STUDY OF PROTOZOA INFECTIONS, ROTAVIRUS, AND CELIAC DISEASE FOR DIARRHEAL CASES OF CHILDREN UNDER FIVE YEARS IN KARBALA PROVINCE [+]

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Abstract:

The aims of this study was concerned on the evaluation the etiology of diarrhea (parasitic (*E. histolytica and G. lamblia)* and viral (Rotavirus)) in children under five years and its effectiveness on the some laboratory investigations which is the complete blood picture (CBP), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP), and sero-prevalence of celiac disease; in which two types of autoantibodies was studied, anti-gliadin (IgG and IgA), and anti-tissutransglutaminase (IgG and IgA); on the other hand the total IgG, IgA, and IgM were also evaluated. A total of 98 children with diarrhea have been diagnostic, 50 children with unknown cause of diarrhea regarded as control positive, and 21 apparently healthy children as a control negative. *E. histolytica* recorded the largest percent 36.7% followed by *G. lamblia* 23.4%, then Rotavirus 17.3% and smallest recorded as Sero-positive celiac disease children 8.1%. On the other hand 14.2% of cases were mixed infection either as *E. histolytica* and *Rotavirus*, *G. lamblia* and Rotavirus, or *E. histolytica* and *G. lamblia*, and all cases with Sero-positive celiac disease were mixed with Rotavirus or *E. histolytica*, or both. Most of Rotavirus infection occur in the age <1 and 1-2 years (23.5% and 47.1%) respectively, Sero-positive celiac disease shows mostly in the age 4-5 years and <1(62.5% and 25%) respectively. But the *E. histolytica* was mostly at 4-5 years (36.1%) and *G. lamblia* was mostly in age 1-2 and 2-3 years (30.4%). we found that most of neutrophilia and eosinophilia cases were in *E. histolytica* and *G. lamblia*, lymphocytosis were mostly recoded in Rotavirus. Hemoglobin concentration show a significant decrease in patients with diarrhea. Abnormal ESR reading has been in Rotavirus cases (30%), and *E. histolytica* (26%), then followed by *G. lamblia* (22%) and Sero-positive celiac disease (8%). CRP titer shows higher mean values with *E. histolytica* (58.18%) and Sero-positive celiac disease (56.29%), then followed by *G. lamblia* (18.89%) and Rotavirus (7.51%). Total IgG show no significant values except for Sero-positive celiac disease (1318.3%) mean value, but all the investigated cases show normal mean value with IgA, this is important for excluding the possibility of IgA deficiency syndrome. IgM show no significant value with the investigated cases.

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دراسة اصابات الاوالي الطفيلية، الفايروس الدوّار، وحساسية الحنطة لحالات الاسهال للأطفال تحت الخمس سنوات في محافظة كربلاء

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المستخلص:

ان الهدف من هذه الدراسة تركز على تقييم اسباب الاسهال عند الاطفال دون سن الخمس سنوات، وتأثيرها على بعض التحاليل المختبرية، كصورة الدم العام (CBP)، معدل ترسيب خلايا الدم الحمراء (ESR)، والبروتين التفاعلي C (CRP)، والانتشار المصلي لحساسية الحنطة، حيت درست نوعين من الاجسام المضادة الذاتية وهي anti-gliadin (IgG, IgA)، و anti-tissutransglutaminase (IgG, IgA)، ومن جانب آخر فقد قدرت النسبة الكلية لل IgG و IgA و IgM. مجموع 98 طفل مصاب بالإسهال قد شُخصوا. الاميبا الحاله للنسيج سجلت اعلى نسبة اصابات حيث كانت 36.7% وياتي بعدها الجياردیا لامبليا وسجل 23.4%، ثم الفايروس الدوّار 17.3%، واصغر نسبة سجلت في الاطفال الحاملين لاضداد حساسية الحنطة 8.1%، من جانب آخر كانت هناك 14.2% من الحالات هي اصابات مختلطة اما اميبا الزحار والفايروس الدوّار او الجياردیا لامبليا والفايروس الدوّار او اميبا الزحار مع الجياردیا لامبليا، وكذلك كانت كل الحالات الحاملة لاضداد حساسية الحنطة مختلطه باصابة الفايروس الدوّار او اميبا الزحار او كلاهما. معظم حالات الاصابة بلفايروس الدوّار كانت في الاعمار اقل من سنة و 1-2 سنة (23.5% و 47.1%) على التوالي، اما الاطفال الحاملين لاضداد حساسية الحنطة كان معظمهم ضمن الاعمار 4-5 سنة واقل من سنة (62.5% و 25%) على التوالي. لكن اصابات الاميبا الحالة للنسيج كان معظمها ضمن الاعمار 4-5 سنة (36.1%) و الجياردیا لامبليا كانت معظمها ضمن الاعمار 1-2 و 2-3 سنة (30.4%). وجدنا ان معظم حالات neutrophilia و eosinophilia كانت في *E. histolytica* و *G. lamblia*، اما lymphocytosis كانت معظمها في حالات Rotavirus. كما لوحظ ان نسبة الهيموغلوبين تقل معنويا عند الاطفال المصابين بالإسهال. ان قراءة اختبار ESR الغير طبيعية وجدت اغلبها في حالات الفايروس الدوار (30%)، و حالات الاميبا الحالة للنسيج (26%)، ثم الجياردیا لامبليا (22%)، و الحاملين لاضداد حساسية الحنطة (8%). مستوى CRP اظهر قيم متوسطة عالية في حالات الاميبا الحالة للنسيج (58.18%) و الحاملين لاضداد حساية الحنطة (56.29%)، ثم تاتي الجياردیا لامبليا (18.89%) و الفايروس الدوار (7.51%). لم تكن هناك فروق معنوية لنسبة IgG الكامل ماعدا في عينات حساسية الحنطة (1318.3%)، ولكن كل الحالات المختبرة كانت طبيعية لل IgA، وهذه نقطة مهمة لاسبعاد احتمالية وجود حالات متلازمة نقص IgA، وكذلك IgM لم يظهر فروق معنوية للحالات المختبرة.

Introduction:

Diarrhea was and stays a leading cause of illness and death among children all over the world especially in developing countries where an estimated about 1.3 billion episodes and 3.2 million deaths occur each year in children under five years old age. Acute diarrhoeal disease is one of the major causes of mortality among infant and young children[1] This is also reported in Iraq [2,3]

Diarrhea refers to the passage of loose or watery stools or an increased frequency of stools for the child and occurs at some point in the life of nearly every child. Diarrhea is not a disease, but is a symptom of a number of illnesses. Although diarrhea is common and rarely life-threatening. Diarrhea can lead to dehydration, which alters the child's natural balance of water, and to electrolyte (sodium, potassium, chloride) imbalance. It can be serious if not treated promptly [4]

Intestinal parasitic infections are considered as one of the most important healthy problems throughout worldwide especially in the tropical and subtropical countries. These infections are regarded as a serious public health problem, the majority of infected persons being children [5] . The incidence of intestinal parasites is closely related to such factors as the socio-economic level of the society, nutritional and hygienic habits, climate, environmental conditions, infrastructure and degree of literacy [6]

In Iraq, many scientific studies; in this field were carried out to show the effect of many factors such as socio-demographic and environmental factors, behavioral habits, economic situations and the educated levels for the mothers [7]

The majority of the intestinal protozoa is considered as non-pathogenic or cause a very mild symptoms. The most important pathogenic protozoa are: *Entamoeba histolytica* which causes amoebiasis and *Giardia lamblia* which causes giardiasis. Almost all of these organisms are transmitted by: feco-oral rout. Transmission involves the ingestion of food or drinking of water contaminated with a mature cyst [8] .The majority of protozoal infections are a symptomatic [9] .

Rotaviruses are the major cause of acute viral gastroenteritis in infants and young children, rotavirus diarrhoea results in significant morbidity and mortality; especially in developing countries [10] .Rotaviruses are estimated to be responsible for approximately 527,000 deaths per year, with more than 85% of these deaths occurring in low-income countries in Africa and Asia, and over two millions are hospitalized each year with pronounced dehydration. Children under five years of age, especially those between 6 months and two years are most vulnerable to the disease [11] .Rotaviruses are most commonly transmitted via faecal / oral route; there is evidence that they can be transmitted in respiratory droplets [12] .

Celiac disease is a digestive disease that damages the small intestine and interferes with absorption of nutrients from food. People who have celiac disease cannot tolerate gluten, a protein in wheat, rye, and barley. Gluten is found mainly in foods but may also be found in everyday products such as medicines, vitamins, and lip balms. Celiac disease is both a disease of malabsorption—meaning nutrients are not absorbed properly—and an abnormal immune reaction to gluten[13,14] .

Aims of study:

1- To study the causative agents in children with diarrhea under five years.

2- To find out the relationship of some demographic data with the causative agents of diarrhea.

3- Focusing on the common laboratory outcome investigations in diarrheal children and their correlation with causative agent.

4- To assess the prevalence of seropositively ofceliac disease among children with diarrhea.

5- Correlation between some hematological tests (ESR and Blood picture) and the cause of diarrhea.

Materials and Methods:

This cross-sectional study was conducted in Karbala teaching hospital of children, in laboratory Department during the period from September 2011 to January 2012. Samples

were collected from 98 children with diarrhea, their ages ranged between one month -five years they were 46 males and 52 females. The criteria for patients selection include, any child older than 5 years was excluded, all children were lived in Karbala city and its surrounding area, all included children were outpatient, and untreated children (new cases) were only included in our study. From all children two types of samples were collected, stool and blood sample, the stool sample used in general stool examination (GSE) macroscopic and microscopic for protozoa detection (*E. histolytica* and *G. lamblia*), and Rotavirus antigen by rabid chromatographic immunoassay. And 3-5 ml blood sample which divided into two tubes, one with anticoagulant (EDTA) used for CBP and ESR, and other without anticoagulant used for CRP titer, anti-gliadin (IgG, IgA), anti-tissutransglutaminase (IgG, IgA), and total IgG, IgA, and IgM. Statistical analysis was done using statistical package for social sciences (SPSS) version 10. ***Descriptive data analysis*** (statistical tables, mean value, standard Deviation, Standard Error, 95% Confidence Interval for population means value, min. and max. readings, contingency causes correlation ship coefficient for association tables, and graphical presentation by using:Bar charts) and ***Inferential data analysis*** (the Chi-Square test, the Binomial test, the One-Way ANOVA procedure, Levene test, and multiple Z-test).

Results:

Table 1 shows that a non significant different at P>0.05 between male and female with overall of the studied samples and they were accounted at males and females 46(46.9%) and 52(53%) respectively.

Table 1 Distribution of gender among the study

Variable	Classes	Freq.	%	C.S.
Gender	Male	46	46.9	Bin. Test P=0.538 (NS)
	Female	52	53	

Table 2 show the distribution of diarrhea with; in which the majority of the overall samples were reported at the age ranged (4 - 5) yrs. and they are accounted 28(28.57%), while the smallest number of individuals was reported at the age ranged <1 yrs., and they are accounted 11(11.22%).

Table 2 Classification of the study samples according to the age

Variable	Classes	Freq.	%	C.S.
Age Group (yrs.)	4 - 5	28	28.57	Chi-sq. test P=0.003 HS
	1 - 2	27	27.55	
	2 - 3	20	20.4	
	3 - 4	12	12.24	
	< 1 yr.	11	11.22	

Table 3 shows that a highly significant different at P<0.01 between urban and rural with overall of the studied samples and they were accounted at urban and rural 19(19.3%) and 79(80.6%) respectively.

Table 3 Distribution of residency in the study

Variable	Classes	Freq.	%	C.S.
Residency	Urban	19	19.3	Bin. Test P=0.000 (HS)
	Rural	79	80.6	

Table 4 show the distribution of feeding according to the cause of diarrhea, the results indicating that a meaningful cause's correlation ships (C.C.=0.474) among the probable outcomes of "Feeding types" and different of the studied samples (*E. Histolytica*, *G. lamblia*, Rota virus, Sero-positive celiac disease, and Controls) , since a highly significant different at P<0.01 had been accounted.

Table 4 distribution of feeding according to cause of diarrhea

Samples	No. and %s	Feeding					C.S. P-value
		Formed	Mixed	Breast	Bottle	Breast and Bottle	
Diagnosis - *Entameba Histolytica*	No.	26	7	0	1	2	C.C.=0.474 P=0.002 HS
	% Samples	72.2%	19.4%	0.0%	2.8%	5.6%	
	% Feeding	44.06 %	21.8%	0.0%	33.3 %	66.6 %	
Diagnosis - *Giardia lamblia*	No.	15	8	0	0	0	
	% Samples	65.2%	34.8%	0.0%	0.0%	0.0%	
	% Feeding	25.42 %	25%	0.0%	0.0%	0.0%	
Diagnosis- Rota virus	No.	5	10	1	1	0	
	% Samples	29.4%	58.8%	5.9%	5.9%	0.0%	
	% Feeding	8.47%	31.25 %	100 %	33.3 %	0.0%	
diagnosis - Mixed infection	No.	8	5	0	0	1	
	% Samples	57.1%	35.7%	0.0%	0.0%	7.1%	
	% Feeding	13.55 %	15.6%	0.0%	0.0%	33.3 %	
Diagnosis - Seropositive celiac disease	No.	5	2	0	1	0	
	% Samples	62.5%	25.0%	0.0%	12.5 %	0.0%	
	% Feeding	8.47%	6.25%	0.0%	33.3 %	0.0%	

Table 5 intended for dividing the causes of diarrhea according to age, we observed that most Rota virus infection occur in the age <1 and 1-2 years (23.5% and 47.1%) respectively. Sero-positive celiac disease show mostly in the age 4-5 years and <1(62.5% and 25%) respectively. But the *E. histolytica* was mostly at 4-5 years (36.1%) and *G. lamblia* was mostly in age 1-2 and 2-3 years (30.4%).

Table 5 Distribution of the studied samples according to age groups

Age Groups	*Entameba Histolytica*		*Giardia lamblia*		Rota virus		Mixed infection		Sero-positive celiac disease	
	No.	%	No.	%	No.	%	No.	%	No.	%
< 1 yr.	3	8.3	0	0	4	23.5	2	14.3	2	25
1 - 2	6	16.7	7	30.4	8	47.1	5	35.7	1	12.5
2 - 3	7	19.4	7	30.4	3	17.6	3	21.4	0	0
3 - 4	7	19.4	4	17.4	1	5.9	0	0	0	0
4 - 5	13	36.1	5	21.7	1	5.9	4	28.6	5	62.5
Total	36	100	23	100	17	100	14	100	8	100
Mean ± SD	2.99 ± 1.46		2.58 ± 1.13		1.59 ± 1.20		2.40 ± 1.71		3.26 ± 2.16	
C.S. ANOVA	F=3.111 P=0.007 HS									

Table 6 shows the distribution of troph an cyst among study groups. During of microscopic examination of stool samples we concerned on the finding of troph or cyst of *E. histolytica* or *G. lamblia* (or some time both).

Table 6 Distribution of Protozoa GSE outcomes among different of the studied samples

Samples	No. and %s	Protozoa GSE			
		Neg.	Troph	Cyst	Troph + Cyst
Diagnosis - *Entameba Histolytica*	No.	0	20	4	12
	% Samples	0.0%	55.6%	11.1%	33.3%
	% Protozoa GSE	0.0%	55.6%	22.2%	52.2%
Diagnosis - *Giardia lamblia*	No.	0	6	14	3
	% Samples	0.0%	26.1%	60.9%	13.0%
	% Protozoa GSE	0.0%	16.7%	77.8%	13.0%
Diagnosis – Mixed Inf.	No.	0	8	0	6
	% Samples	0.0%	57.1%	0.0%	42.9%
	% Protozoa GSE	0.0%	22.2%	0.0%	26.1%
Sero-positive celiac disease	No.	4	2	0	2
	% Samples	50%	25%	0.0%	25%
	% Protozoa GSE	100%	5.6%	0.0%	8.7%

Table 7 show the details of mixed infection group: the group of the mixed infection contains more than one cause of the infected sample of children.

Table 7 Distribution of mixed infections among different of the studied samples

Mixed infection	number	percent
Rotavirus and *E. histolytica*	6	27.2%
Rotavirus and *G. lamblia*	3	13.6%
Rotavirus and Sero-positive CD	4	18.1%
Rotavirus, *E. histolytica* and Sero-positive CD	3	13.6%
E. histolytica and *G. lamblia*	5	22.7%
E. histolytica and Sero-positive CD	1	4.5%

Figure 1 Bar chart for the mean values of Hb g/dl parameter's, shows in the study groups, the mean values full inside of a normal ranges (9.5 – 15.5) g/dl, and however to that some of original readings were reported abnormal status at the down stairs bound with the study groups only.

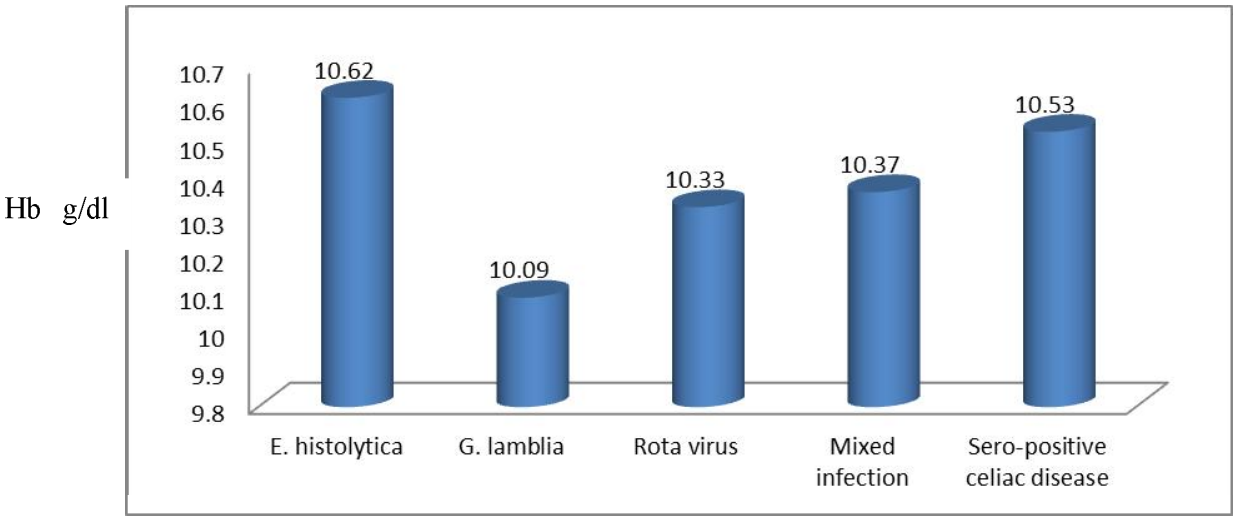

Figure 1 Bar chart for the mean values of Hb g/dl parameter's readings distributed according to different of the studied samples

Figure 2 show the mean values of neutrophils in which the study samples full outside of normal ranges (13 – 45) % in children less than 4 years and <75% in children 4-5 years especially at the upstairs bound to age group, and however to that some of original readings were reported normal status except those whom were diagnosis Rotavirus had normal responding for mean value; however to that some of original readings were abnormal at the upstairs bound.

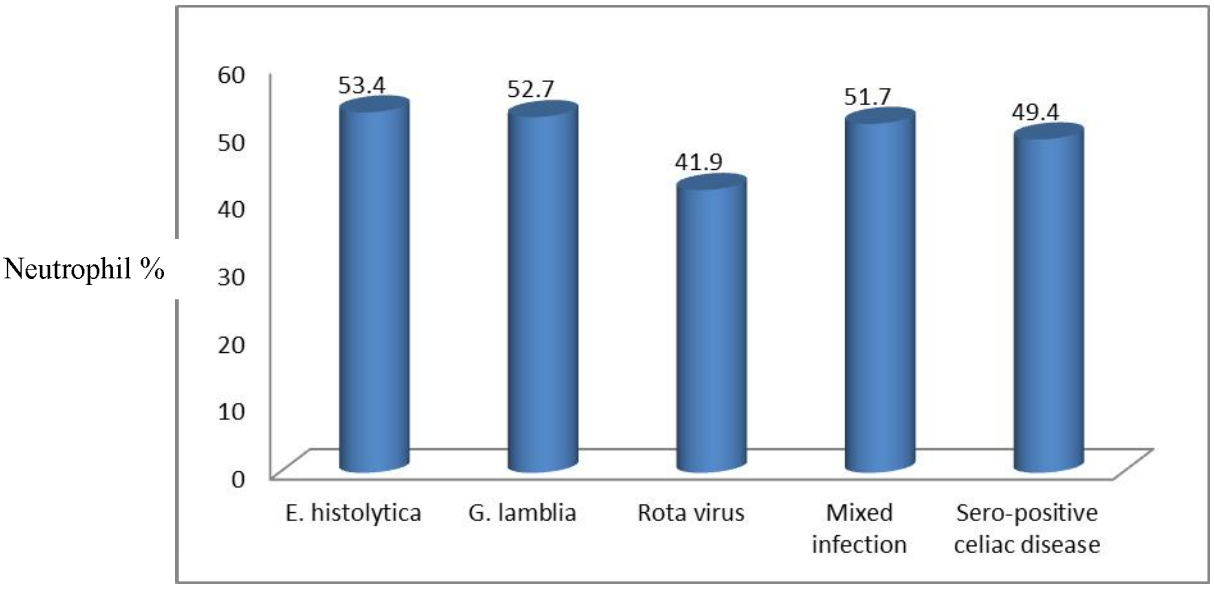

Figure 2 Bar chart for the mean values of Neutrophil parameter's readings distributed according to different of the studied samples

Figure 3 show the frequency of lymphocytosis among study groups, it was recorded in 21 cases in different groups, the largest was in Rotavirus

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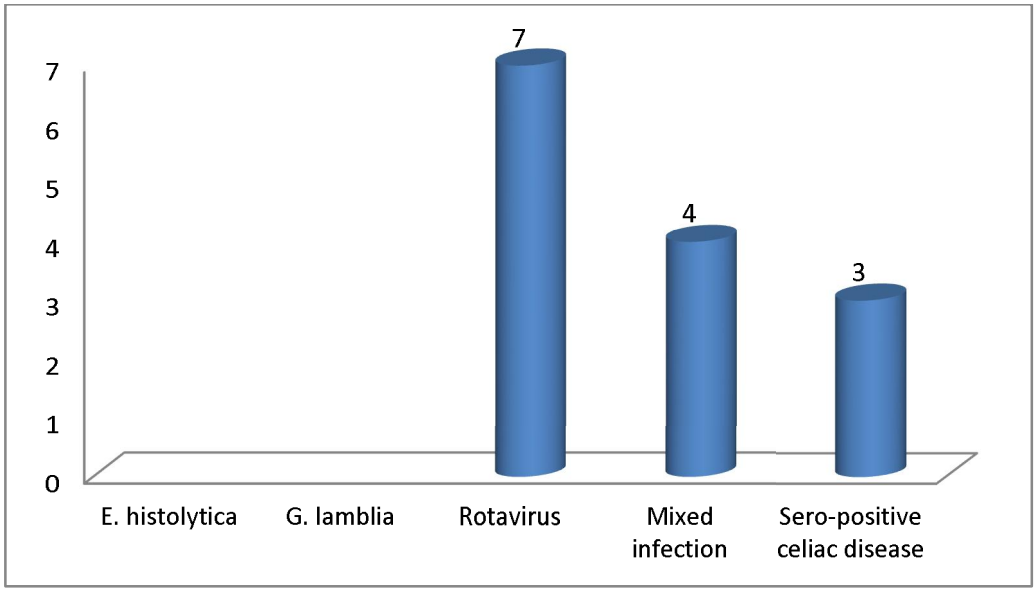

Figure 3 Bar chart for the mean values of Lymphocytosis parameter's readings distributed according to different of the studied samples

Figure 4 show the mean value of eosinophil percent in which the mean values was within normal range (<6%) to age in all study group except for *E. histolytica* in which most of cases complain from eosinophilia. However to that some of original readings were abnormal at the upstairs bound.

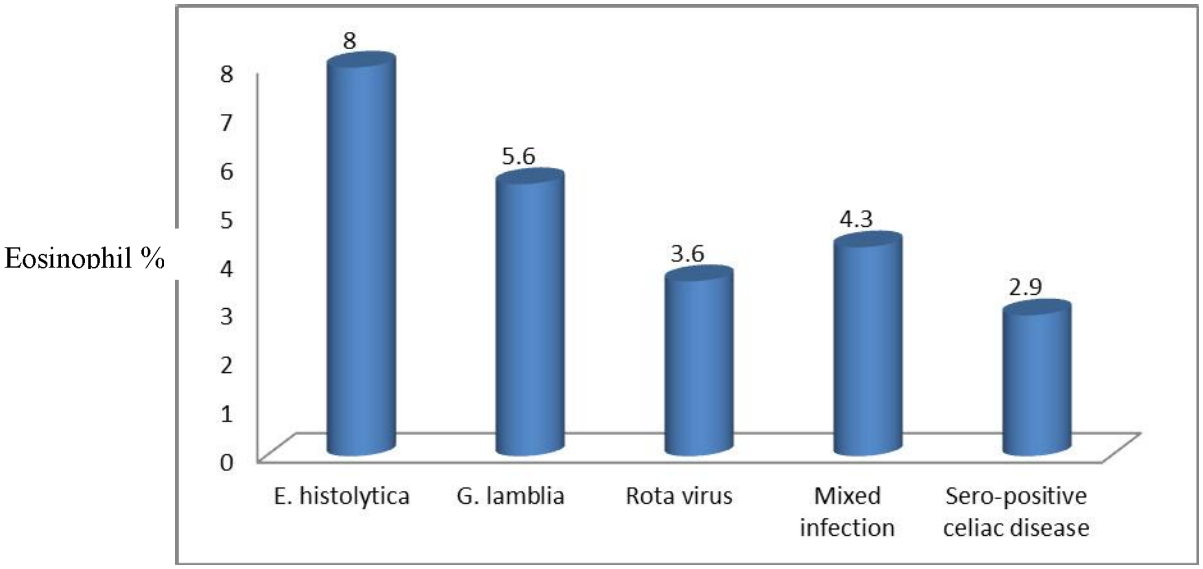

Figure 4 Bar chart for the mean values of Eosinophile parameter's readings distributed according to different of the studied sample

Table 8 show the classification of ESR as normal and abnormal among study groups we observed that most abnormal ESR readings found in Rotavirus *E. histolytica*, and *G. lamblia* (30%, 26% and 22%) respectively. The results indicating that a meaningful cause's correlation ships (C.C.=0.269) between the probable outcomes of " ESR (mm/h) " and different studied samples (*E. histolytica*, *G. lamblia*, Rota virus, and Sero-positive celiac disease) since a significant difference at P<0.05 had been accounted.

Table 8 Distribution of ESR (mm/h) outcomes among different of the studied samples

Samples	No. and %s	ESR mm/h		C.S. P-value
		Normal	Abnormal	
Diagnosis - *Entameba Histolytica*	No.	23	13	C.C.=0.269 P=0.040 S
	% Samples	63.9%	36.1%	
	% ESR mm/h	47.9%	26%	
Diagnosis - *Giardia lamblia*	No.	12	11	
	% Samples	52.2%	47.8%	
	% ESR mm/h	25%	22%	
Diagnosis- Rota virus	No.	2	15	
	% Samples	11.8%	88.2%	
	% ESR mm/h	4.1%	30%	
diagnosis - Mixed infection	No.	7	7	
	% Samples	50%	50%	
	% ESR mm/h	14.5%	14%	
diagnosis – Seropositive celiac disease	No.	4	4	
	% Samples	50%	50%	
	% ESR mm/h	8.33%	8%	

Table 9 show the classification of CRP as positive and negative among study groups, we observed that most positive cases were in *E. histolytica* and *G. lamblia* (43.5% and 26%) respectively. The results indicate that a meaningful cause's correlation ships (C.C.=0.363) between the probable outcomes of "CRP (mg/L) " and different studied samples (*E. histolytica*, *G. lamblia*, Rota virus, and Sero-positive celiac disease) , since a highly significant difference at P<0.01 had been accounted.

Table 9 Distribution of CRP (mg/L) outcomes among different of the studied samples

Samples	No. and %s	CRP mg/L		C.S. P-value
		Neg.	Pos.	
Diagnosis - *Entameba Histolytica*	No.	6	30	
	% Samples	16.7%	83.3%	
	% CRP mg/l	20.7%	43.5%	
Diagnosis - *Giardia lamblia*	No.	5	18	
	% Samples	21.7%	78.3%	
	% CRP mg/l	17.2%	26%	
Diagnosis- Rota virus	No.	11	6	C.C.=0.363 P=0.000 HS
	% Samples	64.7%	35.3%	
	% CRP mg/l	37.9%	8.7%	
Diagnosiss - Mixed infection	No.	3	11	
	% Samples	21.4%	78.6%	
	% CRP mg/l	10.3%	16%	
Diagnosiss – Seropositive celiac disease	No.	4	4	
	% Samples	50%	50%	
	% CRP mg/l	13.8%	5.7%	

Table 10 show the distribution of anti-gliadin IgG, IgA and anti-tissutransglutaminase IgG, IgA among Sero-positive celiac disease children, in which the child regaded as Sero-positive when express two or more types of autoantibodies specific for celiac disease.

Table 10 Distribution of anti-gliadin and anti-tissutransglutaminase among Sero-positive celiac disease children

Sero-positive CD children	Age	Anti-gliadin IgG	Anti-gliadin IgA	Anti-tissutransglutaminase IgG	Anti-tissutransglutaminase IgA
Female	5y	Positive	Positive	Positive	Positve
Female	5y	Positive	Positive	Positive	Positive
Male	5y	Negative	Positive	Negative	Positive
Male	1y	Negative	Positive	Negative	Positive
Female	5y	Positive	Positive	Negative	Positive
Female	3 m	Positive	Positive	Positive	Negative
Male	10m	Positive	Positive	Positive	Negative
Male	4y	Positive	Positive	Positive	Negative

Discussion:

There is no significant association between distributions of disease with gender the diarrhea prevalence in male is more than female; this results was in agreement with Hadi at Babylon (2004) [15] and Hekmat (2010) [16] . While in related to the age distribution of diarrhea with age group we found that most cases were in 1-2 years with a highly significant association. This result was approximately similar to the results of Hekmat (2010) [16] Hind Mahdi (2012) [17] and [18] .

When we take care to patient residency (Urban and Rural) we found there is a highly significant association in and diarrhetic child as in table 3 these results agree with other Iraqi workers like Malak (2001) [19] Hadi (2004) [15] and [20] and disagree with Ali in Hilla city (2004) [21] and Ilham Ayed (2008) in Tikrit [22] .

When we focused on type of feeding, we found most of our cases depended on formed and mixed (formed + milk) because this food either contaminated by itself or during preparation consider as a source of infection, while the lower percent recorded in breast depending feeding because usually its sterile. Within this focus also we noted that most of *E. histolytica*, *G. lamblia*, Rotavirus, and mixed infections cases were formed and mixed feeding while no cases of *E. histolytica* and *G. lamblia* with breast feeding while breast feeding in Rotavirus subgroup recorded the lower percent; also the infection may occur in child with bottle feeding this indicate that the organisms still in milk after preparation. So we noted that there is a highly significant values in feeding type, this result agree with Ali H. Mohammad (2004) [21] Hadi (2004) [15] and [23,24] , and disagree with Abbas (2000) [25] , and Rashidul *et al* (2003) [26] this return to the level of hygiene, and sample size in the comparative study.

When we tried to associate between the disease causation in related to age group we found that most Rotavirus infection occur among age <1 and 1-2 years this result was similar to the study of Herish (2005) [27] ,Ali H. Mohammad (2004) [21] and Abbas (2000) [28] .But most of *E. histolytica* infection occurs among age 3-4 years this is agree with the study of Malak (2001) [19] . and Hind Mahdi (2012) in Basra [17] .The *G. lamblia* occurs among 1-5 years age groups but mostly <3years which represent this result agree with Malak (2001) [19] Hind Mahdi (2012) [17] and Alice *et al* (2007) [29] .

Watery and semi-liquid diarrhea usually indicating acute or subacute infection; most of our cases were acute and subacute diarrhea thus we mostly found troph or troph and cyst; table 6 illustrate the number of troph, cyst, and both as we see that most of *E. histolytica* cases were troph and troph+cyst because the troph mostly appear during acute infection but some of troph can form cyst during subacute infection this explain why we some time see troph and both; [30,31] . In *G. lamblia* this fact in not correctly applying, so even in acute infection the cyst maybe found because the habitat of *G. lamblia* is the duodenal thus there is enough time for encystation [32] .

There is 17 cases pure Rota virus infection, but 9 cases were mixed with *E. histolytica* or *G. lamblia*, and 7 cases with Sero-positive celiac disease, the m.ore acceptable explanation is that the alteration in mucosa structure due to infection and inflammation may facilitate the Rota virus implantation and infection. Conversely Rota virus infection plays a role in developing celiac disease in genetically susceptible children [33,34] .

The (CBP) is important for evaluation on the effects of infectious agent in diarrheal cases in blood components, in addition to confirm the diagnosis of this disease with aid of other investigation. There is a significant association in Hb mean value between groups of the study, this meaning that when child having diarrhea is more susceptible to having anemia than normal, this result is similar to Ammer (2009) [35] , Khawla (2005) [36] .Tawfiq Taki (2012)

[37] and [39] and disagree with Najla'a (2006) [38] because the age was during school stage children.

we found in most of cases of all study groups (except viral cases) were complain from neutrophilia in related to their age this explained that diarrheal disease usually associated by acute inflammation which itself associated with increase with inflammatory cells like neutrophil which play important role in defense against infectious agents like bacteria, or as part of chronic inflammation as occur in celiac disease; this results was the same results of Qahtan (2008) [40] Khawla (2005) [36] and [41] .Lymphocyte plays important roles during viral infections thus higher lymphocytosis cases were found in Rotavirus cases. An increase in eosinophils, and is typically seen in people with a parasitic infestation of the intestines, collagen vascular disease (such as rheumatoid arthritis), malignant diseases such as Hodgkin's disease, etc. [42] .The mean value of eosinophil is higher in *E. histolytica* and slightly elevated in *G. lamblia* as in figure 5 this is mainly because these cell play an important role during parasitic infections; these results agree with Khawla (2005) [36] .

Most of abnormal ESR reading was in Rotavirus, *E. histolytica* and *G. lamblia* because it's mostly increases during acute infection (by increase of fibrinogene concentration) and its value indicating to the severity of inflammation. This results was agree with[43] .

CRP like ESR its mostly elevated during acute inflammation but its elevated faster than ESR, in our cases CRP mostly positive and higher titer in *E. histolytica* and sero-positive celiac disease, then followed by *G. lamblia* and Rotavirus, as in table 10 which explain that there is acute inflammation due to infection processes, CRP is used mainly as a marker of inflammation [44] .

In our study most of children with suspected developing celiac disease show a positive result for two or more autoantibodies of anti-gliadin IgG and IgA and anti-tissuetransglutaminase IgG and IgA, and because of highly specificity and sensitivity of these two autoantibodies especially in children with genetically susceptibility to rising celiac disease; only two of study children in which the physician send a request to lab. for detecting these autoantibodies due to having some symptoms of celiac disease, but the other six children we discover they are susceptible to celiac disease during testing without any suspicious by physician, thus not all child without symptoms of celiac disease meaning he is pure negative, on the other hand not all person with symptoms suspected celiac disease meaning he is positive for celiac disease autoantibodies; there is a screening study in US show that 6% of testing children has positive for autoantibodies [45] especially those with some autoimmune such as type 1 diabetes, autoimmune thyroid disease, and dermatitis herpetiformis [46,47] .This results was agreement with Mohammed (2004) [48] ,and Tawfiq Taki (2012) [37] .

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