogens are identified in more than half of the HIV/AIDS patients with persistent diarrhea [4].

Infection with intestinal parasites, mainly helminths, elicits Th2 immune response [5]. In sub Saharan Africa, where the prevalence of parasitic infections is very high, a dominant Th2 polarized immune response has been reported [6,7] and suggested to increase susceptibility to both intracellular pathogens like *M. Tuberculosis* and HIV. Co-infection also hastens progression of their disease [8-11]. Such an imbalance with an increase in Th2 cells favors IgE production [10] which may have clinical effects such as poor prognosis in co-infected individuals.

Reports on immunological interactions between IgE, intestinal parasitoses and HIV in Ethiopia is too little, a country where the prevalence of these infections is very high. Therefore, this study aimed to investigate the IgE profile *in vivo* in patients with diarrhea and with or without HIV and/or intestinal parasites co-infection at the time of diagnosis.

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Patients and Methods

Study design and subjects

In this cross-sectional study, consecutive diarrheic patients diagnosed at the outpatient department of the University of Gondar Hospital, Gondar, Ethiopia were included. Informed consent was obtained from all subjects and the study was approved by the Research Ethics Committee of the University.

Stool examination

Stool samples were collected and examined on three consecutive days by direct microscopy of samples in saline and iodine. Formalin-ether sedimentation concentration methods were also followed to detect the intestinal parasites [12]. Patients found positive for intestinal protozoa (*Entamoeba histolytica and Giardia lamblia*) were treated with metronidazole or tinidazole. However, such patients were excluded from the study as IgE response is different in protozoal infection than in worm infections. Subjects found positive for intestinal helminths were treated with broad spectrum anthelminthics such as mebendazole, praziquantel, thiabendazole, and niclosamide where appropriate.

Blood collection and screening for HIV

After appropriate pretest counseling, venous blood was collected from each patient before initiation of therapy, and in a sub sample of them at the end of intensive phase of anti-TB chemotherapy. Serum was separated by centrifugation within two hours of collection and kept at -20^{0}C until used. The presence of HIV antibodies was determined by an enzyme linked immunosorbent assay following the manufacturer's instruction (Vironostica HIV Uni-Form II plus O, Organon Teknika, Boxtel, the Netherlands).

Serum IgE determination

The serum IgE levels were quantified by total IgE ELISA kit (IBL Immunobiological Laboratories, Hamburg, Germany) following the manufacturer's instructions. In brief, 10ml serum samples or standard IgE were pipetted in duplicates into wells of micro-titer plates pre-coated with monoclonal mouse antihuman IgE antibody together with peroxidase conjugated antihuman IgE. After incubation for 30 minute at room temperature the plates were rinsed with diluted wash buffer to remove unbound material. Then a substrate solution (tetramethylbenzidine) was pipette and incubated for 15 minute to induce development of color. The reaction was terminated by the addition of stop solution and the resulting dye was measured in a spectrophotometer (Model 680 Micro plate Reader, Bio-Rad Laboratories Inc., Japan) at a wave length of 450 nm against the substrate blank. The IgE concentration of the samples 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.

Statistical analysis

Total IgE levels were expressed as arithmetic or geometric mean and IU/ml. As total serum IgE levels were highly skewed, a logarithmic transformation (Log10) was performed to obtain a Gaussian shape. Correlations were made using Spearman's correlation analysis. The difference of total IgE between two groups was computed using non-parametric Mann Whitney U test. A multivariate linear regression analysis was used to determine the effect of different factors on log (lgE). P-value less than 0.05 were accepted as significant. Statistical computations were performed using Statistical Package for Social Sciences (SPSS).

Parameter		Total	HIV+ (n=109) n (%)	HIV- (n=97) n (%)
Age	<35	136(66.9)	65(59.6)	71(73.2)
	>35	70(34.0)	44(40.4)	26(26.8)
Sex	Male	127(61.7)	65(59.6)	62(63.9)
	Female	79(38.3)	44(40.4)	35(36.1)
Address	Rural	142(68.9)	78(71.6)	64(66.0)
	Urban	64(34.1)	31(28.4)	33(34.0)
Marital Status	Married	100(48.5)	53(48.6)	47(48.5)
	Single	77(37.4)	33(30.3)	44(45.4)
	Divorced	29(14.1)	23(21.1)	6(6.2)
Occupation	Government Employee	47(22.8)	25(22.9)	22(22.7)
	Farmer	63(30.6)	46(42.2)	17(17.5)
	Student	27(13.1)	17(15.6)	10(10.3)
	Housewife	39(18.9)	7(6.4)	32(33.0)
	Other	30(14.6)	14(12.8)	16(16.5)
Income*	Low	126(61.2)	59(54.1)	67(69.1)
	Medium	53(25.7)	34(31.2)	19(19.6)
	High	27(13.1)	16(14.7)	11(11.3)
Diarrhea	Acute	92(44.7)	60(55)	32(33)
	Chronic	114(55.3)	49(45)	65(67)
Weight loss	Yes	18(42.7)	60(55)	28(28.9)
	No	188(57.3)	49(45)	69(71.1)
Intestinal Parasitosis	Yes	66(32.0)	30(27.5)	36(37.1)
	No	140(68.0)	79(72.5)	61(62.9)
Mean IgE†			585.23±447.16	597.32±388.97

†Mean ±SD.
* Low: less than 1000 Ethiopian Birr/month. Medium: 1000 – 5000 Ethiopian Birr, High: more than 5000 Ethiopian Birr/month.
1 Ethiopian Birr = 0.12 US Dollar (at the time of data collection)

Table 1: Demographic and clinical data of diarrheic patients included in the study by their HIV status (n=206).

Parasite	HIV status	
	Positive N (%)	Negative N (%)
Entamoeba histolytica	8(23.5)	12(27.3)
Giardia lamblia	3(8.8)	9(20.5)
Strongyloides stercoralis	9(26.5)	3(6.8)
Ascaris lumbricoides	6(17.6)	8(18.2)
Hookworm	3(8.8)	5(11.4)
Schistosoma mansoni	3(8.8)	5(11.4)
Cryptosporidium spp.	2(5.9)	2(4.5)

Table 2: Type and frequency of intestinal parasites in diarrheic patients by HIV status.

Results

Two hundred and six diarrheic patients were included in the study. Demographic and clinical data of the patients were presented in Table 1. Majority of the patients were males 127(61.7%). One hundred thirty six (66.0%) of the patients were younger than 35 years old. One hundred and nine (52.9%) of the diarrheic patients were seropositive for HIV. Chronic and acute diarrheas were diagnosed in 114(55.3%) and 92(44.7%) of the patients, respectively. Intestinal parasites were detected in 30(27.5%) of the 109 HIV seropositive, two patients was found to have mixed infections and 36(37.1%) of HIV seronegative diarrheic patients with four mixed infections. The prevalence including multiple infections from individual subjects and species of intestinal parasites detected in the patients is shown in Table 2.

The risk of HIV infection was associated with urban residence (OR=

1.98, 95% CI= 0.98-3.99) and age (OR= 1.98, 95% CI= 1.02-3.86). There was no significant difference in the clinical presentation of intestinal parasitosis between HIV-seropositive and seronegative patients (P> 0.1). However, chronic diarrhea (OR= 2.49, 95% CI= 1.30-4.78) and marked weight loss (OR= 2.61, 95% CI= 1.40-4.87) were found to be significantly associated with HIV infection (P< 0.05) (Table 3).

Median IgE concentration found in HIV positive diarrheic patients (618 IU/ml, IQR 107.25-971.25 IU/ml) was not significantly higher than in HIV negative diarrheic patients(618 IU/ml, IQR 304.50-739 IU/ml), *P*=0.3 (Table 4). It was also not significantly higher in patients infected with intestinal parasites than in those without intestinal helminths in HIV seronegative diarrheic patients, *P*=0.3 (Table 4). But, significantly higher association of median total IgE level was obtained in diarrheic male patients (624 IU/ml, IQR 325.50-857.25 IU/ml) than diarrheic female patients (490 IU/ml, IQR 39-835 IU/ml), (*P*<0.001). Adjusted estimates of the effects of HIV, age, residence area and helminth infection on IgE concentration, estimated using linear regression including all these variables, were not significantly different from those in Table 4.

Discussion

The immune profile of individuals living in sub Saharan Africa is characterized by a background of chronic immune activation which has been attributed to exposure to high load of environmental antigens [7-10]. Such exposure has been suggested to impair the host's immune response to HIV and intestinal parasitosis [8] which is highly prevalent in that part of the world [13,14].

The present study was conducted in a tropical country, in sub Saharan Africa, where the prevalence of intestinal parasites, HIV is amongst the highest in the world [15-17]. The results of the study indicated that the level of serum IgE was remarkably high in diarrheic patients. In addition it was revealed that serum IgE was higher in patients irrespective of HIV and/or intestinal parasites.

From HIV positive patients with diarrhea, 55% and 45% had acute and chronic diarrhea, respectively. Enteric pathogens were detected in 30 (27.5) of the 109 patients: 28 patients harboured a single pathogen, and 2 patients had mixed pathogens. Stools of all HIV-positive patients with diarrhea should thoroughly be investigated to identify etiologic agents for proper management; given that, diarrhea is frequently found

Parameter		HIV+ (*n=109*)	HIV- (*n=97*)	OR (95% CI)	Multivariate OR (95% CI)
Age	<35	65	71	1	
	>35	44	26	1.849(1.025, 3.335)	1.979(1.015, 3.857)*
Sex	Male	65	62	1	1
	Female	44	35	1.199(0.682, 2.108)	1.180(0.639, 2.180)
Address	Rural	78	64	1	1
	Urban	31	33	1.297(0.718, 2.343)	1.976(0.979, 3.986)*
Marital Status	Married	53	47	1	1
	Un married	56	50	0.993(0.575, 1.717)	1.306(0.685, 2.488)
Diarrhea	Acute	60	32	1	1
	Chronic	49	65	2.487(1.411, 4.385)	2.492(1.298, 4.784)*
Weight loss	Yes	60	28	1	1
	No	49	69	3.017(1.691, 5.384)	2.606(1.395, 4.869)*
Intestinal Parasitosis	Yes	30	36	1	1
	No	79	61	1.554(0.863, 2.799)	1.535(0.814, 2.896)

*P<0.05
OR, Odds Ratio; CI, Confidence Interval

Table 3: The association of some risk factors with HIV infections.

Variables		Median and IQR	OR (95% CI)	*p-value*
Age	*<35*	618(198-739)	1	0.7
	>35	618(149.75-1028)	1.112 (0.506-2.441)	
Sex	*Male*	624(325.50-857.25)	1	<0.001
	Female	490(39-835)	0.310 (0.151-0.639)*	
Address	*Rural*	611 (159-786)	1	0.7
	Urban	735(307.75-929.25)	0.864 (0.373-2.000)	
Marital Status	*Married*	618(235.75-999.25)	1	0.9
	Un married	614(149.50-739)	1.039 (0.490-2.200)	
Diarrhea	*Acute*	618(178-835)	1	0.6
	Chronic	613(197.50-861)	1.220 (0.556-2.677)	
Weight loss	*Yes*	618(107.25-883.75)	1	0.5
	No	612(241-791.50)	0.805 (0.379-1.711)	
Intestinal Parasitosis	*Yes*	624(322.75-837.25)	1	0.3
	No	611(145-844)	0.695 (0.313-1.545)	
HIV status	*Positive*	618(107.25-971.25)	1	0.3
	Negative	618(304.50-739)	1.420 (0.658-3.067)	

*P<0.05
OR, Odds Ratio; CI, Confidence Interval.

Table 4: Median and interquartile ranges (IQR) for total IgE.

in HIV infection with different clinical stages [18-20]. According to the result of this study; urban residence, age above 35 years old, diarrhea and marked weight loss were significantly associated with HIV infection [21-23].

Intestinal parasitic infections were found to be more prevalent in the rural area and mostly male study subjects were infected. The rarity of infection by multiple parasite species was also notable. Although it is documented that the sensitivity of a single stool examination is only about 40% [24], in most comparable studies in similar developing communities only a single stool sample was examined. Part of the explanation may be an improvement in personal hygiene over the past decades. It is also possible that there has been an increase in the treatment of helminth infections over time in our study communities. Worms are held responsible for all manner of constitutional upsets and use of deworming medicines, which can be freely bought from local drug stores, is very common. The higher prevalence of parasite infections in the rural area could be partly explained by the season of collection since parasitic infections are more common during the wet season [25].

In the present study, significantly higher IgE levels were observed irrespective of HIV sero-status and patients co-infected with intestinal helminths compared to non-infected individuals. This is due to the fact that, intestinal parasitic infections are potent stimulators of IL-4 dependent synthesis of both parasites specific IgE, which is important in the host immune response to parasites, and polyclonal IgE [26]. Parasite specific IgE attaches to high affinity Fc receptors on mast cells, and neighboring specific immunoglobulin cross-link and trigger mast cell degranulation. This process mediates an anti-parasite response via the release of pro-inflammatory cytokines [27]. 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 [28].

In this study, the mean IgE level was increased in most of diarrheic patients irrespective of HIV and intestinal parasitic infection patients and the serum IgE levels were not significantly associated to Age, address, duration of diarrhea. This result was not in line with previous studies on the relationship between IgE and HIV in Western countries which demonstrates the possibility that serum IgE level increases along with the severity of the disease in patients with HIV infection [29,30]. It may due to the fact that, elevated total serum IgE levels also occur in patients with a variety of allergic diseases and non-allergic diseases, including viral infections, atopic dermatitis, and neoplastic disease such as Hodgkin's lymphoma and IgE myeloma [31]. However, this study did not include an exhaustive search for those pathological conditions.

On multiple linear regression analysis, male diarrheic patients (P <0 .001) were independently associated with increased serum IgE levels. Multiple studies have shown that IgE level was elevated in parasite infected patient and mostly male study subjects were infected with parasite [32,33]. There might be a possibility that the higher IgE level found in male was due to a higher ratio of parasite infection in male which is similar to previous report in Ethiopia [35]. The reason for higher intestinal parasitic infection prevalence is not clear but as to schistosomiasis, males may have higher exposure to river water than females. Some parasitologists also suggest that susceptibility to parasitic infections is greater in males and may contribute to male biased mortality [36]. IgE level is also affected by smoking history [37], in Ethiopia male smokers are higher than female smokers [38-39]. In line with our findings, the work of Johnson et al. [34] compared total and allergen specific IgE by gender from birth through 4 years of age and found that total IgE increased with age for boys and girls and was higher in boys. This suggested that differences in IgE development between young boys and girls may partially account for the higher prevalence of asthma in boys than in girls.

The main limitation of this study however is that serum IgE level of the patients with out diarrhea was not documented to make comparisons. The relationship between worm burden and levels of IgE was not also documented due to the nature of study design.

Taken together, the results of this study indicated that, there was a remarkably elevated IgE response in patients irrespective of HIV and/or intestinal parasitic infection; although, this is a small group of study subjects and by no means be representative of the entire Ethiopian population, the findings may be used as a tool to assist further studies in this area. The correlation of IgE level with parasitic infection with or without HIV co infection needs further prospective clinico-epidemiological studies by including non diarrheic subjects. The results suggest further study of IgE response in diarrheic patients in Ethiopia relative to infections other than HIV and on the molecular mechanisms of IgE overproduction and its role in HIV infection are also needed.

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