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Possible correlation between O-antigen serogroup of Enteropathogenic / Enteroinvasive E.coli and co infection with Giardia G.lamblia and Entamoeba histolytica as a possible pathological strategy for acute diarrhea among children of Baghdad governorate

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Abstruct

Diarrheal diseases account for approximately 2 million deaths annually in children under the age of 5years. Disease and death caused by diarrhea is a global problem, but is especially prevalent in developing countries. The aim of the present study is to determine the possible role of *E.coli* mainly Enter pathogenic (EPEC) serogroup *(*O111, O55, O26, O86, O119, O127, O114, and O142*)* and Enteroinvasive *E.coli* (EIEC) serogroup (O124); *Giardia lambleia* and *Entamoeba histolytica* co infections in pathology of acute diarrhea among children's of Baghdad governorate. A total of one hundred seventy presented with diarrhea that proved through clinical investigation were enrolled in this study. After microbiological examinations, Sixty four children that infected with two pathogens were selectively enrolled in this study. The minimum age of infected children was 4 months while maximum age was 116 months. Thirty four (53.1%) % out of Sixty four children were males and females represent the rest 30(46.9%). Stool samples of patients were submitted for direct microscopical examination for detection of *G.lamblia* and *E.histolytica* using wet preparation technique. Gram staining technique was applied for demonstration of gram negative bacilli. Stool culture was done using MaCconky agar for determination of *E.coli*. API 20E system was used for biochemical characterization of *E.coli* .serogrouping of *E.coli* was done by direct slide agglutination technique in to four types (type1 EPEC serogroup (O111,O55,O26), type 2 EPEC serogroup (O86,O119,O127), type3 EPEC serogroup (O125,O126,O128), type 4 EPEC/EIEC serogroup (O114,O124,O142) using Specific trivalent antisera.

In this study the critical age groups were (4-20)month followed by (21-37) month and (38-54) which represent (31.25%) , (28.12%) and (25%) of infected cases . on the other hand the age group (106-122) month was consider less critical age of infection with diarrhea causative agents. Among the age group (4-20) month, infection with ***E.histolytica*** represent (21.87%) while infection with EPEC serogroup (O86, O119, O127)represent (12.5%) out of 64 infected children. infection with ***G.lamblia*** represent (6.25%) while at this age group the low detectable frequency of infection was caused by EPEC */EIEC* serogroup (O114, O124, O142), (3.12 %) with no detectable frequency of infection with EPEC serogroup (O111,O55,O26)and EPEC serogroup *(O125, O126, O128)*. Regarding age group (21-37) month high frequency (25%) of infection with ***E.histolytica*** was recorded compared with (6.25%) for EPEC serogroup (O86,O119,O127), and EPEC */EIEC* serogroup (O114,O124,O142). low detectable frequency (3.12%) of infection was caused by ***G.lamblia*** .At the age group (38-54) month high frequency of infection with ***E.histolytica*** was recorded (18.75%) compared with (3.12%) for EPEC */EIEC* serogroup (O114,O124,O142), ***G.lamblia*** . Equal frequency of infection was recorded for EPEC */EIEC* serogroup (O114, O124, O142) and ***G.lamblia*** (6.25%) in the age group (55-71) month as well as for ***E.histolytica*** (6.25%) in the age group (72-88) month and (3.12%) in the age group (106- 122) month . ***E.histolytica was*** more frequent pathogen that detected in (75%) of diarrheal cases followed by EPEC serogroup (O86, O119, O127), (21.9%), EPEC /EIEC serogroup (O114, O124, O142) and ***G.lamblia*** (18.8%) for each one. This study revealed that co infection with EPEC serogroup (O86, O119, O127) and *E.histolytica* represent (15,62%),while co infection with EPEC / EIEC serogroup (O114,O124,O142) and ***G.lamblia*** represent (9.73%).on the other hand co infection with EPEC / EIEC serogroup (O114,O124,O142)and *E.histolytica* represent (9.73%).This study revealed that there was positive linear relationship between age of infected children and infection with EPEC serogroup (O86, O119, O127) (r=.367;p=.039. strong negative linear relationship between EPEC / EIEC serogroup (O114,O124,O142) and E.histolytica infection(r= -.462;p=.008) as well as between G.lambelia and E.histolytica infection (r=-.832;p=.000) .This study concludes that there is a possible correlation between O-antigen Serogrouping and coinfection with EPEC / EIEC, EPEC, G.lambelia and E.histolytica.

Key word: acute diarrhea, EPEC, EIEC, *Giardia lamblia, Entamoeba histolytica*

الخلاصة

امراض الاسهال مسؤله عن مايقارب مليونا حاله وفاه سنوياً في الاطفال تحت سن الخامسه.المرض و الموت المسبب عن الاسهال هما مشكله عالميه ولكنها تتركز في البلدان الناميه .تهدف الدراسه الحاليه لتحديد الدور المحتمل الاصابه المتزامنه بـ Enteropathogenic (EPEC) serogroup (O111, O55, O26, O86, O119, O127, O114, و O142) and Enteroinvasive E.coli (EIEC) serogroup (O124); Giardia lamblia and Entamoeba histolytica كاستراتيجيه مرضيه محتمله للاسهال الحاد بين اطفال محافظه بغداد . مائه وسبعون طفلاً يعانون من الاسهال الحاد المثبت سريريا تم اختيارهم في هذه الدراسه . بعد اجراء الفحوصات المايكروبايولوجيه على هؤلاء المرضى تم اختيار اربع وستون طفلاً مصابا بمسببين مرضيين .اقل عمر الاطفال المصابين كان اربعه اشهر في حين اعلى عمر كان (116) شهر.اربعه و ثلاثون طفلاً (53.1%)كانوا من الذكور بينما تمثل الاناث (46.9%).. تم اجراء الفحص المجهري المباشر على عينات البراز للمرضى لتحديد الاصابه بـ G lamblia و E. histolytic باستخدام تقنيه التحضير الرطب.تم استخدام تقنيه كرام لصبغ عينات البراز على الشرائح الزجاجيه. زرعت عينات البراز على وسط MaCconky agar لتحديد الاصابه بـ E.coli . نظام API 20E استخدم للتمييز البايوكيميائي لـ E.coli..التقسيم الى مجاميع مصليه جرى باستخدام تقنيه التلازن المباشر على الشريحه الزجاجيه الى اربعه مجاميع مصليه باستخدام مضادات مصول ثلاثيه خاصه (type1 EPEC serogroup (O111,O55,O26), type 2 EPEC serogroup (O86,O119,O127), O114,O124,O142) (type3 EPEC serogroup (O125,O126,O128), type 4 EPEC/EIEC serogroup) . في هذه الدراسه كانت المجاميع العمريه الحرجه للاطفال المصابين هي ((4-20) شهر-38) month and (21-37) ; (54 شهر والتي تمثل (25%) , (28.12%) , (31.25%) بالتعاقب. من جانب اخر اقل الجاميع العمريه حراجه للاصابه بمسببات الاسهال كانت (106-122) شهر.بين المجموعه العمريه (4-20) شهر كانت الاصابه بـ E.histolytica تمثل (21.87%) في حين تمثل الاصابه بـ EPEC المجموعه المصليه (O86, O119, O127)تمثل (12.5%) في حين الاصابه بـ G.lamblia تمثل (6.25%) بينما اقل نسبه اصابه في هذه المجموعه العمريه كانت% 3.12) المسببه عن الاصابه بـ (O114, O124, O142) EPEC /EIEC serogroup. لم يتم تحديد اي نسبه اصابه بـ (EPEC serogroup (O111,O55,O26), EPEC serogroup (O125, O126, O128).فيما يخص المجموعه العمريه (21-37) شهر اعلى نسبه اصابه بـ E. histolytica كانت (25%) مقارنه بـ (6.25%) لكل من EPEC serogroup (O86, O119, O127) و(O114,O124,O142) EPEC /EIEC serogroup في حين كانت نسبه الاصابه (3.12%) لـ G.lamblia .في المجموعه العمريه (38-54) شهر سجلت اعلى نسبه اصابه ب E. histolytica . (18.75%) مقارنه بـ (3.12%) لـ (EPEC /EIEC serogroup (O114,O124,O142) و.G.lamblia. نسبه اصابه متساويه (6.25%) سجلت لكل من EPEC /EIEC serogroup (O114, O124, O142) وG.lamblia في المجموعه العمريه (55-71) شهر كذلك كانت نسبه الاصابه بـ E.histolytica للمجموعه العمريه (72-88) شهر (6.25%)بينما في المجموعه العمريه (106- 122) شهر كانت نسبه الاصابه (3.12%) .اعلى نسبه اصابه بمسببات الاسهال كانت بسبب E. histolytica (75%) بينما(21.9%) حددت نسبه الاصابه بـ EPEC serogroup(O86,O119,O127). حددت نسبه اصابه متساويه (18.8%) لكل من EPEC /EIEC

EPEC serogroup (O86, اوضحت هذه الدراسه بان نسبه الاصابه المتزامنه . (O114, O124, O142) G.lamblia
EPEC / EIEC serogroup (و الاصابه المتزامنه (15,62%),تمثل E.histolytica وO119, O127)
EPEC المتزامنه بـ الاصابه نسبه كانت جانب اخر من . (9.73%) تمثل G.lamblia وO114,O124,O142)
serogroup (O114,O124,O142) وE.histolytica. تمثل (9.73%). بينت هذه الدراسه وجود علاقه خطيه موجبه
بين عمر الاطفال المصابين بالاسهال و الاصابه بـ EPEC serogroup (O86, O119, O127 . كانت هناك علاقه
خطيه سالبه للاصابه بـ Entamoeba histolytica و (EPEC serogroup O114,O124,O142). علاقه
خطيه سالبه للاصابه بـ E. Histolytica و G.lamblia تم تسجيلها.

استنتجت هذه الدراسه احتماليه وجود علاقه بين المجموعه المصليه لـ EPEC / EIEC وتزامن الاصابه بـ E. histolytica
و G.lamblia

Introduction

Diarrhea is usually defined as the passage of loose or watery stools, usually at least three times in 24 hours and the importance is put onto the change in stool consistency rather than frequency, and the usefulness of parental insight in deciding whether children have diarrhea or not [1, 2]. If diarrhea lasts less than 14 days, it is called “Acute diarrhea”. “Persistent diarrhea” is diarrhea of more than 14 days. Some experts refer to diarrhea that lasts > 30 days as “chronic” [3].

In the normal condition, there is a balance in absorptive and secretory functions of intestinal water and electrolytes. Diarrhea results when the balance in electrolytes and water transport is upset in favor of net secretion because of decreased absorption from the intestinal lumen or increased secretion or water loss into the lumen. These are induced by different action mechanisms of enteric pathogens. The outcome is diarrhea. In many cases, the patients have accompanying symptoms such as fever, vomiting, and abdominal pain. Dehydration in diarrhea is the consequence of diarrhea and/or vomiting. .It can be a severe symptom that is life threatening for children. [4] There is a widening range of recognized enteric pathogens such as viruses, bacteria, and parasites that can cause diarrhea. In many studies [5, 6, 7, 8], pathogens are identified in at least about 50 to 60% of stool samples from children with acute diarrhea. Among them, rotavirus and diarrheagenic *Escherichia coli* (DEC) are the most common. Other pathogens such as Campylobacter spp, *Shigella spp, Salmonella spp, and Vibrio cholerae*, *Entamoeba histolytica*, and *Giardia lamblia* also play an important role in many different geographic areas.

Aim of the study

The aim of the present study is to determine the possible role of *E.coli* mainly of Enteropathogenic (EPEC) serotypes *(*O111, O55, O26, O86, O119, O127, O114, and O142*)* and Enteroinvasive E.coli (EIEC) serotype (O124); *Giardia lambleia* and *Entamoeba histolytica* co infections in pathology of acute diarrhea among children's of Baghdad governorate.

1. **Patients ,Materials and Methods :**
2. **2.1. Demography:**

A total of one hundred seventy presented with diarrhea that proved through clinical investigation attended to outpatient's clinic of Baghdad teaching hospital and children care hospital during a period from January, 2008 to March 2009 were enrolled in this study. After microbiological examinations, Sixty four children that infected with two pathogens were selectively enrolled in this study. Thirty four (53.1%) out of Sixty four children were males with mean age (42± 28.77) months, on the other hand females represent the rest 30(46.9%) with mean age (27.13 ± 20.81) months.

2.2. Methods:

2.2.1. Direct microscopic examination:

Stool samples of patients were submitted for direct microscopical examination for detection of *G.lamblia* and *E.histolytica* using wet preparation technique [9] . For parasite detection fresh direct microscopic examination with saline solution and iodine was carried out with recently emitted feces (less than 6 h after collection), allowing the observation of living and moving trophozoite stages of protozoa.

2.2.2. Isolation and identification of *E.coli*

Gram staining technique was applied for microscopic identification of ***gram negative bacilli***. Stool culture was done using MaCconky agar for determination of *E.coli* .positive culture of *E.coli* on MaCconky agar depends on colony morphology which is pinky due to lactose fermentation. [10]

2.2.3. *E.coli* typing:

API 20E system from bio Merieux –France was used for biochemical characterization of *E.coli* according to manufacturer instructions [11]. *E.coli* was typed by direct slide agglutination technique in to four types using Specific trivalent antisera from

Bio-RAD-France, contain (antibodies specific for O111,O55,O26 antigens for *E.coli* type 1 ;antibodies specific for O86,O119,O127 antigens for *E.coli* type 2 ;antibodies specific for O125,O126,O128 antigens for *E.coli* type 3 ; antibodies specific for O114,O124,O142 antigens for *E.coli* type4 .

2.2.3. a .Principle

When a bacterial culture is mixed with a specific antiserum directed against bacterial surface components, the cells are bound together through antigen-antibody bonds to form aggregates (agglutination). This is usually visible to the naked eye as clumps in the suspension. By mixing specific antisera with an E. coli culture, the O- antigens are determined.

2.2.3.b .Procedure

General

Physiological saline is used as a negative control and must be negative. If the negative control is positive (agglutinates), the strain is auto agglutinating, i.e. O rough.

Slide agglutination with O antisera according to statens serum institute procedure ([12])

1. The *E. coli* is grown over night on a suitable agar medium not inhibiting motility.
2. A small drop of antiserum (approximately 20 µl) on a glass slide was applied.
3. Culture from a single colony to each drop of antiserum was Transferred and mixed well. The amount of culture was sufficient to give a distinct milky turbidity. Inoculating loop or a toothpick was used.
4. The slide for 5 - 10 seconds was tilted.
5. The reaction was read with naked eye by holding the slide in front of a light source against a black background (indirect illumination).
6. A positive reaction is seen as a visible agglutination. A negative reaction is persistence of the homogeneous milky turbidity. A late or weak agglutination was considered negative.

2.2. Statistical analysis:

Data analysis was performed using Spearman's test (rho) for correlation for categorical and non categorical data. The level of significance was 0.05(two-tail) in all statistical testing; significant of correlations include also 0.01 (two-tail). The level of confidence limits was 0.095.Statistical analysis was performed using SPSS for windows TM version 14.0. and Microsoft Excel for windows 2007.

Results:

In this study sixty four children with diarrhea were involved as shown in table(1) the minimum age of infected children was 4 months while maximum age was 116 months and the mean age was 35.03 month .among infected children males represent (*53.1%)* and the rest (*46.9%*) were females as illustrated in table (2). Regarding the age group of infected children, the critical age groups were (4-20)month followed by (21-37) month and (38-54) which represent (31.25%) , (28.12%) and (25%) of infected cases . On the other hand this study found that the age group (106-122) month was consider less critical age of infection with diarrhea causative agents. Diarrhea causative agents were not detected among the age group (89-105) month as shown in table (3).

Table (4) shown that among the age group (4-20) month, infection with E.histolytica represent (21.87%) while infection with EPEC serogroup represent (12.5%) out of 64 infected children. on the other hand infection with G.lamblia represent (6.25%) while at this age group the low detectable frequency of infection was caused by EPEC/EIEC serogroup (O114,O124,O142) , (3.12 %) with no detectable frequency of infection with EPEC serogroup and EPEC/EIEC serogroup (O125,O126,O128).Regarding age group ,high frequency (25%) of infection with E.histolytica was recorded in age group (21-37) month compared with (6.25%) for EPEC serogroup (O86, O119, O127), and EPEC/EIEC serogroup (O114,O124,O142). low detectable frequency (3.12%) of infection was caused by G.lamblia .At the age group (38-54) month high frequency of infection with E.histolytica was recorded (18.75%) compared with (3.12%) for EPEC/EIEC serogroup (O114,O124,O142), G.lamblia . Equal frequency of infection was recorded for EPEC/EIEC serogroup (O114, O124, O142) and G.lamblia (6.25%) in the age group (55-71) month as well as for E.histolytica (6.25%) in the age group (72-88) month and (3.12%) in the age group (106- 122) month as shown in table (4).

Table (5) elucidate that E.histolytica was more frequent pathogen that detected in (75%) of diarrheal cases followed by EPEC serogroup (O86, O119, O127), (21.9%), E. coli type 4(O114,O124,O142) and G.lamblia (18.8%)for each one. on the other hand no detectable frequency for EPEC serogroup (O111,O55,O26) and EPEC serogroup (O125,O126,O128) was recorded.Co infection with two pathogens was elucidated in table (6).In EPEC serogroup (O86, O119, O127) -E.histolytica (15,62%),while co infection with EPEC/EIEC serogroup (O114,O124,O142) -G.lamblia as well as EPEC/EIEC serogroup (O114,O124,O142) -E.histolytica represent (9.73%).This study revealed that there was positive linear relationship between age of infected children and infection with EPEC serogroup (O86, O119, O127) (r=.367;p=.039. strong negative linear relationship between EPEC/EIEC serogroup (O114,O124,O142) and E.histolytica infection(r= -.462;p=.008) as well as between G.lamblia and E.histolytica infection (r=-.832;p=.000) as shown in table (7).

Table (1): General description of age for patients that enrolled in the present study

Mean of age(month)	35.03
Std. Error of Mean	3.233
Median of age(month)	30.00
Std. Deviation	25.865
Range of age(month)	116
Minimum of age(month)	4
Maximum of age(month)	120

Table (2): General description of Gender for patients that enrolled in the present study

Gender	**No.(%)**
Male	**34(53.1%)**
Female	**30(46.9%)**
Total	**64(100%)**

Table (3): Distribution of patients according to age groups

Age (month)	4-20	21-37	38-54	55-71	72-88	89-105	106-122	Total
N0. (%)of infected cases	20 (31.25%)	18 (28.12%)	16 (25%)	4 (6.25%)	4 (6.25%)	0 (0%)	2 (3.12)	64 (100%)

Age (months)	children with EPEC serogroup	children with EPEC serogroup	children with EPEC serogroup	with EPEC/EIEC	with *G.lambelia* children	with *E.histolytica* children	No(%)of infected
4-20	0(0%)	8 (12.5%)	0 (0%)	2 (3.12%)	4 (6.25%)	14 (21.87%)	
21-37	0(0%)	4 (6.25%)	0 (0%)	4 (6.25%)	2 (3.12%)	16 (25%)	
38-54	0(0%)	2 (3.12%)	0 (0%)	2 (3.12%)	2 (3.12%)	12 (18.75%)	
55-71	0(0%)	0 (0%)	0 (0%)	4 (6.25%)	4 (6.25%)	0 (0%)	
72-88	0(0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (6.25%)	

89-105	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
106- 122	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	2(3.12%)

Table (5): Distribution of pathogens causing diarrhea in children

Causative agent	**Positive cases**	**Negative cases**
	No. (%)	**No. (%)**
EPEC serogroup (O111,O55,O26)	**0(0%)**	**64(100%)**
EPEC serogroup (O86,O119,O127)	**14(21.9%)**	**50(78.1 %)**
EPEC serogroup (O125,O126,O128)	**0(0%)**	**64(100%**
EPEC/EIEC serogroup (O114,O124,O142)	**12(18.8%)**	**52(81.3%)**
G.lamblia	**12(18.8%)**	**52(81.3%)**
E.histolytica	**48(75%)**	**16(25%)**

Table (6): Distribution co infection with two pathogens among diarrhea cases of children

Diarrhea Causative agents	*No. (%) of cases*
EPEC serogroup ***(O86,O119,O127) +E.histolytica***	***10 (15.62%)***
EPEC serogroup ***(O86,O119,O127) + G.lambelia***	***4(6.25%)***
EPEC/EIEC serogroup ***(O114,O124,O142) +E.histolytica***	***6(9.73%)***
EPEC/EIEC serogroup ***(O114,O124,O142) +G.lambelia***	***6(9.73%)***

Table (7): Correlations among age, gender and pathogens that cause diarrhea of children

Parameters	*rho correlation* ***	*gender*	*EPEC serogroup (O86,O119 ,O127)*	*EPEC/EIEC serogroup (O114,O124 ,O142)*	*E.histolytica*	*G.lambelia*
Age	**r** ***	**-.280**	**.367(*)**	**-.157**	**.032**	**-.066**
	P	**.120**	**.039**	**.390**	**.864**	**.721**
Gender	**r**		**-.109**	**.130**	**-.253**	**.130**
	P		**.553**	**.477**	**.162**	**.477**
EPEC serogroup *(O86,O119,O127)*	**r**			**-.254**	**-.044**	**.133**
	P			**.160**	**.813**	**.468**
EPEC/EIEC serogroup *(O114,O124,O142)*	**r**				**-.462(**)**	**.385(*)**
	P				**.008**	**.030**
E.histolytica	**r**					**-.832(**)**
	P					**.000**

***Correlation is significant (P) at the 0.05 level (2-tailed).**

**** Correlation is significant(P) at the 0.01 level (2-tailed).**

***** Spearman's Correlation coefficient**

Discussion:

The rate of mortality from diarrheal diseases in the world has decreased, mainly because of better therapy and interventions that promote sanitary conditions and that educate inhabitants to encourage them to take part in primary health care activities.(13,14)However, acute diarrheal diseases continue to be one of the major causes of morbidity and mortality in the developing world .An epidemiologic study of an infectious disease in a community is an initial

step toward the introduction of the proper interventions for controlling the disease because the features and the patterns of isolation of etiologic agents of the disease vary from place to place depending on the local meteorology, geography, and socioeconomic elements. (15, 16) The results of the present study come in concordance with Haque et al (2003) (17) ,Ferreccio et al (1991)(18) ;Baqui et al (1992)(1) and Baqui et al (1993)(19) they demonstrate that incidence of acute diarrhea was highest in Bengalian children with age (24-36) months old and lowest for children(48-60) months old. AL-Hamdani (1993)(20) reported that infection in Baghdad city with E.histolytica among children at age one to seventy two months represent (18.9%) in case of G.lamblia at the same age group ,infection represent (27.77%); while at (84-144) months , infection with E.histolytica represent (23.8%) and for G.lamblia represent (21.83%).

The results of the present study come in agreement with numerous local studies such as Al-ani (2008) (21),recorded that highest incidence of acute diarrhea in the age group (4-6)months followed by age group (7-9)months representing (28.8%) and (28.5%)respectively . Arif and Hamody (2007)(22) , stated that highest occurrence of diarrhea in the age group (3-9)months. This might be attributed to the time of introduction of solid food and due to starting of infant crawling with increase ingestion of contaminated materials especially in un hygienic environment (Al-Rafie and Hassouna, 1986) (23). In Saudi Arabia, Al-sekait (1988) (24); In Egypt EL-Gelany and Hamad (2005) (25) and in Bangladesh, pathela and Hasan,(2006) (26) they reported that the peak occurrence of infantile diarrhea was in the age group (6-12) months.

On the other hand regarding gender of infected children AL-Hamdani (1993)(20) elucidated that infection with E.histolytica among males represent (23.07%) compared with (25.35%)among female. Regarding G.lamblia, infection among female was higher (21.12%) than that of male (19.61%). The differences in frequency of infection with G.lamblia and E.histolytica may belong to the study design and the sample size that used for statistical analysis. The results of the present study come in agreement with that reported by Baqui et al (1992) (1); Haque et al (2003)(17) ; Huilan et al (1991)(27);Santosham et al (1995) (28), they reported that the incidence of acute diarrhea in Bengalian children was higher among males than females. Al-ani (2008)(21) found that the majority of diarrhea cases were reported among males which represent (65.5%). Bin Mohanna et al (2005) (29) stated that in Yemen male constituted (76.9%) and female constituted (23%) out of all diarrheal cases. Al-Badri et al (2007) (30) stated that in Iraq

diarrheal cases among males (60%) which was higher than females (40%).This result might be attributed to the deeply rooted preference of many families for males infant which motivate them for quick consultation for male ill infant .El-Zanaty (2002) (31); El-Gilany and Hammad (2005) (25) stated that no sex difference in the occurrence of diarrhea was found as the risk factors associated with diarrhea are environmental and sociodemographical rather than biological factors.

The present study revealed that diarrheal cases caused by E.histolytica represent (75%) of positive cases followed by E.coli type 2(O86,O119,O127) that represent (21.9%); E.coli type 4(O114,O124,O142) and G.lambelia that represent (18.8%) for each one .this result come in agreement with Bin Mohanna et al(2005) (29);they stated that , E.coli was the most important cause of diarrhea in infancy in Yemen .

The result of the present study disagree with that recorded by Al-ani (2008) (21) who stated that the incidence of G.lamblia was (16.2%), E.histolytica (10.8 %); E.coli (6.9%) recovered from diarrheal cases among infant. On the other hand result recorded by Saleem (2003) (32) agree with that recorded in this study, she elucidated that the incidence of E.histolytica among diarrheal cases in Baghdad represent (48.9%) compared with (13.3%) for G.lambelia. Saleem (2003) (32) found that (26.51%) of diarrheal cases in shatrah were infected with G.lambelia. These findings show that the prevalence rates of G.lamblia in Iraq vary from one region to other and from one year to other. Variation in the incidence of infection is probably due to the nature of residence surveyed, the level of personal hygiene and sanitation and socio-economic status and poor community hygiene safety of water consumption from water supplies; it is evident that Giardia cyst can resist chlorination of drinking water (Mehlhorn, 2001) (9).

The result of the present study elucidate that the highest incidence of G.lambelia was recorded among the age group (4-20), (21-37) months. This result comes in agreement with that recorded by saleem (2003) (32) she stated that the highest incidence was recorded among the age group (1-10) years. These finding increases the possibility of oral transmission in this age group which involves the more active individuals and considered as a period of unhygienic habit. On the other hand decreased prevalence of infection suggests acquired immunity after repeated infections. Protective immunity is also suggested by the self –limiting nature of most infections (Abass, 2004) (33).

The importance of classical O-serogroup of Enteropathogenic E.coli (EPEC) was ignored throughout previous studies that focused on diarrheal disease. The result of this study comes in agreement with that recorded by Rodrigues et al (2002) (34), they stated that classical O-serogroup of EPEC (O:55,O:126:O127:O:142 :O125) represent(11.86%) out of all diarrheogenic E.coli (DEC) isolated from acute cases of diarrhea . Haque et al (2003) (17) stated that the incidence of EPEC among diarrhea causative agent was (3.33%) and (1.9%) for EIEC which was O: 124 positive .Torres et al,(2001) (35) mentioned that E.coli pathogenic virotype especially EPEC were the microorganism most frequently associated with diarrhea of infants from low –income families admitted to the public pediatric hospital in Montevideo. Torres et al,(2001) (35) recorded that in Uruguay the incidence of EPEC O:111 (44.31%);O:119 (34. 09%) and O:55 (19.31%) while EIEC O:124 was recovered from (1.13%) diarrheic cases using monovalent and polyvalent EPEC(O:111,O:119,O:55) and EIEC (O:124)rabbit antisera . the result of this study come in agreement with Alvarez et al (1974)(36) they recorded that prevalent serogroups of EPEC in case of children's diarrhea were O:111,O:119,O:55, as has been locally the case for at least 25 years .in this study the prevalence of EPEC(21.9 %)and less frequently EIEC (18.8%) come in agreement with that recorded by Rivas et al (1996) (37) ;Vidotto et al (2000) (38) they recorded that EPEC is not a frequent cause of diarrhea in developed countries, but it is very commonly associated with enteric diseases in developing areas including close Brazilian regions. The similarities between Brazilian regions and our regions may be associated with climate conditions as well as economical status of both societies. (39) Tamaki et al (2005)(40) recorded variable result for the incidence in Japan for EPEC O119 was (74.5%) ;O111was (26.19%);O126 was (1.51%);O86 was(4.54%);O127was (0%); O114 was (4.76%); O142 was (4.54%) and for EIEC of serogroup O124 the incidence was (0%). Paciorek ,(2002) (41) mentioned that in Polish regional sanitary laboratories, stool specimens of children aged up to 2 years with diarrhea are routinely investigated for the presence of E. coli strains belonging to 15 selected serogroups regarded as EPEC. Of these serogroups, the most frequently isolated are O26 (8.2%), O86 (7.1%), O126 (4.8%) and O127 (5.1%).The differences may be attributed to study design as well as to sample size of population under investigation.

This study revealed that co infection with EPEC serogroup (O86, O119, O127) and E.histolytica represent (15,62%),while co infection with EPEC / EIEC serogroup (O114, O124 ,O142) and G.lamblia represent (9.73%).on the other hand co infection with EPEC serogroup (O114,O124,O142) and E.histolytica represent (9.73%).

Until recently there is no available evidence that clearly explain the possible mechanism for coinfection between EPEC and G.lamblia as well as E.histolytica. The possible mechanism can be hypothesized by the pathological lesion that caused by EPEC that may offer favorable nutritional and micro environmental condition for growth and survival of G.lamblia as well as E.histolytica. The alternative scenario may be beneficial effect of attachment of G.lamblia as well as E.histolytica to the intestinal mucosa that may favor the suitable media that enhance the growth and production of pathological effect of EPEC in intestinal epithelial cells. Marie-pierre et al ,(2003) (42) and Lichtman et al (1996)(43) recorded that the possible mechanism can be hypothesized by the role of intestinal mucin glycoproteins that act as an important host-defense by binding of pathogenic microorganisms, thus preventing their attachment to epithelial cells and subsequent cytolysis. However, mucin glycoproteins can also serve as receptors for a wide range of pathogens that colonize the mucus barrier and epithelial cells. Indeed, numerous enteropathogenic microorganisms, including bacteria (e.g. Shigella sp., Salmonella sp., Yersinia enterocolitica, certain strains of Escherichia coli), viruses (rotaviruses), and parasites (Entamoeba histolytica) have been shown to interact with intestinal mucins (44, 45, 46, 47, 48,49). Intestinal mucins of various species, however, may not contain the same binding sites despite of considerable similarity in their overall chemical composition and physical structures. For example, Shigella binds specifically to human colonic mucin and not to rat colonic mucin (50). Thus, animal models are limited in their usefulness to investigate pathogen-host interactions, as appropriate mucin gene expression may directly contribute to the host-restricted enterocolitis that is evident in human intestinal xenografts that are infected with Salmonella typhi (51, 52,53,54), Entamoeba histolytica (55,56).

Devinney et al,(1999) (57) and Frankel et al (1998) (58) mentioned that EPEC are identified by their ability to cause effacement of microvilli and intimate adherence between the bacterium and the epithelial cell (A/E lesion) with polymerized actin accumulation and pedestal-like structures forming beneath adherent bacteria . The locus of enterocyte effacement (LEE)

Pathogenicity Island contains the eae gene, encoding intimin, a bacterial outer-membrane protein (OMP) involved in the intimate bacterial attachment to the gut mucosa. Other properties of EPEC include localised adherence (LA, bacteria form characteristic microcolonies on the surface of the cells) mediated by the EPEC adherence factor (EAF) plasmid-encoded bundle-forming pili (bfp) gene (Baldini et al ,1983(59);Giron et al,1991(60),Paciorek ,2002(61)) However, the EAF plasmid was not detected among many EPEC isolates. Thus, typical EPEC are eae+, possess the EAF plasmid and do not produce Shiga toxins (Kaper, 1996) (62). Atypical EPEC, irrespective of their adherence phenotype, are eae+ and neither possesses EAF plasmid nor produce Shiga toxins.

This study revealed that there was positive linear relationship between age of infected children and infection with EPEC serogroup (O86, O119, O127) (r=.367;p=.039) .The result of the present study come in agreement with that mentioned by Baqui et al (1992)(1) ;Haque et al(2003) (17) ; ,Ferreccio et al (1991)(18) ; and Baqui et al (1993)(19) ; Mandmando et al,(2007) (63) ; Gonzales et al,(1997) (64)they demonstrate that incidence rate of acute diarrhea in Bengalian children was highest for those 24-36 months old and lowest for children 48-60 months old.

Strong negative linear relationship between EPEC /EIEC serogroup (O114, O124, O142) and E.histolytica infection(r= -.462;p=.008) . This may attributed to statistical analysis due to sample size under investigation. Strong negative linear relationship between G.lamblia and E.histolytica infection (r=-.832; p=.000). This may attributed to statistical analysis due to sample size under investigation as well as the site of pathological lesion that differ from small to large intestinal tract. These studies conclude that there is a possible correlation between O serogroup and coinfection with EPEC, EPEC /EIEC and E.histolytica and G.lamblia in acute cases of diarrhea in children.

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