Molecular and Parasitological Study on Selected Opportunistic Intestinal Parasites in Immunocompromised Patients

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Abstract

The common opportunistic parasites which cause morbidity and/or death in immunocompromised populations are predominantly gastrointestinal ones. This study explained the molecular and parasitological aspects on selected opportunistic intestinal parasites in selected groups of immune compromised patients from Al-Anbar hospitals and private laboratories.

In the current study, were taken (130) stool samples from immune compromised patients divided into three groups (50) chemotherapy recipient, (40) chronic renal failures, (40) diabetic Mellitus, and (30) samples apparently healthy as control. A questionnaire was filled out on each subject including all personal and medical history. Stool samples were collected from all groups for detection of the parasites.

The results were displayed the ratio of opportunistic parasites in (55.3)% of patients, and healthy control was (3.3)%. The results showed that the highest group had parasites in those with chemotherapy (64)%, chronic renal failure (57.5)%, and diabetic (42.5)% individually. The highest rate of infection appeared with Blastocystis hominins(26.1%), Cryptosporidium(22.3%), and Cyclospora cayetanensis (8.4%). The age of the patient was ranged between 4-66 years, the Mean was 47.5 and deviation was± 9.12, also, the weight of the patient was ranging between 14-85, the mean was 70.3 and deviation ± 8.07. Three cases of mixed infection were detected.

***Keywords**: Immuno compromised patients, Cryptosporidium, Cyclospora, Blastocystis, Opportunistic parasites, PCR.*

Introduction

The immunocompromised host is commonly defined as an individual who suffered from one or more defects in the normal defense mechanisms which keep the people from infectious, that causes predisposing the individual to an increased risk of severe life-threatening infections [1]. The particular immune response to parasites guides the generation of antibodies. The infection occurs by protozoan parasites was linked with the creation of the IgM and IgG[2]

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Intestinal parasitic infections are considered as a worldwide endemic and have been described as one of the extreme constituting worldwide causes of illness and disease[3]. An estimated about 3.5 billion individuals are affected and nearly 450 million persons suffered from these infections, the children is most of them constitute [4]. Cryptosporidiosis is a significant enteric parasitic infection between infant and children in developing countries [5;6]. Diarrhea and abdominal pain are the most important clinical signs[7]. The infection is acquired by ingestion of mature oocyte[8]. Cyclospora is the protozoan with many species and considered as an important parasite developing cause of diarrhea globally that lead to an important cause of morbidity and mortality [9]. Only Humans are reported to be infected by a single

species of this parasite, C. cayetanensis [7;8]. Blastocystis hominis is a genus containing a single-celled intestinal parasitic protist [11]. The Blastocystis species generally colonize the GIT of humans and a range of the additional animal [12].

Materials and Methods

Our study was applied on (130) of immunocompromised patients, They were categorized into three groups: (50) chemotherapy recipient, (40) chronic renal failure, (40) diabetic Mellitus and (30) control. All of the samples were subjected to direct stool and PCR examinations. The Fresh samples were collected in plastic containers, which were cleaned and labeled

The Samples were tested macroscopically. The stool samples were examined directly by wet mount (normal saline 0.85%) and staining by (MZN), after that each stool sample was kept stored in a deep freeze at (-20) until it used for (DNA) extraction and Polymerase chain reaction (PCR) according to [12;13]

In the present study, we used three sets of primers (forward and reverse) for each parasite. Final concentration according to manufacturing company Integrated DNA technologies (IDT) USA , these primers include:-

Bcowp F (5′-ACC GCT TCT CAA CAA CCA TCT TGT CCT C-3′), Bcowp R (5′-CGC ACC TGT TCC CAC TCA ATG TAA ACC C-3′)for Cryptosporidium, and F (5′ GCAGTCACAGGAGGCATATATCC-3′), R (5′-ATGAGAGACCTCACAGCCAAAC-3′) for Cyclospora , and SB82 F(5 TCTTGCTTCATCGGAGTC-3), SB82 R (5 CCTTCTCGCAGTTCTTTATC-3)for Blastocystis,. The Programs of the PCR generally according to [14] and for Cryptosporidium, Cyclospora, Blastocystis according to [15-17] respectively, with slight modifications.

Result

This study was to clarify the distribution of selected opportunistic parasites among immunocompromised patients was 74(56%) and in control 1(3.33%). According to gender there are 80 male and 50 female, the positive samples was 47(58.7%), and 27(54%) correspondingly .The ratio of the positive opportunistic parasite in immunocompromised patients was 16(26.2%) and 58(84.0%) in those of filter and tap water respectively. According to residential area, the calculation of positive sample was 24(33.3%) in urban and 50(86.2%) rural area. The percentage of the positive cases was 58 (44.6%) and 16 (35.5%), in diarrheic and non- diarrheic respectively.

The prevalence of cryptosporidium in the immunocompromised patient was 29(22.3%) distributed as follow 12(24.0%) in chemotherapy, 9(22.5%) in chronic renal failure, and 8(20.0%) in diabetic patients.

The frequency of Blastocystis hominis in the immunocompromised patient was 34(26.1%) dispersed as follow 15(30.0%) in chemotherapy, 11(27.5%) in chronic renal failure, and 8(2.5%) in diabetic patients.

The occurrence of Cyclospora cayetanensis in the immunocompromised patient was 11(8.4%) distributed as follow 7(14.0%) in chemotherapy, 3(7.5%) in chronic renal failure, and 1(2.5%) in diabetic patients.

Table (1) Total cases of immunocompromised detected by the direct exam.:

immunocompromised * direct exam Cross tabulation						
			direct exam		**Total**	**p value**
			+ve	**-ve**		
immuno-compromised	chemotherapy	Count	14	36	50	0.000
		% within immunocompromised	28.0%	72.0%	100.0%	
	chronic renal failure	Count	11	29	40	0.000
		% within immunocompromised	27.5%	72.5%	100.0%	
	diabetic	Count	10	30	40	0.000
		% within immunocompromised	25.0%	75.0%	100.0%	
Total		Count	35	95	130	
		% within immunocompromised	26.9%	73.1%	100.0%	

Table (2) Total cases of immunocompromised detected by PCR:

immunocompromised * PCR exam Cross tabulation				
	PCR exam		Total	p value
	+ve	-ve		

immuno-compromised	chemotherapy	Count	34	16	50	0.011
		% within immunocompromised	68.0%	32.0%	100.0%	
	chronic renal failure	Count	23	17	40	0.034
		% within immunocompromised	55.0%	45.0%	100.0%	
	diabetic	Count	17	23	40	0.041
		% within immunocompromised	42.5%	57.5%	100.0%	
Total		Count	74	56	130	
		% within immunocompromised	56.9%	43.1%	100.0%	

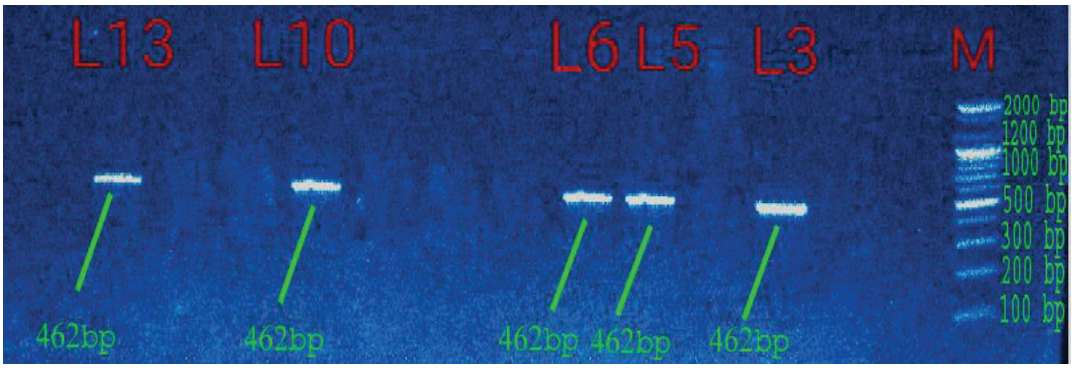

Figure (1): Agarose gel electrophoresis (2%) with ethidium bromide staining. the positive Blastocystis hominis gene (SB82) 462bp, the positive sample in line(L3,5,6,10 and 13) DNA ladder with (100-2000bp) on the right (M) was used as DNA molecular weight marker.

Figure (2): Agarose gel electrophoresis (2%) with ethidium bromide. the positive cryptosporidium

(BCOWP) gene(769bp). the positive s
in line(L3,5,11,12,13,14,and 15). DN
(100-2000bp) on the right (M) was us
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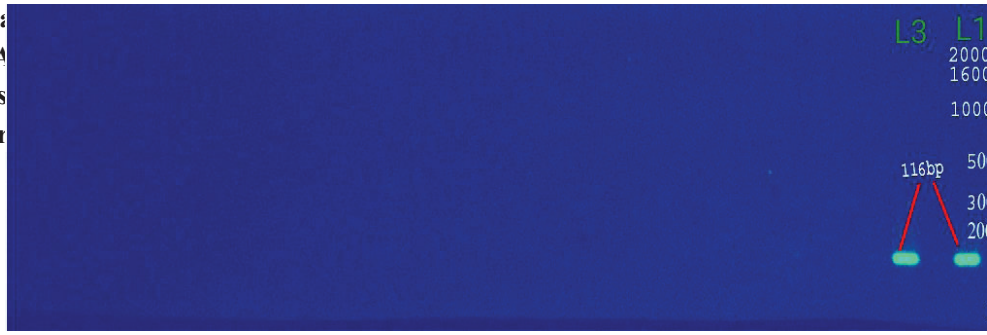

Figure (3): Agarose gel electrophoresis (2%) with ethidium bromide staining. the positive Cyclospora cayetanensis(CCITS2)gene (116bp), the positive sample was shown in the line(L1 and L3) DNA ladder with (100-2000bp) on the right (M) was used as DNA molecular weight marker.

The specificity and sensitivity test was done to determine the test's ability to identify affected and non-affected individuals correctly, the sensitivity and specificity for microscopic examination was (36.4%) , (91.1%) while for PCR examination was (68.2%), (64.4%) respectively.

Discussion

In the current study, we take (130) stool samples from immunocompromised patients were divided into three groups, (50) chemotherapy, (40) chronic renal failures, (40) diabetic Mellitus, and(30) samples from healthy people as a control. Among 130 studied patients, 80 (61.5%) were male and 50 (38.5%) were female. The results of the current study were displayed that the ratio of opportunistic parasites in (55.3)% among immunocompromised patients and (3.3)% in the healthy control. Our study was agreement with [10;18;19] , While disagreement with [20]. The diversity between the result may be due to the way used for diagnosis, group of a patient that selected for analysis, or genetic ethnic diversity.

In our study, the highest group was affected with opportunistic parasites was treated with chemotherapy (68%), and (57),(42.5) in chronic renal failure and diabetic respectively. Our result was agreed with [10;21]. Who reported the high infection with an opportunistic parasite in a patient with cancer followed by renal failure and diabetic respectively, while disagreed with [22] that reported the highest group affected was in patients with corticosteroids, followed by chronic renal failure, malignancy, and diabetic patients. The dissimilarity between results maybe because the differences in the size, number of sample, or because the advance technique used for diagnosis(researchers). In malignant patients treated by chemotherapy, found that suppression of antibody production and prevention of multiplication of the stimulated B-cells were occurred, while the immunoglobulin levels (Igs) were not affected in diabetic patients [23].

In the current study the result according to gender was covenant with [23;24;10;25] whereas disagreement with [26;27], that was found that the prevalence of parasite was higher in female than male .

Our result found that the Blastocystis hominis was the most common parasite in the immunocompromised patient which represented 26.1%, which was an agreement with [28;18] while disagreement with [29;30]. The variation between the results may be due to the difference in the area of study, selected group, difference in the residential area of the patient selected or the way used in the diagnosis (researchers).

In the present study 29 (22.3%) from (130) stool sample of immunocompromised patient was positive for cryptosporidium infection which represented the second parasite detected in our study, the highest group of an immunocompromised patient infected was those in chemotherapy (24%), and (22.5%),(20%) in chronic renal failure and diabetic respectively, the p-value was < 0.05 there was a significant difference between the Cryptosporidium and all group of an immunocompromised patient, The present study was an agreement with [31-33] whereas disagreement with [29;34]. The alteration may be due to the difference in the life style , number of samples (researchers).

In the current study from 130 stool sample found that 11(8.4%) sample was positive for Cyclospora cayetanensis, the rate of infection was distribution as 7,(14%), 3,(7.5%) and 1,(2.5%) in chemotherapy, chronic renal failure and diabetic respectively, the p-value was < 0.05 there was a significant difference between Cyclospora cayetanensis and all groups of immunocompromised patient. This result was agreement with [29;32] although disagreement with [10;30]. The differences may be attributed to environment, genetic diversity and personal hygiene (researchers).

In our study, we detected three cases of mixed infection with cryptosporidium and Cyclospora in patients with chemotherapy and one with chronic renal failure.

From 130 stool sample, the number of all positive sample that detected by (MZN) and wet mount was 35 (26.9%), in chemotherapy 14(28%) chronic renal failure and diabetic 11(27.5%),10(25%) respectively. our result

agreement with the [35 ; 36] whereas discordant with [21].

In this study, the ratio of the positive sample of opportunistic parasite by PCR in all group was 74 sample (56.9%),34(64%) chemotherapy, 23 (57.5%) chronic renal failure patient, and 17(42.5%) diabetic sample . This disagreed with [24], the variance may be due to the difference in the number of primer sequences or specificity of primer, or accuracy of PCR procedure and concentration , purity of DNA(researchers).

This disparity in the results of microscopic examination and PCR assay, Such disappointments might be below DNA levels, and presence of a solid wall prevent the release of DNA from parasite or PCR inhibitors in some of the fecal samples such as lipids, bile salts, polysaccharides from mucus, bacteria and food degradation product or disintegration of parasite during storage [37].

According to our results , we concluded that there was a significant difference between PCR and MZN stain and the most group affected with opportunistic parasites those treated with chemotherapy and Blastocystis hominis was the most prevalent parasite in immunocompromised patients.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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