ORIGINAL ARTICLE

Seroprevalence and associated risk factors of *Entamoeba histolytica* infection among gastroenteritis patients visiting the public healthcare system, Pakistan

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Abstract

Objectives: To investigate the seroprevalence and associated risk factors of *entamoeba histolytica* among patients with gastrointestinal complaints, and to measure the eventual changes in serum biochemical parameters to reflect its pathogenicity.

Methods: The cross-sectional study was conducted in different hospitals of Potohar region in Punjab province and in the Khyber Pakhtunkhwa province of Pakistan from September2015 to February 2017, and comprised individuals of either gender belonging to diverse backgrounds, inhabiting different areas of the country. The patients were enrolled from among those who visited outpatient departments with complaints of vague abdominal pain, nausea, vomiting, indigestion and diarrhoea. Blood samples were screened by using enzyme-linked immunosorbent assay and serum biochemical tests. Data was analysed using SPSS 20.

Results: Of the 356 subjects, 238(66.9%) were females and 118 (33.1%) were males. The overall mean age was 33.4±11.05 years. Seroprevalence of *entamoeba histolytica* was 356(73%). The infection rate did not differ significantly ($p>0.05$) among cities, while the highest infection was recorded in Islamabad 91(25.5%). The participants in rural areas had 2.16-fold higher risk of infection compared to urban areas, while the lowest risk of infection among people aged 50years compared to those aged 40-49 years ($p=0.04$). The amoebiasis was significantly associated with eating unwashed raw vegetables and average toilet facilities. Among clinical complications, haemodynamic changes, jaundice, vomiting, haemoglobin level, loose motion, intolerance to oral feeding, and history of antibiotics were significant associated variables ($p<0.05$ each). Significant elevation in alkaline phosphatase, aspartate aminotransferase, total protein and globulin levels were positively associated with amoebiasis ($p<0.01$ each).

Conclusion: In *entamoeba histolytica* -positive patients ,serum biochemical level was found elevated and the risk factors determined were eating unwashed vegetables, toilet facilities, age, locality, jaundice, vomiting, haemoglobin level, loose motion, intolerance to oral feeding, and history of antibiotics.

Keywords: Amoebiasis, Biochemical parameters, Risk factors, Serology, Pakistan. (JPMA 69: 1777; 2019)
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Introduction

Entamoeba (E.) histolytica is one of the intestinal protozoan that causes a disease known as "amoebiasis", which is the third leading parasitic disease, after malaria and schistosomiasis, causing death in humans. According to World Health Organisation (WHO) estimates, *E. histolyticamay* may infect half-a-billion people annually and is responsible for 40,000 to 100,000 deaths a year.[1] In tropical regions, amoebiasis is more common among the general population and particularly among patients attending hospitals and healthcare centres with diarrhoea, abdominal pain, and fever.[2] E. histolyticais pathogenic amoeba causing damage to intestinal mucosa, amoebic colitis, haematogenous spread,

producing fatal abscesses and extra-intestinal amoebiasis.[3] The inflammatory and necrotic activities of this parasite alter blood parameters, cause anaemia, cholestasis and inflammation, and are marked by appearance of natural anti-parasitary indicators, increase of antibodies, and liver inadequacy.[4]

Amoebiasis is transmitted through the ingestion of mature cysts and is usually acquired from food or water sources contaminated with faeces.[5] Demographic, behavioural, environmental and clinical characteristics that linked with disease are counted among the risk factors. Microscopy, culture analysis and molecular techniques are applied for *E. Histolytica* detection. All these diagnostic techniques have some limitation. Studies on amoebiasis in Pakistan are based on microscopic examination of faeces, which leads to poor sensitivity and confounded with false-positive (FP) results due to misidentification of macrophages as trophozoites, polymorphonuclear leukocytes as cysts and other *entamoeba* species.[6] Polymerase chain reaction (PCR) is a powerful tool, but is technically complex, costly,

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susceptible to cross-contamination, and is affected by faecal components and false-negative (FN) results due to inhibitors of deoxyribonucleic acid (DNA)-polymerase in stool samples.[7] However, the immunodiagnostic assays have so far been the most successful in large-scale epidemiological studies of asymptomatic diseases.[8] The IgG antibody detection assays have the ability to detect both active and chronic infection. The recombinant *E. histolytica* antigens have been recently available at commercial scale, and their usefulness for serodiagnosis of amoebiasis has been reported.[9]

The current study was planned to investigate the seroprevalence and associated risk factors of *E. histolytica* among patients with gastrointestinal complaints, and to measure the eventual changes in serum biochemical parameters to reflect its pathogenicity.

Subjects and Methods

The cross-sectional study was conducted in different hospitals of Potohar region in Punjab province and in the Khyber Pakhtunkhwa province of Pakistan from September 2015 to February 2017, and comprised individuals of either gender from diverse backgrounds, inhabiting different areas including Islamabad, Rawalpindi, Peshawar, Abbottabad, Multan, Muzaffarabad and Gilgit-Baltistan. This study was approved by the ethical committees of Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, and Quaid-i-Azam University, Islamabad. The study was conducted at the Pakistan Institute of Medical Sciences (PIMS), Islamabad, District Headquarters (DHQ) hospital, Rawalpindi, and Ayub Medical Hospital, Abbottabad.

The sample size was determined by using the formula: $n= Z^2 P (1-P)/d^2$,[10] where n was the sample size, Z was the statistic corresponding to level of confidence, P was expected prevalence, and d was precision. According to literature, actual prevalence of amoebiasis may not be more than 37%.[11] Therefore, a minimum sample of 356 gastroenteritis patients was calculated. Using consecutive sampling technique, 700 individuals were approached with a self-generated questionnaire. Those included were patients visiting the various outpatient departments (OPDs) with complaints of vague abdominal pain, nausea, vomiting, indigestion and diarrhoea. Informed written consent was obtained from all the subjects. Those who refused to sign the consent form were excluded.

Blood samples from all the enrolled subjects were obtained for the evaluation of anti-amoebic immunoglobulin-G (IgG) antibody test. The cross-reactivity of the assay was calculated by using control sera including positive controls, negative controls and sera-positive for protozoans and helminthes infection other than amoebiasis.

The questionnaire contained three sections: socio-demographic, environmental and clinical factors. It noted each participant's information on age, gender, locality, socioeconomic status (SES), literacy rate, household members, hygiene, consumption of raw unwashed vegetables, outdoor defecation, exposure to animal/human excreta, source of drinking water, type of water used, eating outside the home, accessibility to toilet, and clinical manifestations like vomiting, diarrhoea, stool with mucous or blood, intolerance to oral feeding, right hypochondriac pain etc. Brief patient history and observation of signs and symptoms were used to obtain clinical data.

After getting the questioner filled, 2ml blood was collected from patients in non-ethylenediaminetetraacetic acid (EDTA) tubes. The sera were separated after centrifugation at 1500rpm for 15 minutes and stored at 20°C until used for anti- amoebic IgG antibody detection assay.

Enzyme-linked immunosorbent assay (*E. histolytica* AccuDiag™) was used to confirm anti-*E. histolytica* IgG antibodies in all sera. The assay was performed according to manufacturer's guidelines, and serum above the cut-off optical density (OD) $\geq$0.5 was considered positive.

Biochemical analysis as secondary test for active amoebiasis was performed on sera positive for anti-*E. histolytica* IgG antibodies (OD $\geq$0.5) compared to control group with OD $\leq$0.1. The studied biochemical parameters were glucose, protein, albumin, globulin, cholesterol and liver enzymes i.e. serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), and alkaline phosphatase (ALP). Biochemical assays were performed according to manufacturer's instructions (Spectrum and Futura diagnostic kits).

The questionnaires were checked regularly for logical errors, missing values, discrepancies, inconsistencies etc. Data was analysed using SPSS 20. Descriptive statistics were presented as mean±standard deviation (SD) for numerical variables and frequencies and percentages for categorical variables. Chi-square test and univariate logistic model to estimate the odd ratio (OR) was calculated to find the independent association between categorical variables with 95% confidence interval (CI). For multiple comparisons, the level of significance was set at $p\leq0.05$ or $p\leq0.01$. Independent sample t-test was applied to analyse biochemical parameters by comparing

the means of cases and controls.

Results

Of the 700 individuals approached, 356(51%) represented the final study sample. Of them, 238(66.9%) were females and 118 (33.1%) were males. The overall mean age was 33.4±11.05 years. The Literacy rate was 272(76.4%) and 147(41.3%) subjects were residing in rural areas. Of the blood samples, 260(73%) were IgG positive for anti-*E. histolytica* IgG antibodies, while 96(27%) were seronegative. The socio-demographic characteristics significantly associated with high risk of amoebiasis were inhabitants of Islamabad (p=0.03), living in rural areas (p=0.002). The lowest risk was among subjects aged 50 years (p=0.04) compared to those aged 40-49 years (Table-1).

Table-1: Seroprevalence of *Entamoeba histolytica* Immunoglobulin-G (IgG) antibodies, according to socio-demographic risk factors among study participants.

Characteristic	Positive n %	Negative n %	χ^2	Odd ratio	95% CI	p-value
District						
Abbottabad	19(5.3)	4(1.1)	15.07	2.23	0.71-7.00	0.166^{NS}
Azad Kashmir	14(3.9)	12(3.4)		0.55	0.23-1.29	0.171^{NS}
Gilgit Baltistan	10(2.8)	0		9.96	0.56-174.09	0.115^{NS}
Islamabad	91(25.5)	23(6.5)		1.86	1.03-3.36	0.038*
Multan	14(3.9)	8(2.2)		0.82	0.32-2.12	0.689^{NS}
Peshawar	25(7)	8(2.2)		1.47	0.61-3.54	0.388^{NS}
Rawalpindi	87(24.4)	41(11.5)		Reference		
Gender						
Male	92(25.8)	26(7.3)	2.18	Reference		
Female	168(47.2)	70(19.7)		0.67	0.40-1.13	0.141^{NS}
Residence						
Peri-Urban	32(9)	6(1.7)	12.5	3.22	1.25-8.27	0.15^{NS}
Rural	147(41.3)	41(11.5)		2.16	1.32-3.56	0.02*
Urban	49(13.8)	81(22.8)		Reference		
Age (years)						
10-19	38(10.7)	10(2.8)	5.86	1.22	0.50-2.93	0.654^{NS}
20-29	62(17.4)	24(6.7)		0.83	0.408-1.689	0.688^{NS}
30-39	86(24.2)	30(8.4)		0.92	0.46-1.80	0.812^{NS}
40-49	56(15.7)	18(5.1)		Reference		
>50	18(5.1)	14(3.9)		0.41	0.17-0.99	0.048 *
Literacy rate						
Higher	17(4.8)	7(2)	2.58	1.21	0.40-3.26	0.701^{NS}
Primary	150(42.1)	48(13.5)		1.56	0.89-2.73	0.117^{NS}
Secondary	37(10.4)	13(3.7)		1.42	0.65-3.09	0.374^{NS}
Uneducated	56(15.7)	28(7.9)		Reference		
Household members						
4-8	92(25.8)	28(7.9)	5.73	1.53	0.90-2.59	0.109^{NS}
9-13	124(34.8)	58(16.3)		Reference		
14-18	28(7.9)	8(2.2)		1.63	0.70-3.81	0.253^{NS}
19-23	16(4.5)	2(0.6)		0.74	0.83-16.81	0.08^{NS}
Infrastructure						
Concrete	193(54.2)	75(21.1)	2.43	0.19	0.01-3.54	0.27^{NS}
Mud brick	61(17.1)	21(5.9)		0.22	0.01-4.07	0.309^{NS}
Mud only	6(1.7)	0		Reference		
Education of Mother						
Primary	12(3.4)	2(0.6)	1.19	2.27	0.5-10.35	0.288^{NS}
Uneducated	248(69.7)	94(26.4)		Reference		
Socio-economic status						
Average	56(15.7)	16(4.5)	1.03	1.37	0.74-2.53	0.311^{NS}
Poor	204(57.3)	80(22.5)		Reference		

χ^2 Pearson's chi-square test; NS: Non-significant difference ($p > 0.05$); * Significant difference ($p < 0.05$);
95% CI 95% Confidence Interval; n: Number of studied samples.

Table-2: Seroprevalence of *Entamoeba histolytica* Immunoglobulin-G (IgG) antibodies according to behavioural and environmental risk factors among study participants.

Characteristic	Positive n %	Negative n %	χ^2	Odd ratio	95% CI	p-value
Household hygiene conditions						
Normal	99(27.8)	31(8.7)	1.01	1.28	0.78-2.11	0.315^{NS}
Poor	161(45.2)	65(18.3)		Reference		
Eating Raw vegetables						
Washed	224(62.9)	90(25.3)	3.88	Reference		
Unwashed	6(1.7)	36(10.1)		2.41	0.98-5.91	0.05*
Food preservation method						
Kept warm	118(33.1)	42(11.8)	4.33	Reference		
Fridge	128(36)	46(12.9)		0.99	0.60-1.61	0.969^{NS}
Both	10(2.8)	8(2.2)		0.44	0.16-1.20	0.11^{NS}
Toilet Facility						
Average	60(16.9)	36(10.1)	7.40	0.50	0.30-0.82	0.007**
Poor	200(56.2)	60(16.9)		Reference		
Type of Latrine						
Dry Latrine	240(67.4)	94(26.4)	4.91	0.101	0.006-1.73	0.1144^{NS}
Wet Latrine	12(3.4)	0(0)		Reference		
No Latrine	8(2.2)	2(0.6)		0.13	0.005-3.20	0.2158^{NS}
Outdoor defecation						
Yes	18(5.1)	2 (0.6)	3.09	Reference		
No	242(68)	94(26.4)		0.28	0.06-1.25	0.097^{NS}
Exposed to human or Animal excreta						
Yes	18(5.1)	6(1.7)	0.05	Reference		
No	242(68)	90(25.3)		0.89	0.34-2.32	0.822^{NS}
Contact with animals						
Yes	32(9)	6(1.7)	2.69	Reference		
No	228(64)	90(25.3)		0.47	0.19-1.17	0.107^{NS}
Use of tap water						
Yes	204(57.3)	80(22.5)	1.03	Reference		
No	56(15.7)	16(4.5)		1.37	0.74-2.53	0.311^{NS}
Use of tube well water						
Yes	16(4.5)	2(0.6)	2.4	Reference		
No	244(68.5)	94(26.4)		0.32	0.07-1.43	0.138^{NS}
Eating away from home						
Yes	176(49.4)	58(16.3)	1.64	Reference		
No	84(23.6)	38(10.7)		0.72	0.44-1.183	0.2^{NS}

χ^2 Pearson's chi-square test; NS: Non-significant difference ($p > 0.05$); * Significant difference ($p < 0.05$);
**Significant difference ($p < 0.01$); 95% CI 95% Confidence Interval; n number of studied samples.

Characteristics related to behavioural and environmental risk factors showed that those eating raw unwashed vegetables (p=0.049) had two times higher risk of amoebiasis compared to those eating washed vegetables. Those with access to toilet facility (p=0.007) were at 50% less risk than those with poor access (Table-2).

Clinical symptoms that showed significant risk association with seropositivity of anti-*E. histolytica* IgG antibodies included low haemoglobin (Hb) level (p=0.047), having no symptoms of loose motion (p=0.014), history of antibiotics (p=0.000) and without haemodynamic changes (p=0.000). Intolerance to oral feeding was a significant (p=0.005) factor. Patients having frequent vomiting (p=0.027) has high risk of testing seropositive for amoebiasis than those with no vomiting (Table-3).

Overall, 80(22.5%) sera were positive for anti-*E. histolytica* IgG antibodies (OD $\geq$0.5) compared to 40(11.23) the control group (OD$\leq$0.1). Serum aspartate aminotransferase (AST) (p=0.005), alkaline phosphate (ALP) (p=0.005), total protein (p=0.002) and globulin (p=0.002) were elevated significantly in E. histolytica IgG-positive cases compared to the controls (Table-4).

Table-3: Seroprevalence of *Entamoeba histolytica* Immunoglobulin-G (IgG) antibodies according to clinical risk factors among study participants.

Characteristic	Positive n %	Negative n %	χ^2	Odd ratio	95% CI	p-value
Fever						
Yes	252(70.8)	96(27)	3.02	Reference		
No	8(2.2)	0(0)		6.49	0.37-113.6	0.2^{NS}
Haemoglobin Level						
Low	166(46.6)	72(20.2)	3.39	0.58	0.34-0.99	0.04*
Normal	94(26.4)	24(6.7)		Reference		
Stool with mucous or blood						
Yes	26(7.3)	8(2.2)	0.22	Reference		
No	234(65.7)	88(24.7)		0.81	0.35-1.87	0.6354^{NS}
Right hypocondriac pain						
Yes	103(28.9)	39(11)	0.03	Reference		
No	157(44.1)	57(16)		1.04	0.64-1.68	0.8629^{NS}
Dysentery						
Yes	32(9)	18(5.1)	2.41	Reference		
No	228(64)	78(21.9)		1.64	0.87-3.09	0.123^{NS}
Loose motion						
Yes	180(50.6)	53(14.9)	6.09	Reference		
No	80(22.5)	43(12.1)		0.54	0.33-0.88	0.014*
Vomiting						
Frequent	179(50.3)	54(15.2)	4.91	1.71	1.06-2.78	0.027*
Sometimes	81(22.8)	42(11.8)		Reference		
Intolerance to oral feeding						
Yes	210(59)	64(18)	7.86	Reference		
No	50(14)	32(9)		0.47	0.28-0.80	0.005**
History of Antibiotics						
Yes	254(71.3)	84(23.6)	15.17	Reference		
No	6(1.7)	12(3.4)		0.16	0.060-0.45	0.000**
Haemodynamic changes						
Yes	18(5.1)	22(6.2)	17.98	Reference		
No	242(68)	74(20.8)		3.99	2.03-7.85	0.0001**
Weight loss						
Yes	141(39.6)	45(12.6)	1.52	Reference		
No	119(33.4)	51(14.3)		0.74	0.46-1.19	0.2182^{NS}
Jaundice						
Yes	24(6.7)	14(3.9)	2.10	Reference		
No	236(66.3)	82(23)		1.67	0.82-3.39	0.15^{NS}
Hepatomegaly						
Yes	8(2.2)	2(0.6)	0.25	Reference		
No	252(70.8)	94(26.4)		0.67	0.13-3.21	0.6168^{NS}

χ^2 Pearson's chi-square test; NS: Non-significant difference ($p > 0.05$); * Significant difference ($p < 0.05$);
**Significant difference ($p < 0.01$); 95% CI 95% Confidence Interval; n number of studied samples.

Discussion

To our knowledge, this is the first study on sero-epidemiology and associated risk factors of *E. histolytica* in relation to serum biochemical parameters among patients with gastrointestinal complains. In Pakistan, data for amoebiasis based on microscopic analysis of faeces suggests that *E. histolytica* infection may be endemic to rural areas. The estimated prevalence of *E. histolytica* so far in Pakistanis 13.6%-63.8%.[12,13] Furthermore, the present prevalence of *E. histolytica* infection in Pakistan among patients visiting healthcare centres with clinical manifestation associated with amoebiasis is not yet known. However, the current higher occurrence of anti *E. histolytica* IgG is due to targeting risk population positive for amoebiasis and limitation of detection assays is not to differentiate between active and past infection. In the current study, biochemical analysis was coupled with ELISA to determine acute and extra-intestinal amoebiasis.

Table-4: The overall biochemical changes (mean ± SD) of *Entamoeba histolytica* infected and control group.

Variables	Infected Group (n=80) Mean ± SD	Control Group (n=40) Mean ± SD	t-value	p-value
Liver Enzymes				
AST (U/l)	53.645 ± 28.991	29.914 ± 18.707	3.171	0.005**
ALT (U/l)	61.895 ± 20.711	48.364 ± 18.859	1.989	0.065^{NS}
ALP (U/L)	100.035 ± 125.821	39.650 ± 12.144	2.98	0.005**
GGT (U/L)	19.551± 19.595	13.664 ± 13.011	1.143	0.266^{NS}
Serum protein				
Protein (g/dL)	10.153 ± 2.524	7.620 ± 1.885	3.529	0.002**
Albumin (g/dL)	4.209 ± 0.161	4.234 ± .0654	-0.734	0.468^{NS}
Globulin (g/dL)	5.943 ± 2.467	3.386 ± 1.860	3.623	0.002**
Cholesterol (mg/dl)	193.251 ± 66.076	187.144 ±55.289	0.3	0.768^{NS}
Glucose (mg/dl)	58.378 ± 51.369	46.726 ± 68.242	0.505	0.623^{NS}

NS: Non-significant difference ($p > 0.05$); * Significant difference ($p < 0.05$); **Significant difference ($p < 0.01$); n number of studied samples
SD: Standard deviation
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
ALP: Alkaline phosphatase
GGT: Gamma glutamyltransferase.

Regarding gender, the level of anti-*E. histolytica* IgG antibodies were found higher in females compared to males and the difference was not significant (p>0.5). This could be attributed to the fact that due to the socio-cultural lifestyle of the area, females are more likely to interact with contaminated environment, food, water than males.[14] Among age groups of 30-39 and 20-29 years the infection rate was higher, and it was the lowest among patients aged >50 years. The higher infection in young participants may be due to less immunity, and lower infection in adults due to well-developed immunity against parasitic diseases.[15]

Amoebiasis was found higher in uneducated participants than the educated, but the difference was not significant. The low infection in educated people may be due to adequate knowledge about diseases, good hygiene and health practices.[16] Similarly educated mothers are aware of the importance of sanitation, cleanliness and are able to inculcate better sense of hygiene.

SES revealed that participants living in households with a poor SES had high infection rate, consistent with previous findings.[16] The toilet facility was found to be a significant contributing factor for amoebiasis and participants with poor toilet facility were mostly found infected. This may be due to the fact that the areas brought under study had poor sanitation and short water supply which affects the safety of toilets. The finding was contrary to studies which reported that amoebiasis is common in people irrespective of access to a toilet.[17] An important aspect analysed was outdoor defecation in the river or bushes. This unhygienic activity of defecation in river enhances the faecal pollution of domestic water supply that may introduce a variety of intestinal pathogens resulting in spread of many pathogenic diseases.[18]

In the present study, no significant association was found between infection and contact with animals. Similarly, reports on sporadic zoonotic transmission have never been seen, although there is possible risk of transmission of *E. histolytica* cysts from animals to humans by close contacts.[19] Globally, the companion animals have been considered significant causes of health problems due to their role as reservoirs for zoonotic diseases. The probable zoonotic risk of *E. histolytica* is of great concern to public health as amoebiasis is not a zoonotic disease.

Eating unwashed/ raw vegetables found an important contributing risk factor for disease. Other epidemiological studies have shown higher risk of amoebiasis in subjects who ate raw unwashed vegetables.[20] The present study emphasises the potential of unwashed vegetables in the transmission of *E. histolytica* infections, as people are frequently involved with consumption of raw/unwashed vegetables. Source of drinking water showed a non-significant relation with prevalence, in accordance with previous findings.[21] Among clinical symptoms vomiting and diarrhoea showed significant association between anti-*E. histolytica*

IgG antibodies. Amoebiasis is associated with 80-98% symptomatic illness and 2-20% with invasive disease. Patients with no haemodynamic changes were found at significant risk of amoebiasis. The results are not in agreement with previous study where 38.89% amoebic patients showed haemodynamic changes.[22] Jaundice was found significantly associated with E. histolytica infection. The results are in agreement with some studies where jaundice is described as an unusual presentation of amoebic liver abscess and reported in only 5% cases.[23] The causes of jaundice may either be hepatocellular dysfunctioning or intrahepatic biliary obstruction.

Liver enzymes i.e. AST, alanine aminotransferase (ALT) and ALP showed significant elevation in the current study when compared to the controls. The elevation in AST, ALT and ALP is the result of extensive degradation of hepatic parenchyma leading to hepatic necrosis that occurs during migration of trophozoites.[24] Elevated AST, ALT and ALP levels were also reported in patients with amoebic liver abscess. The systematic complications associated with liver dysfunctioning found an increase in all liver enzymes. A study conducted on animal model showed increase in AST, ALT levels, when liver cells were invaded by trophozoites. Injury to hepatocytes results in spilling of these enzymes into the blood stream, hence increasing the serum enzyme level. Similarly, elevated level of ALP was recorded due to obstruction or damage of bile duct of liver.[25] The current results matched with the findings of elevated levels of liver enzymes in the serum of patients infected with the amoebiasis.[26]

Serum total protein and globulin were significantly elevated in the present study. The concentration of proteins such as C-reactive protein (CRP), alpha1 acid glycoprotein, fibrogen, haptoglobin, alpha 1 proteinase inhibitor and immunogloblins such as IgG, IgA, IgM and IgE always increases during parasitic infections and provide early protection and preparation for a prolonged defense.[27] The cholesterol level did not show significant difference. In contrast, studies on relationship of serum cholesterol levels in *E. histolytica* infected patients found significant lower levels of lipid profile, which shows that parasite remodels host lipids for their growth and to generate phospholipids membrane.[28]

Conclusions

Gaining an overview of the seropositivity of *E. histolytica* infection in different regions of Pakistan coupled with biochemical tests provided a more accurate measure for intestinal and extra-intestinal prevalence of amoebiasis, thereby facilitating better resource allocation to protect the population from public health concerns.

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