ORIGINAL ARTICLE

Relationship between *Entamoeba histolytica* and Fecal Calprotectin in Patients with Gastroenteritis in Kirkuk City-Iraq

[1]Ola Salih Ali, [2]Shihab Ahmad Mohammad, [3] Yahya Jirjees Salman*

[1]College of Education for Pure Science-Tikrit University.
[2]Parasitology. College of Education for pure science-Tikrit University.
[3]Medical Microbiology. Faculty of Medicine-Kirkuk University.

ABSTRACT

Background: *Intestinal amoebiasis due to Entamoeba histolytica in association to gastroenteritis in Kirkuk city was recently reported.* ***Objective:*** *To measure fecal calprotectin (FC) also during intestinal amoebiasis. The relation between Ent.histolytica and gastroenteritis was investigated.* ***Methodology:*** *A total of 419 patients attend to the gastroenteritis clinics in two Hospital Kirkuk center. They suffer from gastrointestinal disorders (GITDs), such as diarrhea, vomiting, nausea, diarrhea altered by constipation. The collected stool samples were tested for: parasitological tests and fresh portion was extracted for fecal calprotectin using ELISA technique. Blood samples were tested for some hematological tests. For each stool sample direct double wet preparations were performed then confirmed by flotation technique. Overnight extracted fecal sample was tested for FC using ELISA-copro kit. Neutrophil count and white blood cells were calculated using samples using automated machine.* ***Results:*** *A total of 82.81 % of parasitic infection was recorded, and Entamoeba histolytica contributed rate was 24.85 %.Inflammatory bowel disease (IBD) due to intestinal amoebiasis were highly recorded among patients aging from 31to 40 years (74.34 %) followed by 71.42 % among patients aging from 11 to 20 years. Compare to high rate in irritable bowel syndrome (IBS) patients aging from 41 to over than 60 years old. Samples from females positive for Entamoeba histolytica in relation to IBD and IBS exert significance compare to males. Fecal calprotectin positive level above than 50 ng/ml was recorded in 50 stool samples versus to 37 negative mean levels as Fc was below 50 ng/ml. Frequency of FC positive was higher among females than males. Leucopenia and neutrophilia were dominant among positive FC samples contrary to normal neutrophil count among FC negative samples. High rate 59.22 % of stool sample pH was ranged from 6.1 to 7.* ***Conclusions:*** *Entamoeba histolytica rate is high among patients with gastroenteritis particularly among IBD patients. ELISA Fecal calprotectin is good marker for detecting the injury caused by the parasites to the host.*

INTRODUCTION

Amoebiasis, is a ubiquitous parasitic infection affecting approximately 10% of the world's population and causing more deaths every year (100,000 deaths) than any other parasitic infection, with the exception of malaria and schistosomiasis[1,2]. Most often *E. histolytica* infection is asymptomatic, but some develop severe invasive diseases such as amoebic dysentery. Extra-intestinal types included pulmonary, cardiac or brain involvement, In chronic cases, non-dysenteric syndrome of diarrhea, abdominal pain and weight loss that can last for years and mimic IBD[3]. The following rates for intestinal amoebic infections were recorded: 65.20% and 30.11% in Basra, 36.26% in Nasiriya,54.6% in Najaf, 21.11% in Karbala, 12% in Diala, recorded by[4,5,6,7, 8] respectively. Whereas the following rates 5.11%.4.57%, 4.9%, 3.5%, 7.8% and 21.2% were recorded in Arab neighbor countries by[9,10,11,12,13, 14] respectively.

Abdominal pain and diarrhea are sharing syndrome between patients with IBD and IBS, accordingly IBD and IBS cannot be separated from each other exclusively. Recently, colonoscopy was required to rule out IBD, as a result negative endoscopy finding was diagnosed as IBS, whereas half of patients with symptoms suggesting IBD.[15]

Following sentinel gastrointestinal infection, post infectious IBS (PI-IBS) can be focused as infectious gastroenteritis [16,17] The occurance of PI-IBS following infectious gastroenteritis ranges from 4% to 31%, with a pooled incidence of 10% [18.19]. Several parasites such as *E. histolytica, Giardia spp*., *B. hominis*, and *Trichinella*

spp. have been discussed as contributing factors to the development of post-IBS [20,21,22].

The relationship between gastroenteritis (GITDs) and infectious agents was studied in details; for example; in Jordon bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from North Jordon were studied, they state that protozoan infections contribute 3.5%[12]. In Korea in separate study, young patients experienced high risk of IBS after 3 years from *Shigella sonnei*, and modestly increased risk until 8 years [23].

Fecal calprotectin (FC) antibody is produced by the neutrophils when they were in challenges with the pathogens causing IBD [24]. These antibodies bound to serum albumin can be assessed serologically as antigen antibody-complex color formation, to be easily determined by ELISAreader. FC antibody level above 50 ng/ml indicates severe damage in GIT which require interference of endoscopy for detecting the causative agents[25]. Furthermore FC degranulation inside the intestinal lumen occurs as a response to local inflammation[26]. Recently FC is a considerable trial in gastroenterology practices for distinguishing inflammatory bowel disease from other non-inflammatory ailments. In addition it can be considered as a validated marker for disease activity and response to treatment [27,28], especially in infectious diarrhea of both viral and bacterial etiologies[29].

In Kirkuk Province-Iraq; via gastrointestinal practices the finding of leukocytosis and neutrophilia were recorded among patients with gastroenteritis, this finding was explained and referred to acute infections particularly by *Entamoeba histolytica*. Whereas high mean FC level among IBD patients refers to good challenges from the host against intestinal protozoan parasite [3].

METHODOLOGY

The study was approved by the Kirkuk Research and Publication Committee of Kirkuk Health directorate (document number639 in March 2017), and by the respective Hospital Authorities at the two study hospitals. Written informed consent was taken from elderly patients and from the guardian on behalf of all the young patients enrolled in the study.

Study design:

A cross-sectional study was carried on from the 15th of March 2017 till the 1st February of 2018; stool samples were collected from 419 patients attending into Gastroenterology and Endoscopy Unit in Azadi teaching Hospital, Kirkuk General Hospital, Private clinics and Ibn-Nafees private medical laboratory in Kirkuk city. The diagnosis and patients selection were done under the supervision of gastrenterologist. Samples were examined in Department of Medical Microbiology, Faculty of Medicine, Kirkuk University and Ibn-Nafees Private Medical Laboratory. Tis patients included 216 males and 203 females of different ages ranging from 1 year to over than 60 years. According to the physical examination; the following patients were enrolled in the study: 174 as IBS and 173 as IBD.

Laboratory investigations :

Macroscopic examination:

This involved stool consistency, color, odor and presence of blood or mucous.

Microscopic examination:

Direct wet mount smear and Iodine-stained smear were performed according to [30], while concentration technique (Formal-ether) was performed according to [31].

Fecal calprotectin assessment (Quantitative fecal calprotectin ELISA test):

This ELISA kit was purchased from Epitope Diagnostic Int (EDI) company-USA. The kit is quantitative for measurement FC in stool samples using Sandwich technique with two selected antibodies that bind to different epitopes of human calprotectin. Assay standards, controls and patient samples were added directly to the wells of a micro-titer plate that is coated with antibodies to FC. After a short incubation period, the plate was washed and horseradish peroxidase (HRP) –conjugated human calprotectin specific monoclonal antibody was added to each well. After the second incubation period, human calprotectin – HRP-conjugated monoclonal antibody was formed. The buffer matrix and unbounded antibodies were expelled by an excess rinsing with wash solution .then after 100μl of substrate was added and incubated for obtaining immune-complex, ;then measured in a spectrophotometric micro-plate reader. The enzymatic activity of the immune-complex bound to the wall of each micro-titer well is directly proportional to the amount of human calprotectin in the test sample. A standard curve was done and the concentration of Fc in test samples was assessed from this standard curve. Results for FC below 50 ng/ml was considered negative.

Blood sample collection and processing:

Five ml of blood were withdrawn and transferred in to EDTA tubes, inverted several times to prevent clots. Blood samples were used for checking complete blood count (CBC), including total white blood corpuscle count (WBCs) and differential count involving neutrophils percentage using automated hematology machine XP-sysmex.

Statistical analysis:

Values were expressed as percentages, averages and mean± SE were arranged in tables. Variances between study parameters were checked by using chi-square test and t-student test as significant when the differences between two analyzed parameters were at P<0.05.

RESULTS

From the examination of a total 419 stool samples, the overall rate of *Entamoeba histolytica* was 26.96 % compared to 73.04 % of negative sample, p<0.05, (table-1) .

Table 1: Incidence of *Entamoeba histolytica* among gastroenteritis patients, positive   and negative rates.

Total number examined	Positive		Negative		P
	%	**No**	**%**	**No**	
419	24.58	103	5.42	313	<0.05

In table 2; the rate of intestinal amoebiasis among males was 55.33 % versus 44.66% in females, (P<0.05). A rate 19.2 % was recorded among patients aging from 51 years to 60 years and , 17.54% was recorded among patients in age groups (1 year to 10 years) and in (11years to 20 years) in comparison to lowest rate 10.2 % among patients aging 61 years and over in male group. Fluctuated rates were recorded among female groups ;a rate of 28.25 % was recorded among females aging from 41 to 50 years compare to 15.15 % among patient aging from 11 years to 20 years, (P<0.05). Statistically the rate of intestinal amoebiasis was higher in males than in females, (p<0.05).

Table 2: Positive and negative rates of stool samples for *Entamoeba   histolytica* according to genders and ages.

Entamoeba histolytica	Males		Females	
	Positive	Negative	Positive	Negative
Age groups	No %	No %	No %	No %
1----10	10 (17.54)	3 (8.57)	7 (21.21)	5 (13.51)
11----20	10 (17.54)	5 (12.28)	5 (15.15)	4 (10.81)
21----30	8 (14.03)	6 (17.14)	6 (18.18)	8 (21.62)
31----40	9 (15.78)	8 (22.85)	6 (18.18)	8 (21.62)
41----50	3 (5.26)	2 (5.71)	13 (28.25)	2 (5.40)
51-----60	11 (19.2)	2 (5.71)	2 (6.06)	4 (10.81)
61------above	6 (10.52)	9 (25.7)	7 (21.21)	6 (16.21)
Total =103	57 (55.33)	35 (48.61)	46 (44.66)	37 (51.28)

Total number examined=175 , males= 92 females=83

In table 3 gastroenteritis as IBD and IBS was correlated with the frequencies of intestinal amoebiasis according to age group and patient's gender. From a total of 103 positive cases; positive IBD was found among 49 (47.57 %) versus to 54(52.43 %).In males an IBD rate was 49.13 %   compared to 45.66 % among females, p<0.05. Higher number of intestinal amoebiasis among IBD patients was recorded among young males aging from 1 year to 10 years, the rate was 28.57 % and the lower rates were recorded among elderly patients, (P<0.05).In general IBD positive for amoebiasis was high among young aged patients and up to 40 years. Moreover high rates of intestinal amoebiasis among IBS patients (68.75% ,69.24% and 85.72 %) were recorded among patients aging from (40 years over than 61 years) respectively .

Table 3: Frequencies of *Entamoeba histolytica* in regard of types of gastrointestinal disorders (IBD and IBS), patient genders and ages.

Entamoeba histolytica	Males		Females		Total	
Age groups	IBD +ve No %	IBS +ve No %	IBD +ve No %	IBS + ve No %	IBD + ve No %	IBS +ve No%
1----10	8 (28.57)	2 (6.89)	3 (14.28)	4 (16)	11 (64.70)	6 (35.30)
11----20	6 (21.42)	3 (10.43)	4 (19.04)	1 (40)	10 (71.42)	4 (28.38)
21-----30	3 (10.71)	5 (17.24)	3 (14.28)	3 (12)	6 (42.85)	8 (57.14)
31----40	6 (21.42)	3 (10.43)	5 (23.80)	1 (4)	11 (73.34)	4 (26.66)
41-----50	1 (3.57)	2 (6.89)	4 (19.04)	9 (36)	5 (31.25)	11 (68.75)
51-----60	3 (10.71)	8 (27.58)	1 (4.76)	1 (4)	4 (30.76)	9 (69.24)
60----above	1 (3.57)	6 (20.68)	1 (4.76)	6 (25)	2 (14.28)	12 (85.72)
Total	28 (49.13)	29 (50.87)	21 (45.66)	25 (54.34)	49 (47.57)	54 (52.43)

* Total positive female number=180 .   Total positive male number= 167 .
All positive number =347.   Total number examined= 419

Correlation between *Entamoebia histolytica* infection and stool consistency is reported in table 4; 96 positive patients had diarrhea compare to 7 patients with constipation. From a total of 103, only 54 males (94.73 %) had diarrhea compare to 42 females (91, 30 %), p<0.05). In contrast to 8.69 % of the females have constipation versus 5.26 % in males . Statistically the relationship between stool consistency and intestinal amoebiasis occurrence was high.

Table - 4 distribution of *Entamoeba histolytica* according to stool consistency in Relation to patient gender.

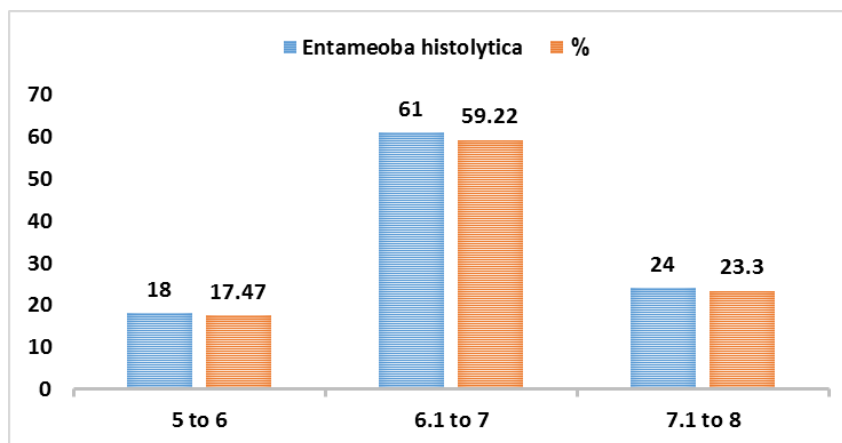| Genders		Males		Females	
Diarrhea /constipation		Constipation	Diarrhea	Constipation	Diarrhea
Entamoeba histolytica	*No*	3	54	4	42
	%	5.26	94.73	8.69	91.30

Total positive = 103.

Fig. 1: Distribution of *Entamoeba histolytica* according to stool pH

A high rate of positive stool samples 59.22% was recorded for *Entamoeba histolytica* according to pH of the specimen, in 61 stool samples has been recorded in pH range(6.1 to 7.0), followed by 23.3 % and 17.47 % in specimens with pH ranges (7.1 to 8) and (5 to 6) respectively, (p< 0.05).

Positive fecal calprotectin was significantly higher 84.40% in males than females 54.73%, p<0.05.Significant leucopenia was reported in both males and females .Neutrophilia was reported in Fc positive males 76.16 % compared to 64,04 % in feces negative for FC.   Contrary to slightly neutropenia 48.21% among females FC positive; meanwhile normal neutrophil rate.

Table 5: Fecal calprotectin levels, mean of total white blood cells count and neutrophils count among patients with intestinal amoebiasis.

Entamoeba histolytica	Fecal calprotectin FC		Males		Females	
	Males *	Females	Mean Total WBC	Mean Total Neutrophils	Mean Total WBC No%	Mean Total Neutrophils
	No. mean Fc	No. mean Fc	Cell/mm³	%	Cell/mm³	%
>50 ng /ml	18 84.40	32 54.73	3811.93	76.16	3738.4	48.21***
<50 ng / ml	12 36.71	25 37.18	7934.18 **	64.05	3835	62.12

In table-6 the mean Hb levels was 10.58 gm/dl in IBD males compare to normal level (12.8gm/dl gm/dl). The mean PCV level in IBD patients was 32.59 % compare to normal level(38.4 %).The difference was statistically insignificant (P>0.05). Also the difference was not significant between both genders.

Table 6: Determination of Haemoglobin and PCV mean levels among IBS and IBD positive for *Entamoeba histolytica* in regard to patients gender compare to control.

Types of Gastroenteritis		IBD		IBS		Total (IBD+IBS)	
Genders		Male	Female	Male	Female	Male	Female
Test group *Entamoeba histolytica* positive	*Hb (g/dl)*	10.58	11.18	12.47	11.93	11.52	11.55
	PCV (%)	32.59	33.08	37.41	35.65	35	34.36
Control	*Hb (g/dl)*	12.8	12.2	13.2	12.9	13	12.55
Not infected	*PCV (%)*	38.4	36.6	39.6	38.6	39	37.6

Normal range of Hb for female: 13 to 15 gm/dl and for male is 13.5 to 18 gm/dl.
Normal range of PCV for female is from 38 to 48 %, while for male is from 42 to 54 %.

DISCUSSION

Parasitic infections has a strong impact on the gastrointestinal tract status particularly when the causative agent is an invasive parasite like *Entamoeba histolytica* .This parasite can invade mucosa and reaches to lateral portion of sub mucosa of the intestine leading to flask shape ulcer formation, it was mostly associated with blood and mucus with possible role of secondary bacterial infection [32]. Continuous trophozoites migration through portal vein to vital organs such as liver lung and brain ends with ulcer formation; this phenomenon is known as extra-intestinal cycle mostly fatal among young individuals. Consequently a rate of 24.58 % of positivity for intestinal amoebiasis is critical and prone to dangerous outcome if the cases were left without treatment. Also this high rate highlights the shadow on this parasite among peoples in Kirkuk province [33], and reflecting the rate of contamination particularly water quality because this Province since about 20 years suffers from shortages in water supply in addition to old governmental pipes unground, most of them were broken, consequently it may lead to the increasing the rate of intestinal parasitic infection particularly *Entamoeba histolytica.*

The result of the current study did not agree with that recorded in the same Province by Salman et al , [34]

and Salman et al; [35]. Basra and Nasiriya by [5,6]. Moreover the following rates 5.11%.4.57%, 4.9%, 3.5%, 7.8% and 21.2% recorded in Arab neighbor countries [9,10,11,12,13,14] did not agree with the result of the current study. Variance might be factorial such as size of samples, type of the laboratory methods, study designs, study duration and continuous war impacts on infra-structure, and health condition.

The high rate of amoebic infection in males compared with female patients could be explained by the fact that males most often spend time outdoors than females, so they are more exposed to pathogens by eating food than females. Moreover the continuous war particularly the usage of different kinds of weapons by collation force may had role in causing environmental and bad psycatric conditions which had a role in increasing the rate of infection among males than in females. This finding agrees with that recorded in Benha-Egypt [35] .

In our work, the female samples revealed significant difference in the infection by *Entamoeba histolytica* with IBS than IBD. This might be due to in Iraqi communities females are in long contact with water and with vegetables during food preparation, so females are more susceptible to infection. But high rate among females with IBS, may be due to IBS is a predisposing factor for increasing infection with *Helicobacter pylori* [3], and *Blastocystis hominis* [36] intestinal protozoan infections[37.] These findings are in agreement with that recorded in Kirkuk city by [3,30,36,37] and with that in Basra by[28].

Moreover considering high incidence of IBD for *Entamoeba histolytica* among patients from 1 year to 20 years, could be interpreted by the fact that patients in this age are most often primary school children, so they acquire infection by contact; Kirkuk Province is very crowded due migration of the peoples(refuges) from neighboring cities due to war[33].

On the other hand, the high rate of IBD positive for intestinal amoebiasis among patients aging from 31 to 40 years may be related to environmental factors, stress and other gastrointestinal co-infection and co-impact of environmental factors[22,26]. Considering the high incidence of *Entamoeba histoltytica* among IBS elderly patients than in other ages, this finding is critical, because old people are susceptible to infection due immune suppresion ,diablitiang diseases and in this case most of the parasitic infections become opportunistic such as giardiasis, cryptosporidiosis and cyclosporiasis[34].

The optimum pH within which the parasites survive is vital to predict the type of causative agents of diarrhea. In a recent study a high incidence of intestinal amoebiasis 59.22 % of pH range between (6.1 to 7.0) mostly related to the habitat of the parasite in large intestine while 23.24 % occurrence in alkaline pH due to the presence of red blood corpuscles and pus cells

due to ulceration; this finding may refer to progression of the intestinal amoebiasis [38].

Fecal calprotectin mean level at or higher than 50ng/ml in males than females may be related to male occupations, in addition to other causes such as logical rate of co-infections records among these patients with *Helicobacter pylori, Giardia lamblia* and *Blastocystis hominis*[3].

Regarding the FC negative (below 50 ng/ml) in both genders must be considered because if the cases were not followed by the physicians, it may show a conversion to increasing levels of FC. So it is from wisdom to take in consider among patient with gastroenteritis, the FC and monitoring its levels interval to optimize the doses of treatment or to perform endoscopy to fix progression of the disease [24].

In the current study leucopenia and neutrophilia among *Entamoeba histolytica* FC positive was refers to acute infections of protozoan infection. Whereas leucopenia and mild neutropenia among females may refer to other factors such as GIT bleeding (occult blood)[26], drug interference and adverse action, genetic disorder such as Crohn's disease.[3,22,39]

The Impact of *Entamoeba hstolytica* on complete blood count, leucopenia and nuetrophilia refer to acute infection and challenge of the immune system against amoebiasis, The adverse effects extend to involve other parameters such as the mean level of haemoglobin(Hb) and packed cell volume(PCV) in infected patients compared to people in the control group. Due to the high level of FC in males, so Hb and PCV reveal dropping compared to the control group. This finding refers to damages caused by the parasite through its invasion via intestine layers among IBD patients. Contrary to IBS patients positive for *Entamoeba histolytica*, they show slight decrease in the levels of Hb and PCV compare to IBD patients. In general in both conditions of IBS and IBD were parallel to *Entamoeba histolytica*, the signs of microcytic, hypochromic anemia can be suggested. Pancytopenia is a typical term that could be applied to the patients with gastroenteritis due to intestinal amoebiasis as the three main blood compartments were at lower levels. Another explanation to anemia might be due to continuous diarrhea that may influence trace elements levels such as: Iron, copper, zinc In addition to other co-infectious associated agents Like *H. pylori* [17,18]. The result of the CBC in our study was in agreement with those recorded in the same province by Salman et al; 2017 [3] and with Abdul_razaq, 2017[37].

In conclusion: *Entamoeba histolytica* rate is high in patients with gastroenteritis particularly among IBD patients in association with diarrhea. ELISA Fecal calprotectin is a good marker for detecting the injury caused by the parasites to the host .Complete blood count assessment during gastroenteritis due to intestinal amoebiasis is advisable to prevent anemia. According to

our information and literatures available in Iraq; the current study was the first in Kirkuk-Iraq concerning with FC, GITDs with *Entamoeba histolytica* infections. So this study can stimulate other scientific worker to carry on further studies on FC in relation to gastroenteritis due to bacteria, viruses and candidiasis.

REFERENCES

1. Lehmann, FS, Burri E and Beglinger C. The role and utility of faecal markers in inflammatory bowel disease. Therap Adv Gastroenterol, 2015 Jan; 8(1): 23–36.

2. Annaházi A, Molnár T, Farkas K, Rosztóczy A, Izbéki F and Gecse K. Fecal MMP-9: a new noninvasive differential diagnostic and activity marker in ulcerative colitis. Inflamm Bowel Dis ,2013; 19: 316–320.

3. Salman, Y J; Ali,CH E and Abdul-Razaq , A.A. Fecal calprotectin among patients infected with some protozoan infections .*Int.J.Curr.Microbiol.App.Sci* , 2017; *6*(6): 3258-3274.

4. Shenin, W.DH. Epidemiology of intestinal parasites among primary school and peoples in ALfohood-Jebayiesh town-Nasiriya governorate. M.Sc. thesis. Coll.Sci. Basrah Univ. 2005.

5. Al-Nassiry, M.A. Study of epidemiology of *Entamoeba histolytica* in Beji town with the study of some herbal extract on the parasite. M.Sc. thesis. Coll. Edu. Tikrit Univ. 2007.

6. Sayel YK. Prevalence of intestinal parasites among children in Baghdad. M.Sc. thesis. Coll.Sci. Bagh dad Univ. 2005

7. Al-Zufri KAS. Epidemiological study of some pathogenic aspects of intestinal parasite among in and out patients attending Al-Najaf Hospitals. M.Sc. Coll. Edu. Kofa Univ. 2004.

8. AL-Zubaydi, BJ, Mawlood NA and Abdullah N A. Study the causative agents of diarrhea among children below five years in some regions of Diyala-Iraq. J. Sci Al-Mustanseryia Univ, 1997; 8(3): 18-24.

9. Morsy T A , Farrag AM , Sabry A , Salama M M. and Arafa M A. Ecto and endo-parasites in low primary schools in Qualyob city. Egypt. J.Soc.Parasitol, 1991; 21: 391-401.

10. Ismaiel M T. Detection of intestinal parasites among primary shool children in Hama-Syria. J. Med.Lab. Diag, 2011; 10: 1---9.

11. Hamzy M, Dabboussi F, AL-Ali K and Ourabi L. Prevalence of intestinal parasites in Northern Lebanon. Estr.Miditerian Health J, 2003; 10(3): 343-346.

12. Youssef M, Shurman A, Bougnoux M, Rawashdeh M, Bretange S and Strockbine N. Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from North Jordon. FFMS Immunol.Med. Microbiol,2000; 28: 257-263.

13. Azazy AA and Al-Tair A S. Study of intestinal and blood parasites among children in Sana Province-Yaman. Saudia Med. J,1999; 20(6): 422-424.

14. Al-Nakkas E, AL-Mutar E M, Shweiki H M , Sharm P N and Rihan S. Parasitic infection in Kuwait. A study based on primary care center.Med.East. J. Fam.Med,2004; 3:7.

15. Hammood A M , Ahmed B A and Salman Y J. *Blastocystis hominis* Detection among Gastrointestinal Disorders' Patients in Kirkuk Province Using Three Different Laboratory Methods. *Int.J.Curr.Microbiol.App.Sci* ,2016 ; *5*(7): 883-901.

16. Rhodes D Y and Wallace M. "Post-infectious irritable bowel syndrome," Current Gastroenterology Reports , 2006 ; 8(4): 327–332,

17. Thabane M and Marshall J K "Post-infectious irritable bowel syndrome," World Journal of Gastroenterology, 2009; 15 (29): 3591–3596.

18. Halvorson H A, Schlett C.D and Riddle M S ."Post infectious irritable bowel syndrome—a meta-analysis," Amer J Gastroenterol, 2006 ;101(8) 1894–1942.

19. Thabane M, Kottachchi D T and Marshall J K. "Systematic review and meta-analysis: the incidence and prognosis of post infectious irritable bowel syndrome," Alimentary Pharmacology and Therapeutics, 2007; 26(4): 535–544.

20. Broadhurst M J,. Leung J M Kashyap V and et al., "IL-22+ CD4+ T cells are associated with therapeutic Trichuris trichiura infection in an ulcerative colitis patient," Science Translational Medicine, 2010; 2 (60) :60-88.

21. Wensaas K A, Langeland N, Hanevik K , Mørch K, Eide G E and Rortveit G. "Irritable bowel syndrome and chronic fatigue 3 years after acute giardiasis: historic cohort study," Gut, 2012 ; 61(2) : 214–219.

22. Ximenez C, Mor P,´. Rojas An Valadez A and´ omez A G. "Reassessment of the epidemiology of

amebiasis: state of the art," Infection, Genetics and Evolution, 2009; 9(6): 1023– 1032.

23. Park Ji S, Lee H, Song D, Choi Y K and *et al*. Post-infectious irritable bowel syndrome in patients with Shigella infection. *J. Gastroenterol. Hepatol , 2005;* 20: 381–386.

24. Quail MA, Russell RK, Van Limbergen JE, et al., Fecal calprotectin complements routine laboratory investigations in diagnosing childhood inflammatory bowel disease. Inflamm Bowel , 2009; 15(5):756 –759.

25. Van Rheenen P F, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. BMJ, 2010; 341.

26. Foell D, Wittkowski H, Roth J. Monitoring disease activity by stool analyses: from occult blood to molecular markers of intestinal inflammation and damage. Gut ,2009; 58: 859–868.

27. Komraus M, Wos H, Wiecek S, Kajor M, Grzybowska-Chlebowczyk U. Usefulness of faecal calprotectin measurement in children with various types of inflammatory bowel disease. Mediators Inflamm ,2012: 608249.

28. Sherwood RA. Fecal markers of gastrointestinal inflammation. J Clin Pathol,2012; 65: 981–985.

29. Chen CC, Huang JL, Chang CJ, Kong MS. Fecal calprotectin as a correlative marker in clinical severity of infectious diarrhea and usefulness in evaluating bacterial or viral pathogens in children. J Pediatr Gastroenterol Nutr,2012; 55: 541–547.

30. Salman Y J. Detection of *Blastocystis hominis* among Peoples in Kirkuk Province Using ELISA and Direct Microscopy*".* Int. J. Curr. Microbiol. App. Sci.,2015 4(10): 686-695.

31. World Health Organization (2003)Manual to medical laboratory investigations. WHO Geneva.

32. Paniker C K.J. Text book of medical parasitology,6th edit. Jaypee Brother publisher India: 20 to 37.

33. Salman Y J, Sadek W S and Rasheed Z KH. Prevalence of *Cryptosporidium parvum* among Iraqi displaced people in Kirkuk city using direct microscopy, flotation technique and ELISA copro-antigen. Int.J.Curr.Microbiol.App.Sci, 2015 4(11): 559-572.

34. Salman YJ, Al-taee AA and Abid AM. Prevalence of *Giardia lamblia* among Iraqi Displaced People in Kirkuk Province . *Int.J.Curr.Microbiol.App.Sci, 2016 , 5(1): 753-760.*

35. Abdel-Magied A A, Elahwel A M. Factors associated with intestinal parasitic infection among school children in Sirt, Libya. Benha Med J, 2006;23:821–32.

36. Hamood A M , Salman Y J and Ahmed B A. Possible Association between *Blastocystis hominis* and Bowel Disorders among Iraqi Patients in Kirkuk Province. Int.J.Curr.Res.Aca.Rev.2016; 4(7): 50-56 .

37. Abul-Razaq A A. Detection of some physiology and immunological parameters related to for some intestinal parasites among patients with gastro-intestinal disorders in Kirkuk/Iraq M.Sc.thesis. Coll. Sci. Tikrit Univ.2017.

38. Salman, Y.J., Mustafa, M.I. Evaluation of the employment of four laboratory diagnostics for Giardia lamblia among children in Kirkuk city. J.Kirkuk Med. Coll, 2013; 1(2):52 - 60.

39. Sands B E. Crohn's disease. In M Feldman, LS Friedman, LJ Brant, MH Sleisenger, *Sleisenger and Fordtran's gastrointestinal and liver disease, Pathophysiology Diagnosis Management*, 8th Ed., Saunders Elsevier, USA, 2006: 2459-2490.