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Fecal calprotectin among patients infected with some protozoan infections

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A B S T R A C T

Intestinal parasitic infections have had a strong impact on people with gastro-intestinal tract disorders (GITDs) particularly those with irritable bowel syndrome (IBS) and inflammatory disease (IBD). Fecal calprotectin g(FC) test had a role in detecting the causes of GITDs as indicated to the damages caused by the infectious agents to the host; particularly the parasitic infections. The aim of the current study was to assess a relationship between parasitic infection and fecal calprotecting. To achieve that, 266 patients were chosen by the gasto-enterologist in two main Hospitals in Kirkuk city, they were referred for general stool examination and to determine fecal calprotectin. Direct microscopy was used in preparation of double wet amount of 0.9 % of NaCl and 1 % of Lugols iodine. Whereas for FC special ELISA sandwich kit was applied. The overall rate of parasitic infection was 64.28 % in 171 stool samples, this rate was contributed five following protozoan parasites: *Entamoeba histolytica Giardia lamblia, Blastocystis hominis, Dientamoeba fragilis* and *Chilomastix mesnili*, the rates were: 94 (34.21 %), 47(17.66 %), 31(11.65 %), 1(0.37 %) and 1(0.37 %) respectively. Eighty one patients (30.45 %) have IBD versus to 43(16.16 %) for IBS; high rates 43.95%, 57,44%and 38.70 % have: *Entamoeba histolytica Giardia lamblia, Blastocystis hominis* among IBD were recorded compared to 27.47 %, 21.27 % and 25.80% respectively, P<0.05. Fecal calprotectin positive mean levels (above than 50 ng/ml) were found as followings: 38(41.75 %) for *Entamoeba histolytica,* followed by 10(21.27 %) for *Giardia lamblia* and 1(3.22 %) for *Blastocystis hominis*; whereas FC levels were at negative level for *Dientamoeba fragilis* and *Chilomastix mesnili*. Co-existence of *Helicobacter pylori (H. pylori)* was seen with protozoan infections, 122 (71.34 %), with high dominant incidence 38(41.45 %) was for *Entamoeba histolytica*. According to patients age high mean level 86.89 ng/ml and 51.75ng/ml of FC were found among patients aging above than 70 years and those between 61 to 69 years. Correlation among FC, protozoan infections, white blood cell total count and neutrophil % was significant, P<0.05. Relationship between patient's occupation and positive level of FC was significant, high rates 46.05 % and 41.86 % were recorded among house makers and workers respectively. The rate of intestinal protozoan infections among patients with GITDs was high. Fecal calprotectin has had high efficacy for revealing the damages in GIT due toprotozoan infections and H. pylori infections among patients with GITDs.

Introduction

Gastrointestinal tract (GIT) is a vital system in the body of human being, several assessor organs are joined with it. Maintaining GIT healthy from any infectious agents give raises healthy body. The more famous mysteries to this system are factorial involving;

inflammation that can be seen as inflammatory bowel disease (IBD), either organic or inorganic types (Kaser *et al.*, 2010). Additionally irritable bowel syndrome (IBS) that is resembles somewhat to IBD also considered as the second health problem to GIT (Fengming Y, Jianbing, 2014).Irritable bowel syndrome (IBS) is a highly prevalent gastrointestinal disorder of unknown cause with the same above symptoms (Wilson *et al.*, 2004). Inflammatory bowel disease (IBD) is a disease of unknown cause associated with diarrhea and colonic lesions that are identified by endoscopy. However, other studies state an opposing viewpoint and it is believed that other factors probably are the causing agents of these symptoms (Kaya *et al.*, 2007).

The etiology of each one is varying may involve some microbial agents such as invasive *Entamoeba histolytica* that causes ulceration of the mucosa of the large intestine (Friedman and Blumberg, 2008).

Previously Amoebiasis in Kirkuk city was described as bloody dysentery and the first scientific record of the causative agent as *Entamoeba histolytica* was in 1989 by (Salman and Kadir, 1999).

The rates of intestinal amoebiasis in Iraq were fluctuated depending on the number of samples, laboratory methods, seasonal assessments and other factors; the following rates 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 (Shenin, 2005, Al-Nassiry, 2005, Sayel, 2005,Al-Zufri, 2004 and AL-Zubaydi *et al.*,1997) 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 (Morsy *et al.*, 1991, Ismaiel, 2011, Hamze *et al.*, 2004, Youssef *et al.*, 2000, Azazy and Al-Tair 1999 and Al-Nakkas *et al.*, 2004).

Infection with *Giardia lamblia* has been shown to lead to an increased prevalence of irritable bowel syndrome, as well as chronic fatigue syndrome. In a historical cohort study of patients with *G lamblia* infection as detected by stool cysts, the prevalence of irritable bowel syndrome was 46.1% as long as 3 years after exposure, compared with 14% in controls (Wensaas *et al.*, 2011).

Giardiasis is caused by a flagellate protozoan parasite, *Giardia lamblia* (order Diplomonadida, family Hexamitidae) and affects people worldwide, including people living in developing countries, but is more prevalent in areas with inadequate sanitary conditions (El-Safi,*et al.*, 2013). It is one of the most important non-viral infections causing diarrheal illness in humans (Meyer 1990; Dib *et al*. 2008).

Giardia lamblia is recognized as the most common intestinal protozoan parasite infecting humans in Iraq (Abd-Alzahra *et al.*, 2012) particularly in Kirkuk Province (Salman and Mustafa, 2013). These diseases are often overlooked during routine parasitological or serological testing of intestinal parasites (Salman and Salih, 2013). Depending on availability of equipment, reagents, technical experience, considerations of time, and cost, there are several methods for the detection of giardiasis (Ndao, 2010). Microscopic examination of stool samples, either direct or concentrated, for the recovery of *G.lamblia* both stages trophozoites and cysts (CLSI,2005).

The recent crisis and violence lead to massive population movement, and presence of over 1,500,000 internal displaced in Iraq (Xiao *et al.*, 1999). The displacement of populations from different communities often brings people into proximity either dueto increased concentrations of displaced populations and also increased density in terms of the living

environment (Salman *et al.,* 2015 a). If one group is a carrier of illness, disease outbreaks reflecting endemic pathogens circulating within the community may occur (Watson *et al.,* 2007). Because of the internally displaced persons are at high risk for emerging parasitic infections, since in most cases, they have a history of poor utilization of medical care and vaccination, living conditions of low socioeconomic status and a high possibility to be carrying symptomless diseases, so it is important to carry out an assessment for diarrheal risk factors particularly intestinal parasites with an emphasis on *Giardia lamblia* and to avoid any health risks.

Blastocystis hominis is the most common intestinal parasite in humans and many other animals (Windsor *et al.,* 2002). *Blastocystis*, an unusual anaerobic, single-celled stramenopile, is a remarkably successful intestinal parasite of a vast array of host species including humans (Stensvold, 2013). Infections with this organism are spread worldwide and it is often the most frequently isolated protozoan in parasitological surveys (Boorom *et al.,* 2008; Chandramathi *et al.,* 2010; Roldan *et al.,* 2009). In developing countries, *B. hominis* has a higher prevalence (30 to 50 %) in comparison with developed countries (1.5 to 10 %) (Li *et al.,* 2007). The pathogenicity of *B. hominis* still has been debated. A report supporting the pathogenic potential of this parasite (Ok *et al.,* 1999); that can be found in patients with or without gastrointestinal symptoms. Some of the symptoms associated with *Blastocystis* infection include abdominal pain, constipation, and diarrhea, alternating diarrhea and constipation and others (Qadri *et al.,* 1989).

Morphologically the parasite has four phases: vacuolar, granular, amoebic and cystic phase. The later phase has been considered a dominant phase found in the environment (soil and water) so, it acts as a vehicle for transmitting the parasite into the host. Human to human and human to animal transmission was not obvious (Yoshikawa *et al.,* 2004). Diagnosis of *B. hominis* overlaps with other causatives of diarrhea specially the size of the cysts that measures 3 to 10 µm which is close to oocyst of *Cyclospora*, *Entamoeba histolytica* and other protozoan parasites (Tan, 2004). Routinely direct microscopy by preparing of wet preparation of Lugol's iodine, fecal smear staining with trichrome stain can demonstrate *B. hominis* (Tan, 2008),

Information about *B. hominis* in Iraq was not clear, except the study was carried out in south part of Baghdad by Raof and Abdul-Raham (2011) and in Kirkuk karyaghdi (2013) when they carried out diagnostic study on some intestinal parasites, via which *B. hominis* contributed 3.6 %.In Kirkuk city-Iraq Salman in 2015was carried on a comparative study using direct microscopy and ELISA-copro antigen test for detecting *Blastocystis hominis* rate among peoples with irritable bowel syndrome, he found the all rate of Blastocystosis 58.22% This rate was contributed 59.44% and 58.99% for direct microscopy finding and ELISA testing. Statistically the differences between two methods was not significant.

Dientamoeba fragilis is a species of single-celled excavates found in the gastrointestinal tract of some humans, pigs and gorillas. It causes gastrointestinal upset in some people, but not in others. *Dientamoeba fragilis* is a type of trichomonad. Trichomonads are flagellated organisms, but *D. fragilis* lacks flagella (Chudnovesky, 2016) having secondarily 'lost' them over evolutionary time. Thus, it is an amoeba of flagellate ancestry. In point of ultrastructural and antigenic view, Dientamoeba is reclassified as a flagellate. It is an important cause of travelers' diarrhea, chronic diarrhea, fatigue and, in children,

failure to thrive. There is a continuous debate where *D. fragilis* is considered to be a harmless organism or a pathogenic parasite (Windsor JJ, Macfarlane, 2005).

Infection with *D. fragilis*, called dientamoebiasis, is associated variously with symptoms of abdominal pain, diarrhea, weight loss, nausea, fatigue and fever. In one study, *D. fragilis* was identified in 0.9% of patients observed. Its coincidence with *enterobiasis*, caused by *E. vermicularis*, has been reported (Stark *et al.,* 2005) in another study, eosinophilia was present in half of the infected children participating in the case. *D. fragilis* does not penetrate the host tissue directly; therefore, some of these symptoms may be caused from irritation which then leads to colonic motility (Johnson, 2016). Infection can occur at any age; however, the most common ages that have been reported are children 5-10 years old (Mack, 2016)

In order to diagnose the parasite, patients are required to provide (multiple) fresh stool samples that have been preserved for parasite examination. The multiple samples are required because of parasite detection being difficult, therefore, a sample might be obtained each day to help increase the sensitivity Patients can also be tested for *E. vermicularis* since the two parasites are known to coincide. (Stark *et al.,* 2005)

Unlike the majority of parasitic infections, *D. fragilis* is more prevalent in well-developed countries as opposed to disadvantaged and resource poor nations. (Lagacé-Wiens *et al.,* 20006) The parasite is also endemic in crowded communities (i.e institutions), populations with unsatisfactory sanitation conditions, and individuals who travel to underprivileged countries (Mack, 2016). Globally, the prevalence of *D. fragilis* ranges from 0.3% to 90%, occurring in multiple countries, including many urbanized cities such as Los Angeles, California and Sydney, Australia. Recently, *D. fragilis* was considered to be more prevalent than *Giardia*, thus leading to better diagnostics (Lagacé-Wiens *et al.,* 20006).

The lifecycle of this parasite has not yet been completely determined, but some assumptions have been made based on clinical data. Recently, a cyst stage has been reported (Munasinghe, 2013) although it is yet to be independently confirmed. If true, *D. fragilis* is probably transmitted by the fecal-oral route. Prior to the report of this cyst stage in the lifecycle of *Dientamoeba*, transmission was postulated to occur by helminth eggs (e.g., *Ascaris*, *Enterobius* spp.). The rationale for this suggestion was that *D. fragilis* is closely related to the turkey parasite *Histomonas*, which is known to be transmitted by the eggs of the helminth *Heterakis*. Since *D. fragilis* is known to frequently coinfect with *E. vermicularis*, this leads to the assumption that *E. vermicularis* is a possible vector and mode of transmission (Lagacé-Wiens *et al.,* 2006).Reports on *Dientamoeba fragilis* were very rare in Iraq, only one record of this parasite was recorded in Kirkuk Province by (Salman, *et al.,* 2015 b), who report 0.12 % from a total of 780 stool samples collected from Iraqi displaced peoples.

Chilomastix mesnili is of cosmopolitan distribution, although found more frequently in warm climates. It is thought to be non-pathogenic although the trophozoite has been associated with diarrhoeic stool. The cost is 6-9μm contains a large single nucleus with a large karyosome. It has a prominent side knob which gives it a characteristic lemon shape. The cytosome is evident with a curved shepherd's crook fibril. The trophozoites of C. mesnili are pear shaped and measure 10-20μm in length. It has 1 large nucleus with a

small karyosome and 3 flagella which extend from the nucleus at the anterior end of the parasite. A distinct oral groove or cytosome can be seen near the nucleus. It moves in a directional manner. Laboratory diagnosis is based on finding lemon shaped cysts can be seen in a formol-ether concentrate stool samples. Motile organisms can be seen in a wet preparation of a fresh stool however the characteristic morphology is evident in a permanently stained preparation. The records of this parasite were not obvious, because mostly it was considered as non-pathogen, so it was often missed, the two following rates: 0.239 % and 0.126 % of *Chilomastix mesnili* were recorded in Kirkuk by (Salman *et al.*, 2016 and Salman *et al.*, 2015 b).

Helicobacter. Pylori (H. pylori) is a gram-negative bacterium that is estimated to infect approximately half of the world population. It colonizes the gastric mucosa of its human host where it may give rise to symptoms such as recurrent peptic ulcers and chronic gastritis, and has also been associated with gastric cancer (Suerbaum and Josenhans, 2007) The prevalence of *H. pylori* is high in low-income countries and it was recently shown to colonize 46% of children age 1<3 years in an area of urban Kampala, Uganda (Hestvik *et al.*, 2007).

Fecal calprotectin (FC) antibody is that antibody produced by the white blood cell neutrophils when it was in challenges with the pathogens that causes IBD (Quail *et al.*, 2009). That antibodies bound to serum albumin can be assessed serologically as antigen antibody-complex color formation, that to be easily determined by Elisa reader. Some authors stated that the FC antibody level above than 50 ng/ml was indicating to severe damage in GIT which demands endoscopy for getting more details about the causative agents. While FC levels below 50 ng/ml may indicate other etiological agents that not demands endoscopy application (van Rheenen *et al.*, 2010).

Furthermore Calprotectin is a neutrophil cytoplasmic calcium-binding protein that is also found in monocytes and early stage macrophages. Its degranulation inside the intestinal lumen occurs as a response to local inflammation (Foell *et al.*, 2009). Detection of calprotectin in stool is currently used across gastroenterology practices to aid diagnostically in distinguishing between inflammatory bowel disease and other non-inflammatory ailments, thereby decreasing the number of unnecessary endoscopies performed. It is also used as a validated marker for disease activity and response to treatment (Komraus ET: 2012) and (Sherwood, 2012). Its use in enteric infections is gaining recognition, particularly as a correlative marker for clinical severity in infectious diarrhea from both viral and bacterial etiologies (Chen *et al.*, 2012).

From geographical view, Kirkuk Province is located in the North of Iraq and its location was very important because it acts as a passage from Northern to capital and other Provinces. Due to war in 2013 in Al-Hawija town and consequence war in other neighboring Provinces, most of peoples were migrated and attends to Kirkuk city, which becomes a more crowd. The migrated or displaced Iraqi people were, habitat old buildings, under construction, schools, they lifted under very poor hygienic condition. Moreover to continuous water interruption in this Province since about 10 years ago. All of these factors were worsening the doctors and scientific workers in this Province to predict the increasing in the rate of infectious agents and increasing the incidence of some diseases in particular diarrheal, respiratory, cutaneous and parasitological diseases. Diagnostic laboratory studies concerning GIT in particular IBD and IBS among patients in

Kirkuk province are very rare, except two studies carried on by(Salman,2015) on the relationship between IBS and *Blastocystis hominis* and (Al-Jubori *et al.*,2015) who studied the possible role of *Blastocystis hominins* during IBD. On the other hand, there is no real data or studies about protozoan parasitic infection prevalence among IBD and IBS patients and for the first time FC antibody in Kirkuk city was undertaken as a laboratory test. For these reasons the current study was conducted to assess gastro-intestinal disorders (GIDs), to determine the rate of some common protozoan infections and to detect the relationship between fecal calprotectin and protozoan infections.

Materials and Methods

Ethics statement

The study was approved by the Kirkuk Research and Publication Committee of Kirkuk Health directorate, and by the respective Hospital authorities at the two study hospitals. Written informed consent was obtained from the parents or guardian on behalf of all the patients enrolled in the study.

Study population

From the 1st of July/ 2016 till the 31th of January /2017, a total of (266) Stool and blood samples from 145 male and 121 female were collected, who attends gastro-enterology clinics in both Azidi teaching Hospital and Kirkuk general Hospital in addition to patient selection from private clinics and medical labs in particular Ibn-Nafies private medical lab in Kirkuk city. Patients' ages were ranged from 1 year to over than 70 years. According to occupations, they were classified into five groups. Most of them were with gasto-intestinal disorders (GIDs); they divided into IBS, IBD and control group. Their compliances were abdominal cramps, epigastric pain, and dailydiarrhea, diarrhea altered with constipations, weight loss, and fatigues. Diarrhea was defined as three or more watery stools within 24 hours. An episode of diarrhea was considered over when two consecutive days pass without diarrhea. An episode of acute diarrhea was defined as duration between 24 hours and less than 14 days. Persistent diarrhea was defined as diarrhea for 14 days or more. Control (N = 51) was patients with no history of diarrhea during the last month prior to enrollment. A standardized questionnaire and patient files were used for collection of demographic and clinical information.

Samples collections

From each patient stool and blood samples were collected. Stool samples were collected in clean and dry containers. Each specimen was divided into three fractions: first was kept in the cold box for H. pylori investigation, second fraction was mixed 5ml of sterile NaCl solution then kept for protozoa motility investigation. Whereas 5 ml of Potassium chloride solution was added to the third fraction for complete parasite microscopy. All the stool samples were kept in refrigerator till to processing, this procedure was done according to (Salman, *et al.*, 2015 a). Five ml of venous blood was aspirated and transferred in to EDTA tube (Contains anti-coagulant)., 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. Also the same sample was used for detecting ABO blood group and Rhesus factor for each patient using the tube agglutination test., In case predicting delay sample processing blood samples were kept in refrigerator to less than 24 hours.

Samples Processing

Stool examination

Direct wet, double preparation of NaCl 0.9% and 1 % of Lugols iodine was applied for each stool specimen. For each specimen 3 trials of repeated examination were achieved in the same day to exclude negative results. This method was done according to (Salman, 2015a) and (WHO, 1991).

The second fraction

The stool samples was introduced for direct agglutination between Helicobacter pylori in the stool as antigen with immobilized monoclonal antibodies bound on the chromatography pad inside the special cassette containing enzymatic conjugate (HRP) and Substrate as (TMB)., the method briefly started by transporting small portion of stool sample in the small container containing buffer, mixed thoroughly then about 2 drops (80 microliters) were inserted in to specific hole. Before 10 minutes the pink to red bands (control and test) mean positive for H.pylori while appearing only control band and means negative result. This method was done according to the leaflet of Bio-zek company-Netherland.

Quantitative fecal calprotectin ELISA test

This ELISA kit was purchased from Epitope Diagnostic Int (EDI) company-USA. This ELISA is designed, developed and produced for the quantitative measurement of human calprotectin in stool samples. The assay utilizes the two-site (Sandwich) technique with two selected antibodies that bind to different epitopes of human calprotectin.

Assay standards, controls and patient samples are added directly to the wells of a micro-titer plate that is coated with antibodies to calprotectin. After a short incubation period, the plate is washed and horseradish peroxidase (HRP) –conjugated human calprotectin specific monoclonal antibody is added to each well. After the second incubation period a sandwich of solid – phase antibody –human calprotectin – HRP-conjugated monoclonal antibody is formed. The unbound monoclonal antibodies and buffer matrix are removed in the subsequent washing step. For the detection of this immune-complex, the well is then incubated with a substrate solution in a timed reaction and 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 is generated by plotting the absorbance versus the respective human calprotectin concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of fecal human calprotectin in test samples was determined directly from this standard curve. Result below 50 ng/ml was considerd negative.

Statistical analysis

Values are 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 and Discussion

Five species of intestinal parasites were recorded during the examination of a total of (266) stool samples using direct wet, double preparations of 0.85 % of NaCl and 1 % of lugols iodine stain, table(1). The common recorded parasites involve: *Entamoeba*

*histolytica*in 91 specimens, the rate was 34.21 %, followed by *Giardia lamblia* 47(17.66%), *Blastocystis hominis*in 31 (11.65 %) and 1(0.37 %) for each of the following equally *Dientamoeba fragilis* and *Chilomastix mesnilli*, P<0.05. Co-existence of protozoan infection was highly associated to *H.pylori* positivity. The following rates of *H.pylori* 75 (82, 41 %), 37 (78.72 %) and 10(32.25 %) were recorded among the positive samples for *Entamoeba histolytica*, *Giardia lamblia* and *Blastocystis hominis* respectively, P<0.05

In the same table, the frequencies of positive FC were assessed via which the following results were recorded: 38(41.75 %) of FC were positive among stool samples positive for *Entamoeba histolytica*, followed by 10(21.27 %) for *Giardia lamblia*and 1(3.22) for *Blastocystis hominis*, p<0.05. Samples positive for *Dientamoeba fragilis* and *Chilomastix mesnilli* reveal negative for FC.

Frequency of *protozoan parasites*, according to GITDs was clarified in the table -2 via which from a total of 171(64.28 %) of samples were positive for protozoan parasites; only 81 (30.45%) patients were with IBD, 43(16.16) with IBS and 47(17.17 %) were with other etiology. Also from a total of 47(17.66 %) samples positive for *Entamoeba histolytica*, 40 (43.95 %) have IBD versus to 25(27.47 %) and 26(28.57 %) with other etiology or normal. Regarding *Giardia lamblia*; 27(57.44%), 10(21.27 %) and 10(21.27 %) were IBD, IBS and other etiology or normal respectively. While *Blastocystis hominis* were recorded as IBD 12(38.70 %) followed by 8 (25.80 %) and 11(35.48 %) for IBS and for other etiology or normal. Moreover *Dientamoeba fragilis* and *Chilomastix mesnili* both were recorded among IBD. Statistical analysis was showed strong significant relationship between types of GITDs and Protozoan infections, P<0.05.

The correlation between the mean of white blood cell numbers in cells/mm^3 and the high and low fecal calprotectin levels was illustrated in figures -2, the results of the present study clarified that the mean numbers of white blood cells in specimens with high calprotectin concentration was 12119 cell/mm^3 versus to 5629 cell/mm^3 in specimens with low calprotectin concentration, P<0.05.

The relationship between patients age and the mean of fecal calprotectin levels was shown in figure - 1, via which the result of the present study showed that higher FC 86.89 ng/ml was among patients agingover, 70 years, 51.75 ng/ml among 61-70 years, and 40.62 ng/ml among 31-40 years.Followed by 40.03 ng/ml, 38.35 ng/ml, and 38.17 ng/ml among 41-50 years, 0-10 years and 51-60 years respectively. And in the same figures the mean of fecal calprotectin levels 37.16 ng/ml detected in 11-20 years, followed by 32.47 ng/ml in 21-30 years.

The correlation between the mean number of neutrophil cells/mm^3 and the high and low fecal calprotectin levels was arranged in figures -3; the results showed that the mean numbers of neutrophils in high calprotectin concentration were 6638 cells/mm^3 and the versus 4892 cells/mm^3 was found to be low calprotectin concentration.

The mean rate of fecal calprotectin in relation to patients' occupation was showen in figure – 4, via which the specimens belong to house makers show the highest average rate 46.05 ng/ml of FC, followed by the workers that the mean levels was 41.86 ng/ml, and 38.51 ng/ml, 36.08 ng/ml, 33.76 ng/ml in children, students and officers respectively. P<0.05.

Intestinal parasitic infections have had a strong impact on the status of the GIT. Symptoms of amoebiasis can overlap with symptoms of the inflammatory bowel disease (IBD). It can lead to difficulties in the diagnosis and treatment of IBD (Hansen and

Lund 1998). In that case the diagnosis and management of inflammatory bowel disease can be challenging as certain infections can mimic IBD and lead to a misdiagnosis. Because of the increasing use of corticosteroids, immunosuppressive drugs and biological agents in the risk of opportunistic infection including amoebiasis are also higher in IBD patients. The role of the physician lies not only in the diagnosis and management of IBD but also in the ability to prevent, recognize and treat infections.

Amoebiasis can exacerbate symptoms of IBD and has unfavorable influence on course of the disease and therapy. Inadequate mucosal immune response on the intraluminal antigenic components is essential in the IBD pathogenesis (Kaser *et al.,* 2010). Finding of 43.21 % of amoebiasis in the current study was not in agreement with those 65.20 % and 54.6% recorded in Basra and Al-Nasiriya-Iraq respectively, also it was higher than those recorded in neighboring country as the following rates 5.11%.4.57%,4.9 %, 3.5 %, 7.8 % and 21.2 % were recorded in Arab neighbor countries by (Morsy *et al.,* 1991, Ismaiel,2011,Hamze *et al.,*2004,Youssef *et al.*, 2000, Azazy and Al-Tair 1999 and Al-Nakkas *et al.,* 2004). This high rate of intestinal amoebiasis reflects the highest level of contamination in this Province, particularly of water. On the other hand, this rate may not refer to real infection with *Entamoeba histolytica*, because in the nature water mostly contain high numbers of *Entamoeba dispar* as it seems very similar to *Entamoeba histolytica*, except it was not invasive. This explanation should be excluded here because most of Amoebic cases in the current study were IBD(Invasive) 43.95 %.Variances in the rates might be due to differences in sample size, type of laboratory method, type of the study group and design of the study, because current study was designed for GIDs not for general population.

Table.1 Distribution of intestinal protozoan parasites among patients positive for *Helicobacter pylori* in relation to calprotectin positive levels

Parasite species	Positive No. (%)	H.pylori +ve No. (%)	Calprotectin antibody +ve *
Entamoeba histolytica	**91 (34.21)** **	**75 (82.41)** **	**38 (41.75)** **
Giardia lambilia	**47 (17.66)**	**37 (78.72)**	**10 (21.27)**
Blastocystis hominis	**31 (11.65)**	**10 (32.25)**	**1 (3.22)**
Dientamoeba fragilis	**0.37) (1**	**0 (0.00)**	**0 (0,00)**
Chilomastix mesnilli	**1 (0.37)**	**0 (0.00)**	**0 (0.00)**
Total	**171 (64.28)**	**122 (71.34)D**	**49 (28.65)**

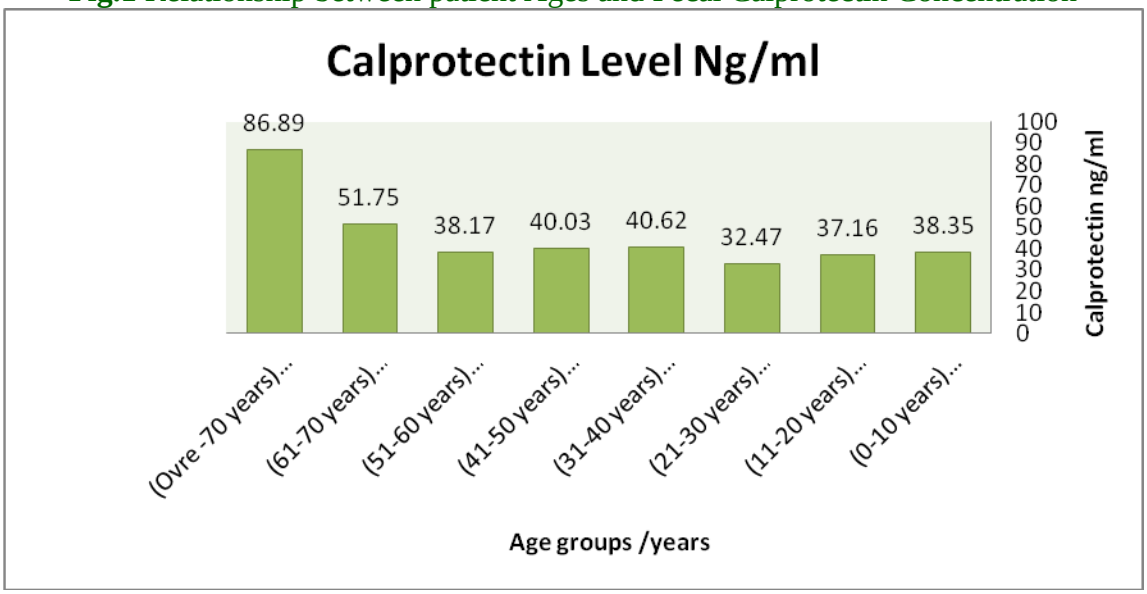
* Calprotectin +ve level is from 50 ng/ml up to above. Total examined stool samples =266. **: P<0.05[(*E. histolytica* P=0.864, P <0.05). (*G. lamblia* P =0.718, P <0.05). (*B.hominis* P =0.498, P>0.05.Not Significant). (*D.fragilis & Ch. mesnili* / No significant due to unique value).]

Table.2 Frequencies of Protozoan parasites and type of Gastro-intestinal disorders

Protozoan parasites	IBD		IBS		Normal or other etiology		Total	
	No	% +ve	No	% +ve	No	% +ve	No	% +ve
Entamoeba histolytica	40	43.95	25	27.47	26	28.57	91	34.21
Giardia lamblia	27	57.44	10	21.27	10	21.27	47	17.66
Blastocystis hominis	12	38.70	8	25.80	11	35.48	31	11.65
Dientamoeba fragilis	1	100	0.0	0.0	0.0	0.0	1	100
Chilomastix mesnili	1	100	0.0	0.0	0.0	0.0	1	100
Total	81	30.45	43	16.16	47	17.66	171	64.28

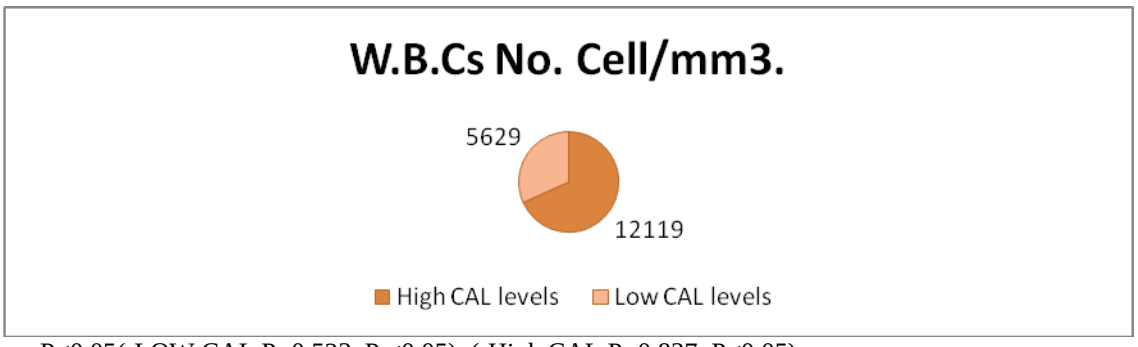
Total examined stool samples: 266 P<0.05

Fig.1 Relationship between patient Ages and Fecal Calprotectin Concentration



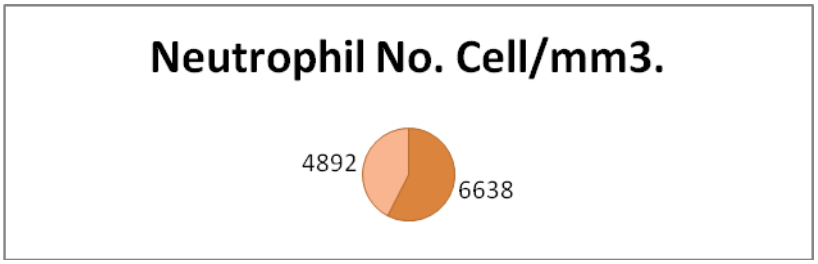
Over -70 years P=0.934, P<0.05. -61-70 years P=0.84.2, P <0.05.

Fig.2 Correlation between number of white blood cell / mm^3 and high and low fecal Calprotectin levels



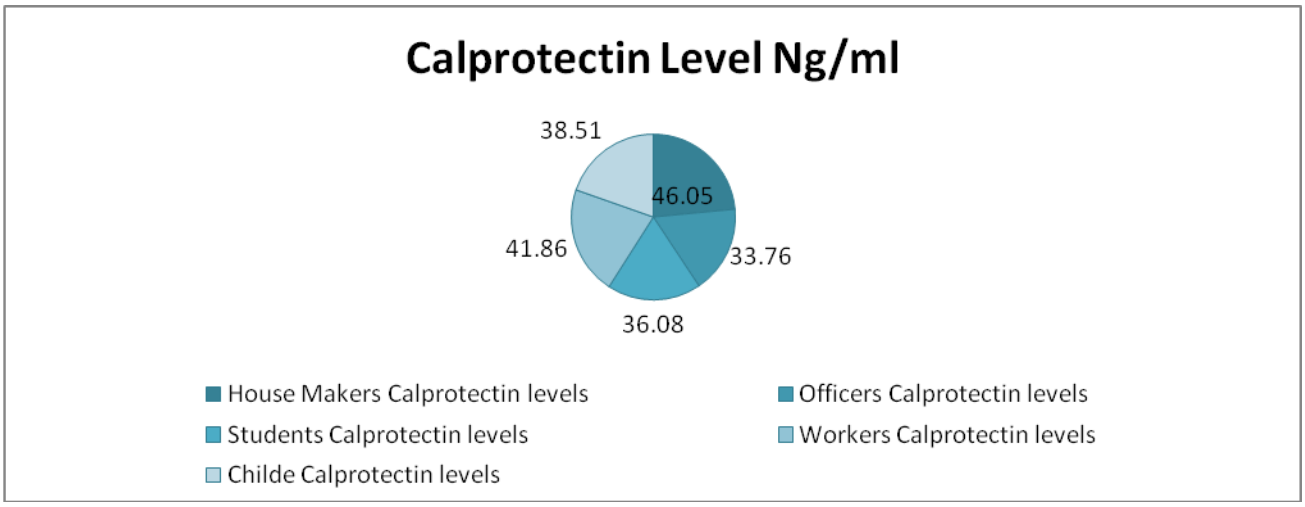
P<0.05(-LOW CAL P=0.523, P <0.05). (-High CAL P=0.837, P<0.05)

Fig.3 Correlation between Number of Neutrophil cell / mm^3 and High Calprotectin levels Results and Low Calprotectinlevels Results



Low FC level, P= 0.588, P<0.05 High FC level, P= 0.721=p<0.05;

Fig.4 The Correlation between Fecal Calprotectin Concentration and the Patients Occupations



P<0.05[(H.M. P=0.913, P <0.05), (Officer P=0.497, P > 0.05),
(Students P=0.643, P <0.05), (Workers P=0.885, P<0.05).and (Children P=0.855, P <0.05)].

The second protozoan parasite that found in 47 samples with the rate 17.66% was *Giardia lamblia*. In acute cases the picture of liquid diarrhea is mimic to that of IBS, color and even the odor of the positive *Giardia* stool samples are the same. (Salman *et al.*, ;2016).The finding rate of giardiasis in current study was not agreed those 7.06 % and 10.31 % recorded in Kirkuk by Salman, *et al.*, 2015b and Salman *et al.*, 2016 respectively in the same Province. Interpretation to this high rate might be due to type of the patients as 21.27% of patients has IBS, while in their studies they take general patients. This finding was lower than 46.3 % recorded among IBS patients by Wansaas *et al.*, 2012). Giardiasis rates in other studies (Abd-Alzahra *et al.*, 2012, Salman and Mustaf, 2013. Salman and Salih, 2013, whom they record high rates of giardiasis also not agreed the Giardia rate in the current study.

While in chronic cases giardiasis is resemble to IBD during which epi-gastric pain is common in addition to other sequels. Pathogenic infections such as *Clostridium difficile* and parasites such as *Giardia lamblia* are reported in 5.7% and 6.5%, respectively, of people with symptoms attributable to IBS (Clayton *et al.*,2012.) and (Grazioli *et al.*, 2006) and are readily detected in the fecal specimens using established techniques such as culture and light microscopy.

Blastocystis hominis, the most common human intestinal parasite, was long thought to be non-pathogenic. (Poirier *et al.,* 2012) Some (but not all) recent studies, however, have demonstrated a significant increased prevalence of *Blastocystis hominis* in IBS patients compared with controls, and at least one authority has recommended treatment with metronidazole in the face of a positive identification of the organism and a symptomatic patient.(Grazioli *et al.,* 2006)

In the present study, parasites as a whole accounted for 11.65% of abnormal values. The single most commonly-identified organism was *Blastocystis hominis*, which until recently was regarded as a non-pathogenic organism.(Ramirez-Miranda *et al.,* 2011)and (Jimenez-Gonzalez *et al.,* 2012) Several recent studies, however, point to a moderately strong association between *B hominis* and symptomatic IBS, with some variation between geographic areas.(Yakoob *et al.,* 2010a) and (Grazioli *et al.,* 2006). Certain genotype 1 of the organism shows the closest correlation with IBS.(Yakoob *et al.,*2010 b) In light of growing evidence for an etiologic role for the organism, it appears reasonable to include *B hominis* in a screening test seeking treatable underlying conditions capable of producing IBS symptoms, particularly because treatment with metronidazole is curative.(Coyle *et al.,* 2012). The finding rate was not agreed 37.75 % that recorded by Hammood *et al.,* 2016 in the same Province, also they recorded high Blastocystis positive rate among IBD than IBS. The variance in the rate mostly due high number of sample 608 stool samples and employee of five lab methods compare to two methods in current study.

Fecal calprotectin is known to be present in stool in neutrophil-mediated inflammation of the intestinal mucosa.(Konikof. 2007) Conversely, in functional disorders such as IBS, calprotectin levels are typically much lower than those found in inflammatory bowel disease (IBD) and not significantly different from those found in healthy controls.(Tursi *et al.,* 2011) and(Sydora *et al.,* 2012).Van Rheenen *et al.,* 2013 in a meta-analysis of 13 studies from the primary literature, found that in adults being evaluated for IBD, screening by measuring calprotectin levels would produce a 67% reduction in the number of adults undergoing endoscopy, while only 3 of 33 adults in every 100 who do undergo endoscopy will not have IBD (but would likely have a different condition for which endoscopy is nonetheless inevitable). Conversely, 6% of adults would have a delay in diagnosis of IBD because of a false negative results.

Remarked GITDs was obvious in current study because 3 of 5 species of protozoan parasites in samples show co-existence with *Helicobacter pylori*. The invasive *Entamoeba histolytica* + *H. pylori* leads for producing IBD among some patients enrolling the study. Whereas *Blastocystis homini* + *H. pylori* and *Giardia lamblia* + *H. pylori* Co-existence reveal IBS among some patients.

With the protozoan infection co-exists with *H. pylori* the FC positive level or cutoff were fluctuated, it was shown higher with IBS cases versus to slightly lower FC among IBD patients. Controversy to *Entamoeba histolytica* + H.pylori samples exert FC levels higher than 50 ng/ml. This finding reflects a high grade of injury caused by both microorganisms on GIT.

High mean FC levels close to FC cutoff recording with *Entamoeba histolytica* and *Giardia lamblia*, particularly the former parasite which was invasive than the other 3 protozoan parasites in the current study was highlighting alarm of vital impact of parasitic infections in GITDs and exposure the light on

the role of FC to determine the injury caused to the host by the most infectious agents.

Considering high white blood cell count and neutrophila was indicated to acute infections particularly by *Entamoeba histolytica*. Whereas high mean FC level among IBD patients was referring to good challenges from the host against intestinal protozoan parasite. According to our information and literatures available in Iraq; the current study was the first in Kirkuk-Iraq concerning with FC, GITDs with protozoan infections and *H. pylori*. So this study was preliminary and the base for future studies.

GITS were health criteria facing scientific workers and gastro-enterologist in Kirkuk Province in particular of IBD and IBS; intestinal protozoan infections may have a role in increasing the rate of gastroenteritis. FC ELISA was good lab tool to assess the degree of injuries in GIT and can be used for predicting GIT cases before and monitoring after endoscopy.

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