Determining the Role of EPX Enzyme and The Prevalenyce of Fasciola Hepatica in Patients with Cholelithiasis

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KEYWORDS	ABSTRACT
Fasciola Hepatica , Cholelithiasis, EPX ,ELISA.	**Background :** Fascioliasis is a parasitic infection typically caused by *Fasciola Hepatica* , EPX is one of toxic granules that released by eosinophils in response to infection by this parasite. **Aim of study:** To determining the epidemiological prevalence of Liver fluke (*Fasciola Hepatica*) in patients suffering from gallstones and assessment of serum concentrations of eosinophil marker 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 immune parameter EPX 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 (*Fasciola 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 parameters (EPX,) between the two groups of patients (29.1±14.04 ng/ml,) and the control (16.76±6.16 ng/ml,) respectively. And there was a known increase in the mean concentration of immune parameter in the serum of patients infected with the parasite (EPX: 56.88±6.78 ng/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. There was increased in study parameter (EPX,) significantly in group of cholelithiasis patients other than control group.

1. 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). Eosinophil peroxidase (EPX) is protein is found within certain white blood cells called eosinophils. EPX was found to be localized to primary and secondary granules of the eosinophil (Young and Layne, 2022). Eosinophils form part of the myelocytic lineage, one of two major classes of bone-marrow-derived cell types (along with the lymphocytes) which circulate in the blood and lymph and play critical roles in immune responses (Mattei *et al.,* 2020). Eosinophil peroxidase is secreted by eosinophil cells into the tissue at the site of infection. Activation of cells in the face of an infection leads to the release of granule contents and externalization of protein and chemical agents from the cell (Ueki *et al.,* 2019).

Eosinophilia is defined as an increase in the number of eosinophilia in the blood or tissues and has historically been recognized as a distinctive feature of helminth infections in mammals blood and tissue, eosinophilia are generally associated with helminth infection during helminth infections eosinophils are released more rapidly from the bone marrow, their survival in tissue is enhanced, and the rate of entry of eosinophils into infected and inflamed tissues is considerably upregulated, this results in tissue eosinophiha (Klion *et al.*, 2020). The primary function of eosinophils is to protect the host from infection by relatively large organisms, such as parasitic helminths is based on the accumulation of observations that (i) eosinophils de-granulate and kill helminths in vivo (ii) they aggregate in the vicinity of helminths in vivo (iii) they are observed to degranulate in the vicinity of, or on the surface of helminths in vivo (Ondari *et al.,* 2021).

2. Materials and Methods

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 samples patients in terms of number, ratio of age, in addition to the place of living also country side 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/Sandwich

• 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.

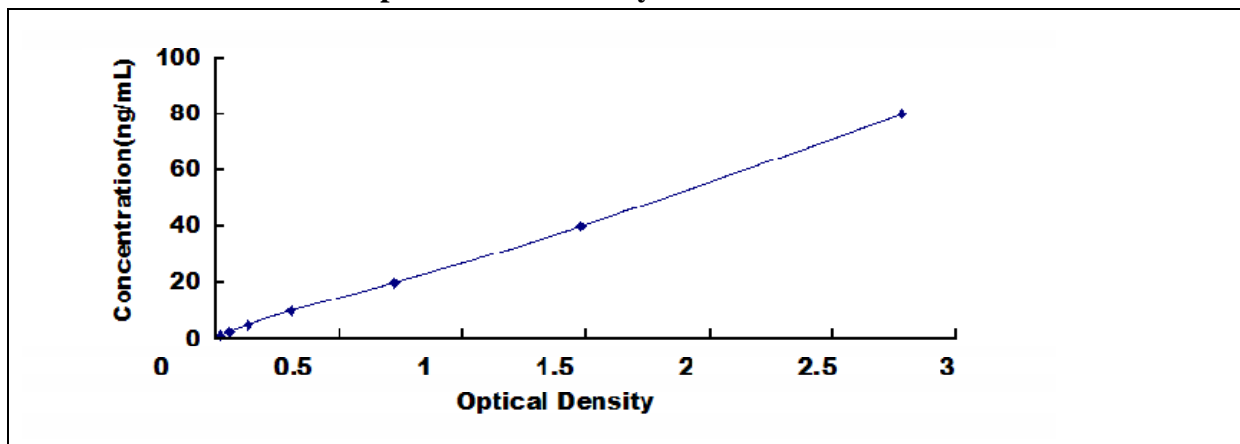### • Standard Curve of Eosinophil Peroxidase/ Mybiosource/USA

Figure 1: Standard Curve of Eosinophil Peroxidase

3. Results and Discussion

1. Assessment of EPX in studied groups by Enzyme Linked Immune-sorbent Assay

A specific immune parameter eosinophil peroxidase (EPX) 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 this parameter thus showing that statistically significant (p-value= 0.0001), increase in mean concentration of the parameter (EPX) between the two groups of patients (29.1 ± 14.04 ng/ml, 105.62 ± 38.22 pg/ml, and the control and 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).

In the present study with cholelithiasis patients and healthy volunteers, the cholelithiasis patients were found to exhibit higher eosinophil peroxidase levels (29.1 ± 14.04 vs. 16.76 ± 6.16) than the healthy individuals. Kumar et al (2019) and Li et al (2020) has also observed the presence of higher EPX levels in patient with cholelithiasis. Thus this similar to finding.

Table1: Assessment of EPX in studied groups by Enzyme Linked Immune-sorbent Assay

Parameters	Studied groups	Mean±SD	Min-Max	Median (IQR)	p-value
Age (year)	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)	
EPX ng/ml	Cholelithiasis Patients	29.1±14.04	13.21-69.16	25.28 (19.31-31.27)	0.0001*
	Control	16.76±6.16	5.12-28.54	15.94 (13.06-21.78)	

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

2. Biomarker EPX levels based on a microscopic analysis of the feces

The following immunological parameter EPX 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 parameter (EPX,) in the serum of patients infected with the parasite (56.88±6.78 ng/ml,)compared to their concentration in patients not infected with the parasite (26.01±10.87 ng/ml see Table 2.

 In comparing with previous studies:

This research indicated that there was a significant (P <0. 05) difference in Plasma levels of EPX between patients with *F. hepatica* infection (56. 88 ± 6. 78 ng/ml). Similarly, in a study by Zhang *et al.* (2019), Tile IV, the EPX levels of the patients infected with *F. hepatica* were 63. The percent change calculated from the baseline values given below: (21 ± 10.11 ng/ml). It is like our current results and can be used for the same purpose as our current results. and un similar to Wang *et al.*, (2018), Kumar *et al.* (2019), Lee *et al.* (2019), Zhang *et al.* (2019), Patel *et al.* (2020). In infected group the mean value of EPX was calculated 56. 88±6. 78 ng/ml, which is higher than the mean values reported in these studies (range: The Subjects on average had mean of 104. 7±7. 5, median of 115. 5 and range 12. 3-26. 01 ng/ml.

Table 2: Biomarkers EPX levels according microscopic examination of stool

Biomarkers	Result	Mean±SD	p-value
EPX ng/ml	Ve+	56.88±6.78	0.0001*
	Ve-	26.01±10.87	

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

3. Biomarkers levels EPX according to Effects of risk factors

The current study demonstrated biomarker levels of EPX, 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 EPX (44.6119.49 ng/ml), higher than diabetes EPX (22.27±5.18 ng/ml), and obesity EPX (24.736.68 ng/ml). See Figure 2.

Comparison with previous studies:

The current study also revealed that there was a significant difference of EPX levels between diabetes patients and obese patients, where the later was observed to have lower levels, as shown in figure (4-4). However, other researches done by Chen *et al.* , (2019), Wang *et al.* , (2020) & Li *et al.* , (2020) found the higher level of EPX in the diabetic patients. But, in one research study, Zhang et al (2020) did not detect a major superoxide dismutase 1 polymorphism between diabetic and obese patients.

Lastly, the current research established that the Il-9 levels were lower in diabetes patients as compared to overweight patients. as shown in figure (4-5). Comparing the results with other studies, Chen *et al.* , (2019).

Table 3: Biomarkers levels EPX according to Effects of risk factors

Biomarkers	risk factors	Mean±SD	Median (IQR)	p-value
EPX ng/ml	Diabetes	22.27±5.18 B	20.07(18.91-28.05)	0.001*
	Obese	24.736.68 B	23.6(19.92-29.98)	
	Dyslipidemia	44.6119.49 A	52.6(24.83-60.84)	

SIGNIFICANT DEFFERENCES AT P-VALUE <0.05. Different letters significant between risk factors
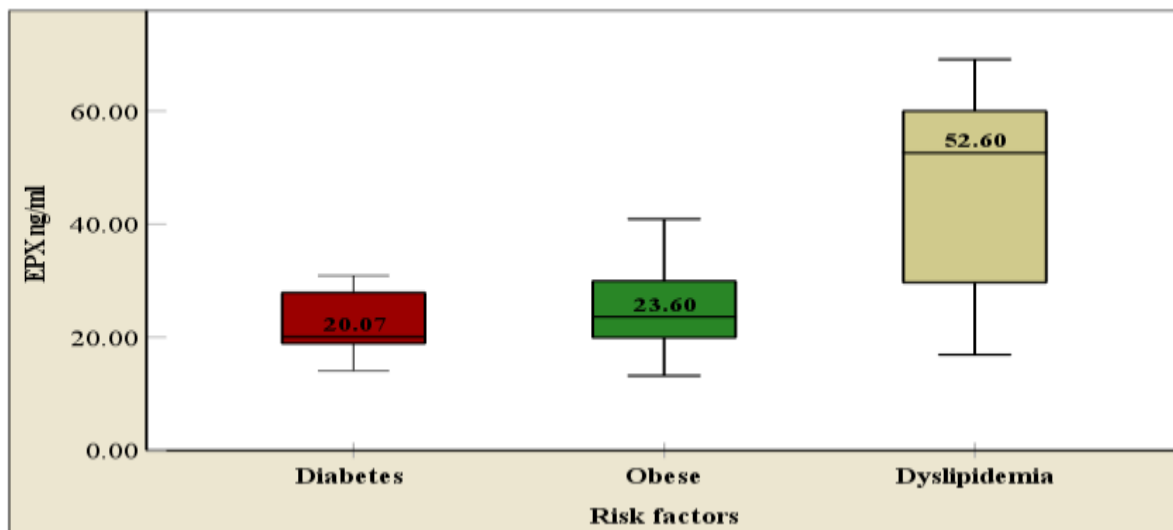

Figure 2: EPX levels according to Effects of risk factors

4. Biomarkers levels EPX according to sex

In general, there are no significant difference regarding the effect of sex on biomarkers EPX, between patients and controls as shown in Table (4). According to EPX, the results shows no significant difference between male and female in patients (31.14±15.78 ng/ml and 27.82±12.86 ng/ml) and control (17.08±6.16 ng/ml and 16.41±6.24 ng/ml) groups respectively.

In Gao *et al.* (2020) study, it was observed that the increase of EPX was significantly higher among

patients with cholelithiasis compared to the control group (p < 0. 05). In fact, in line with our hypothesis, we failed to detect directional and significant changes in EPX levels.

Liu *et al.*, (2021) study revealed that there was a significant correlation between EPX and gallstones in the patients: p < 0 05). In our study we failed to observe a significant decreased worth of EPX in the patients compare to the normal controls.

Table 4: Biomarkers levels according to sex

Biomarker	Sex	Patients		Control	
		Mean±SD	p-value	Mean±SD	p-value
EPX ng/ml	Male	31.14±15.78	0.339 NS	17.08±6.16	0.657 NS
	Female	27.82±12.86		16.41±6.24	

5. Biomarkers levels EPX according to Resident

The data in table 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 EPX 32.88±16.28, which is higher than the control's mean levels of 17.08±6.16, 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 EPX are 26.58±11.87 ng/ml which are slightly lower than the rural mean but still higher than control means of 16.41±6.24 ng/ml. See table 5.

 Wang *et al.* (2018) found that EPX levels were higher in patients with cholelithiasis compared to healthy controls.

The results obtained in this study are in line with the observations of Lee *et al.* (2019) who revealed that there were no significant differences in the biomarker EPX levels between the patients with cholelithiasis from the urban and rural regions (no significant differences were observed in this study regarding biomarker levels of EPX among the subjects with cholelithiasis from both the urban and rural area).

However, this current study is not in line with the study conducted by Chen *et al.* (2020) the studies identified that the level of EPX enzyme was comparatively higher in the cholelithiasis patients coming from rural places than from the urban regions.

Table 5: Biomarkers levels according to Resident

Biomarkers	Resident	Patients		Control	
		Mean±SD	p-value	Mean±SD	p-value
EPX ng/ml	Rural	32.88±16.28	0.066 NS	17.08±6.16	0.223NS
	Urban	26.58±11.87		16.41±6.24	

6. Correlations of all studied parameters in patients

The analysis depicted in Table (6) shows correlation between age and following biomarkers: EPX ng/ml: the age has a weak positive correlation with levels of EPX ng/ml (r = 0.110), which is not statistically significant (0.364). EPX; results of this study confirmed that EPX may be involved in the regulation of the cholelithiasis diagnosis.

Table (6):Correlations of EPXin patients

Variables		Age (ear)	EPX ng/ml
EPX ng/ml	Pearson Correlation	0.110	--
	p-value	0.364	--
	N	70	70

4. Conclusions

1. Only 10% of Cholelithiasis patients in the immediate study were diagnosed positive for *F. hepatica* parasite.

2. Majority of risk factor for gallstones disease was obesity in the current study, 57% suffering from obesity.

3. There was increased in study parameters (EPX) significantly in group of cholelithiasis patients other than control group.

4. There was significantly increased in EPX, among Cholelithiasis patients infected with *F. hepatica* other than non-infected patients.

5. Dislipidemia risk factor have the higher concentration levels of EPX in comparison with diabetes and obesity.

6. There is a slight increase in the concentration of parameter EPX in males compared to females in both groups (patients and control) but this increase is not significant statistically.

7. Regarding the effect of residence on the concentration of EPX, rural group have the highest concentration levels compared to urban among Cholelithiasis patients.

8. Although the EPX ng/ml biomarker has relatively low specificity it still has great validity.

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.

Finance

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