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Association between Sudanese Serum Total IgE and Schistosoma Mansoni Infection

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Abstract: *This study aimed to associate between Sudanese serum total IgE and S. mansoni infection. A cross sectional study was conducted in the period between March-October 2011. The study was conducted on 764 serum samples (110 were positive for S. mansoni and 654 were negative for S. mansoni) and 4 serum samples as European control were examined. The age ranged between 4-85 years, mean age was 23 years old. Faeces samples were collected from all the subjects included in the study and examined by using Kato-Katz technique. Also, serum samples were taken from all subjects and examined by using Human IgE Ready-SET-Go! ELISA (eBioscience, Frankfurt, Germany) technique was used. The results showed that there was a statistically significant relationship between the mean of total IgE and S. mansoni infection (p=0.000). The results demonstrated that the relationship between the mean of total IgE and age-groups was fluctuated. The results reflected that there was a statistically significant relationship between the mean of total IgE and the gender (p=0.000). In addition to, there was a statistically significant relationship between the mean of total IgE and treatment (p=0.000). The results revealed that the relationship between the mean of total IgE and intensity of S. mansoni infection was fluctuated while there was a statistically significant relationship between the mean of total IgE and Kato technique (p=0.000).*

Keywords— Association; *Schistosoma mansoni*; Sudanese; Total IgE

1. INTRODUCTION

Schistosoma mansoni is causes intestinal and hepatic schistosomiasis in more than 100 million individuals that live in sub-Saharan Africa, the Caribbean and South American areas [1]. Most infected individuals with *Schistosoma mansoni* in endemic areas are asymptomatic or have mild clinical manifestations. However, in a minority of infected individuals, infection with this parasite can lead to severe hepatosplenic schistosomiasis, characterized by periportal fibrosis, portal hypertension, gastrointestinal bleeding and death [2]. Most of the morbidity related to chronic schistosomiasis is associated with hepatic and intestinal granulomatous inflammation induced by the parasite eggs that become trapped in these tissues. Granulomatous inflammation is dependent on CD4+T cells, leading to tissue eosinophilia and the activation of alternatively activated macrophages [3]. There are many factors might influence both the development and level of morbidity in an exposed population, among them the degree and length of exposure, the intensity of the infection, concurrent pathologies, host and parasite genetics and nutritional status, which have all been associated with disease severity [4]. However, because granuloma formation is an immune-mediated process, factors that influence the induction and modulation of the immune response against parasite egg antigens could also be determinants in the progression of severe schistosomiasis. Epidemiologic studies have indicated that *S. mansoni* infected patients presenting with severe fibrosis have elevated levels of the chemokine CCL3, tumor necrosis factor (TNF)-alpha, IL-5 and IL-13 [5], whereas patients with low levels of fibrosis present with high levels of IFN-gamma and IL-10 [6]. Regarding the role of cytokines in granuloma formation and their association with disease severity, the participation of antibody responses against *Schistosoma* infection on the progression of clinical disease has been poorly investigated. The importance of B cell and antibody responses in the pathology associated with schistosomiasis has been suggested from experimental infections of *S. mansoni* in B cell deficient mice [7]. In human populations, immunoepidemiologic studies have indicated that increased levels of anti-schistosome IgE are closely correlated with resistance to re-infection and that high levels of anti-schistosome IgG4 are correlated with increased susceptibility to the parasite [8]. In contrast, there are very few clinical studies showing the relationship between specific antibody production and schistosomiasis severity. These studies have demonstrated a positive association between anti-schistosome IgG responses, particularly IgG4, and severe schistosomiasis [9]. The main objective of the present study was to associate between total IgE and *S. mansoni* infection in the study area.

2. Materials and methods

2.1 Study design:

It is a cross-sectional study.

2.2 Study area and study duration:

The study was conducted in different villages (Tiba, Tabark Allah, AlQadesia, AlGamhoria and AlWehda) in New Halfa city in Eastern Sudan., during the period between March to October 2011**.**

2.3 Study population:

The study was conducted on the inhabitants of Al Qadesia, Tiba, Tabark allah, AlGamhoria and Alwehda villages in New Halfa city, males and females with different ages and occupations, suffering from schistosomiasis were recruited to the study.

2.4 Sample size:

764 serum samples (110 were positive for *S. mansoni* and 654 were negative for *S. mansoni*) and 4 serum samples as European control were examined.

2.5 Sample collection:

Faeces and serum samples were collected from studied population.

2.6 Data collection:

Designed and previously prepared questionnaires were filled by participants.

3. Methods

3.1 Original Kato-Katz technique:

The faecal specimen was forced through the screen (sieve) by a spatula to separate faecal material from large debris. Then the sieved faecal material was transferred into the hole of a template (approximately 41.7mg) that previously placed on a clean and dry microscope slide. The template hole was completely filled with screened faecal material and leveled to the surface of the template and the template was removed gently leaving the sample to take its form. A cellophane strips (25x 35mm) were soaked in 50% glycerol-malachite green solution for at least 24 hours before use (as a clearing agent) was laid on top of the sample, and gently pressed to print a thin film on the cellophane lower surface. The preparation then kept for an hour, before it will be examined microscopically; using the 10x objective lens for search and the 40x will be used for identification. The number of eggs observed was multiplied by 24 to obtain the number of eggs per gram of faeces (WHO, 2001) [10]. Then the intensity of infection was determined as described by (WHO, 2001) [10] as follows:

Mild	$\leq$ 50 eggs per gram of faeces
Moderate	51- 200 eggs per gram of faeces
Severe	201-300 eggs per gram of faeces
Hyper infection	$\geq$ 400 eggs per gram of faeces

3.2 Human IgE Ready-SET-Go ELISA (eBioscience):

Serum samples from surveyed populations were investigated for immunoglobulin total IgE, using the Human IgE Ready-SET-Go! ELISA (eBioscience, Frankfurt, Germany). In brief, ELISA plates (Greiner Bio-One, Germany) were coated with 50 µl/well of capture antibody (diluted 1:250) in coating buffer (diluted 1:10 in distilled water), plates were sealed and incubated overnight at 4°C. Wells were aspirated and washed twice with 400 µl/well wash buffer (PBS, 0.05% Tween 20), time was allowed for soaking (~1 minute) during each wash step to increases the effectiveness of the washes. Wells were block with 125µl of blocking buffer (1:10 diluted assay buffer) and then incubated at room temperature for 2 hours. Wells were aspirated and washed twice with 400 µl/well wash buffer. Afterwards, 50 µl of standards, blanks and of undiluted serum samples were added to the appropriate wells. Plates were sealed and incubated at room temperature for 2 hours. Wells were aspirated and washed 4 times with 400 µl/well wash buffer. Next, 50 µl of the detection antibody (1:250 diluted in 1x assay buffer) were added to each well. Plates were then sealed and incubated at room temperature for 1 hour. Wells were aspirated and washed 4 times with 400 µl/well wash buffer. Finally, 50 µl of the substrate solution were added to each well and the plates were incubated at room temperature for approximately 15 minutes until the enzymatic reaction was stopped with 25 µl 2N H_2SO_4 per well. Plates were then read at 450 nm.

3.4 Data analysis:

Data was analyzed using Statistical Package of Social Sciences (SPSS) for windows, version 15 and the p values of less than 0.05 were considered statistically significant. Data presented in graphs using Microsoft Excel and PRISM 5 programme (Graph Pad Software, Inc., Jolla, USA) after analysis by SPSSResults were analyzed using the computerized program of statistical package of social science (SPSS). Then data were presented in tables.

3.4 Ethical consideration:

Informed consents will be taken from study subjects or their gardeners after explaining the study purpose. Ethical clearance will also be taken from the ethical committee of Sudan University of Science and Technology and Ministry of Health-Kassala State Department of Preventive Medicine Office of the anti-bilharzia and intestinal worms New Halfa City.

4. Results

For detection of human total IgE, 764 serum samples (110 were positive for *S. mansoni* and 654 were negative for *S. mansoni*) and 4 serum samples as European control were examined. The result of infected individuals from 0.00-14053.36 ng/ml, results of non-infected from 0.00-11061.99 ng/ml and result of European control from 15.5- 43.88 ng/ml (figure 1). The mean of total IgE was 2464.11 ng/ml. The relation between total IgE in an infected and uninfected individuals was significant (p value was less than 0.05, p=0.000). Among 764 serum samples, 110 were positive for *S. mansoni* and 654 were negative for *S. mansoni*. The mean of total IgE was higher in infected individuals (4481.26 ng/ml) than uninfected (2123.66 ng/ml) (figure 2). The relation between the mean of total IgE and *S. mansoni* infection was significant (p value was less than 0.05, p=0.000), the correlation was positive (r=0.000). 764 serum

samples were examined for total IgE, 472 were male (45 were positive and 427 were negative) and 292 were female (65 were positive and 227 were negative). The mean value of total IgE was found to be higher in female (2942.86 ng/ml) than in male (2166.31 ng/ml) (figure 3). The value of total IgE was significantly different between gender (p value was less than 0.05, p=0.000) and the correlation was positive (r=0.001). Among 764 serum samples, 256 had previous infection with *S. mansoni*, 243 of them received treatment and 13 did not. Among those received treatment the level of total IgE was found to be lower (3043.63 ng/ml) compared to that reported among non treated individuals (4240.19 ng/ml) (figure 4). The difference was found to be significant (p value was less than 0.05, p=0.000) and the correlation was positive (r=0.236). The relation between the mean of total IgE results and age-groups was fluctuated (figure 5). Also, The relation between the mean of total IgE and intensity of *S. mansoni* infection was fluctuated (figure 6), while The relation between the mean of total IgE and Kato technique was significant (p value was less than 0.05, p=0.000) (figure 7).

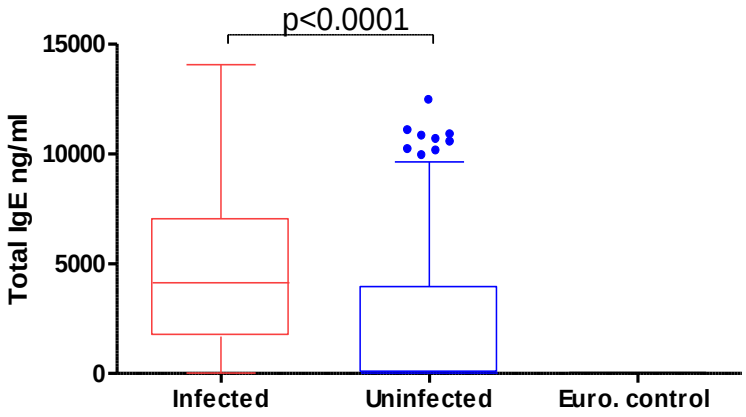

Figure 1: Result of Total IgE

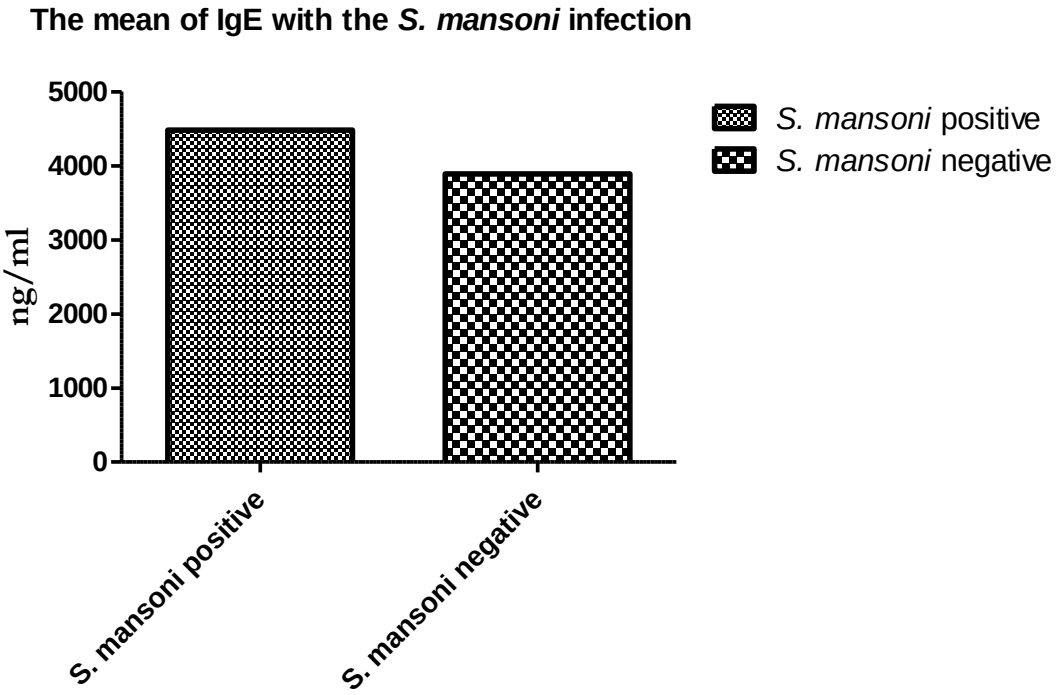

Figure 2: The mean of IgE with the *S. mansoni* infection

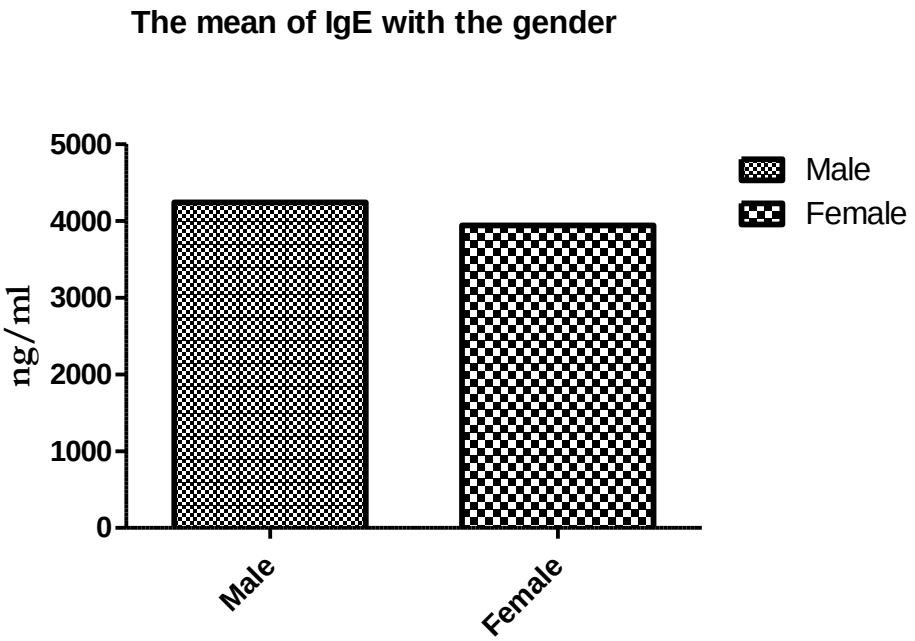

Figure 3: The mean of IgE with the gender

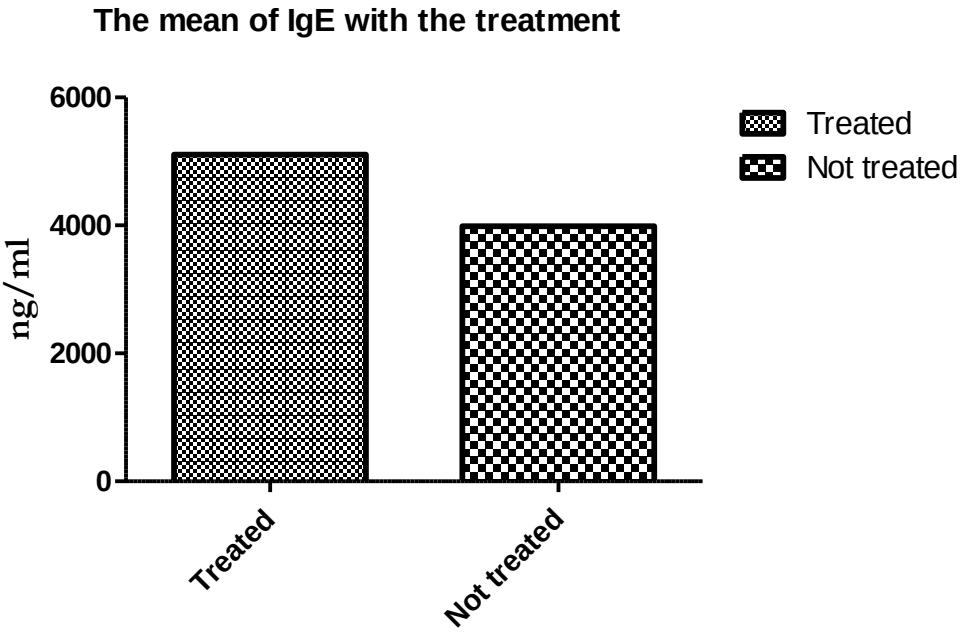

Figure 4: The mean of IgE with the treatment

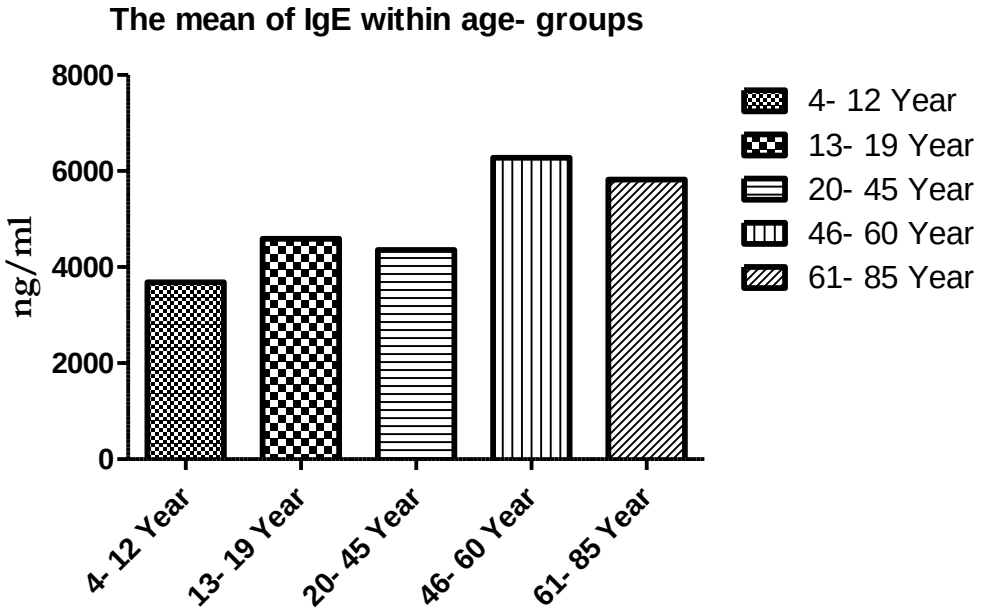

Figure 5: The mean of total IgE within age-groups

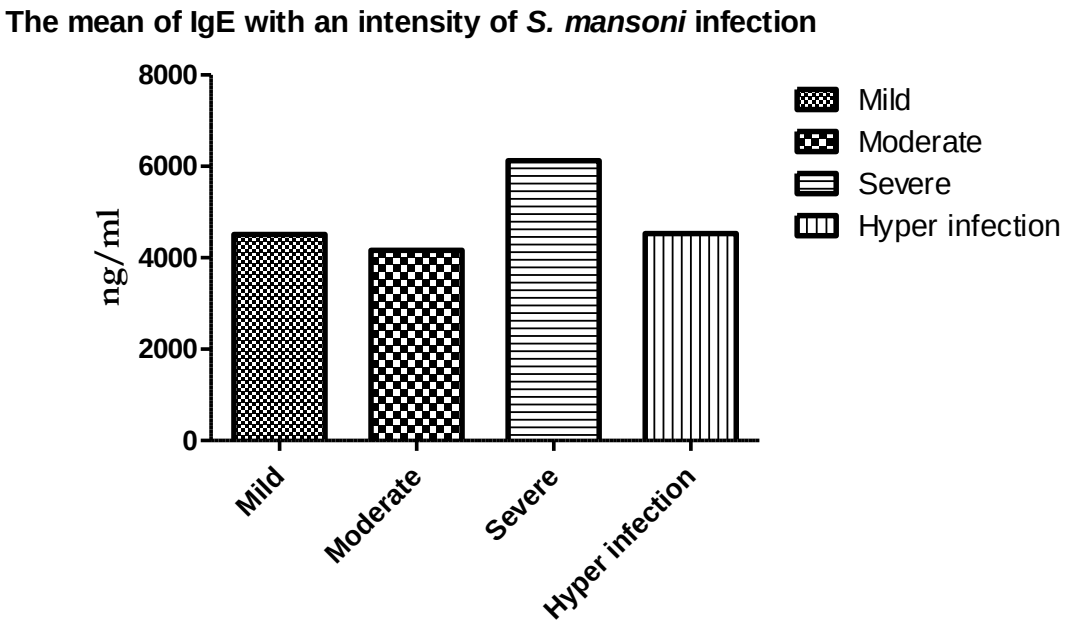

Figure 6: The mean of total IgE with an intensity of *S. mansoni* infection

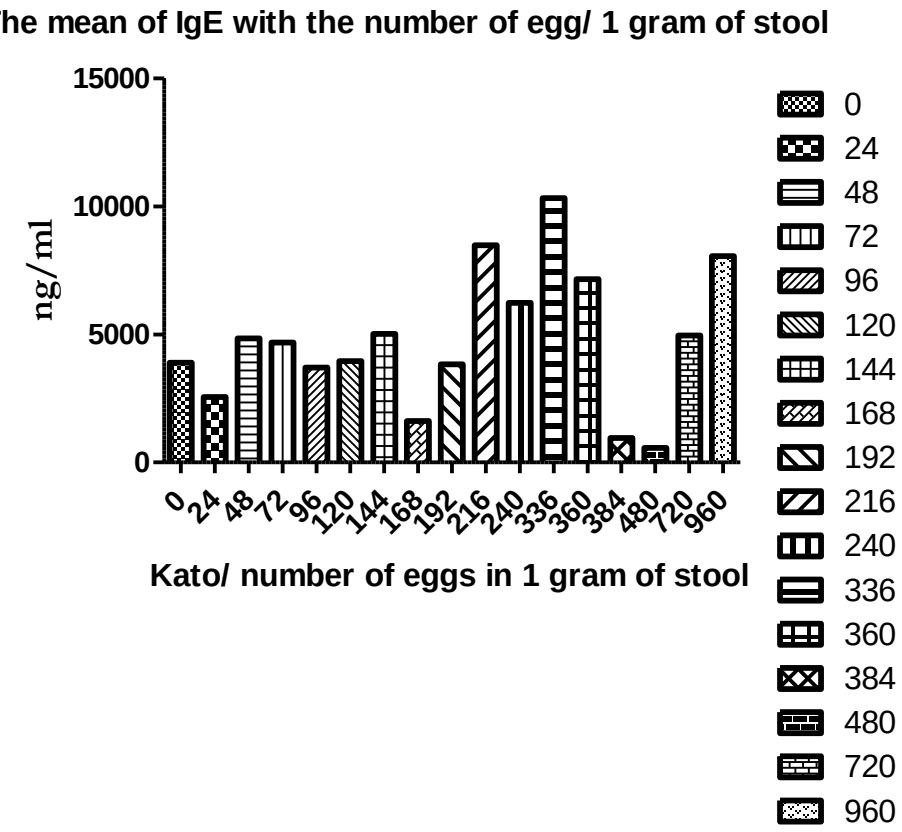

Figure 7: The mean of IgE with the number of egg/ 1 gram of stool

5. Discussion

The present study showed that the relation between the mean of total IgE and *S. mansoni* infection was significant (p value was less than 0.05, p=0.000). The relation between the mean of total IgE results and age-groups was fluctuated. The relation between the mean of total IgE and the gender was significant (p value was less than 0.05, p=0.000). The relation between the mean of total IgE and treatment was significant (p value was less than 0.05, p=000). The relation between the mean of total IgE and intensity of *S. mansoni* infection was fluctuated. The relation between the mean of total IgE and Kato technique was significant (p value was less than 0.05, p=0.000). These findings were in agreement with the findings obtained by Negra˜o-Corre^a *et al.* (2014) [11].

6. Conclusion

The study concluded that there was association between total IgE and *Schistosoma mansoni* infection (Serum total IgE higher in individuals infected with *S. mansoni* than uninfected). In addition to, total IgE was correlated with the gender, Kato technique and the treatment of infection.

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