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EVALUATING THE ROLE OF INTERLEUKIN-9 IN CHOLELITHIASIS PATIENTS AND DETERMINE PREVALENCE OF FASIOLA HEPATICA AMONG THESE PATIENTS

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ABSTRACT

Background: *Fascioliasis is a parasitic infection typically caused by Fasciola hepatica, which is also known as "the common liver fluke" or "the sheep liver fluke. People usually become infected by eating raw watercress or other water plants contaminated with immature parasite larvae.* ***Aim of study:*** *To determining the epidemiological prevalence of Liver fluke (Fasciola hepatica) in patients suffering from gallstones and assessment of serum concentrations of immunological marker IL-9 in these patients and control groups by ELISA technique.* ***Methodology:*** *Case control study design included 140 participants, 70 of them included as cases (suffered from Cholelithiasis), and 70 of them included as controls. History of the study was from beginning of October 2023 to end of January 2024, all participants were attending to Al-Sader Medical City/Digestive System Unit, Al-Hakeem Hospital and Al-Haidarya Hospital. Two samples (stool and 5 ml serum) were taken from each participant for microscopic detection of F.hepatica and for measure some immune parameters by ELISA, respectively.* ***Results:*** *It was found that there is a statistically significant difference between patients with liver fluke (fasciola hepatica), where the percentage of patients with liver fluke (f. hepatica) was 10%. Only 90% of participants suffering from gallstones were negative for this parasite. There was statistically significant (p-value= 0.0001), increase in mean concentration of the parameter (IL-9,) between the two groups of patients (105.62±38.22 pg/ml, ml) and the control (38.86±10.1pg/ml,) respectively. And there was a known increase in the mean concentration of immune parameters in the serum of patients infected with the parasite (, IL-9: 193.79±13.59 pg/ml,) compared to their concentration in patients not infected with the parasite.* ***Conclusions:*** *Only 10% of 90% of Cholelithiasis patients in the immediate study were diagnosed positive for F.hepatica parasite. Majority of risk factor for gallstones disease was obesity in the current study, 57% suffering from obesity .There was increased in study parameters (, IL-9,) significantly in group of cholelithiasis patients other than control group.*

INTRODUCTION

Fascioliasis is a parasitic infection typically caused by Fasciola hepatica, which is also known as "the common liver fluke" or "the sheep liver fluke. People usually become infected by eating raw watercress or other water plants contaminated with immature parasite larvae. The young worms move through the intestinal wall, the abdominal cavity, and the liver tissue, into the bile ducts, where they develop into mature adult flukes that produce eggs. The pathology typically is most pronounced in the bile ducts and liver (Oleiwi *et al.*, 2017).

In the chronic phase the parasite, now in bile ducts, is matured into adult flukes and produces eggs. During this phase the patient may be asymptomatic or present the following symptoms: cholangitis, biliary obstruction, cholecystitis, gallstones, pancreatitis and cirrhosis (Hawash, 2020).

Gallstone disease is prevalent in the general population, affecting 5% to 15% of adults worldwide. The incidence has increased in recent years (Rebholz *et al.,* 2018).

Some inflammation-related conditions, such as obesity, diabetes, and infections (e.g. *F.hepatica*), are also associated with risk of cholesterol gallstones. Therefore, inflammation plays a role in the development of gallstone (Zeng *et al.,* 2019).

IL-9 is type 2 inflammatory cytokine, drive the characteristic features of immunity against parasitic worms, IL-9 serves an essential role in the initiation of host-protective against liver flukes. IL-9 represents a local effector cytokine that, directly or indirectly, promotes activation of mucosal cells in the gallbladder, thereby contributing to efficient of these parasite expulsion (Zaiss *et al.,* 2023).

IL-9 is produced by activated Th2 cells, mast cells, eosinophils and Th9, these cells have been shown to enhance inflammatory responses. Interestingly, IL-9 inhibits Th1 cytokine production and this may be because uncontrolled Th1 responses can lead to necrosis and tissue damage (Malik, S., & Awasthi, A. (2018).

IL-9, which plays an important role in regulating inflammation and antitumor responses. Through its receptor (IL9R), IL-9 acts by altering signaling and protein expression in different cell types such as T and B lymphocytes, mast cells, neutrophils and macrophages (Pajulas *et al.,* 2023).

METHODOLOGY

Patients Group

Case-control study design involve patients suffered from gallstones (70 participants) were chosen. Ultrasound scan examination will be performed for these patients to confirm gallstones and diagnosis of Liver fluke (*Fasciola hepatica*) parasite by find eggs in stool test. All of these patients were involved in this study and during the period starting from October 2023 to the end of January 2024, from all ages of patient from both sex (Males and Females). Every patient was reported though a specifically prepared questionnaire which included name, gender, age, living, risk disease.

Control Group

The control group was 70 Patients attending the gastrointestinal unit who suffer from diseases other than gallstones. The control group was used only for comparing parameter. The control samples were approximately similar with the sample's patients in terms of number, ratio of age, in addition to the place of living also countryside and city.

Samples Collections

Serum Samples

Five ml of venous blood were withdrawn from each subjects by vein puncture using sterile syringe with needle gauge 23,than the blood sample was transfer in to coagulate gel tubes ,then centrifuged for 5 minutes at 4000 (rpm) to separate serum were transferred to another sterile Eppendorf tubes, labeled with Serial Number together with the patient name, and frozen at (-20°C) until used.

Stool Samples

Stool specimens were collected in a suitable, clean and dry container, all samples were introduced for conducting sedimentation method then examined under light microscope.

Inclusion and Exclusion Criteria

1. Individuals aged >18 years
2. Patients diagnosed with gallstones through imaging techniques (such as ultrasound or CT scan).
3. Patients presenting with symptoms of gallstones, such as abdominal pain, nausea, vomiting, and jaundice.
4. Patients who have not undergone cholecystectomy (gallbladder removal) prior to the study.
5. Patients willing to provide informed consent to participate in the study.

ELISA Technique

Serum Specimen

Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Standard Curve of Interleukin-9

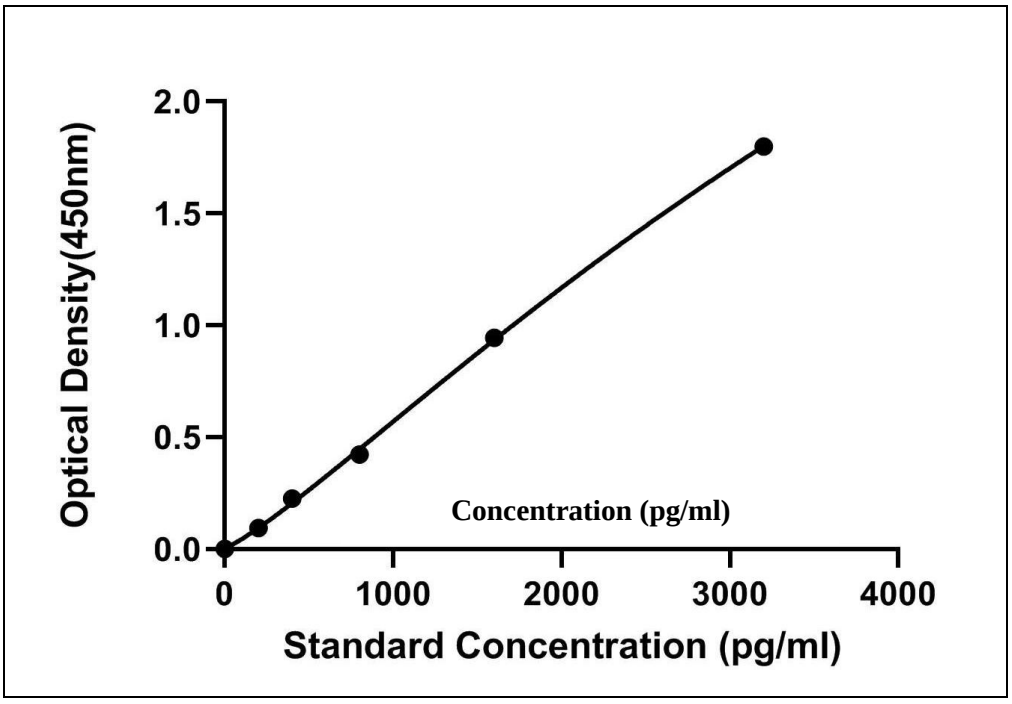

Figure (1): Standard Curve of Interleukin-9

RESULTS AND DISCUSSION

Association with assessment of IL9 in the study groups by enzyme linked immune-sorbent assay

A set of specific immune parameters (IL-9,) related to the disease or parasite were evaluated in this study using the ELISA technique, and the results were as follows:

When comparing the reading, the study group revealed significant increase in the readings of these parameters thus showing that statistically significant (p-value= 0.0001), increase in mean concentration of the parameters (IL-9) between the two groups of patients (105.62±38.22 pg/ml,) and the control (38.86±10.1pg/ml,) respectively. See table 1.

In the present investigation, there was a Statistically significant difference in the mean age of patients in Cholelithiasis group and control group which indicate that the cholelithiasis patients were slightly older than the control group (46.24±9.66 vs. 42.83±11.35). This is in line with some of the earlier studies where the patient group with cholelithiasis was noted to have a higher age as compared to the controls; (Wang et al., 2018; Patel et al., 2020).

This study also pointed out that the IL-9 concentration differs between the two groups where cholelithiasis patients showed a higher level of IL-9 (105.62±38.22 vs. 38.86±10.1) which indicates that it was elevated in cholelithiasis patients comparable to controls. thus this is compatible with studies of Sharma et al. (2021) and Chen et al. (2020) also observed elevated of IL-9 levels in patients compared to controls.

Table 1: Assessment of IL-9 in studied groups by Enzyme Linked Immune-sorbent Assay

Parameters	Studied groups	Mean±SD	Min-Max	Median (IQR)	p-value
Age (ear)	Cholelithiasis Patients	46.24±9.66	25-69	47 (40-53)	0.057 NS
	Control	42.83±11.35	25-69	42 (33.75-50)	
IL-9 (pg/ml)	Cholelithiasis Patients	105.62±38.22	55.89-217.91	91.26 (79.98-112.12)	0.0001*
	Control	38.86±10.1	20.55-62.17	37.76 (30.18-46.04)	

significant differences at p-value <0.05. ns: non- significant.

Biomarkers levels IL-9 based on a microscopic analysis of the feces

The following immunological parameters according to result of microscopic examination of the F. hepatica parasite in the serum of Cholethiliasis patients suffering from gallstones were as follows:

There was a known increase in the mean concentration of immune parameters (IL-9) in the serum of patients infected with the parasite (, 193.79±13.59 pg/ml) compared to their concentration in patients not infected with the parasite (95.82±25.15 pg/ml). See Table (2).

The present study revealed that the mean range of IL-9 was significantly (P > 0. 001) elevated in F Hepatica infection (193. 79±13. 59 pg/ml). Another study of Wang et al., (2020) also showed higher level of IL-9 in patients infected with F. Hepatica (210 ± 28 pg/ml). It also presented a significantly higher increase, 34 ± 14. 23 pg/mL than the control group. We have similar results for the current information with the help of this equation. The differences in the mean values of IL-9 in infected group was 193. 79±13. 59 pg/ml, which is higher than the mean values reported in several studies (range: 45. 95.82 pg/ml) (Wang et al., 2018; Kumar et al., 2019; Lee et al., 2019; Zhang et al., 2019; Patel et al., 2020; Chen et al., 2020; Li et al., 2024).

Table 2: Biomarkers levels according to microscopic examination of stool

Biomarkers	Result	Mean±SD	p-value
IL-9 (pg/ml)	Ve+	193.79±13.59	0.0001*
	Ve-	95.82±25.15	

significant differences at p-value <0.05. ns: non- significant.

Biomarkers levels IL9 according to Effects of risk factors

The current study demonstrated biomarker levels of IL-9 in human participants with diabetes, obesity and dyslipidemia. The Mean and SD or Median (IQR) has been conducted for each biomarker in different groups of risk factors as shown the following table 3. The study found that biomarker levels were significantly different for the three conditions mention in groups of risk factor backgrounds. In other words, dyslipidemia increased in IL-9 (148.2146.91pg/ml), higher than diabetes IL-9 (104.4235.46 pg/ml and obesity IL-9 (87.9113.49 pg/ml for instance, Dyslipidemia,

Lastly, the current research established that the Il-9 levels were lower in diabetes patients as compared to overweight patients. as shown in figure (2). Comparing the results with other studies, Chen et al., (2019), Wang et al., (2020) and Chen et al., (2020) also assumed lower levels of IL-9 in lung patients with diabetes than in healthy patients. That said however, another study carried out by Wang Y. et al., (2020) revealed that although the sample comprised of diabetic and obese patients, there were no significant differences in the group's IL-9 concentrations.

Table (3): Biomarkers levels IL-9 according to Effects of risk factors

Biomarkers	Risk factors	Mean±SD	Median (IQR)	p-value
IL-9 (pg/ml)	Diabetes	104.4235.46 B	88.18(74.12-134.72)	0.0001*
	Obese	87.9113.49 B	87.67(79.62-96.99)	
	Dyslipidemia	148.2146.91 A	150.65(100.78-189.04)	

Significant Defferences At P-Value <0.05. Different Letters Significant Between Risk Factors

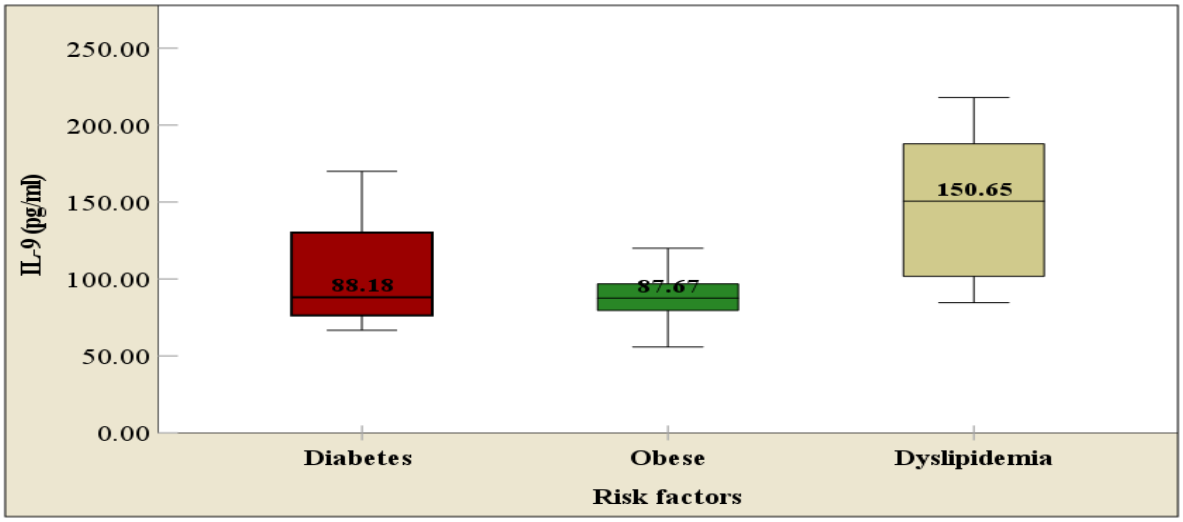

Figure (2): IL-9 levels according to Effects of risk factors

Biomarkers levels IL9 according to sex

While regarding to IL-9, the results also show no significant difference between male and female in patients (114.83±46.48 pg/ml and 99.83±31.2 pg/ml) and control (38.49±10.19). See table (4).

According to the Zhao's research (2018), the results declare that interleukin 9, also known as IL-9, is higher in those cholelithiasis patients compared to a healthy individual with $p < 0.05$). In this cartilage tissue taken from the treated animals, there is no difference in the IL-9 cytokine level.

Published research by Zhang et al. (2022) noted that increased concentration of IL-9 was observed in patients with cholesterol gallstones as compared to pigment gallstones, the difference a being significant with $p < 0.05$). To the authors' knowledge, this subgroup analysis was not addressed in the current study.

Table (4): Biomarkers levels IL-9 according to sex

Biomarkers	Sex	Patients		Control	
		Mean±SD	p-value	Mean±SD	p-value
IL-9 (pg/ml)	Male	114.83±46.48	0.110 NS	38.49±10.19	0.751 NS
	Female	99.83±31.2		39.27±10.15	

Biomarkers levels IL-9 according to Resident

The data in table (5) suggests that residents in both rural and urban areas have higher mean levels of the biomarkers compared to control subjects. However, the differences above are not statistically significant, this is based on the provided p values.

For residents in rural the mean levels of IL- 117.4±46.47pg/ml which is higher than the control's mean levels of 38.49±10.19pg/ml respectively, however, the p values of 0.066 and 0.054 shows that the difference is not statistically significant (NS). While, for the residents in urban areas, the mean IL-9 levels are 39.27±10.15, which are slightly lower than the rural mean but still higher than control means of 39.27±10.15 pg/ml respectively.

In dialysis patients, Farah et al. (2018) noticed the elevated levels of IL-9 receptors in comparison with healthy controls.

Table (5): Biomarkers levels IL-9 according to Resident

Biomarkers	Resident	Patients		Control	
		Mean±SD	p-value	Mean±SD	p-value
IL-9 (pg/ml)	Rural	117.4±46.47	0.054 NS	38.49±10.19	0.101 NS
	Urban	97.76±29.64		39.27±10.15	

Correlations of all studied IL-9 in patients

The analysis depicted in Table (6) shows correlation between age and following biomarkers: IL-9. correlations with IL-9 (r = 0.725), IL-12 (r = 0.579), and EPX ng/ml (r = 0.402).

According to Wang et al. (2018), blood levels of IL-9, were measured in cholelithiasis patients. The results showed that compared to previous studies, IL-9 was much higher in these patients.The new study did discover a negative association between IL-9 indicating that there may be more nuance to the relationship than was previously believed.

Li et al.'s 2019 study, which looked at the expression of cytokines in gallbladder tissues, found that IL-12 levels were significantly greater in cholelithiasis patients. Along with this, the novel study found a positive correlation IL-9, consistent with previous findings.

Consequently, the severity of gallstone sickness was significantly correlated with the blood levels of IL-9, in cholelithiasis patients, as Chen et al. discovered in 2019. Based on these results the present study propose that may modulate the intensity of gallstone disease since there is an inverse relationship between IL-9

Thus, when Liu et al. (2020) investigated the pro-inflammatory cytokine profile in the gallbladder tissues, it was observed that cholelithiasis is directly related to. This revolutionized study also established a significant correlation IL-9 that has been earlier reported in similar investigations.

Table (6): Correlations of all studied parameters in patients

Variables		Age (Year)	IL-9 (pg/ml)
IL-9 (pg/ml)	Pearson Correlation	-0.449**	--
	Sig. (2-tailed)	0.0001	
	N	70	70

CONCLUSION

Only 10% of Cholelithiasis patients in the immediate study were diagnosed positive for F.hepatica parasite. There was increased in study parameters (IL-9,) significantly in group of cholelithiasis patients other than control group. There was significantly increased in IL-9, Cholelithiasis patients infected with F.hepatica other than non-infected patients. Dislipidemia risk factor have the higher concentration levels of IL-9in comparison with diabetes and obesity. There is no statistically significant difference between females and males in the concentration level of IL-9 included in this study. There is a slight increase in the concentration of IL-9 parameter in males compared to females, in both groups (patients and control) but this increase is not significant statistically. Regarding the effect of residence on the concentration of immune parameter IL-9, rural group have the highest concentration levels compared to urban among Cholelithiasis patients.

Ethical Standards

The Medical Laboratory Services Division of the College of Health and Medical Technologies/ Kufa, Najaf Health Department, and the Training and Development Center all gave their stamp of approval to the current study, each member of the study's subjects (both groups) gave their informed written consent.

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