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Interleukin-1β (IL-1β) and Their Associated Level Factors in Sudanese Patients Infected with *Schistosoma Mansoni*

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Abstract: *This study aimed to detect Interleukin-1β (IL-1β) and their associated level factors in Sudanese patients infected with Schistosoma mansoni. A cross-sectional study was conducted in New Halfa City-Eastern Sudan during the period from March to October 2011. Participants in this study included 305, 177 were males (45 were positive and 132 were negative) and 128 were females (65 were positive and 63 were negative). Faeces samples were collected from all the subjects included in the study and examined by using direct wet mount and Kato-Katz technique. Also, serum samples were collected from all subjects and examined by using the Th1/Th2/Th9/Th17/Th22 13plex Kit FlowCytomix (eBioscience) technique. The results showed that S. mansoni-infected individuals have differences in their levels of IL-1β when was compared in two groups, p=0.0279 and in three groups, p=0.009. Also, when was compared infected with uninfected p=0.001. Currently S. mansoni-infected individuals have more IL-1β than uninfected. The relation between the mean of IL-1β and the gender was significant (p value was less than 0.05, p=0.000). The relation between the mean of IL-1β and age-groups was significant (p value was less than 0.05, p=0.000). The relation between the mean of IL-1β and intensity of S. mansoni infection was significant (p value was less than 0.05, p=0.000) while the correlation was positive (r=0.759). The relation between the mean of IL-1β and Kato technique was significant (p value was less than 0.05, p=0.000). The relation between the mean of IL-1β and treatment was significant (p value was less than 0.05, p=0.000). The results obtained from the current study showed that the relation between the mean of IL-1β and the tribes was insignificant (p value was more than 0.05, p=1.000).*

Keywords— *IL-1β*; Level Factors; *Schistosoma mansoni*; 13plex Kit FlowCytomix (eBioscience) Technique

1. Introduction

Schistosoma mansoni is caused by a worm of the trematode family. These flukes or flat worms require an intermediate host from a fresh water snail and do not replicate in their definitive human host. The infection and its clinical consequence caused by this worm depend on interactions between the distribution of the intermediate hosts, and the social and cultural behaviour of humans [1]. The infection has been well documented during classic epidemiology. *S. mansoni* exhibit market age dependency in infection patterns and prevalence of infections increases throughout childhood to relatively stable plateau or show a slight decrease in adulthood [2]. *S. mansoni* is the major health risk in the rural areas of China and Egypt and other developing countries and the disease substantially affects children's growth and school performance [3]. Diagnosis is aided by a history of exposure, but depends on the finding of eggs or a positive serological result [1]. Eggs of *S.mansoni* are often excreted in stool samples, and detection rates may be increased by various concentration technique (e.g. the Kato-Katz method). Also, biopsy of affected tissues can demonstrate eggs [1]. Serological tests are based on the detection of circulating antibodies using egg antigens in an enzyme-linked immuno sorbent assay (ELISA). These tests are sensitive, but remain positive for long time after infection. They are not useful for diagnosis of active infection in endemic areas, where many individuals have been infected in the past, but are useful for detecting infection in returning travelers. Newer serological tests to detect adult worms may distinguish active from past infection, but are still under development [1]. Interleukin-1β (IL-1β) is defined as a potent pro-inflammatory cytokine that is crucial for host-defense responses to infection and injury [4]. It is also the best characterized and most studied of the 11 IL-1 family members. A variety of cell types is produced and secreted IL-1β, although the vast majority of studies have focused on its production within cells of the innate immune system, such as monocytes and macrophages. IL-1β is produced as an inactive 31 kDa precursor, termed pro-IL-1β, in response to molecular motifs carried by pathogens called pathogen associated molecular patterns (PAMPs). PAMPs act through pattern recognition receptors (PRR's) on macrophages to regulate pathways that control gene expression [5]. The objective of this study was to detect Interleukin-1β (IL-1β) and their associated level factors in Sudanese patients infected with *Schistosoma 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:

305 serum samples and 305 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 Methods of stool examination

3. 1. 1 Direct wet mount

Stool specimens were collected in clean and dry containers. A drop of normal saline was placed in the centre of the left half of the slide. A small piece of stool was added by using applicator stick. Mixed well and then covered with a cover glass. Slides were examined under microscope (×10 and ×40 lenses objectives) with a reduced condenser aperture [6]. Three slides were prepared for each stool specimen.

3. 1. 2 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 [7].

3. 1. 3 Determination of intensity of infection

Mild	≤ 50 eggs per gram of stool
Moderate	51- 200 eggs per gram of stool
Severe	201-300 eggs per gram of stool
Hyper infection	≥ 400 eggs per gram of stool [7].

3.2 Methods of IL-1β examination

For detection of IL-1β in serum from surveyed populations the Th1/Th2/Th9/Th17/Th22 13plex Kit FlowCytomix (eBioscience) was used which is a bead based analyte detection system for quantitative detection of human IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 p70, IL-13, IL-17A, IL-22 and TNF-α by Flow Cytometry. First, the 1x assay buffer was prepared by diluting 1 part of the concentrate in 9 parts of distilled water. Standards were reconstituted with distilled water according to the label on each vial and incubated for 30 minutes at RT. Afterwards, a standard pool consisting of 10 µl of each reconstituted standard was prepared and filled up with 70 µl of 1x assay buffer and a 1:3 serial dilution was performed. In-between, a bead mixture was prepared which consist of 12.5 µl of each bead fraction for one sample. The bead mixture was pipetted and centrifuged for 5 minutes at 3000 x g. Supernatant was discarded and the beads were filled up with the same amount of reagent diluent. Then, 12.5 µl of the bead mix were transferred to each fluorescence activated cells sorting (FACS) tube (BD, Heidelberg, Germany). Afterwards, 25 µl of blank, standard mixture and samples were added to the designated tubes. Additionally, 25 µl of biotin-conjugate mixture (1x) were added to all tubes, including the blank tubes. Then the contents of each tube were mixed well and incubated at RT for 2 hours and protected from light with an aluminium foil. After the incubation, samples were washed twice with 500 µl of assay buffer (1x) with each centrifugation at 200 x g for 5 minutes. The supernatant was carefully discarded from each tube leaving approximately 100 µl of liquid in each tube. A 25 µl of Streptavidin-PE solution were added to all tubes including the blank tubes. The contents of each tube were mixed well and incubate at RT for 1 hour and protected from light with an aluminium foil. Wash steps were repeated as described above. Samples were dissolved in 200 µl assay buffer (1x) and afterwards analyzed using the FACS Canto II (BD). Data were analyzed with FlowCytomix Pro 3.0 software (eBioscience).

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 differences in their levels of IL-1β when was compared in two groups, p=0.0279 (figure 1) and in three groups, p=0.009. Also, when was compared infected with uninfected p=0.001 (figure 2). Currently *S. mansoni*-infected individuals have more IL-1β than uninfected. 305 serum samples were examined for IL-1β, 177 were males, within those the mean was 79.16 pg/ml and 128 were females, within those the mean was 89.95 pg/ml (table 1). The relation between the mean of IL-1β and the gender was significant (p value was less than 0.05, p=0.000) while the correlation was positive (r=0.454). In the age-group (4-12 year) 151 were examined, the mean was 52.67 pg/ml, in group (13-19 year) 71 were examined, the mean was 112.19 pg/ml, in group (20-45 year) 53 were examined, the mean was 109.92 pg/ml, in group (46-60 year) 15 were examined, the mean was 107.39 pg/ml and in group (61-85 year) 15 were examined, the mean was 144.67 pg/ml (table 2). The relation between the mean of IL-1β and age-groups was significant (p value was less than 0.05, p=0.000) while the correlation was

positive (r=0.000). Among 305 serum samples, 110 were positive for *S. mansoni*, 41 were mild infection mean of IL-1β was 88.45 pg/ml, 56 were moderate infection the mean was 87.55 pg/ml, 10 were severe infection the mean was 103.41 pg/ml and 3 were hyper infection the mean was 127.67 pg/ml (figure 3). The relation between the mean of IL-1β and intensity of *S. mansoni* infection was significant (p value was less than 0.05, p=0.000) while the correlation was positive (r=0.759). Among 305 serum samples, 195 were negative for *S. mansoni*, the number of eggs were zero the mean of IL-1β was 79.90 pg/ml, 110 were positive for *S. mansoni*, among those 6 had 24 eggs/1gram of stool the mean was 62.92 pg/ml, 35 had 48 eggs/1gram of stool the mean was 92.82 pg/ml, 15 had 72 eggs/1gram of stool the mean was 57.96 pg/ml, 21 had 96 eggs/1gram of stool the mean was 117.27 pg/ml, 8 had 120 eggs/1gram of stool the mean was 82.54 pg/ml, 8 had 144 eggs/1gram of stool the mean was 45.41 pg/ml, 1 had 168 eggs/1gram of stool the mean was 11.44 pg/ml, 3 had 192 eggs/1gram of stool the mean was 178.47 pg/ml, 2 had 216 eggs/1gram of stool the mean was 12.66 pg/ml, 4 had 240 eggs/1gram of stool the mean was 149.42 pg/ml, 1 had 336 eggs/1gram of stool the mean was 94.90 pg/ml, 1 had 360 eggs/1gram of stool the mean was 224.29 pg/ml, 2 had 384 eggs/1gram of stool the mean was 45.96 pg/ml, 1 had 480 eggs/1gram of stool the mean was 201.06 pg/ml, 1 had 720 eggs/1gram of stool the mean was 51.51 pg/ml and 1 had 960 eggs/1gram of stool the mean was 130.43 pg/ml (table 3). The relation between the mean of IL-1β and Kato technique was significant (p value was less than 0.05, p=0.000) while the correlation was positive (r=0.369). Among 305 serum samples, 103 had previous infection with *S. mansoni*, among those 97 were treated the mean of IL-1β was 100.74 pg/ml while 6 not treated the mean was 131.10 pg/ml (table 4). The relation between the mean of IL-1β and treatment was significant (p value was less than 0.05, p=0.000) while the correlation was positive (r=0.600). Among 305 serum samples, 6 were taken from Bagara the mean of IL-1β was 0.00 pg/ml, 2 were taken from Bandawa mean was 0.000 pg/ml, 13 from Bany Aamir mean was 72.92 pg/ml, 9 from Barno mean was 15.44 pg/ml, 5 from Danagla mean was 24.71 pg/ml, 2 from Flata mean was 0.000 pg/ml, 1 from Fong mean was 94.30 pg/ml, 14 from Foor mean was 22.06 pg/ml, 19 from Gaalia mean was 72.22 pg/ml, 1 from Gamoaia mean was 0.00 pg/ml, 1 from Garara mean was 152.43 pg/ml, 2 from Gawamaa mean was 0.00 pg/ml, 3 from Hamar mean was 146.60 pg/ml, 3 from Kawahla mean was 0.00 pg/ml, 2 from Khawalda the mean was 109.22 pg/ml, 1 from Kinana mean was 0.00 pg/ml, 1 from Masaleet mean was 0.00 pg/ml, 9 from Misaria mean was 2.44 pg/ml, 1 from Noba mean 0.000 pg/ml, 2 from Rashida mean was 0.00 pg/ml, 1 from Rufaa mean was 0.000 pg/ml, 4 from Shigia mean was 0.00 pg/ml, 1 from Taaisha mean was 186.80 pg/ml, 192 from Tama mean was 110.89 pg/ml and 10 from Zagawa mean was 23.01 pg/ml (table 5). The relation between the mean of IL-1β and the tribes was insignificant (p value was more than 0.05, p=1.000).

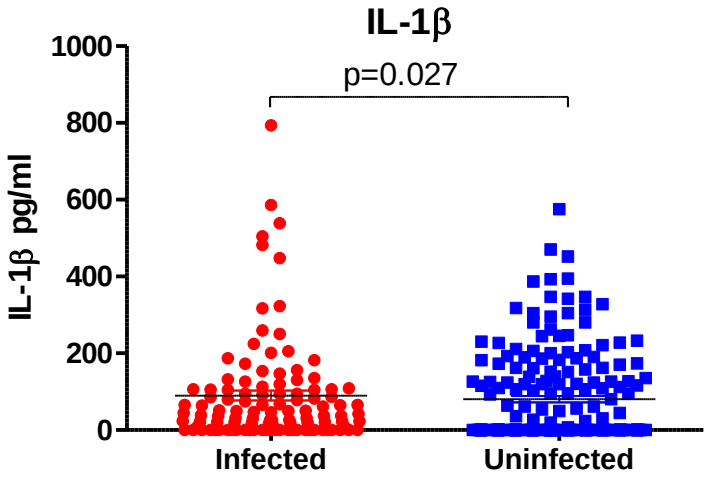

Figure 1: IL-1β level in infected and uninfected individuals

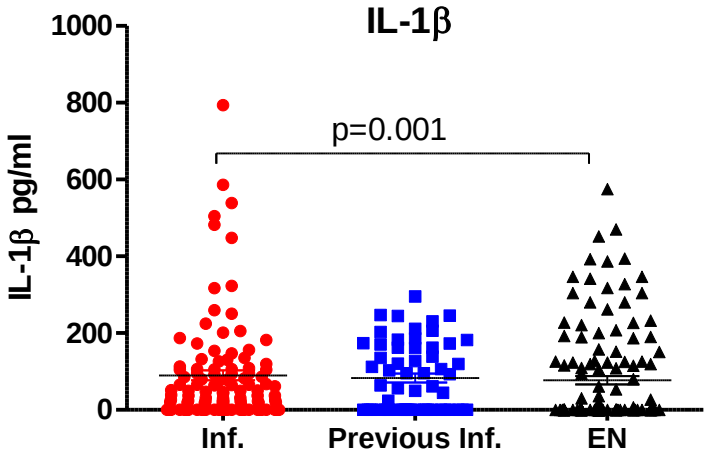

Figure 2: IL-1β in infected, previously infected and uninfected individuals. Currently *S. mansoni*-infected individuals have more IL-1β than uninfected.

Table 1: The relationship between the mean of IL-1β and the gender

Gender	Mean	N
Male	79.1642	177
Female	89.9485	128
Total	83.6901	305

Table 2: The relationship between the mean of IL-1β and age-groups

Age-groups	Mean	N
4-12 Year	52.6679	151
13-19 Year	112.1928	71
20-45 Year	109.9247	53
46-60 Year	107.3913	15
61-85 Year	144.6693	15
Total	83.6901	305

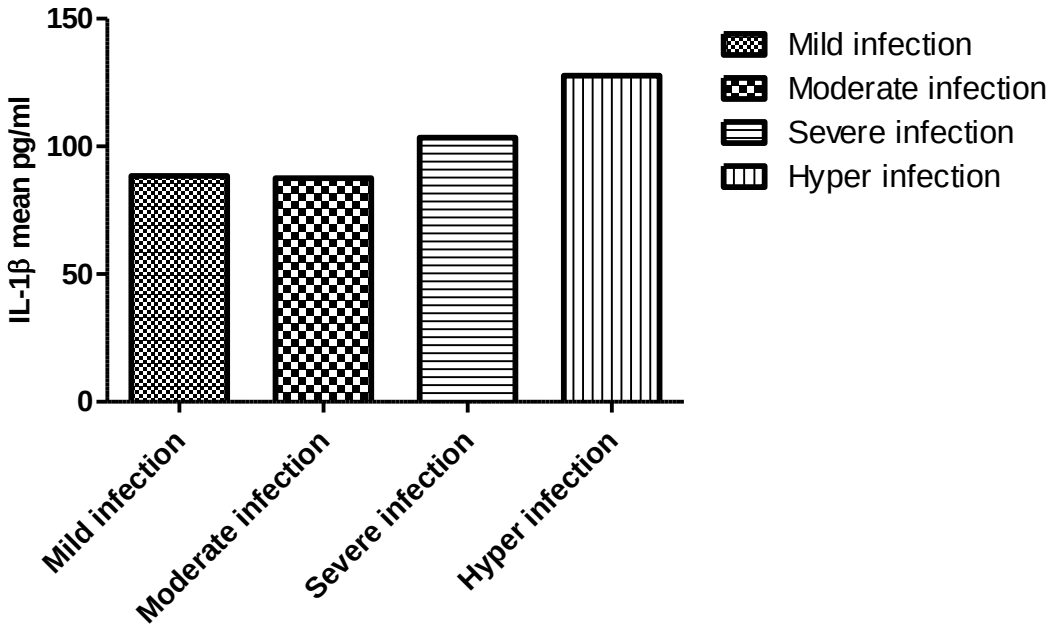

Figure 3: The relationship between the mean of IL-1β and intensity of *S. mansoni* infection

Table 3: The relationship between the mean of IL-1β and Kato technique (number of eggs/1gram of stool)

Kato/1gram of stool	Mean	N
0	79.8949	195
24	62.9150	6
48	92.8249	35
72	57.9627	15
96	117.2690	21
120	82.5387	8
144	45.4088	8
168	11.4400	1
192	178.4700	3
216	12.6600	2
240	149.4150	4
336	94.9000	1
360	224.2900	1
384	45.9550	2
480	201.0600	1
720	51.5100	1
960	130.4300	1
Total	83.6901	305

Table 4: The relationship between the mean of IL-1β and treatment

Treatment	Mean	N

Treated	100.7418	97
Not treated	131.0967	6
Total	102.5100	103

Table 5: The relationship between the mean of IL-1β and tribes

Tribe	Mean	N
Bagara	.0000	6
Bandawa	.0000	2
Bany Aamir	72.9177	13
Barno	15.4444	9
Danagla	24.7060	5
Flata	.0000	2
Fong	94.3000	1
Foor	22.0607	14
Gaalia	72.2153	19
Gamoaia	.0000	1
Garara	152.4300	1
Gawamaa	.0000	2
Hamar	146.5967	3
Kawahla	.0000	3
Khawalda	109.2150	2
Kinana	.0000	1
Masaleet	.0000	1
Misaria	2.4378	9
Noba	.0000	1
Rashida	.0000	2
Rufaa	.0000	1
Shigia	.0000	4
Taaisha	186.8000	1
Tama	110.8868	192
Zagawa	23.0120	10
Total	83.6901	305

5. Discussion

The current study showed that *S. mansoni*-infected individuals have differences in their levels of IL-1β. The results obtained from the present study illustrated that IL-1β showed significant differences when was compared with gender and treatment. Besides, this cytokine showed significant differences with age in different age groups. These findings were in disagreement with the findings obtained by Milner *et al*. (Milner *et al*., 2010). Also, the current study showed that IL-1β levels were showed significant differences with intensity of *S. mansoni* infection using Kato-Katz technique. These findings were in agreement with the findings obtained by Milner *et al*. (2010) [8].

6. Conclusion

The study concluded that *S. mansoni*-infected individuals have more IL-1β than uninfected. And there was a statistically significant relationship between IL-1β 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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