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Serum Interleukin (IL)-5 and Their Associated Level Factors in Sudanese Patients Infected with Human Schistosomiasis Mansoni

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Abstract: *This cross-sectional study was conducted in New Halfa City-Eastern Sudan to evaluate serum interleukin (IL)-5 and their associated level factors in Sudanese patients infected with human Schistosomiasis mansoni during the period from March to June 2011. The study was conducted on 55 subjects (33 were positive for S. mansoni and 22 were negative for S. mansoni). The age ranged between 4-60 years old, and the age was divided into 4 groups 4-12, 13-19, 20-45 and 46-60 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 IL-5 Immunoassay (R&D Systems) technique. The results showed that the results of IL-5 were ranged from 0.00-52.84 pg/ml; the mean was 3.12 pg/ml. The results reflected that the relation between the mean of IL-5 and the gender was significant (p=0.000). The results demonstrated that the relationships between the mean of IL-5 results and age-groups, treatment of S.mansoni infection, Kato-Katz technique, intensity of S. mansoni infection and tribes were insignificant. The results revealed that the mean of serum IL-5 in S. mansoni infection illustrated positive correlation with gender, treatment, Kato technique, intensity of infection, tribes and age groups.*

Keywords — Serum IL-5; *Schistosoma mansoni*; Sudanese; R&D Systems Technique

1. INTRODUCTION

Schistosoma mansoni (S. mansoni) caused intestinal schistosomiasis where the large intestine and rectum are typically involved with polyposis, papules, abscesses, ulcers, papillomata, fistulae and ova in faeces. The bladder is sometimes involved [1]. There can be ectopic lesions; the liver is frequently involved, receiving eggs via portal vein with inflammatory reaction and fibrosis leading to periportal fibrosis with portal-hypertension and oesophageal varices [1]. Because organ size is related to egg output, hepatic manifestation permits differentiation from the genuinely cirrhotic process [2]. Congestive splenomegaly may result from portal hypertension, lymphoid hyperplasia, or secondary hyper splenism [3]. Anemia, ascites, anasarca from low albumin, and gall bladder thickening from adjacent liver fibrosis are signs of advanced disease [4]. During infection with schistosomiasis, a large proportion of the eggs laid by the parasite become trapped in host organs such as the intestine and liver, inducing a Th2-dominated inflammatory reaction that contributes to the development of hepatic fibrosis, portal hypertension, and eventually, fatal hematemesis [5]. Although the immunological factors important in generating the Th2 response are slowly being identified, the genetic machinery responsible for regulating the development of severe liver pathology during schistosomiasis remains virtually uncharacterized. Schistosomiasis-related liver pathology is limited to the immunologically mediated phase induced by the Th2-associated cytokines interleukin-4 (IL-4) [6], transforming growth factor beta (TGF-β) [7], and IL-13 [8] and inhibited by the Th1-associated cytokines interferon gamma (IFN-γ) [9] and IL-12 [10]. Nevertheless, the exact mechanisms by which egg-induced cytokines stimulate the development of fatal liver pathology remain largely unknown. Therefore, understanding the genetic events induced in hepatic tissue during *S. mansoni* infection may reveal novel immunological pathways that can be targeted in disease intervention strategies for schistosomiasis, as well as other chronic inflammatory diseases. Acutely lethal forms of schistosomiasis that develop in mice genetically predisposed to develop highly polarized type-1 or type-2 cytokine and antibody responses. Polarized Th1- and Th2-type immune responses dramatically increase the severity of egg-induced immunopathology in *S. mansoni* infection [11]. In the murine model of schistosomiasis, type 2-associated cytokines, including interleukin-4 (IL-4), IL-5, and IL-13, contribute to granuloma formation and the presence of eosinophils in these lesions [12]. However, in human schistosomiasis, studies have high levels of tumor necrosis factor alpha (TNF-α) produced by peripheral blood mononuclear cells (PBMCs) stimulated with *Schistosome* antigen are significantly associated with the presence of hepatosplenomegaly, while gamma interferon (IFN-γ) has a protective effect in severe fibrosis of the liver [13]. As hepatoesplenic disease is a long-term complication of *Schistosomiasis mansoni* and is considered to be indicative of severe hepatic and periportal fibrosis, it is conceivable that the immune mechanisms responsible for this lesion occur much earlier during infection and precede the downstream development of hepatosplenomegaly. Consequently, it is important to evaluate the immune

response in the early events of hepatic fibrosis. The main objective of the present study to evaluate serum interleukin (IL)-5 and their associated level factors in Sudanese patients infected with human *Schistosomiasis 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 different villages in New Halfa city in Eastern Sudan., during the period between March to June 2011**.**

2.3 Study population:
The study was carried out in inhabitants from different villages, males and females with different ages.

2.4 Sample size:
55 serum samples and 55 faecal samples were examined.

2.5 Sample collection:
Serum samples and faecal samples were collected from studied population.

2.6 Data collection:
Designed and previously prepared questionnaires were filled by participants.

3. Methods

3.1 Kato-Katz technique:
Kato-Katz technique was used for the quantitative assessment of eggs in 1gram of stool as described by Berhe *et al*. (2004) [14]. The intensity of infection was obtained by counting the number of *S. mansoni* eggs per gram (epg) of stool. Results were expressed as (≤ 50 epg) presented as mild infection, (51-200 epg) as moderate infection, (201-300 epg) as severe infection and (≥ 400 epg) as hyper infection.

3.2 Human IL-5 Immunoassay (R&D Systems)
A 100 µl of assay diluent RD1W were added to each well. A 100 µl of standard, control, or sample were added per well and then covered with the adhesive strip provided. The plate was incubated for 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout was provided and standards and samples assayed were recorded. Each well was aspirated and washed; the process was repeated three times for a total of four washes. Each well was filled with wash buffer (400 µl) a squirt bottle, manifold dispenser, or autowasher was used. After the last wash, any remaining wash buffer was removed by aspirating or by decanting. The plate was inverted and blotted against clean paper towels. A 200 µl of IL-5 conjugate were added to each well, plate was covered with a new adhesive strip and then incubated for 2 hours at room temperature on the shaker. The aspiration/wash was repeated as in above. A 200 µl of substrate solution were added to each well, plate was incubated for 30 minutes at room temperature and then protected from light. A 50 µl of stop solution were added to each well. The color in the wells was changed from blue to yellow. The optical density of each well was determined within 30 minutes; a microplate reader was used and 450 nm was set.

3.3 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 SPSS.

3.4 Ethical consideration:
Approval of the study was taken from the College of Medical Laboratory Science-Sudan University of Science and Technology. Permission for the samples collection was taken from study subjects or their gardeners after explaining the study purpose. Ethical clearance will also 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
For detection of human IL-5, 55 serum samples (33 were positive for *S. mansoni*, 22 were negative for *S. mansoni*) were examined. The results were ranged from 0.00-52.84 pg/ml (figure 1); the mean was 3.12 pg/ml. In the age-group (4-12 year) 10 were examined, the mean was 2.11 pg/ml, in group (13-19 year) 15 were examined, the mean was 3.47 pg/ml, in group (20-45 year) 22 were examined, the mean was 3.39 pg/ml and in group (46-60 year) 8 were examined, the mean was 2.96 pg/ml (figure 2). The relation between the mean of IL-5 result and age-groups was insignificant (p value was more than 0.05, p=0.383) while the correlation was positive (r=0.804). Among 55 serum samples, 26 were previously infected with *S. mansoni*, among those 24 were treated; the mean of IL-5 was 2.26 pg/ml while 2 were not treated; the mean was 0.01 pg/ml (figure 3). The relation between the mean of IL-5 and treatment was insignificant (p value was more than 0.05, p=0.353) while the correlation was positive (r=0.170). Among 55 serum samples, 22 were negative for *S. mansoni*, the number of eggs were zero with the mean of IL-5 was 2.63 pg/ml, 33 were positive for *S. mansoni*, among those 1 had 24 eggs/1gram of stool with the mean was 2.70 pg/ml, 14 had 48 eggs/1gram of stool with the mean was 5.74 pg/ml, 3 had 72 eggs/1gram of stool with the mean was 1.02 pg/ml, 8 had 96 eggs/1gram of stool with the mean was 1.08 pg/ml, 3 had 120 eggs/1gram of stool with the mean was 0.49 pg/ml, 1 had 144 eggs/1gram of stool with the mean was 4.67 pg/ml, 1 had 192 eggs/1gram of stool with the mean was 8.77 pg/ml, 1 had 240 eggs/1gram of stool with the mean was 0.65 pg/ml and 1 had 360 eggs/1gram of stool with the mean was 3.26 pg/ml, (figure 4). The relation between the mean of IL-5 and Kato technique was insignificant (p value was more than 0.05, p=0.089) while the correlation was positive (r=0.908) (figure 5). Among 55 serum samples, 33 were positive for *S.*

mansoni, 15 were mild infection with the mean of IL-5 was 5.53 pg/ml, 16 were moderate infection with the mean was 1.66 pg/ml and 2 were severe infection with the mean was 1.96 pg/ml (figure 6). The relation between the mean of IL-5 result and intensity of *S. mansoni* infection was insignificant (p value was more than 0.05, p=0.411) while the correlation was positive (r=0.281). Among 55 serum samples, 2 were taken from Bagara; the mean of IL-5 was 0.58 pg/ml, 6 were taken from Bany Aamir; the mean was 2.88 pg/ml, 1 from Foor; the mean was 52.84 pg/ml, 2 from Gaalia; the mean was 10.87 pg/ml, 1 from Khawalda; the mean was 1.6 pg/ml and 43 from Tama; the mean was 1.79 pg/ml (figure 7). The relation between the mean of IL-5 and the tribes was insignificant (p value was more than 0.05, p=0.624). 55 serum samples were examined for IL-5, 32 were male (16 were positive and 16 were negative) and 23 were female (17 were positive and 6 were negative). The mean value of IL-5 was found to be higher in male (4.24 pg/ml) than in female (1.55 pg/ml) (figure 8). The value of IL-5 was significantly different between gender (p value was less than 0.05, p=0.000) and the correlation was positive (r=0.197).

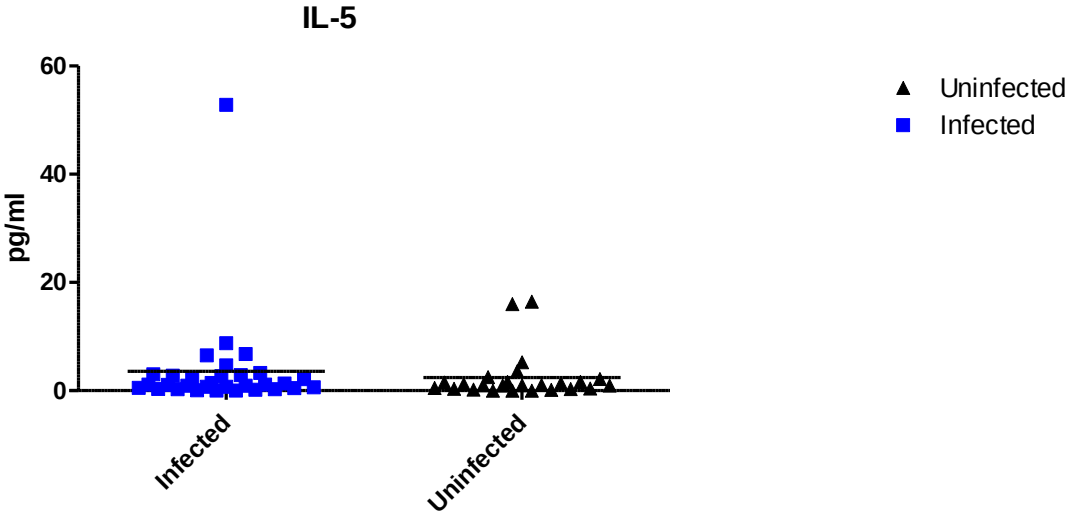

Figure 1: The result of IL-5

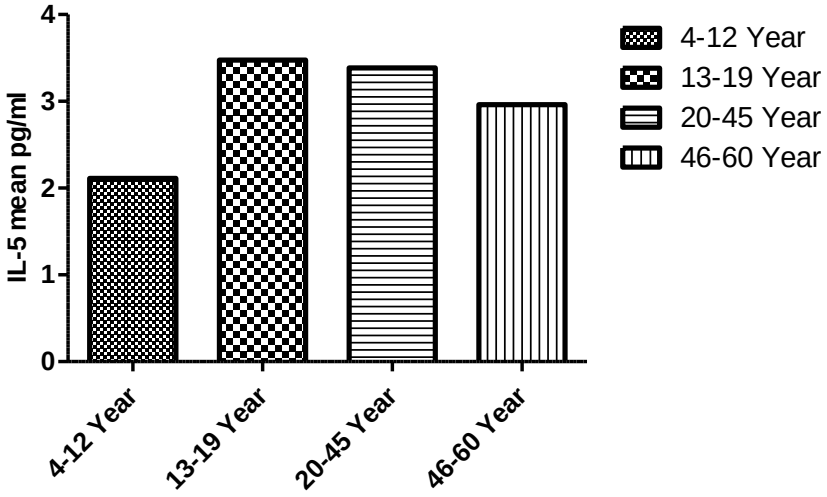

Figure 2: The relationship between IL-5 mean and age-groups

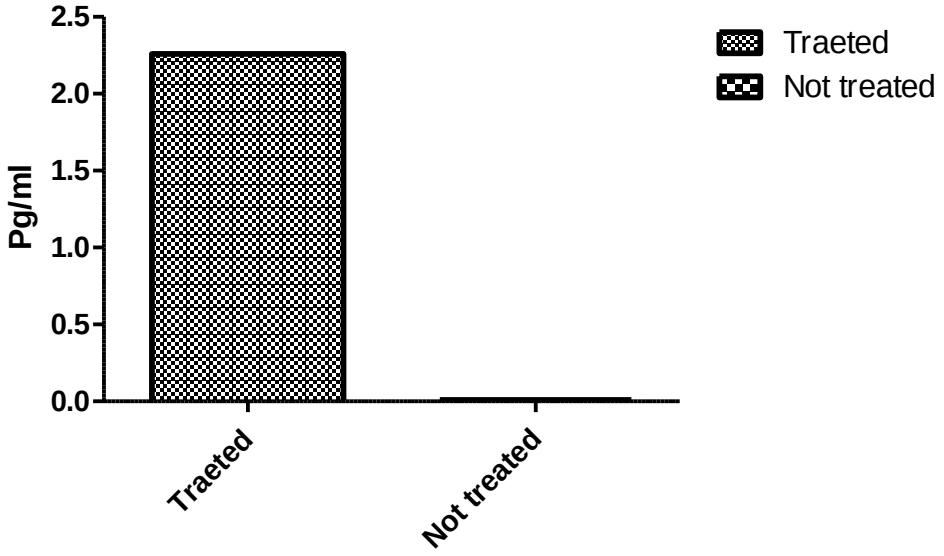

Figure 3: The relationship between IL-5 mean and treatment

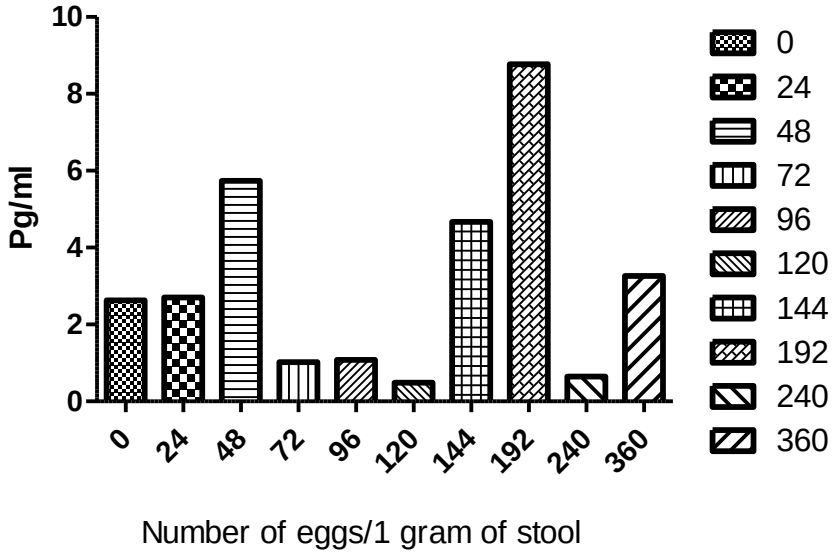

Figure 4: The relationship between the mean of IL-5 and Kato technique (number of eggs/1 gram of stool)

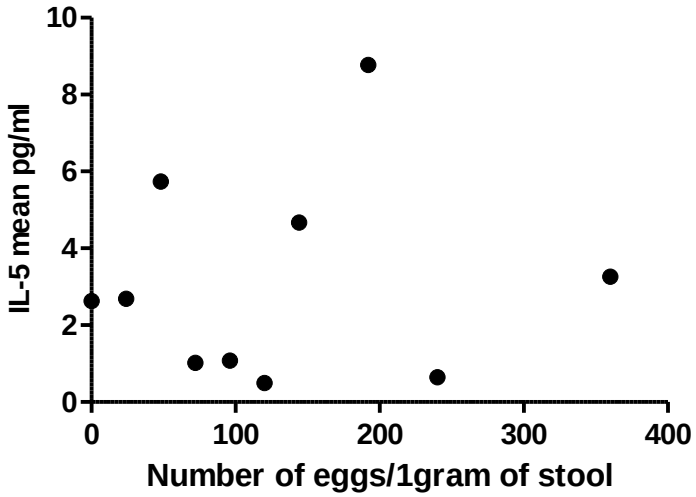

Figure 5: The correlation between the mean of IL-5 and Kato technique (number of eggs/1gram of stool)

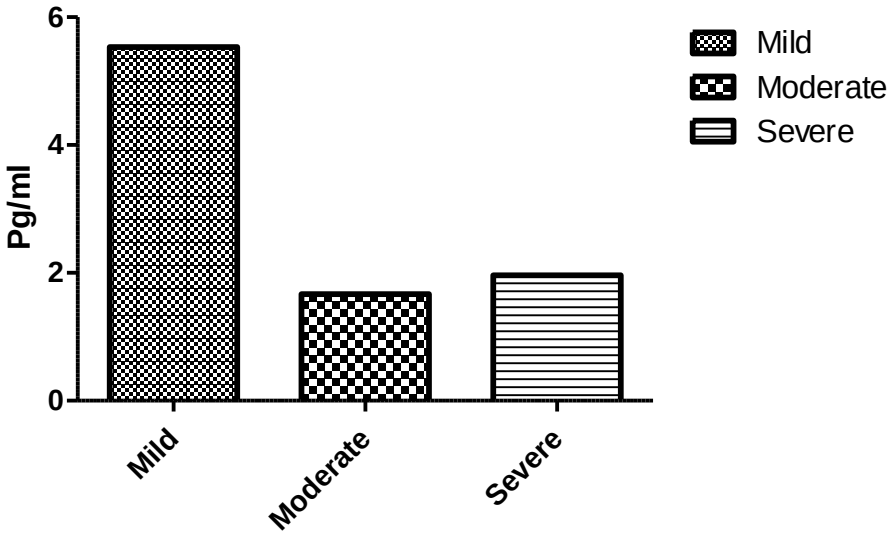

Figure 6: The relationship between the mean of IL-5 and intensity of *S. mansoni* infection

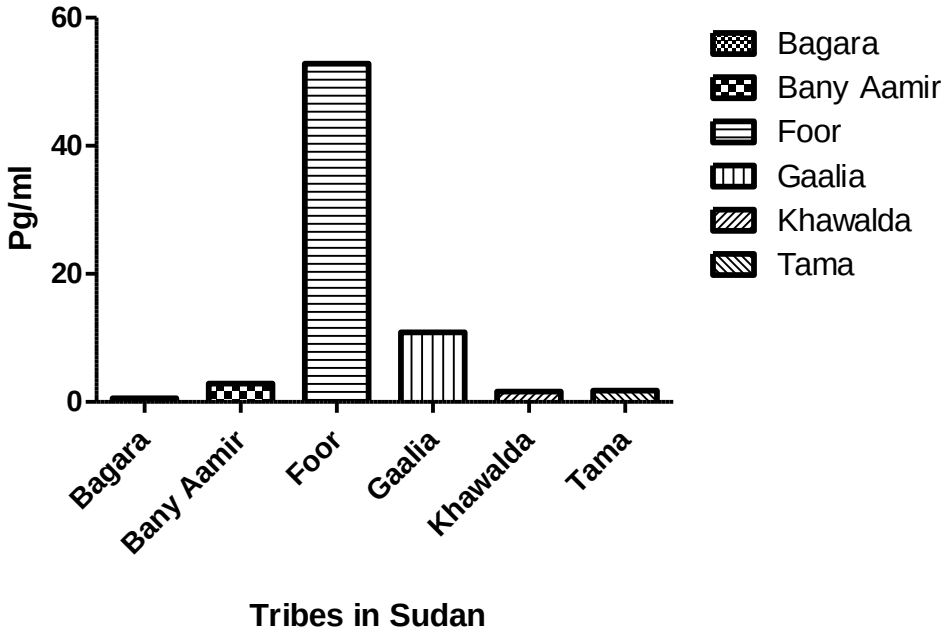

Figure 7: The relationship between the mean of IL-5 and tribes

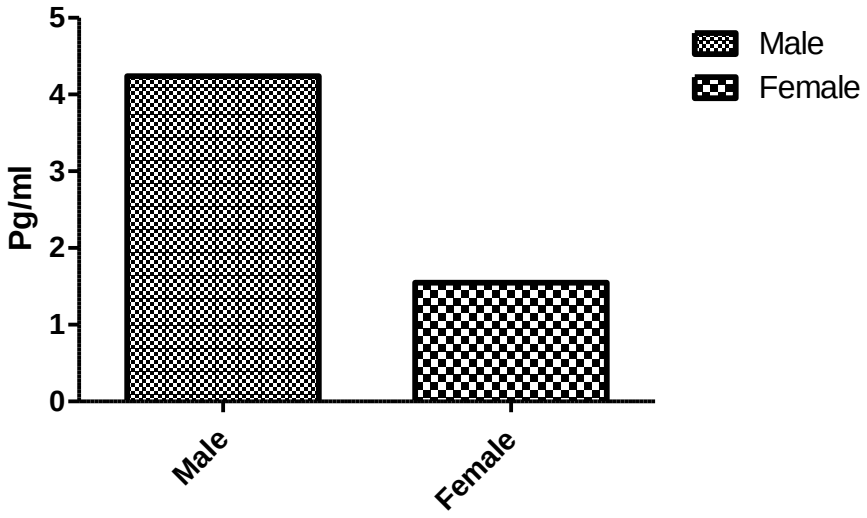

Figure 8: The relationship between the mean of IL-5 and the gender

5. Discussion

From the results, it was obvious that the mean of serum IL-5 in patients infected with *S.mansoni* in the study area was 3.12 pg/ml more than a 2.63 pg/ml in uninfected. When this cytokine was compared with factors such as age, gender, treatment, Kato-Katz technique, intensity of infection and race which were associated with cytokines levels, there was a statistically significant relationship between this mean and gender (p=0.000) while there was a statistically insignificant relationship between this mean and age, treatment, Kato-Katz technique, intensity of infection and race. These findings were in agreement with the findings obtained by Montenegro *et al.* (1999) [15] who reported that there was significantly increased IL-5 production in detected samples from patients with both acute and chronic schistosomiasis. Also, the findings from the present study were in agreement with the findings obtained by Magalhães *et al.* (2004) [16]

who reported that there were significantly higher levels of IL-5 in the study subjects.

6. Conclusion

The study concluded that the mean of serum IL-5 from patients infected with *Schistosoma mansoni* was 3.12 pg/ml and there was a statistically significant relationship between IL-5 mean and the gender.

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