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Association Between Two Types of Heat Shock Proteins with Cryptosporidiosis Infection

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Abstract

THE STUDY aimed to determining the prevalence of Cryptosporidium spp. among different groups of residents in Al-Anbar Province. The Fallujah Teaching Hospital, Al-Ramadi Maternity, Children's Hospital, IDP camps, prestigious medical facilities in Al-Anbar Province, and privately held laboratories were among the medical facilities from which these individuals sought care. Children, adults, boys, and girls were represented in the 420 stool and blood serum samples that were collected and sent to private laboratories. A 14.3% Cryptosporidium spp. infection was discovered in 60 of the individuals who had been examined. Based on microscopic, 360 individuals tested negative for Cryptosporidium spp. indicating an 85.7% non-infection proportion. The study comprised 28 (46.7%) men and 32 (53.3%) women. Notably, the greatest infection incidence was seen in the age category of 1 to 14 years, with 21 people (15.3%) afflicted. Regarding residence, 37 patients (15.7%) were from rural regions, whereas 23 (12.5%) were from metropolitan areas. The study also found that Cryptosporidium spp. infections are seasonal, with the largest infection rates (31.80%) occurring throughout the summer months of April 2022, July 2022, and August 2022. Furthermore, the study used ELISA testing, which found that 22 serum samples (22.9%) were positive. IgM ELISA findings showed a 10.40% positive rate, whereas IgG results showed a 12.50% positivity rate for Cryptosporidium spp. These data were acquired by the ELISA technique.

Keywords: Cryptosporidiosis, Heat Shock Proteins, ELISA, Al-Anbar province.

Introduction

One protozoan parasite that only infects the intestinal system and is known to cause gastrointestinal disorders is Cryptosporidium. Although it was first discovered in the stomach lining of mice in 1907, it wasn't until 1976 that it was linked to infections in humans. It is ubiquitous in the environment and across many geographical locations over 40 species have been discovered and at least 20 of them have been linked to infections in humans. Nonetheless, most human cases are caused by *C. hominis* and C. *parvum* [1].

Prior until recently, the identification of Cryptosporidium oocysts in environmental, water, food, fecal or tissue samples was done under a microscope. This approach is labor-intensive and sluggish, while being economical and a vital diagnostic tool for many parasites [2]. Furthermore, when oocyst concentrations are low or when oocysts are damaged its accuracy decreases. Additionally, because of the brief, irregular shedding of oocysts, it is hard to assess the incidence of prior infections. Because ELISA targets oocyst antigens and has a better sensitivity than microscopy, it thus offers a more accurate diagnostic approach [3].

C. parvum parasites infiltrate the microvillus brush border of enterocytes in the intestines of both humans and domestic animals. The immune system which is made up of both innate and adaptive parts is essential for fighting *C. parvum* and getting rid of primary or secondary infections. The immunological response to cryptosporidiosis usually includes systemic helper T1 cells (Th1 type) activation. By starting Th1 responses that regulate the infection and using cytotoxic T-cells to eradicate the parasites, intestinal intraepithelial lymphocytes are essential in the fight against cryptosporidiosis [4].

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It was previously thought that proinflammatory cytokines including interleukin (IL)-1β and tumor necrosis factor (TNF)-α, as well as interferon (IFN)-γ, might directly affect intestinal epithelial cells (IECs) to control the proliferation of parasites [5]. IECs are becoming more and more known for their crucial function as infection sensors because of the range of proinflammatory cytokines, chemokines and antimicrobial peptides that they release. These substances may also have autocrine effects on the epithelium. Moreover, evidence shows that epithelial cells, including IECs, may generate IL-18 [6].

The induction of a protective Th1 response against a wide range of pathogens is significantly aided by IL-18. Additionally, research suggests that IL-18 may have a unique proinflammatory role as it increases the synthesis of IL-1β, TNF-α, and IL-18 in macrophages and neutrophils that have been exposed to IL-18. Prior studies have also demonstrated that macrophages produce more IL-18 in response to direct infection, and epithelial cells may express different amounts of this cytokine after infection [7].

German researchers employed HSP70 as a marker in a study on the survivability of *C. parvum* oocysts. In heat-stressed oocysts, the induction ratio of HSP70 mRNA synthesis was measured. Findings revealed a small rise in viable oocysts after 20 minutes of heat shock at 45°C suggesting that the HSP70 mRNA induction ratio changed depending on the viability of the oocysts [8]. No prior research has demonstrated a connection between the proportion of heat shock proteins (HSP40 and HSP60) and cryptosporidiosis infection. Thus, the goal of the current work is to demonstrate how infection affects the amount of heat shock proteins.

Material and Methods

Sample collection

Encompassed collecting 420 stool and blood serum samples from a heterogeneous group of patients including adults, children, males and females. These samples came from private labs well as many healthcare facilities data gathering was extended from March 2022 until February 2023. Samples of stool weighing approximately 20 grams apiece were carefully gathered and put in sterile plastic containers with tight-fitting lids to preserve moisture content and avoid drying out.

Patients were also given a questionnaire to complete to provide data for the study on their place of residence, gender, age group, occupation and any pertinent seasonal changes. The stool samples were then inspected as soon as possible after they were collected but not more than thirty minutes. Optical microscopy was used to detect several intestinal parasites using direct smear methods [9].

Blood serum samples

Approximately five milliliters of blood were drawn from subjects who were chosen at random from those from whom stool samples were taken. Plastic medical syringes were used to obtain these blood samples. After the drawn blood was put into sterile plastic test tubes and left for fifteen minutes to encourage the body's natural clotting process. Once the clotting process was complete, the blood samples were centrifuged for 10 minutes at 4000 revolutions per minute (rpm).

The blood serum was separated from other blood components using centrifugation. After carefully transferring the acquired blood serum into Eppendorf tubes, the tubes were promptly refrigerated at a temperature of -20°C in order to preserve the blood serum for further immunological testing as specified in the research [10].

Direct Wet Method

Direct smear procedures were used to examine the stool samples. For this use clean glass slides were used. A little drop of iodine stain or regular saline solution (0.9%) was placed on the glass slide. This solution was thoroughly combined with a small amount of the stool sample using a wooden stick. The produced samples were inspected under a microscope at magnifications of 40X and 100X after coverslips were put on the slides [11].

Ziehl-Neelsen Staining

A drop of distilled water was added to a little quantity of excrement, about the size of a matchstick head, on a glass slide to prepare the sample for analysis. After uniformly spreading this mixture over the slide, it was left for about ten minutes to air dry. The smear was fixed by immersing it in 100% pure methanol for five minutes and allowing it to dry. Submerging the slide in carbol-fuchsin for three minutes was the next step. Acidic alcohol was administered for 30 seconds to remove the red color, and then the area was rinsed again with tap water. The slide was then exposed to methylene blue for two minutes, followed by another rinse under tap water and drying time. Lastly, an oil immersion objective lens with 100x magnification was used to view the prepared slide under a microscope [12].

Measurement of antibodies (IgG and IgM) by ELISA

We tested these antibodies to diagnose infection, adhering to the technique as per the supplier's (Sunlong, China) instructions.

Estimate levels of Interleukins (HSP40, HSP60)

By following the manufacturer's instructions, the ELISA method was used to determine the quantities of these proteins in the blood serum samples.

Ethics approval

The authors confirm that the present work complied with all necessary protocols and has received approval from the Faculty of Applied Science, University of Fallujah. The researcher collected the blood samples from the patients in accordance with the earlier mentioned ethical standards ethics approval number; 577.

Statistical analysis

The gathered information was statistically analyzed using the Analysis of Variance (ANOVA) to identify mean differences and the Chi-square test for categorical variables. The SPSS software was used to conduct the analysis [13].

Results

In the current study, we have proposed a comprehensive method to detect Cryptosporidium spp. in blood serum and stool samples collected from a heterogeneous population comprising males, females, children, and adults. This method integrates conventional immunological techniques. These samples came from a variety of medical facilities. An overview of the total prevalence of Cryptosporidium spp. infection in Al-Anbar province is given in Table 1 of our study.

Sixty of the people who were tested had an illness, translating to an infection rate of 14.3%. On the other hand, 360 people had negative Cryptosporidium spp. tests, which means that 85.7% of them were not infected according to microscopic analysis. The gender distribution of the research participants with 32 (53.3%) female participants and 28 (46.7%) male individuals.

This pattern is clearly where children and primary school students within the age range of 1 to 14 years old have the highest infection rate shown in Table 2. However, with 7 people (17.5%), the 45–59 age group had the lowest infection rate. The P value of indicates that this difference did not reach statistical significance ($P < 0.05$). The study determined that 37 patients (15.7%) were from rural regions and 23 patients (12.5%) were from urban areas based on where they lived.

Figure 1 summarizes the study analysis of the seasonal variance in illnesses caused by Cryptosporidium spp. remarkably, the summer months of April 2022, July 2022, and August 2022 had the greatest infection rates, which totaled 31.80%. The statistical significance of this seasonal fluctuation ($P<0.05$) underscores the impact of seasonal influences on the incidence of Cryptosporidium infection.

Ninety-six blood samples were taken from people who were suspected of having a specific illness (those with digestive symptoms) and who ranged in age and gender. An ELISA analysis was performed on these samples. Only 22 serum samples, or around 22.9% of the total tested positive for the applied test, according to the data.

Additionally, when ELISA was used to evaluate the results particularly for IgM, it was discovered that almost 10.40% of the samples tested positive, whereas roughly 12.50% of the samples tested positive as shown in Fig. 2.

Moreover, a study was carried out to investigate the relationship between IgG concentration and gender and age groups. According to the data, males in the age ranges of 1–14, 15–29, and 30-44 had the greatest IgG concentrations. Furthermore, as shown in Table 3, there were statistically significant variations in IgG concentration across the 30-44 and 60-74 age groups.

Furthermore, as shown in Table 4, the connections between IgG concentrations, gender and age groups. It's interesting to note that we found that two distinct age groups those between the ages of 1, 14 and 60, 74 had rather higher IgG concentrations. Notably, compared to other age groups, we found substantial disparities in IgG concentration.

In the current study, we compared the concentration of secreted HSP40 between age groups. We found that patients in the 45–59 age group had higher concentrations with a rate of 518.455 ± 229.693) pg/ml, while those in the 60–74 age group had lower concentrations with a rate of 113.157 ± 44.462) pg/ml.

There was no significant difference between the patient and control groups in any age group, as shown in Table 5. The observed differences in concentration between age groups may be related to children's and middle-aged adults heightened immune responses, which strengthens their tolerance to heat stresses. Elderly people's immune systems are compromised, which makes them less able to react to heat stress and produce heat shock proteins and as a result, there is little heat shock protein secretion; this is what our investigation revealed.

Numerous environmental stressors, including heavy metals, ethanol, amino acid analogs, anoxia, and substances that can alter protein structure, have been shown via studies to elicit comparable reactions. The present investigation demonstrated the correlation between age groups and HSP60 concentration. Table 6 elucidates this correlation indicating that patients in the (1<-14) and (15-29) age groups had higher concentrations of HSP60, with rates of (5.690 ± 2.997) pg/mL and (6.300 ± 5.850) pg/mL respectively, while those in the 45-59 age group had lower concentrations without significant differences. The control group showed significant differences across all age groups, but not between the patient and control.

Discussion

The rates found in this study are significantly lower than the rates found in many other studies conducted in Iraq, as evidenced by earlier research in Wasit province and other locations in Iraq, which found an 18.3% parasite infection rate, and in Basra Province, which found a 23.8% parasite infection rate [14, 15]. The parasite infection rate recorded in this study is higher than the results of several other studies conducted in Iraq; previous studies in the Erbil City-Kurdistan region of Iraq reported an infection rate of 14%. It was discovered that the infection rate in Basra was 5% and in the Mid-Euphrates region it was 8.35% [16, 17].

The disparity in environmental and climatic conditions across the study areas and the presence or absence of specific animal reservoir hosts for various parasites, can significantly influence the infection rates. Additionally, factors such as the sample size, examination methods employed, and the comprehensive assessment of both pathogenic and non-pathogenic parasites play crucial roles in determining infection percentages [18].

These results align with other studies conducted in the Wasit region of Iraq, where the infection rate was 41.2% for women and 4.8% for men, with no significant differences [3]. Similarly, studies conducted in China revealed that 51.2% of women and 48.8% of males were infected [19]. The disparity in infection rates across genders maybe elated to the sorts of jobs hat men and women perform differently.

When working with domesticated animals, women may be more exposed to risk factors and may also be less conscious of the importance of maintaining good personal hygiene. Moreover, compared to men, females may be more exposed to untreated water sources, which might explain why they have a greater infection rate. This disparity may also be caused by frequent interaction with high-risk sources of infection, such as managing food waste, domestic garbage and gardening [6]. These findings are consistent with those of earlier research carried out in Taiz District [2].

Other countries, such as Yemen, where the highest prevalence of infection was observed in specific age groups, have also demonstrated comparable trends of increased infection rates, with the age group of 2 to 6 years registering at 40.3%. Furthermore, a study conducted in Ethiopia's North Shewa Zone found that people who had direct contact with animals had a higher infection rate (8.5%).20 However, it is essential to remember that some research resulted in contradicting findings. One such study, conducted in the Venda district of the Limpopo Province, South Africa, looked at the prevalence and genotype distribution of the condition in school-age children and hospital patients. The study's sample of participants in the 50–59 age bracket exhibited a remarkably high infection rate, with a frequency of 50.0% [18]. The observed discrepancies in the results may be explained by a greater vulnerability to Cryptosporidium spp. infections among those who may not be aware of appropriate food and water practices.

Certain children's behaviors raise their chance of contracting Cryptosporidium infection. These include touching contaminated food or drink, participating in activities in sewage- and soil-polluted water, frequently being near fecal-oral transmission channels and not washing your hands properly before eating. Furthermore, a significant portion of village children's drinking supplies are untreated well water, particularly in the summer when piped water is in short supply. This winter-stored untreated well water creates an environment that is favorable for the survival of Cryptosporidium oocysts and other diseases. In addition, these kids frequently eat outside of their homes and might be exposed to the parasite when working hard or in dusty conditions [14, 21].

These results are consistent with other studies carried out in different nations, including one in the Iraqi region of Wasit [20]. These, however, are not consistent with the results of esearch conducted in Iran, where the infection rate was greater in urban regions (66.7%) than in rural areas (33.3%) [17]. There are many reasons why infection rates differ between rural and urban locations.

People living in rural areas may be more exposed to environmental contamination due to the grazing of sheep and cattle, as well as the existence of farm animals that contaminate water supplies. Significant precipitation, particularly in the rainy season, may accelerate the spread of Cryptosporidium oocysts from tainted animal excrement, contaminating drinking water supplies. To lower the risk of developing a Cryptosporidium infection, preventive actions must be taken. Examples of these include limiting the grazing of farm animals near sources of drinking water and utilizing water filtration systems. These actions are required to prevent the spread of Cryptosporidium and safeguard the quality of drinking water [22–23].

The study findings demonstrated that the proportion of individuals with Cryptosporidium spp. infection changed according to heir occupation, with children having a greater infection rate than those in other jobs. As shown in Table 2, the improved acid-fast staining approach specifically revealed an infection incidence of 18.4% in preschool-aged children. On the other hand, the incidence of *Cryptosporidium* spp. infection did not change statistically significantly according to occupation. These findings are consistent with other studies carried out in the West Bank, Palestine, which found that children had an infection rate of 11.6%, and

another study carried out in Bethlehem, West Bank, which found that children with diarrhea had a prevalence rate of 13.5% [24, 25].

They do not, however, align with esearch conducted in Qatar that found that primary school pupils had a greater infection rate (7.1%) than younger children (1%). This disparity might be explained by the difficult living circumstances that kids in certain places have to deal with, as well as things like a lack of self-awareness, poor personal hygiene habits, and poor cleanliness at this crucial developmental period. Children who have these diseases are more likely to have cryptosporidiosis Some research produced different results, in the New York City Watershed, summertime C. parvum prevalence was greater (26%) than wintertime prevalence (11%), as reported in our results [26].

However, were in direct opposition to those of some other studies, such as one conducted in Jiangsu Province, China, which found that the infection rate was greater in the autumn 29.1% in that region [27]. These variations in outcomes and numerous variables might be to blame for the year-round fluctuation in Cryptosporidium infection rates.

April, July, and August are the months with the highest infection rates. Variable epidemiological variables and generally warm temperatures may be at play during these months. These variables might include slight variations in humidity and temperature as well as more readily available water, all of which provide an environment that is conducive to the sewage and animal waste contaminating drinking water sources. The ability of Cryptosporidium oocysts to survive and spread can be enhanced by warmer temperatures.

On the other hand, the low infection rates seen in January and February during the winter may be related to the sharp decrease in temperature that occurs during this time of year, which frequently reaches or falls below freezing (0°C or below). Extremely low temperatures can negatively affect Cryptosporidium oocyst viability and infectivity, decreasing the organisms' capacity to spread illnesses. It's important to remember that rotavirus infections can potentially be affected by climate change. According to other research, the months of December through April are when rotavirus gastroenteritis is most common. Summertime also brings with it an increase in leisure activities that may lead to harm and maybe even aid in the spread of illnesses, such as swimming, traveling, and park visits.[28]. In the villages investigated in the province of Al-Anbar, the overall point prevalence of IgG antibodies against *Cryptosporidium* was determined to be 12.50%. It is noteworthy that the existence of IgG antibodies against *Cryptosporidium* does not imply an ongoing infection; rather, it implies that members of the communities investigated have had a history of *Cryptosporidium* [4].

This result suggests that most people in these areas have at least one instance of *Cryptosporidium* infection. It's interesting to note that a greater incidence of Cryptosporidium antibodies than predicted has been found in certain other investigations. *Cryptosporidium* antibodies were detected in 86% of randomly sampled Australian blood donors 26% of randomly selected sera from two diagnostic laboratories in Great Britain and 17% of hospital workers (who were presumed to have had little contact) at a U.S. hospital. After the initial infection, different individuals may experience the specific IgG antibody response for varying lengths of time. In one study, for example, four participants' IgG responses disappeared after a year [29, 30].

These proteins participate in the folding, assembly, translocation, and control of host and parasite proteins mediated by chaperones. The findings of our investigation corroborated those of Munera *et al.* study on the impact of heat shock proteins on Apicomplexa in which they saw a rise in HSP40. Another study found that intestinal parasite exposure in children, young and adult results in a modest increase in HSP40 and HSP70. Exposure to the malaria parasite and other members of the same family can cause this rise [31].

Furthermore, exposure to the *Leishmania* parasite boosted the body's amount of HSP40 and HSP70 according to another study conducted in 2022. Additionally, he proved that this protein has a stronger correlation than any other microbe with the presence of parasites in the body [32]. The HSP40 family, often referred to as the DNAJ family, is a subset of the HSP family that works as a co-chaperone with the well-researched HSP70 proteins [33].

The highly conserved J domain, which HSP40 proteins use to attach to HSP70s and control their ATPase activity, is a defining feature of HSP40 proteins.34Our results were consistent with the study by Ahmed and Hussan (2009) on the immune response to heat shock protein 60 and its relationship to enteric reactive arthritis, which found that middle-aged patients with the condition had slightly elevated levels of the protein [35].

Moreover, our results were consistent with other studies that found a reduction in parasite counts in the small intestine after heat shock protein treatment, as well as the 2011 study by Kadhim and Mohaymen, which found that all patients had elevated levels of HSP60 expression and that there was no significant difference between acute and chronic cases of atherosclerotic coronary heart disease [34].

Moreover, the outcomes were inconsistent with the study conducted which found that older individuals had higher HSP60. The amount and concentration of heat shock protein are taken into consideration in a few recent studies and research projects that emphasize the importance of this protein in intestinal parasite-infected people [35].

Conclusion

The findings of our study provide valuable information on the prevalence of Cryptosporidium spp. in the province of Al-Anbar. We could not find ppreciable variations in infection rates across the age categories of patients infected with Cryptosporidium. However, we found that the infection rate in rural regions was greater than in metropolitan areas. Moreover, we discovered noticeably higher infection rates for seasonal fluctuations in April, July, and August. It's important to note that the age group of the subjects seems to have an impact on the concentration of antibodies, including IgG, IgM, and heat shock proteins.

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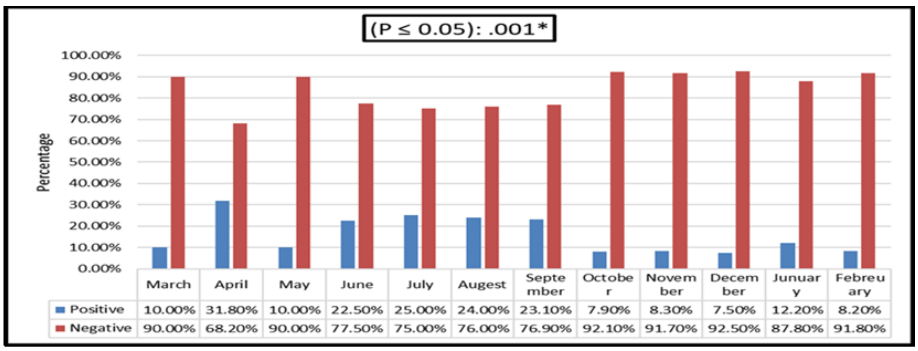
Conflict of Interest

There are no conflicts of interest.

Author's contributions

All Authors designed the entire work and worked simultaneously to collect data and statistically analyses it.



	March	April	May	June	July	Augest	September	October	November	December	January	February
Positive	10.00%	31.80%	10.00%	22.50%	25.00%	24.00%	23.10%	7.90%	8.30%	7.50%	12.20%	8.20%
Negative	90.00%	68.20%	90.00%	77.50%	75.00%	76.00%	76.90%	92.10%	91.70%	92.50%	87.80%	91.80%

Fig. 1. Illustrates the prevalence of Cryptosporidium spp. in relation to seasonal variations

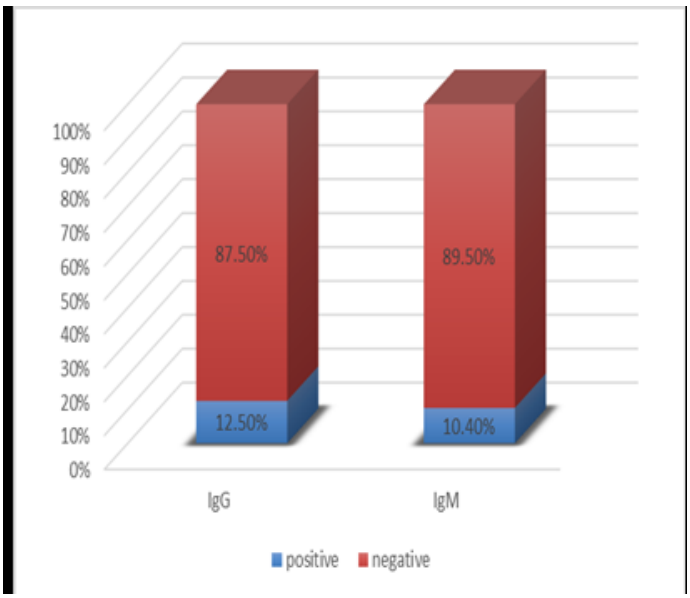


Fig. 2. The rates of IgG and IgM by ELISA test

TABLE 1. The occurrence of Cryptosporidium spp. in the Al-Anbar province

Number of samples	420
Positive (%)	60(14.3%)
Negative (%)	360(85.7%)

TABLE 2. Statistical information describing the study's participant group.

Age group (year)	**Microscopic diagnosis**	**Positive**	**Negative**	**Total**
	1<-14	21(15.3%)	116 (84.7%)	137
	15-29	10 (11.0%)	81(89.0%)	91
	30-44	13 (13.4%)	84(86.6%)	97
	45-59	7 (17.5%)	33(82.5%)	40
	60-<74	9 (16.4%)	46 (83.6%)	55
	Total	60 (14.3%)	360 (85.7%)	420 (100%)
	***P value* (P ≤ 0.05).**	.823^{NS}	.817^{NS}	.706^{NS}
Occupation	**Microscopic diagnosis**	**Positive**	**Negative**	**Total**
	Child	18 (18.4%)	80 (81.6%)	98
	Primary school	2 (6.9%)	27 (93.1%)	29
	Student	7 (12.7%)	48 (87.3%)	55
	Officer	15 (18.1%)	68 (81.9%)	83
	Housewife	10 (11.4%)	78 (88.6%)	88
	Wage earner	1(2.7%)	36 (97.3%)	37
	Retired	7 (23.3%)	23 (76.7%)	30
	Total	60 (14.3%)	360 (85.7%)	420 (100%)
		.110^{NS}	.062NS	.468^{NS}
Gender	**Microscopic diagnosis**	**positive**	**negative**	**Total**
	Male	28 (46.7%)	155 (43.1%)	183 (43.6%)
	Female	32 (53.3%)	205 (56.9%)	237(56.4%)
	Total	60 (14.3%)	360 (85.7%)	420 (100%)
	***P value* (P ≤ 0.05).**	.601^{NS}	.703^{NS}	.602^{NS}
	ODD (CI95%)	1.157 (.669 – 2.003)		
Residency	**Microscopic diagnosis**	**Positive**	**negative**	**Total**
	Rural	37(15.7%)	199(84.3%)	236
	Urban	23(12.5%)	161(87.5%)	184
	Total	60 (14.3%)	360 (85.7%)	420 (100%)
	***P value* (P ≤ 0.05).**	.356^{NS}	.434^{NS}	.353^{NS}
	ODD (CI95%)	1.302 (.743 - 2.279)		

NS: A chi-square test indicates a non-significant difference at the 0.05 level.

TABLE 3. Correlation between IgG and age groups and gender

Patients		Gender			Age group
		Male	Female	Total	
IgG concentration	Mean	1.41304	.38009	1.07885	1<-14
	Std. Deviation	.505589	.057730	.350261	
	P value ($p \leq 0.05$)	.171 NS			
IgG concentration	Mean	.63100	.60300	.61175	15-29
	Std. Deviation	.047109	.128718	.088261	
	P value ($p \leq 0.05$)	.889 NS			
IgG concentration	Mean	1.31800	.38719	.67048	30-44
	Std. Deviation	.689987	.049258	.221372	
	P value ($p \leq 0.05$)	.050*			
IgG concentration	Mean	.42733	.066884	.62825	45-59
	Std. Deviation	.69522	.220600	.167245	
	P value ($p \leq 0.05$)	.514 NS			
IgG concentration	Mean	.52767	.21460	.38536	60-<74
	Std. Deviation	.075719	.053618	.067204	
	P value ($p \leq 0.05$)	.010*			

*One-way ANOVA with $p \leq 0.05$ indicates a significant difference; NS indicates a non-significant difference.

TABLE 4. Association between IgM levels and age categories and gender.

	Patients	IgM concentration		P value
Age group	Gender	Mean	Std. Deviation	($p \leq 0.05$)
1<-14	M	0.82239	0.261547	0.937 NS
	F	0.86409	0.522556	
	Total	0.83588	0.240068	
15-29	M	0.50160	0.123325	0.331 NS
	F	0.38900	0.052033	
	Total	0.42419	0.051865	
30-44	M	0.34614	0.084680	0.718 NS
	F	0.42688	0.139427	
	Total	0.40230	0.099390	
45-59	M	0.24600	0.060086	0.260 NS
	F	.58389	.157337	
	Total	.49942	.124952	
60-<74	M	.76250	.222322	.121 NS
	F	.33080	.060966	
	Total	.56627	.137027	

NS: One-way ANOVA results indicate no significant difference under $p \leq 0.05$.

TABLE 5. The concentration of HSP40 in patients according to age groups

Age groups	HSP40 Concentration Mean ± Standard Deviation		P value ($p \leq 0.05$)
	Patient	Control	
1<-14	291.744 ± 124.557	209.787 ± 91.713	0.718 ^{NS}
15-29	500.784 ± 216.817	107.693 ± 28.495	0.224 ^{NS}
30-44	233.664 ± 111.841	107.260 ± 29.262	0.468 ^{NS}
45-59	518.455 ± 229.693	74.766 ± 1.357	0.126 ^{NS}
60-<74	113.157 ± 44.462	76.133 ± 1.656	0.218 ^{NS}
Total	313.827 ± 69.412	121.044 ± 18.295	
P value ($p \leq 0.05$)	0.503 ^{NS}	0.044 *	

* One-way ANOVA with a significant difference below $p < 0.05$; NS stands for non-significant difference.

TABLE 6. The levels of concentration of HSP60 in patients according to age groups

Age groups	Concentration of HSP60 Mean ± Standard Deviation		P value ($p \leq 0.05$)
	Patient	Control	
1<-14	5.690 ± 2.997	0.365 ± 0.014	0.333 ^{NS}
15-29	6.300 ± 5.850	1.355 ± 0.337	0.550^{NS}
30-44	0.940 ± 0.207	0.513 ± 0.0185	0.204 ^{NS}
45-59	0.680 ± 0.330	0.440 ± 0.026	0.405 ^{NS}
60-<74	0.983 ± 0.365	0.490 ± 0.065	0.309 ^{NS}
Total	3.818 ± 1.588	0.616 ± 0.107	
P value ($p \leq 0.05$)	0.695 ^{NS}	0.003*	

* One-way ANOVA with a significant difference below $p < 0.05$; NS stands for non-significant difference.

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العلاقة بين نوعين من بروتينات الصدمة الحرارية والإصابة بداء البويغات الخبيئة

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الملخص

هدفت الدراسة الحالية إلى تحديد مدى انتشار طفيل البويغات الخبيئة بين مجموعات مختلفة من سكان محافظة الأنبار. حيث جمعت العينات من المرضى المراجعين لمستشفى الفلوجة التعليمي، ومستشفى الرمادي للنسائية والأطفال، ومخيمات النازحين، بالإضافة الى بعض المراكز الصحية الطبية الموجودة في محافظة الأنبار، والمختبرات الخاصة. وجمعت العينات من الأطفال والبالغين والفتيان والفتيات في 420 عينة من البراز ومصل الدم تم جمعها وإرسالها إلى المختبرات الخاصة. وكانت نسبة الإصابة بالطفيل 60(14.3%) بناءً على الفحص المجهري، تم اختبار 360 شخصًا سلبيًا لطفيلي البويغات الخبيئة ـ مما يشير إلى نسبة عدم الإصابة بنسبة 85.7%. شملت الدراسة 28 (46.7%) من الذكور و 32(53.3%) من الأناث كانت نتيجة الفحص لديهم موجبة. والجدير بالذكر أن أكبر معدل للإصابة بالمرض شوهد في الفئة العمرية من سنة إلى 14 سنة، حيث كانت نسبة الاصابة 21 شخصًا (15.3%). وفيما يتعلق بالمنطقة السكنية، كان 37 مريضاً (15.7%) من المناطق الريفية، في حين كان 23(12.5%) من المناطق الحضارية. ووجدت الدراسة أيضًا أن طفيل البويغات الخبيئة ينتشر بصورة اكبر في موسم الصيف حيث تحدث أكبر معدلات الإصابة (31.80%) طوال أشهر الصيف في أبريل 2022، ويوليو 2022، وأغسطس 2022. علاوة على ذلك، استخدمت الدراسة فحص ELISA لاختبار العينات الموجبة والسالبة بالنسبة لهذا الطفيلي، الذي وجد أن 22 عينة مصل (22.9%) كانت إيجابية. أظهرت نتائج IgM ELISA نسبة إيجابية (% 10.40)، بينما أظهرت نتائج IgG نسبة إيجابية 12.50% لطفيلي البويغات الخبيئة. وكذلك تم اختبار مستويات نوعين من بروتينات الصدمة الحرارية عند الإصابة بهذا النوع من الطفيليات.

الكلمات الدالة: داء البويغات الخبيئة ، بروتينات الصدمة الحرارية ، تقنية الممتز المناعي المرتبط بالأنزيم ،محافظة الأنبار