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Investigation of the Parasitic Protozoa that Cause Diarrhea and their Effect on GOT, GPT and ALP

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ABSTRACT: The current training was accompanied throughout the dated from December 2021 until June 2022 to study the spread of parasitic protozoa in children under the age of six and to study the biochemical changes, this current training was accompanied at the College of Science, Al-Qadisiyah University, in cooperation with the Maternity and Children's Hospital in Kut and the Maternity and Children's Hospital in Diwaniyah. 350 microscopic stool samples from children aged (0-6) years were examined. The samples that were diagnosed under a microscope and containing the study parasites (50 samples), Venous blood (5ml) from all samples was collected to measure some biochemical parameters.

The results of the current training, when conducting microscopic examination, showed that the highest infection with protozoan parasites was in the month of July, and the parasite Entamoeba histolytica recorded the highest infection among the rest of the protozoan parasites. It included 13 out of 85 samples with a ratio of (15.29) positive for parasite infection, while our current study recorded the lowest infection in March, as it included that 5 of 38 (13.15) were positive for intestinal parasites.

The study showed that there were changes in the biochemical parameters that were studied paralleled to the results of the controller group, Changes in the three liver enzymes GOT, GPT and ALP were studied. The results of the showed a high concentration of enzymes the three livers GOT, GPT and ALP.

Keywords: parasitic protozoa, ALP, GOT, diarrhea, PCR

1. INTRODUCTION

Diarrheal diseases constitute a substantial source of mortality in developing nations, with their control and prevention standing as key objectives for the (WHO) within the Separation of Diarrheal and Acute Respiratory Sickness Control programs in those regions (1). Accurately estimating the economic significance of parasitic diseases proves challenging due to considerable variations between countries and regions (2). Throughout the world, intestinal parasites are prevalent and represent some of the most widespread human infections, with particularly high prevalence rates observed in developing countries (3).

Children with compromised immune systems are susceptible to numerous infections, comprising those disturbing the gastrointestinal tract, which may present as austere extended diarrhea, long-lasting malabsorption, letdown to thrive, and malnourishment (4). Among the array of pathogens causing gastrointestinal infections, parasites are likely to play significant roles as either the primary cause or a co-morbidity of diarrhea in immunocompromised children, as indicated by previous studies on this population (5) , Intestinal parasitic infections are among the most prevalent infections globally, impacting approximately 3.5 billion people and contributing to over 450 million cases of ill health annually (6) .

Diarrhea is characterized by the passage of loose or watery stools exceeding the normal for a child or an increase in stool frequency. While not a disease in itself, it serves as a symptom for various underlying conditions, posing a significant risk as the second leading cause of death in children under five years. Annually, approximately 760,000 children under five succumb to diarrhea (7) . Diarrhea can be acute, lasting less than two weeks, or chronic, persisting for two to three weeks. Recurrent diarrhea involves the repeated occurrence and resolution of large volume or frequent loose stools (8), Because of the spread of intestinal parasites among children in Iraq and this problem is exacerbated and its impact on the activity and vitality of these children, which facilitates some of them with diseases that accompany

parasitic infections such as anemia, hepatitis, Biochemical and physiological changes in blood components came the idea of the current study.

1.1 THE AIM OF THE RESEARCH

The isolation and diagnosis of the parasite causing diarrhea in children under the age of six microscopic, Confirm the diagnosis of cause by molecular methods, measuring some of the Standards for functions liver and kidneys in children: Got, Gpt, AlKaline phosphats.

2. METHODS

2.1 Primers

The PCR primers designed for the finding of E. histolytica, G. intestinalis, B. hominis, and C. parvum were developed in this study. The design process involved utilizing NCBI-Genbank and Primer 3 Plus scheme tools. The primers, providing via Scientific Researcher Co. Ltd, Iraq, are detailed in the table (1):

Table 1.- The PCR primers for detection *Entamoeba histolytica*, *Giardia intestinalis*, *Blastocystis hominis* and *Cryptosporidium parvum*

Primers	Sequence 5'-3'		Product size	GenBank
Entamoeba histolytica	F	ACGAGGAATTGGGGTTCGAC	457bp	OM780324.1
	R	CCCCTCAGCATTGTCCCATG		
Giardia lamblia	F	GAATCAGGGTTCGACTCCGG	239bp	OM618754.1
	R	CCAACTACGGGCGTTTCAAC		
Blastocystis hominis	F	GGGACAGTTGGGGGTATTCA	588bp	ON248458.1
	R	GCAGCCCAGGACATCTAAGG		
Cryptosporidium parvum		AGACGGTAGGGTATTGGCCT	784bp	AH006572.2
		TCAGCCTTGCGACCATACTC		

2.2 Study area

This training the existing has been approved out at the College of Science, Al-Qadisiyah University in cooperation with the Maternity and Children Hospital in Al-Kut and the Maternity and Children Hospital in Diwaniyah -in the city of Diwaniyah for the period from December 2021 to June 2022. For the purpose of determining the pathological conditions associated with the infection of the parasites after the diagnosis.

2.3 Collection of specimens

A total of 350 microscopic stool samples of children between the ages of (0-6) years have been examined, samples that were diagnosed under the microscope and that contains on the parasites of the study (50) samples, venous blood (5 ml) of all samples was collected for measuring some biochemical parameters, Stool testers stayed composed in sterilized, leak-free common flasks, each labeled with the respective contributor training numbers. The samples were transported to the laboratory on the alike day as gathering. Upon coming at the laboratory, the stool samples were promptly stored at -20°C to facilitate subsequent molecular analysis.

2.4 Method of diagnosis parasites using PCR technique

The PCR technique was employed for the finding of *E. histolytica*, *G. intestinalis*, *B. hominis*, and *C. parvum*. This detection was based on the amplification of the minor subunit ribosomal RNA gene using stool testers obtained from patients. This technique stayed approved out rendering as next steps:

1- Stool DNA Extraction

Stool DNA was extracted from stool testers by means of the Presto™ Stool DNA Extraction Kit, following the instructions provided by the company.

2- Genomic DNA assessment

The removed genomic DNA from stool testers stood assessed by means of a Nano-drop spectrophotometer (THERMO. USA) to determine its purity. The assessment involved reading the absorbance at 260 nm of wavelengths and 280 nm.

3- PCR master mixture grounding

The mPCR master mix was prepared by using (**GoTaq® Green PCR Master mix**), Prepared by the Korean company Bioneer

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3 BLOOD TESTS

3.1 Serum Alanine aminotransferase (ALT)

A-Principle: α-oxoglutarate + L-alanine $\xrightarrow{ALT}$ L-glutamate + oxaloacetate

The concentration of pyruvate hydrazine produced with 2, 4- dinitrophenyl-hydrazine is used to determine ALT.

Pyruvate + (DNPH) $\longrightarrow$ Pyruvate 2, 4- dinitrophenyl hydrazone [9]

B-Procedure:

Pipette into test tubes	Reagent Blank	Sample
Serum		0.1ml
Buffer (R1)	0.5 ml	0.5 ml
Distilled water	0.1ml	
Mixed, incubated for exactly 30 minutes at 37 °C		
2,4dinitrophenylhydrazine (R2)	0.5ml	0.5ml
Mixed and let to stand for exactly 20 minutes at a temperature of 20 to 25 °C		
Sodium hydroxide (R3)	5.0 ml	5.0 ml
After 5 minutes, the absorbance of the sample was measured at (546) nm against the reagent blank (530-550 nm)		

C: Calculation:

$$\text{ALT in serum (IU/liter)} = \frac{\text{sample} - \text{control}}{\text{Standard} - \text{Blank}} * 133$$

3.2 Serum Aspartate Aminotransferase (AST)

A- Principle

α-oxoglutarate +L-aspartae $\xrightarrow{AST}$ L-glutamate + oxaloacetate

AST is measured by monitoring the concentration of oxaloacetate hydrazine. formed with 2, 4-dinitrophenyl-hydrazine [10].

B-Procedure:

Pipette into test tubes	Reagent Blank	Sample
Serum		0.1ml
Buffer (R1)	0.5 ml	0.5 ml
Distilled water	0.1ml	
Mixed, incubated for exactly 30 minutes at 37°C		
2,4dinitrophenylhydrazine (R2)	0.5ml	0.5ml
Mixed, allowed to stand for exactly20 minutes at 20 to 25°C		
Sodium hydroxide (R3)	5.0 ml	5.0 ml
Mixed, the absorbance of sample was recorded against the reagent blank after 5 minutes at 546 nm (530-550 nm)		

C: Calculation:

$$\text{AST in serum (IU/liter)} = \frac{\text{Sample – control}}{\text{Standard - Blank}} * 67$$

3.3 Alkaline Phosphatase (ALP)

In the presence of magnesium ions, *p*-nitrophenylphosphate is hydrolysed by phosphatases to phosphate and *p*-nitrophenol. The rate of *p*-nitrophenol liberation is proportional to the ALP activity and can be measured photometrically [11].

4. STATISTICAL ANALYSIS

The outcomes stayed stated as the mean minus the standard error of the mean (SEM). To compare unpaired values among all groups, a two-way investigation of alteration (ANOVA2) and Newman-Keuls post hoc examination were working. A importance flat of $P < 0.05$ was considered to conclude statistical importance. The statistical investigates stayed done by means of software (SPSS Institute, Inc., USA), following the methodology described by [12] .

5. RESULTE & DISCUSSION

5.1 Epidemiological study

The results of the current study, when conducting microscopic examination, showed that the highest infection with parasites protozoa was in the month of July, and the parasite Entamoeba histolytica recorded the highest infection among the rest of the protozoan parasites. It included 13 out of 85 samples with a ratio of (15.29) positive for parasite infection distributed among 7 (8.23) *E. histolytica*, 3 (3.52) *G. lamblia*, 2 (2.35) *B. hominis* and 1 (1.17) *C. parvum*.

While our current study recorded the lowest infection in March, as it included that 5 of 38 (13.15) were positive for intestinal parasites distributed as 2 (6.66) *E. histolytica*, 1 (5.33) *G. lamblia*, 1 (2.66) *B. hominis*,1 (2.66) *C. parvum* as shown in table (4).

These results were consistent with his findings [13] in his study on intestinal parasites that infect children in the city of Mahmoudiya, which dates back to and the capital, Baghdad, showed that the highest infection rate was during the month of July, when it reached (41%), and in the month of August. It reached (38%), but it does not agree with what [14] said in the study she conducted on children under the age of majority. At the age of eight, those who visited some health centers in some neighborhoods of the city of Diwaniyah recorded the highest infection rate. And it was during the month of March, when it reached (66.3%), while the lowest infection rate was during the month of January, when it reached. Also, (9.18%) do not agree with the study of [15] that was conducted on people who frequented Al-Hussein Hospital. year in the city of Nasiriyah, Dhi Qar Governorate, where it showed that the month of June is the highest percentage, so the percentage reached (3.96%), while the lowest percentage was in August, when it reached (19.48%).

It does not agree with the study of [16], which was conducted in the city of Diwaniyah and residents of Ghams district, we showed that the highest percentage was in the month of April when It was (5.51%) and (18.68%) in the two regions, respectively, while the lowest infection was recorded in January. They amounted to (28.14%) and (81.21%), respectively.

The reason may be the high temperatures during the summer months, when suitable conditions are created for its growth parasites and their infective stages, as well as eating juices and other foodstuffs that may be contaminated with the stages Infectious to parasites that are active in the summer.

Table 4.- It shows the distribution samples for month year

Months	Diagnosed Parasites	No. tested	No positive (%)	Total (%)	X^2	P value
March	*Entamoeba histolytica*	38	2 (5.26)	5 (13.15)	0.620	0.892
	Giardia lamblia		1 (2.63)			
	Blastocysts sp.		1 (2.63)			
	Cryptosporidium sp.		1 (2.63)			
April	*Entamoeba histolytica*	50	3(6)	7 (14)	0.211	0.646
	Giardia lamblia		2 (4)			
	Blastocysts sp.		1 (2)			
	Cryptosporidium sp.		1(2)			
May	*Entamoeba histolytica*	50	3(6)	7 (14)	2.40	0.791
	Giardia lamblia		2(4)			
	Blastocysts sp.		1(2)			
	Cryptosporidium sp.		1 (2)			
June	*Entamoeba histolytica*	52	4 (7.69)	8 (15.38)	3.12	0.373
	Giardia lamblia		2 (3.84)			
	Blastocysts sp.		1 (1.92)			
	Cryptosporidium sp.		1 (1.92)			
July	*Entamoeba histolytica*	85	7 (8.23)	13 (15.29)	6.63	0.084
	Giardia lamblia		3 (3.52)			
	Blastocysts sp.		2 (2.35)			
	Cryptosporidium sp.		1 (1.17)			
August	*Entamoeba histolytica*	75	4 (5.33)	10 (13.33)	2.06	0.558
	Giardia lamblia		3 (4)			
	Blastocysts sp.		1(1.33)			
	Cryptosporidium sp.		2 (2.66)			
Total		350	50 (14.28)			
X2	0.224					
P value	0.999					

5.2 Biochemical changes

The current study showed, after conducting a molecular examination of the positive samples, changes are found that there were changes in the results the three liver enzymes GOT, GPT and ALP that were studied compared to the control group as shown in table (5,6,7).

Table 5. - The effect of parasites at the level of GOT

Type of parasites	GOT
Entamoeba histolytica	108.6±14.5A
Giardia lamblia	74.73±10.8B
Blastocysts sp.	134.6±15.2C
Cryptosporidium sp.	90.13±12.8B
Control	29.8±9.12D
LSD(P<0.05)	19.8

***Different letters between any two means denote to the significant difference at P<0.05**

The results of the current study showed a high concentration of enzymes the three livers GOT, GPT, and ALP, as shown in table (6) and (7), This study agrees with [17] in his study on children with diarrhea coming to Al-Mansour Teaching Hospital in

the capital, Baghdad, to find out the effect of Giardia on some biochemical parameters, as he confirmed an increase in the values of the three liver enzymes, GOT, GPT, and ALP.

Table 6.- The effect of parasites at the level of GPT

Type of parasites	GPT
Entamoeba histolytica	64.4±10.6A
Giardia lamblia	40.2±19.11B
Blastocysts sp.	130.4±24.2C
Cryptosporidium sp.	105.7±24.9D
Control	28.6±4.3B
LSD(P<0.05)	21.6

***Different letters between any two means denote to the significant difference at P<0.05**

The reason for the rise in GOT and GPT enzymes may be due to the fact that some parasites, such as *Giardia lamblia*, cause diarrhea that leads to malabsorption and digestion [18]. The energy in food is not used properly, and therefore the energy stored in the liver is resorted to, which causes an increase in the level of these enzymes to replace it [19].

Table 7.- The effect of parasites at the level of Alk. Phosphatase

Type of parasites	Alk. phosphatase
Entamoeba histolytica	270.6±70.06AB
Giardia lamblia	208.13±35.1A
Blastocysts sp.	293.93±93.2B
Cryptosporidium sp.	245.9±133.8A
Control	133.2±17.08C
LSD(P<0.05)	65.9

As for the high ALP enzyme, it could be the result of diarrhea and malabsorption, which in turn leads to the loss of many minerals and salts such as calcium and sodium, and because this enzyme has a role in transporting these minerals, so its secretion increases to compensate for the deficiency in those minerals and salts [20].

5.3 Molecular diagnosis

Some pictures show the results of the molecular examination using PCR technology for samples infected with parasitic protozoa for the current study.

Genomic DNA from stool samples were extracted by using gSYAN DNA kit extraction kit (Frozen stool) Geneaid, and checked by using Nano drop spectrophotometer at (260/280 nm), it was ranging between 1.8- 2.2, with purity average equal 2, and DNA concentration mean was 20 ng/µl.

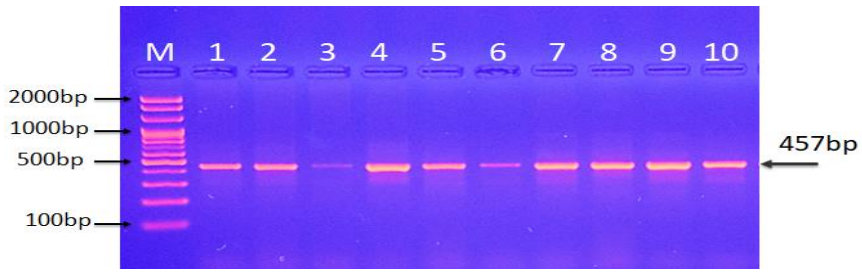

FIGURE 1.- Agarose gel electrophoresis image that show the PCR product analysis small subunit ribosomal RNA gene for detection in *Entamoeba histolytica* from Human stool isolate, where ladder (2000-100bp), and lane (1-10) were showed only positive PCR at (457p) PCR product size.

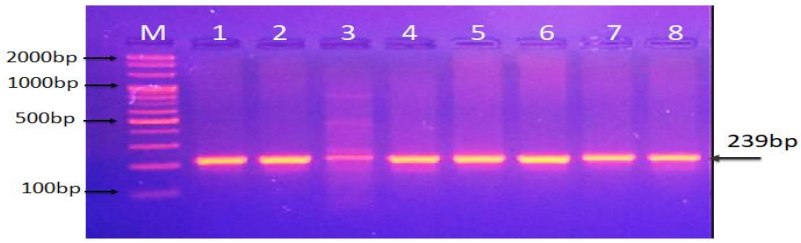

FIGURE 2.- Agarose gel electrophoresis image that show the PCR product analysis small subunit ribosomal RNA gene for detection in *Giardia lamblia* from Human stool isolate, where ladder (2000-100bp), and lane (1-8) were showed only positive PCR at (239p) PCR product size.

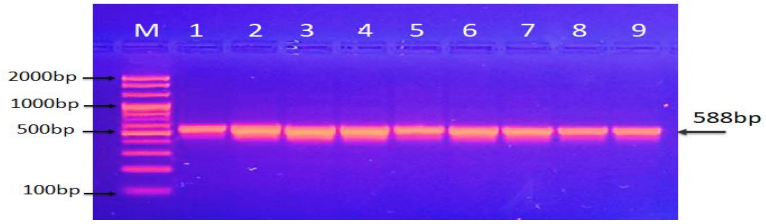

FIGURE 3.- Agarose gel electrophoresis image that show the PCR product analysis small subunit ribosomal RNA gene for detection in *Blastocystis hominis* from Human stool isolate, where ladder (2000-100bp), and lane (1-9) were showed only positive PCR at (588p) PCR product size.

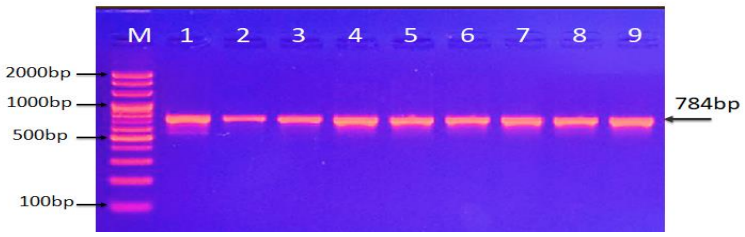

FIGURE 4.- Agarose gel electrophoresis image that show the PCR product analysis small subunit ribosomal RNA gene for detection in *Cryptosporidium parvum* from Human stool isolate, where ladder (2000-100bp), and lane (1-9) were showed only positive PCR at (784p) PCR product size.

6. CONCLUSIONS

1- The outcomes of the current study showed that the highest infection with parasites protozoa was in the month of July, and the parasite *Entamoeba histolytica* recorded the highest infection among the rest of the protozoan parasites, while our current study recorded the lowest infection in March.

2- Our results showed an increase in the concentration of the three liver enzymes GOT, GPT, and ALP.

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