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Factors to Consider in Measuring Serum Interleukin (IL)-8 Levels in Sudanese Patients Infected with Schistosoma Mansoni

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Abstract: *The study was conducted to evaluate factors to consider in measuring serum Interleukin (IL)-8 levels in Sudanese patients infected with S. mansoni. A cross-sectional study was conducted in New Halfa City-Eastern Sudan during the period from March to October 2011. The study was conducted on 575 subjects, 332 were males (45 were positive for S. mansoni and 287 were negative for S. mansoni) and 243 were females (65 were positive for S. mansoni and 178 were negative for S. mansoni). The age ranged between 4-85 years old, and the age was divided into 5 groups: 4-12, 13-19, 20-45, 46-60 and 61-85 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 collected from all subjects and examined by using Human IL-8 ELISA Ready-SET-Go! (eBioscience) technique. The results showed that S. mansoni-infected individuals have no differences in their levels of IL-8 when was compared in two groups (infected and uninfected by using Mann-Whitney test), p=0.4331 and in three groups (infected, uninfected and previously infected by using Kruskal-Wallis test), p=0.0656. But, when `was compared infected with uninfected p=0.0139 and previously infected with uninfected p=0.0074. The results reflected that there was a statistically significant relationship between the mean of IL-8 and gender, age-groups, treatment of S.mansoni infection, Kato-Katz technique and intensity of S. mansoni infection and there was a statistically insignificant relationship between the mean of IL-8 and the tribes. The results revealed that the mean of serum IL-8 in S. mansoni infection reflected positive correlation with gender, treatment, Kato technique, intensity of infection, tribes and age groups.*

Keywords— Factors; IL-8; *Schistosoma mansoni*; Human IL-8 ELISA Ready-SET-Go! (eBioscience) Technique

1. INTRODUCTION

Schistosoma mansoni is the most prevalent of the schistosome species that affect the intestines and liver. An estimated 62 million persons are infected worldwide. *S.mansoni* is known to occur in 52 countries, including sub-Saharan Africa (where around 85% of the global burden is concentrated), North African and Eastern Mediterranean countries and South American countries as well as several Caribbean countries [1]. Humans enter the water where snails have become infected by the miracidia of one species of *Schistosoma*. The miracidia, which develop into cercariae, are released from the snail and penetrate the skin of the human host [2]. Repercussions of schistosomiasis infections include the initial inflammatory reaction at the site where the metacercariae penetrate the skin and is commonly called swimmer's itch. Abdominal pain and weight loss are common and bloody diarrhea may occur along with eosinophilia and hepatosplenomegaly (enlargement of the liver and spleen) [2]. Cytokines are low molecular weight, soluble proteins produced by immunocompetent cells that communicate with other cells to regulate immune function. Cytokines act via specific receptors and, depending on the particular cytokine and the cell that it binds to, they can up-regulate or down-regulate the activity of other immune cells. Most cytokines have short half-lives and are effective locally, acting on cells in close proximity to their release. Thus, many cytokines are only detectable in peripheral circulation in response to pathology. In contrast, a few cytokines are present at measurable levels in the peripheral blood of most healthy individuals [3]. Cytokine levels can be measured in a number of body fluids, including serum, plasma, urine, supernatants, mucus, and saliva. A range of techniques is available to measure cytokine levels, including enzyme-linked immuno sorbent assay (ELISA), multiplex technology, ELISPOT and flow cytometry. Below are basic descriptions of some of these methods [4]. The objective of this study to evaluate factors to consider in measuring serum Interleukin (IL)-8 levels in Sudanese patients infected with *S. mansoni*.

2. Materials and methods

2.1 Study design:

It is a cross-sectional study.

2.2 Study area and study period:

The study was conducted in New Halfa city in Eastern Sudan., during the period from March to October 2011**.**

2.3 Study population:

The study was carried out in inhabitants from New Halfa City-Eastern Sudan.

2.4 Sample size:

575 serum samples and 575 faecal samples were examined.

2.5 Sample collection:

Serum samples and faecal samples were collected from all the study subjects.

2.6 Data collection:

The primary data were collected by using self-administrated per-coded questionnaire which was specifically designed to obtain information that helped in the study.

3. Methods

3.1 Kato-Katz technique:

Small amount of sieved faecal material was transferred into the hole of a template (approximately 41.7mg) that previously placed on a clean and dry microscope slide. Carefully the hole was filled 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 was counted per gram of faeces [5]. Then the intensity of infection was determined as follows:

Mild ≤ 50 eggs per gram of faeces
Moderate 51- 200 eggs per gram of faeces
Severe 201-300 eggs per gram of faeces
Hyper infection ≥ 400 eggs per gram of faeces

3.2 Human IL-8 ELISA Ready-SET-Go! (eBioscience)

Serum samples from surveyed populations were investigated for IL-8, using the Human IL-8-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 5 times 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 100µl of blocking buffer (1:5 diluted assay diluent) and then incubated at room temperature for 1 hour. Wells were aspirated and washed 5 times 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 5 times with 400 µl/well wash buffer. Next, 50 µl of the detection antibody (1:250 diluted in 1x assay diluent) were added to each well. Plates were then sealed and incubated at room temperature for 1 hour. Wells were aspirated and washed 5 times with 400 µl/well wash buffer. A 50 µl of streptavidin solution (1:250 diluted in 1x assay diluent) were added to each well and the plates were incubated at room temperature for 30 minutes. Wells were aspirated and washed 7 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

3.3 Data analysis:

Data was analyzed using Statistical Package of Social Sciences (SPSS) for windows, and the p values of less than 0.05 were considered statistically significant. Data presented in graphs using PRISM 5 programme (Graph Pad Software, Inc., Jolla, USA) after analysis by SPSS. Mann-Whitney test and Kruskal-Wallis test were used.

3.4 Ethical consideration:

Permission for the samples collection was taken from study subjects or their gardeners after explaining the study purpose. Ethical clearance will be taken from Ministry of Health-Kassala State Department of Preventive Medicine Office of the anti-bilharzia and intestinal worms New Halfa City.

4. Results

S. mansoni-infected individuals have no differences in their levels of IL-8 when was compared in two groups (infected and uninfected by using Mann-Whitney test), p=0.4331 (figure 1) and in three groups (infected, uninfected and previously infected by using Kruskal-Wallis test), p=0.0656. But, when `was compared infected with uninfected p=0.0139 and previously infected with uninfected p=0.0074 (figure 2). 575 serum samples were examined for IL-8, 332 were male (45 were positive and 287 were negative) and 243 were female (65 were positive and 178 were negative). The mean value of IL-8 was found to be higher in male (4483.84 pg/ml) than in female (4476.11 pg/ml) (figure 3). The value of IL-8 was significantly different between gender (p value was less than 0.05, p=0.000) and the correlation was positive (r=0.988). The level of IL-8 was found to increase with age in different age groups. In the age-group (4-12 year) 244 (67 were positive and 172 were negative) were examined, the mean was 3589.34 pg/ml, in age group (13-19 year) 91 (21 were positive and 70 were negative) were examined, the mean was 3575.20 pg/ml, in age group (20-45 year) 148 (18 were positive and 130 were negative) were examined, the mean was 5650.13 pg/ml, in age group (46-60 year) 49 (3 were positive and 46 were negative) were examined, the mean was 5374.18 pg/ml and in age group (61-85 year) 43 (1 was positive and 42 were negative) were examined, the mean was 6410.13 pg/ml (table 1). The relation between the mean of IL-8 and different age-groups were significant (p value was less than 0.05, p=0.000). Among 575 serum samples, 207 had previous infection with *S. mansoni*, 200 of them received treatment and 7 did not. Among those received treatment the level of IL-8 was found to be lower (5336.95 pg/ml) compared to that reported among non treated individuals (7015.93 pg/ml) (table 2). The difference was found to be significant (p value was less than 0.05, p=0.000) and the correlation was positive (r=0.526). Among 575 serum samples, 110 were positive for *S. mansoni*, 41 were mild infection mean of IL-8 was 6163.47 pg/ml, 56 were moderate infection the mean was 4238.70 pg/ml, 10 were severe infection the mean was 3322.67 pg/ml and 3 were hyper infection the mean was 4412.97 pg/ml (table 3). The relation between the mean of IL-8 and intensity of *S.*

mansoni infection was significant (p value was less than 0.05, p=0.000) while the correlation was positive (r=0.153). Among 575 serum samples, 465 were negative for *S. mansoni*, the number of eggs were zero the mean of IL-8 was 4386.66 pg/ml, 110 were positive for *S. mansoni*, among those 6 had 24 eggs/1gram of stool the mean was 2093.13 pg/ml, 35 had 48 eggs/1gram of stool the mean was 6861.24 pg/ml, 15 had 72 eggs/1gram of stool the mean was 3819.99 pg/ml, 21 had 96 eggs/1gram of stool the mean was 6122.70 pg/ml, 8 had 120 eggs/1gram of stool the mean was 3800.15 pg/ml, 8 had 144 eggs/1gram of stool the mean was 845.92 pg/ml, 1 had 168 eggs/1gram of stool the mean was 32.84 pg/ml, 3 had 192 eggs/1gram of stool the mean was 4763.01 pg/ml, 2 had 216 eggs/1gram of stool the mean was 268.08 pg/ml, 4 had 240 eggs/1gram of stool the mean was 5081.06 pg/ml, 1 had 336 eggs/1gram of stool the mean was 3138.70 pg/ml, 1 had 360 eggs/1gram of stool the mean was 600.82 pg/ml, 2 had 384 eggs/1gram of stool the mean was 4313.40 pg/ml, 1 had 480 eggs/1gram of stool the mean was 831.92 pg/ml, 1 had 720 eggs/1gram of stool the mean was 11892.06 pg/ml and 1 had 960 eggs/1gram of stool the mean was 514.92 pg/ml (table 4). The relation between the mean of IL-8 and Kato technique was significant (p value was less than 0.05, p=0.000) while the correlation was positive (r=0.800) (figure 4). Among 575 serum samples, 13 were taken from Bagara the mean of IL-8 was 165.73 pg/ml, 2 were taken from Bandawa mean was 477.86 pg/ml, 54 from Bany Aamir mean was 2292.35 pg/ml, 15 from Barno mean was 4473.64 pg/ml, 7 from Danagla mean was 1149.7577 pg/ml, 4 from Flata mean was 520.43 pg/ml, 1 from Fong mean was 20.88 pg/ml, 29 from Foor mean was 4853.3923 pg/ml, 32 from Gaalia mean was 1092.93 pg/ml, 1 from Gamoaia mean was 171.64 pg/ml, 1 from Garara mean was 2215.26 pg/ml, 4 from Gawamaa mean was 23.7840 pg/ml, 4 from Hamar mean was 2674.77 pg/ml, 1 from Hasania mean was 15.50 pg/ml, 6 from Kawahla mean was 642.5867 pg/ml, 4 from Khawalda the mean was 1430.83 pg/ml, 3 from Kinana mean was 660.58 pg/ml, 1 from Masaleet mean was 6110.44 pg/ml, 11 from Misaria mean was 946.01 pg/ml, 1 from Noba mean was 8.27 pg/ml, 6 from Rashida mean was 6645.44 pg/ml, 1 from Rufaa mean was 374.43 pg/ml, 4 from Shigia mean was 814.77 pg/ml, 1 from Taaisha mean was 2494.34 pg/ml, 353 from Tama mean was 5784.63 pg/ml and 13 from Zagawa mean was 4981.24 pg/ml (table 5). The relation between the mean of IL-8 and the tribes was insignificant (p value was more than 0.05, p=1.000).

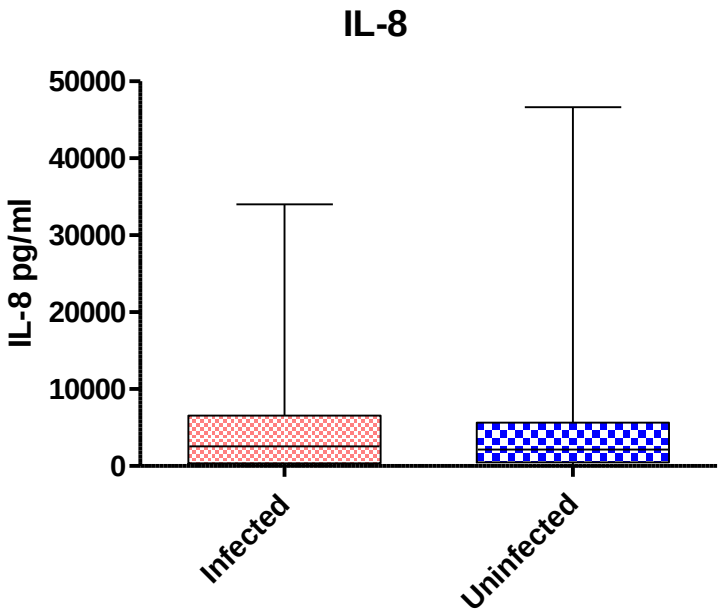

Figure 1: IL-8 level in infected and uninfected individuals

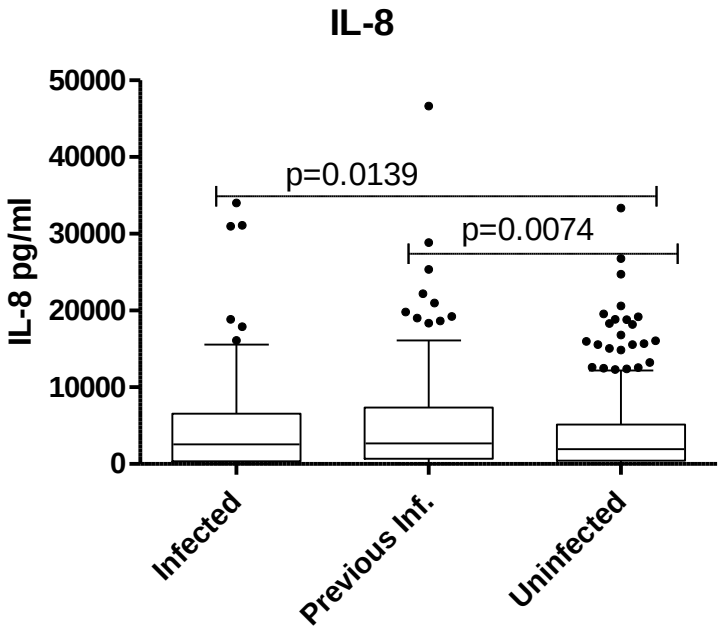

Figure 2: IL-8 level in infected, previously infected and uninfected individuals

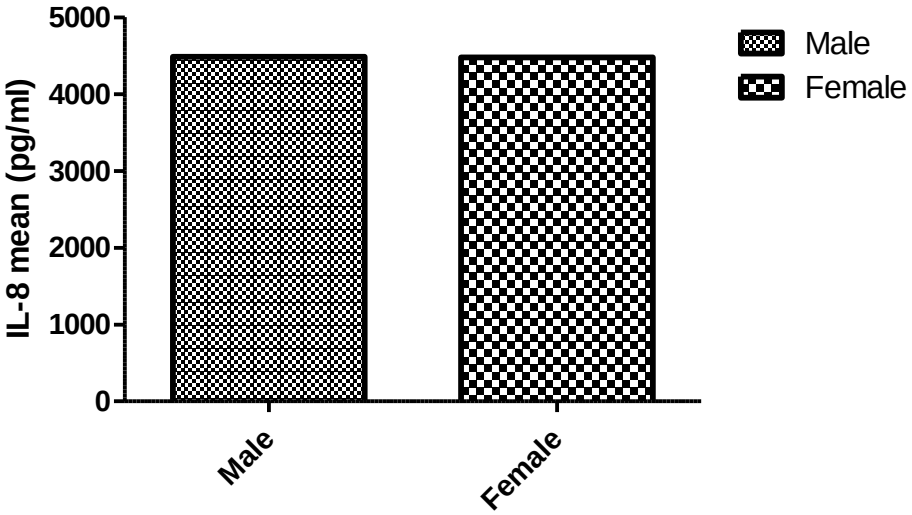

Figure 3: The relationship between the mean of IL-8 and the gender

Table 1: The relationship between the mean of IL-8 and age-groups

Age-groups	Mean (pg/ml)	N
4-12 Year	3589.3404	244
13-19 Year	3575.1970	91

20-45 Year	5650.1268	148
46-60 Year	5374.1812	49
61-85 Year	6410.1278	43
Total	4480.5758	575

Table 2: The relationship between the mean of IL-8 and treatment

Treatment	Mean (pg/ml)	N
Treated	5336.9486	200
Not treated	7015.9326	7
Total	5393.7258	207

Table 3: The relationship between the mean of IL-8 and intensity of *S. mansoni* infection

Intensity of *S. mansoni*	Mean (pg/ml)	N
Mild infection	6163.4638	41
Moderate infection	4238.6981	56
Severe infection	3322.6704	10
Hyper infection	4412.9680	3
Total	4877.5884	110

Table 4: The relationship between the mean of IL-8 and Kato technique (number of eggs/1gram of stool)

Kato/1gram of stool	Mean (pg/ml)	N
0	4386.6589	465
24	2093.1293	6
48	6861.2354	35
72	3819.9893	15
96	6122.7036	21
120	3800.1520	8
144	845.9230	8
168	32.8400	1
192	4763.0133	3
216	268.0800	2
240	5081.0560	4
336	3138.7040	1
360	600.8160	1
384	4313.4000	2
480	831.9200	1
720	11892.0640	1
960	514.9200	1
Total	4480.5758	575

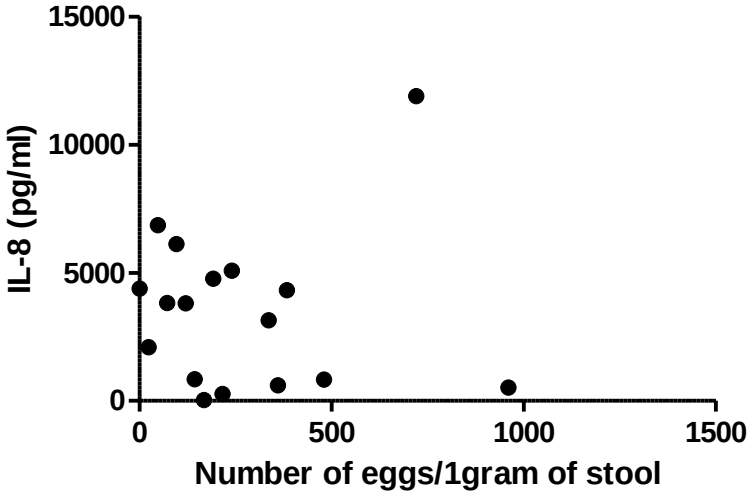

Figure 4: The correlation between the mean of IL-8 and Kato technique (number of eggs/1gram of stool)

Table 5: Relationship between the mean of IL-8 and tribes

Tribe	Mean (pg/ml)	N
Bagara	165.7268	13
Bandawa	477.8640	2
Bany Aamir	2292.3516	54
Barno	4473.6427	15
Danagla	1149.7577	7
Flata	520.4300	4
Fong	20.8800	1
Foor	4853.3923	29
Gaalia	1092.9315	32
Gamoaia	171.6400	1
Garara	2215.2560	1
Gawamaa	23.7840	4
Hamar	2674.7700	4
Hasania	15.4960	1
Kawahla	642.5867	6
Khawalda	1430.8260	4
Kinana	660.5787	3
Masaleet	6110.4400	1
Misaria	946.0138	11

Noba	8.2720	1
Rashida	6645.4387	6
Rufaa	374.4320	1
Shigia	814.7669	7
Taaisha	2494.3360	1
Tama	5784.6268	353
Zagawa	4981.2431	13
Total	4480.5758	575

5. Discussion

The present study showed that *S. mansoni*-infected individuals have no differences in their levels of IL-8 when was compared in two groups (infected and uninfected), p=0.4331 and in three groups (infected, uninfected and previously infected), p=0.0656. But, when `was compared infected with uninfected p=0.0139 and previously infected with uninfected p=0.0074. When the IL-8 level was compared with factors such as age, gender, treatment, Kato-Katz technique, intensity of infection and tribes which were factors to consider in measuring IL-8 level and these factors should be taken into consideration in interpreting findings; there was a statistically significant relationship between IL-8 level and gender, age, treatment, Kato-Katz technique, intensity of infection. But there was a statistically insignificant relationship between IL-8 and tribes. These findings were in agreement with the findings obtained by Silveira-Lemos *et al.* (2010) [6] who reported that there was significantly increased IL-8 secretion in detected samples from patients with schistosomiasis. But the findings from the present study were in disagreement with the findings obtained by Turner *et al.* (2013) [7] who reported that there were decreased levels of IL-8 in subjects from an area endemic for *S. mansoni*.

6. Conclusion

The study concluded that there was a statistically significant relationship between IL-8 level and the age, gender, treatment, Kato-Katz technique and intensity of infection among the patients infected with *S. mansoni* in the study area.

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