

Frequency of Parasitic, Bacterial and Fungal Enteropathogens among Children and Adults Patients with Diarrhea and Association Affecting Factors

قياس تكرار الممرضات المعوية الطفيلية، البكتيرية والفطرية لدى الاطفال والبالغين
المصابين بالإسهال وبعض العوامل المؤثرة ذات العلاقة

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الخلاصة:-

الهدف: أجريت هذه الدراسة للتحري عن مسببات المايكروبية لمرض الاسهال و ايجاد الفروق المعنوية ذات الدلالة الاحصائية لعدة عوامل (محل الإقامة ، العمر و الجنس) ومدى تأثيرها على زيادة نسبة الاصابة و تنوع المسببات المرضية والتباين في نسبها لدى المرضى الوافدين لمستشفى الصويرة العام والمراكز الصحية لمدينة الصويرة وضواحيها.

المنهجية : وشملت الدراسة 596 مريضاً من كلا الجنسين ومن مختلف الاعمار وللفترة من بداية تشرين الثاني 2013 ولغاية نهاية ايلول 2014. حيث تم جمع عينات البراز في حاويات معقمة وتم اجراء الفحوصات العيانية والمختبرية المختلفة لتحديد نوع العامل المسبب سواء كان طفيلي او بكتيري او فطري وتم ملء استمارة استبيان المعلومات الخاصة بكل مريض والتي تضمنت العمر ،الجنس،ومحل الإقامة واخضعت هذه البيانات للتحليل الاحصائي باستخدام نضام SPSS وتم تحليل النتائج باستخدام الوسائل الاحصائية (النسبة المئوية والتكرارات)والوسائل الاستنتاجية (مربع كاي).

النتائج: وكانت نسبة العينات الموجبة 376(63%) منها 177(47.07%) اصابة طفيلية تضمنت اصابات بالاميبيا الحالة للنسيج 100(26.5%) واصابات بالجيارديا لامبلا 77(20.4%) ، بينما بلغت نسبة الاصابات البكتيرية 156(41.4%) تضمن كل مما يلي 49(13.03%) اشريشيا كولاي، 38(10.1%) سالمونيلا، 36(9.5%) شكيلا بينما كانت اقل نسبة اصابة للعدلات الفطرية وبلغت 43(11.4%). وكانت غالبية العينات الموجبة هي ضمن الفئة العمرية اقل من 5 سنوات 119(31.6%) وشملت 17.7% ذكور و 13.8% اناث. بينما اقل نسبة كانت ضمن الفئة العمرية 35-26 (11.7%) وكانت اعلى نسبة للعينات الموجبة لدى الذكور 200(53.1) مقارنة بالاناث 176(46.8) وكذلك كانت اعلى نسبة في المناطق الريفية 233(39%) مقارنة بالمدينة 143(23.9%).

الاستنتاجات : كشفت هذه الدراسة ان المسببات الطفيلية هي الاكثر شيوعاً لدى مرضى الاسهال في مستشفى الصويرة والمراكز الصحية التابعة لمدينة الصويرة وضواحيها حيث كانت اعلى نسبة للاميبيا الحالة للنسيج تليها الجيارديا لامبلا بينما تمثلت المسببات البكتيرية بكل من الاشريشيا كولاي، واجناس السالمونيلا والشكيلا واقل نسبة كانت للمبيضات الفطرية.

التوصيات:- واوصت الدراسة باستخدام الاختبارات المصلية والتحليل الجيني وغيرها من الفحوصات الحديثة لتحديد العامل المسبب لمرضى الاسهال في البحوث المستقبلية.

Abstract:-

Objective: The frequency of bacterial , parasitic and fungal enteropathogens among 596 children and adults patients who attended to Al- Sweara hospital and to health centers in Al- Sweara city were investigated. And the possibility of the influence of some factors such as residence, Sex and Age on the incidence variation of diarrhea and the diversity of its microbial etiology.

Methods: the study achieved from the beginning of November 2013 until the end of September 2014 and involved 596 patients from both sexes and deferent ages, all stool samples were collected in sterile containers and conducting to macroscopic and microscopic examination and various laboratory tests to determine the type of the causative agent, whether parasitic or bacterial or fungal ,also an informative questionnaire form was fill for each patient which included age, sex, place of residence and subjected these data for statistical analysis using spss.

Results : Total number of positive samples were 376(63%) of these 177 (47.07%) had parasitic etiology, of which *Entamoeba histolytica* was found in the majority of cases 100(26.5%) while *Giardia lambila* represent 77(20.4%). Bacteria represent 156(41.4%) which of 49(13.03%) were *Escherichia coli*, 38(10.1%) were *Salmonella* species, 36(9.5%) were *Shigella* species and only 43(11.4) of the samples were positive for yeast. The majority of positive samples in this study 119(31.6) were under the 5 years. While the lost positive samples 44(11.7) mad up the 46 years old. Male members were affected more than the female, where are the infection percent in male was 200(53.1) while in female 176(46.8). The rate of positive samples was 143(23.9%) in urban. While the rate of positive sample was higher 233 (39%) in rural area.

Conclusion : The result of this study concluded that most of the causative agents of diarrhea in the subjected patients in Al- Sweara hospital and to health centers in Al- Sweara city are of parasitical origin, where *Entamoeba histolytica* ranks first followed by *Giardia lambila* and *Escherichia.coli* ,*Salmonella*Spp. and *Shigella* Spp. represented the bacterial etiology and *Candeda albicans* was the fungal cause.

Recommendations:- For future studies, we recommend further investigations such as: prevalence of various serotypes, electrophoretotypes and genomic analysis of enteropathogens ,and ELISA methods for all patients with diarrhoea to identify the causative agent involved.

Key Words: diarrhea, enteropathogen, *Entamoeba histolytica*, mortality, rural.

INTRODUCTION:

Diarrhea is a medical condition characterized by inflammation of the gastrointestinal tract and can have many causes, which may be infectious or non-infectious. Infectious diarrheal diseases are a great problem throughout the world and are responsible for considerable morbidity and mortality, especially in developing countries ⁽¹⁾. Infectious diarrhea also remains an important problem in industrialized countries, but the course of the disease is generally mild, and mortality has decreased tremendously over time⁽²⁾.

Knowledge of the etiology of diarrhea is important for epidemiological surveillance but also, in many cases, for correct treatment, however recently one of the major challenges in the infectious diarrhea is the recent increase in the number of probable etiological agents⁽³⁾, in addition to The main etiology of the diarrhea which related to a wide range of bacteria (such as, *Shigella* spp., *Salmonella* spp., *E. coli* and *Vibrio cholera* etc.) entero-parasites (*Giardia* spp. and *Entamoeba histolytica* etc.), and viruses (Rotavirus, Adenovirus and Norwalk virus etc.) ^(1, 4). Also there are some factors that increase an individual's susceptibility to acquired infectious diarrhea which probably include poor personal and food hygiene, lack of clean drinking water⁽⁵⁾. WHO predicts that there will be about 5 million deaths in children younger than five years by 2025, of which 97% will be in the developing countries and mostly caused by infectious diseases, within which diarrhea will continue to play a prominent role⁽⁶⁾.

Objective: The frequency of bacterial , parasitic and fungal enteropathogens among 596 children and adults patients who attended to Al- Sweara hospital and to health centers in Al-Sweara city were investigated. And the possibility of the influence of some factors such as residence, Sex and Age on the incidence variation of diarrhea and the diversity of its microbial etiology.

MATERIAL AND METHODS :

Time and location (data collection).

The present study was achieved on (596) children and adult including (371) males and (225) females and they were attended to Al- Sweara hospital and to health centers in Al-Sweara city between the beginning of November 2013 to the end of September 2014 for detection of the microbial causative agents of diarrhea then informative questionnaire form was fill for each patient which include data such as age, sex, residence (urban, rural).

Collection of stool samples:-

The stool specimens were collected in sterile wide necked containers labeled with names of patients and brought to the laboratory for macroscopic and microscopic examination. All samples were subjected to several diagnostic laboratory tests for detection of bacterial, parasitic and fungal agents.

Laboratory methods:-

A macroscopic examination was done upon receiving the sample. The fresh stool samples were examined under the microscope using the saline solution by adding a small quantity of the selected fresh stool to one or two drops of normal saline (0.9% NaCl)on the slide with an applicator stick and covered with a cover slip⁽⁸⁾. For trophozoites, ova, cysts etc.

A portion of stool sample was aseptically transferred to a sterile selective enrichment broth to study the incidence of bacterial enteric pathogens present in the diarrheal stool samples. Further, the enriched culture was transferred to selective agar medium for the isolation of different bacterial genera. Starch ampicillin agar (SAA), Eosine methylene blue agar, Brilliant green agar (BGA), *Salmonella Shigella* agar (SS), Cetrimide agar, Nutrient agar and Mannitol salt agar (MSA) were used for the selective isolation of deferent kind of bacteria. The biochemical identification was performed by oxidase reaction, fermentation of sugars, and presence of lysine, gelatine liquefaction, arginine decarboxylation and absence of ornithine decarboxylase, proposed by the method outlined by⁽⁹⁾.

Initially lactophenol cotton blue stain identification and scoring for yeast was made with each fecal sample. Yeast overgrowth was defined as growth of 10^5 or more colonies\ routine inoculation was done in to look for the growth of a sufficient number of colonies. Culturing on Sabourauds dextrose agar and corn meal agar for the isolation of the organism was performed by the standard technique.

The identification of yeast was based on a morphological criteria that were included cultural characteristic (shape, size, texture, etc), asexual structure (shape, size, budding pattern, presence\ absence of arthroconidia, ballistoconidia, germ tubes, hyphae or pseudo hyghae formation) and on the biochemical and physiological characteristic that were used in identification included assimilation and fermentation of sugars, nitrogen utilization, urea hydrolysis, cyclohexamide resistance and temperature studies⁽¹⁰⁾.

Statistical analysis:-

The results were statistically analyzed by calculating the Chi square, frequency, percent and p value, and distributed according to age, residence and gender differences using SPSS, version 13.0. P values less than 0.05 was considered significant.

RESULTS :

Table 1:- prevalence rat of isolated enteropathogen that are associated with diarrhea.

Organism	No	%	Species	frequency	Percent %
Parasites	177	47.07	<i>Giardia lambila</i>	77	20.4
			<i>Entamoeba histolytica</i>	100	26.4
Bacteria	156	41.4	<i>Salmonella Spp.</i>	38	10.1
			<i>Shigella Spp.</i>	36	9.5
			<i>E. coli</i>	49	13.03
			<i>Staphylococcus Spp.</i>	19	5.05
			<i>Streptococcus Spp.</i>	14	3.7
Fungi	43	11.4	<i>Candida ssp.</i>	43	11.4
Total	376	63		376	100

This table shows that the total number of positive samples was 376(63%) of these 177(47.07%) had Parasitic etiology, of which *Entamoba histolytica* was found in the majority of cases 100(26.5%) *Giardia lambila* was detected in 77(20.4%) of the isolates, 156(41.4%) were of bacterial origin of which 38(10.1%) were *salmonella Spp.* 36(9.5%) were *Shigella Spp.*, 49(13.03%) were *E. coli* and only 43(11.4%) of the samples were positive for fungi.

Table 2:- The number of diarrheal cases in urban and rural region distributed was according to gender.

This table shows that a higher percentage of diarrhea cases in rural area 167(63.3%) in comparison with that of urban area 97(36.7%), Also shows that the percent of diarrheal cases among female was higher (19.4%) than male from urban area which give contrast result with rural area were infected male (34.5) more than infected female (27.3%). but this differences was not statistically significant (p value =0.5 level).

Table 3:- distribution of positive stool samples according to age differences and gender.

No of positive samples						M= male F= femal X ² =19.3 X ² _T =22.3 d.f= 15 p< 0.005	
Age(years)	gender				total		%
	M		F				
	No	%	No	%			
<-5	67	17.8	52	13.8	119		31.6
6-15	25	6.6	33	8.7	58		15.4
16-25	36	9.5	25	6.6	61		16.2
26-35	20	5.3	10	2.6	30		7.9
36-45	14	3.7	31	8.2	45		11.9
≥ 46	38	10.1	25	6.6	63		16.7
Total	200	53.9	176	46.8	376		63

This table shows that the children with age group < 5 years more susceptible to acquired infectious diarrhea 119(31.6%) followed by the individual with age >46, while the lowest percentage appear to be in the age group 26-35 were represent 30(7.9%), also result reveal that males with age group <5 years represent a high percentage of infection (17.8%) when compared to female (13.8%) at the same ages, which also similar to the deference's between males and females at the age group (26-35)(5.3%, 2.6%) respectively. this difference was statistically not significant (p value =0.5) .

DISCUSSION :

Infectious diarrhoea are the most important cause of morbidity and mortality in developing countries ⁽¹¹⁾. The prevalence rate of etiological agent of diarrhea in this study defer in comparison ,with other parts of Iraq such as Tikrit by Alrifai *et al* (2009) who reported that the rate of bacteria was higher (67.9%) than parasitic (11.1%) and fungal represent (2.5%)⁽¹²⁾. Our prevalence rate of etiological agent of diarrhea was in agreement with study of Prakash,(2008) in Omman who reported that parasitic isolation rate (18%) was higher than bacterial isolation rat (11.4%)⁽¹³⁾. A prevalence of (38.8%) bacterial pathogen

residence	Diarrheal cases												Total	%	X ² =4.8 X ² _T =6.2 d.f=3 p<0.005
	+ve Sables						-ve samples								
	Gender														
	M		F		total	%	M		F		total	%			
	No	%	No	%			No	%	No	%					
Urban	70	18.6	73	19.4	143	23.9	53	24	14	6.3	95	15.9	268	44.9	
Rural	130	34.5	103	27.3	233	39	100	45.4	25	11.3	125	20.9	328	55	
Total	200	53.1	176	46.8	376	63	153	69.5	39	17.7	220	36.9	596	100	

isolation from stool cultures fits in the wide range of bacterial pathogen prevalence of 4.8-45% ^(14, 15-16). Our results shows that *E.coli* was the most prevalence bacterial isolates 13.03% followed by *Salmonella Spp.* 10.1% and *Shigella Spp.* 9.5% which was in agreement with

other study whose reported that 59.3% were identified as *E. coli*, (7.4%) were *Salmonella species*, while (9%) were *Shigella species*⁽¹⁶⁾. These result may be due to lack of supervision, poor infection prevention practices, Lack of good hygienic conditions, Lack of proper treatment of sanitation level and lack of preventive health system as well as on the presence of insects that help to spread opportunity microbial infections⁽¹⁷⁾.

The sex distribution among the examined patients in our study showed a slight preponderance of diarrhea in males 53.1% compared with females 46.8% similar finding have been reported by(Johargy *et al* (2010) who found that the prevalence in male 58% higher than females 42%.⁽¹⁵⁾ , Also our result was in agreement with other studies in the world for instance , in a study in Nepal Sherchand *et al* (2009) reported that the Higher prevalence of diarrhea in male 56.4%, in Missouri Klein, E.J. *et al.*, 2006) was 57% for mal and in Tanzania Moyo (2011) 61.4% .^(18,19,20). This was due to the fact that males are more prone to infection through their presence in places an exposure to contaminated environmental pollution unlike females spend most of their time inside the house and thus the chance of exposure to pollution is lowest⁽¹⁹⁾.

Different studies have shown that diarrheal infection was more prevalent in children below 5 years of age than other age groups. For instance Bissoume *et a*, (2013) how reported that the higher incidence of infection was among children under 5 years old (50.2%)⁽²¹⁾ which in agreement with our study. While other study report that the children less than 5 years had a lower rate of infection (19%)⁽²²⁾ which contrast with our study. The increased incidence in this age is most likely because of the large numbers and crowding of kindergartens, as well as the absence of health awareness and the quality of the food used in these places, making them more exposure to enteropathogens plus the immaturity of the immune system of the new born and the decline in passive immunity after age 6 months⁽²³⁾.

CONCLUSIONS :

Our study concluded that most of the causative agents of diarrhea in the subjected patients in Al- Sweara hospital and to health centers in Al- Sweara city are of parasitical origin, where *Entamoeba histolytica* ranks first followed by *Giardia lamblia* and . *Escherichia.coli* , *Salmonella Spp.* and *Shigella Spp.* represented the bacterial etiology and *Candida albicans* was the fungal cause. Also the patients with age group less than 5 years old were more exposed to infect with infectious diarrhea regarding the gender of patients which doesn't represent an ideal factor for increase the infection.

RECOMMENDATIONS :

- 1- Further investigations such as: prevalence of various serotypes, electropherotypes and genomic analysis of enteropathogens, and ELISA methods for all patients with diarrhea to identify the causative agent involved.
- 2- The necessity of activating the program of primary health care in rural areas to increase awareness of the health of the people and thus avoid the spread of diarrheal disease and other morbidity epidemic in children and adults as well as to Improve more effective methods to sterilize drinking water and the disposal of stray animals and rancid water pools which serve as incubators for insects and pathogenic microorganisms

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