Measuring Concentration of Heat Shock Protein Hsp90 and Cysteine Proteinase in Patients with Cryptosporidium and Giardia Lamblia

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Summary

The current study was conducted during 8 months and for the period from the beginning of November 2020 until June 2021, and 212 samples were collected for the auditors to Salah El-Din General Hospital and private laboratories in the city of Tikrit, and for age groups ranging from 1-60 years to investigate the spread of the parasite *Giardia lamblia* and *Cryptosporidium*, and the results showed that the infection rate of intestinal parasites was (81.13%), and the percentage of infection with intestinal parasite species was 43.86% for *G. lamblia* and 37.26% for *Cryplosporidium.,* And the rate of infection with *Cryptosporidium* was with highest percentage for age groups (40.31) (30-41) years, which amounted to 41.66%, and lowest percentage was in age group (1-10) by (30.5%), The rate of infection with Giardiasis was 43.86%, and the highest percentage of infection was 55.26% in age group (11-20) years, and the lowest percentage was in age group (51-60) years, and it amounted to 33.33., The rate of infection with *Giardia* increased during January (2021) reaching 51.42%, while percentage decreased during May, reaching 36%, and with *Cryptosporidium*, the infection increased during May, reaching 48%, and decreased in January 2021, reaching 25.71%. and recorded 34% for females and 59% for males when infected with **Giardia**, and 40.65% for females and 34.71% for males when infected with *Cryptosporidium. ,* the concentration of heat shock protein HSP90 in serum of those infected with *Giardia* was (20.5 + 4.8) compared with control group (24.8 +4.96), as well as for the concentration of heat shock protein HSP90 in serum of infected patients with *Cryplosporidium* was (18.0 + 4.6) compared with control group (24.0 + 4.89), while percentage of cysteine in serum of infected people was (6.07 + 3.45) compared with control group (7.53 + 1.08) as well. As for *Cryplosporidium*, the incidence of cysteine was (6.55 + 3.36) compared with control group (7.02 + 1.48z

Keywords: Hsp90 heat shock protein, Cysteine proteinase, Cryptosporidium, Giardiasis.

1. Introduction

Cryptosporidiosis is a widespread endemic disease common between humans and animals and Zoonotic diseases in various parts of the world (Radostits et al., 2000). Infection occurs when ingesting cysts of one of members of this genus that belongs to the coccidain, which causes severe diarrhea that may be dangerous of patient life, especially immunosuppressed patients (Huntor and Nichols, 2003).

Heat shock proteins (HSPs) play a role in pathogen resistance and are molecular chaperones necessary to maintain cellular functions by preventing misfolding of protein and facilitating protein folding (Hartl and Hayer-Hartl, 2002) not only for the folding of newly synthesized proteins. In the cell, but also to protect proteins when exposed to stressful situations, such as exposure to heat shock, where the heat shock protein helps single cells to deal with this to maintain the smooth functioning of cellular functions in the face of unfavorable conditions (Srivastava, 2008) where heat shock protein has been shown to provide the link between the innate and acquired immune systems, and its presence in the circulation serves as a danger signal to the host of foreign bodies (Srivastava, 2002).

(Mohandes et al., 2002) conducted a study on the prevalence of intestinal parasites among AIDS patients in northern India, and by examining 120 patients (53 of whom had diarrhea), it was found that the rate of infection with the Cryptosporidium parasite is 10.8%, and for G. lamblia 8.3% for dysentery amoeba 1.7%.

(Diaz et al., 2003), by examining 272 children in Mexico aged 4-2 years suffering from intestinal disorders, noted that the rates of infection with Cryptosporidium 4%, Giardia 18%, and dysentery amebiasis 10%

A study (AL-Yasaree, 2004) of some intestinal protozoa of patients and patients visiting hospitals in the city of Hilla showed a rate of infection with the Cryptosporidium parasite of 5.6%.

The study aimed to measure concentration of heat shock protein Hsp90 and Cysteine proteinase in patients with Cryptosporidium and Giardia to determine the effect of infection with these two parasites on the concentrations of proteins

2. Materials and Methods

1- Epidemiological study

The current study was conducted in Tikrit district in Salah al-Din Governorate, for period from November

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2020 to May 2021. Information was collected from the questionnaire form, which included the name, research, age and location of the included housing. Date of collection of faeces samples randomly, both sexes and age groups from 1-50 years, the number of male samples was 121 samples, while number of female samples amounted to 91 samples from patients who were confirmed to be infected with one of the intestinal parasites. During the collection, the Tourniquet and sterile medical syringes with a capacity of 5 ml were used, through which 5-4 ml of venous blood was drawn from the injured, as well as blood was drawn from healthy people used as a control group. the blood was left for 10-15 minutes at room temperature to coagulate and then the tubes were placed in a centrifuge at a speed of 3000 revolutions/min for the purpose of isolating serum and then transferring the serum to an Ependerf tube and keeping the serum at -20 degrees percentage until examinations under study are conducted

2- Laboratory tests for parasitic instars

Stool Examination: This method included the description of the shape and consistency of stool samples as soft, watery or well-formed, as this is an indication of the type of parasitic organisms available in it

Fecal samples were examined in two ways

A- Direct method: A drop of locle-iodine was placed on the glass slide, then a small amount of excrement was transferred with a wooden stick mixed together on the glass slide, the sample was spread to obtain a thin smear and left for several minutes to dry, then the cover slide was placed quietly at an angled angle, then the slide was transferred to the microscope for microscopy Using 40x and 100x magnification power (Singh et al., ., 2009).

B- The indirect method (the flotation technique): Flotation technique was used to determine the cysts of the protozoa, which depends on the difference in the specific gravity of the cysts according to the method (Dryden et al., 2005).

C - Permanent swabs dyed with Modified Zeihl - Neelson Staining Technique, following the method (Henrikson & pholen,1981)

3- Detection of Heat shock protein 90 (HSP 90)

In the current study, the ELISA test for the detection of heat shock protein (HSP 90) was used, and a test kit (Bioassay ELISA Kit, U. S. A HSP90) was used

4- Statistical analysis

The results were analyzed by applying the Minitab statistical program and the analysis of variance (ANOVA) and the T-test were used to analyze the biochemical results. As for the results of bacterial and parasitic infections for age groups, sex, months of study, location of residence and educational level, they were analyzed according to the chi-square test (X2) and the significant differences were compared. Using Duncan's polynomial test with significance levels of 0.05 and 0.01 (Al-Rawi, 2000)

3. Results and Discussion

The study showed that percentage of infection with intestinal parasites was (81.13%) of the total sample examined, and the percentage of infection with intestinal parasite species was 43.86% for G. lamblia and 37.26% for Cryplosporidium of total number of samples examined. The results of the statistical analysis showed that there were no significant differences between the infection of the parasitic species under study (Table 1).

With regard to G. lamblia parasite, the infection rate during the current study was 43.86% of total samples, and it was consistent with the study conducted by (Pam et al., 2013), and recorded (El-Ghareeb et al., 2015) in Egypt. The infection rate was 49.4%, and the study differed with what was recorded in Baghdad (Raof and Abdaal Rahman, 2011) of 17.5%, and a study conducted by (AL-Ammash, 2015) in the city of Samarra when studying the epidemiology of G. lamblia showed a difference in the infection rate. It is 22.12%.

As for Cryplosporidium, the infection rate during the current study was 37.26%, and it was consistent with the study conducted by (Pam et al., 2013), and (El-Ghareeb et al., 2015.) in Egypt recorded an infection rate of 49.4. %, and the study differed with what was recorded in Baghdad (Raof and Abdaal Rahman, 2011) in terms of infection rate of 17.5%.

The high incidence of intestinal parasites in general may be attributed to the lack of attention to general and personal hygiene, such as not washing hands well and not paying attention to washing vegetables and fruits, with the possibility of eating them without washing, water pollution, differences in geographical nature from one region to another, different climatic conditions, in addition to examination methods and age groups in The study samples, as for the similarity between the recorded infection rates and those close to the percentages that we reached in the current study, may be due to the similarity between these areas in terms of health, cultural and social levels (Long et al., 2007).

The study showed that the rate of infection with Cryptosporidium was 37.26%, and the highest percentage was for the age groups (40.31) (30-41) years, which amounted to 41.66%, and the lowest percentage was in the age group (1-10) it amounted to (30.5%),The rate of infection with Giardia disease was 43.86%, and the highest percentage of infection was 55.26% in the age group (11-20) years, and the lowest percentage of infection was in the age group (51-60) years and it amounted to 33.33 (Table 2).

As for infection with G. lamblia, a study was conducted (Ciragil et al., 2003) in Turkey, where it was found that the age groups most exposed to infection (1-14) years.

The current study showed that the rate of infection with Giardia increased during January (2021), reaching 51.42%, while the percentage of parasitic infection decreased during May, reaching 36%, and with Cryptosporidium, the infection increased during

May, reaching 48%, and the infection rate decreased in January is 2021, reaching 25.71% (Table 3).

These results were similar to what was mentioned by (Mahdi et al., 1996) At higher temperatures, the chance of infection is less because it leads to killing the active parasite, and the inability of the cysts to resist high temperatures for more than 3 days. Cryptosporidium sp is spread in all countries of the world and affects all age groups. Studies and research conducted to study the structure of this parasite around the world have shown that the prevalence rates of the parasite vary from one country to another and from one region to another according to the geographical difference of those countries, where the spread of the parasite was by 1-4% in the continent of Europe and North America, while it was 3-20% in the continents of Africa, Asia, Australia and in South and Central America (WHO 2009).

The difference in infection rates among the target age groups was clear. In this study, a decrease in infection rates was observed among children as the child's age increased until no infection was recorded for children over the age of 7 years, due to the increase in the effectiveness of the immune system in eliminating the parasite as it progressed Individual by age, identical to what was published by Al-Braiken (2013), where it was mentioned that the highest rate of infection with the Cryptosporidium parasite is in children under the age of 5 years in the Kingdom of Saudi Arabia.

The results recorded 34% for females and 59% for males when infected with Giardia, and 40.65% for females and 34.71% for males when infected with Cryptosporidium (Table 4).

The current study did not agree with the study (Hamad and Ramzy,2012) who recorded a higher incidence of intestinal parasites in females than males.

The results of the current study showed that there were significant differences between parasite infections at a probability of P≤ 0.01 between the total samples infected with parasites and the control group, as the concentration of heat shock protein HSP90 in the serum of those infected with Giardia was (20.5 + 4.8) compared with the control group (24.8 +4.96), as well as for The concentration of heat shock protein HSP90 in serum of infected patients was (18.0 + 4.6) compared with control group (24.0 + 4.89), while the percentage of cysteine in blood of infected people was (6.07 + 3.45) compared with control group (7.53 + 1.08) as well. As for Cryplosporidium, the incidence of cysteine in blood of patients was (6.55 + 3.36) compared with control group (7.02 + 1.48). The results of study showed a significant difference at the probability level of P≤ 0.01. Among the infected group with (Giardia and Cryptosporidia) and control group with the rate of HSP90 and cysteine, as shown in Table (5)

Table (1): Percentage of infection with Giardia and Cryplosporidium

Parasite's name	No. samples examined	+ samples	%
Giardia lamblia	212	93	43.86
Cryplosporidium	212	79	37.26
total	212	172	81.13
statistical analysis	Ns chi-square = 1.917 p-value = 0.166		
ns There were no significant differences between infection with parasitic species			

Table (2) Parasitic Infections by Age Groups

Age group	No. samples examined	+no. *Cryplosporidium*	%	+no. *G. lamblia*	%
10-1	48	18	37.5	23	47.91
20-11	38	13	34.21	21	55.26
30-21	39	12	30.76	16	41.02
40-31	36	15	41.66	13	36.11
50-41	24	10	41.66	11	45.83
60-51	27	11	40.74	9	33.33
total	212	79	37.26	93	43.86
statistical analysis	Ns chi-square = 2.330 p-value = 0.802				
ns There were no significant differences between infection with parasitic species					

Table (3) Distribution of infection with *Giardia* and *Cryptosporidia* according to the months of the study

months	No. samples examined	No. infected by *Cryptosporidia*	%	No. infected by *Giardia*	%
November 2020	32	12	37.5	13	40.62
December 2021	27	10	37.03	11	40.72
January 2021	35	9	25.71	18	51.42
February	39	13	33.33	16	41.02
March	29	11	37.93	15	51.27
April	27	12	44.44	11	40.74
May	25	12	48	9	36
total	212	79	37.26	93	43.86
statistical analysis	Ns chi-square = 2.330 p-value = 0.802				
ns There were no significant differences between infection with parasitic species					

Table (4): percentage of infection with *Giardia* and *cryptosporidia* by gender					
gender	No. samples examined	No. infected by Cryptosporidia	%	No. infected by Giardia	%
female	91	37	40.65	34	37.36
male	121	42	34.71	59	48.76
total	212	79	37.26	93	43.86
statistical analysis	Ns Chi-square = 1.861 p-value = 0.173				
ns There were no significant differences between infection with parasitic species					

Table (5): Measurement of shock protein and cysteine in patients with *Giardia* and *Cryptosporidia* and its comparison with control group				
group	No. infected by *Cryptosporidia*		No. infected by *Giardia*	
	Cysteine	HSP-90	Cysteine	HSP-90
infected	3.36 + 6.55	4.6 + 18.0	3.45 + 6.07	4.8 + 20.5
control	1.48 + 7.02	4.89 + 24.0	1.08 + 7.53	4.96 + 24.8
statistical analysis p-value	** 2.01- 0.0007	Ns -1.24 0.208	** 2.84- 0.0007	Ns -1.30 0.212
ns There were no significant differences between infection with parasitic species. ** There are significant differences between parasitic infections at the probability of P 0.01				

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