

# Use of immunological methods to the detection of toxoplasmosis and heat shock protein HSP70 in men

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**Abstract** Toxoplasmosis is a contagious infection caused by the parasite *Toxoplasma gondii*, affecting many different body organs, and its effect varies depending on the location of the infection and the condition of the host's immune system, this study was the first experiment in Al-Qadisiyah governorate to detect toxoplasmosis and thermal shock protein in males. 200 male students between the ages of 18–23 years at University of Al-Qadisiyah were persuaded to perform the examination, the immunological method ELISA showed to presence of *T. gondii* in 39/200 (19.5%), (10%) positive IgG antibodies and (6%) positive for IgM, statistical analysis indicates that there are significant differences between antibody presence rates between control and infection group, due to the inaccuracy of serological tests in some cases due to the reliance on the presence of antibodies, the present study used molecular methods to examine the samples again as a step to confirmation and to reach the most accurate results, depending on the gene B1 special for the parasite by using real-time PCR technique, and the results indicated a positive proportion was 15/200 (7.5%) as modern infection, the study also assessed the level of the heat shock protein HSP70 in the positive samples of infection and showed a high concentration of HSP70 in the blood samples for infected males (28.21) ng/ml compared with control group (6.96) ng/ml, statistical analysis showed significant differences between them.

**Keywords** Real-time PCR · HPS70 · Toxoplasmosis · ELISA · Al-Qadisiyah

## Introduction

Toxoplasmosis is a contagious disease that spreads all over the world, it's a serious disease common to humans and animals (Zoonotic disease) caused by a parasite known as *Toxoplasma gondii*, it's obligate intracellular parasite and has the ability to infect various tissues in many mammals and birds (Al-abodi 2018a, b; Al-Ammash et al. 2018), the infection occurs to humans due to eating not well-cooked meat or foods contaminated with egg cysts, drinking contaminated unpasteurized milk with tachyzoites, also dealing with soil contain feces of infected cats, addition the infection occurs in other transmission methods example blood transfusion or organ transplantation (Torrey and Yolken 2013), Cats and all species belonging to a family Felidae are the final hosts of the parasite, whereas the intermediate hosts include species belonging to the class primates as human, the importance of human infection lies in the occurrence of severe cases the symptoms appear on the patient, including fever, tremors, and lymphatic dysfunction hepatitis and encephalitis that caused birth defects (Liesenfeld et al. 2001). Transplantation this parasite from the infected mother to the fetus during the placenta considered the most serious because it causes Congenital malformation of the fetus (Fallahizadeh et al. 2018), the parasite can be passed through sexual contact in warm-blooded animals such as sheep, dogs and rats, but its transmission in humans has not been established yet (Wallon and Peron 2018), the diagnosis of this disease depend on examination the body fluids by serological,

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biological and molecular methods (Singh et al. 2014; Al-abodi 2017).

Serological tests may fail to give real results due to antibodies to the parasite do not form in the host's body until several weeks, or because host's body fails to form it (Hashoosh and Majeed 2014). So molecular tests which are the latest technology are used to investigate the most accurate parasitic genetic material, the basis of these test is to detect DNA in body fluids and tissues and in case of *T. gondii* the basis according to amplify the B1 or P30 gene for parasite DNA (Belaz 2016). Real Time PCR is the most accurate and sensitive in diagnosis and it has proven to be effective in quantitative diagnosis of parasite (Su et al. 2010; Al-abodi 2018a, b), this technique uses current study to give a realistic results of the incidence of parasitic infection depending on presence of parasite.

Heat shock proteins (HSPs) are known as molecular chaperone, which preserves cellular functions by helping the newly formed peptide complexes to fold into their true protein form, it also prepares an immune response against pathogens by providing the immune system with a complete and accurate description of the body's invasive alien organism (Pramod 2008), by a formation of fingerprint antigenic, they are present in the bloodstream as hazard signals to hosts (Hori et al. 2010). These proteins are associated with the peptides derived from pathogenic antigens, whether they are cancer antigens, antiviral, viral or bacterial antigens, to form the (protein HSPs-peptide) complex that transports the antigen and on lymphocytes (T) identified with the help of MHC antigens and thus lymphocytes (T) multiply and destroy the underlying pathogen (Pramod 2008). The current study targeted men only because of the paucity of studies on the detection of parasitic infection in men in Iraq, in addition to the absence of any study of men infected with this disease in Al-Qadisiyah governorate, which made the current study is exclusively for men Donna for females, This study aimed to investigate *T. gondii* parasites in men in Al-Qadisiyah governorate, by using serological methods known as Enzyme-Linked Immune Sorbent Assay (ELISA) and molecular methods by Real -Time PCR, and to detect heat shock protein to know it's role when the disease occurs by using the serological.

## Materials and methods

*A-Collection of blood samples* (200) blood samples were collected for a random sample of males university students who volunteered to be examined at University of Al-Qadisiyah at 5 ml per sample, blood samples were collected from male students at university after wearing protective medical bra that prevents leakage of liquids, sterile gloves

and gags to avoid infection of any potential illness by donors, the samples was divided as follows: (3 ml) was isolated for extracted the serum by using a centrifuge at 3000 cycles/10 min then stored in Eppendorf-tube at temperature  $-20^{\circ}\text{C}$  until the time of ELISA testing and investigation of Hps40 (Firouz et al. 2014), (2 ml) of venous blood put in a test tube containing an EDTA and store at  $-80^{\circ}\text{C}$  until DNA extraction for molecular diagnosis (Darweesh et al. 2018; Al-abodi 2018a, b).

*B-1-Samples examinations* ELISA test was used to investigate of *T. gondii* by detect IgM and IgG immunoglobulins of the parasite in the serum depending on ELISA kit produced by USA company (Biocheck) and were according to the manufacturer's instructions.

*2-Real-time PCR technique* DNA was extracted from the whole blood samples by using *ExiPrep*™ Plus Genomic DNA Kit from (Bioneer Company. Korea), performed according to the manufacturer's instructions, after extraction DNA we went to real-time PCR (TaqMan probe) was performed for detection of *T. gondii* according to Fernanda et al. (2010), we used real-time PCR TaqMan probe and primers for amplification of conserved region B1 gene in *T. gondii* when it found in samples DNA extraction of patients, the primers and probes performed according to Al-Mosawi et al. (2015) and it's provided by (Bioneer Company. Korea), as in the following Table 1.

Real-time PCR amplification done by (AccuPower® DualStar™ qPCRPreMix Bioneer. Korea), the qPCR master mix were prepared according to company instruction.

*3-Commercial kit (HSP70 Bioassay) ELISA Kit USA* used for to estimate the concentration of heat shock protein (HSP70) According to the method ELISA Sandwich which involves the addition of samples and Standards to the drilling of the plate containing (Monoclonal antibody specific for HSP70) Taking into account the double examination of each sample, the ELISA device give values of Optical Density (OD), which represents a concentration (HSP70) and compare with the control group (Al-Mosawi 2015).

*C-Statistical analysis* Data were analyzed by the Statistical Package to Social Sciences (SPSS), Chi square (X<sup>2</sup>) were used for compare the significant differences.

## Results and discussion

The results showed that positive samples with *T. gondii* were 39/200 (19.5%) in males students under study, 20(10%) for antibodies IgG and 12(6%) for antibodies IgM, this results refers to the ancient infection more than modern infection, because when results show to IgM in samples, that's refers to (modern infection) present the

**Table 1** Real-time PCR (TaqMan) probe and primers for *Toxoplasma gondii*

Primer	Sequence		Product size
B1 primer	F	TCCCCTCTGCTGGCGAAAAGT	94 bp
	R	AGCGTTCGTGGTCAACTATCGATTG	
B1 probe	5-6FAM-TCTGTGCAACTTTGGTGTATTTCGCAG-TAMRA-3		

parasite in the time of examination, while IgG show to ancient infection (Darweesh et al. 2018), as explain in a Table 2.

This results nearly (Al-Masoudi 2015) when using molecular and serological methods to detect *T. gondii* in healthy students in Babylon province and recorded (21.4%) positive infection with toxoplasmosis by using ELISA method and (13.8%) positive for IgG and (8%) positive of IgM antibodies, recorded (27.6%) by Latex agglutination test (LAT) and (16.9%) by nested PCR, while the current results less than Bassad and Al-aboody (2015) in Thi-Qar when recorded (38.04%) positive infection (35%) for IgG and (2.7%) positive for IgM antibodies in study of them about prevalence *T. gondii* among males blood donors, and Mohamed et al. (2013) in their study about prevalence of *T. gondii* among males blood donors in Baghdad governorate and showed to (30.25%) positive infection in married men and (30%) for unmarried men, as well as Abdul-Lateef (2011) when recorded positive infection of toxoplasmosis in men reached (77.5%) for IgG and (22.5%) for IgM. All these studies agree with the current result about sovereignty the ancient infection with toxoplasmosis in men on modern infection, it is expected to show a clear difference in the rates of parasitic infection recorded by other studies due to different environmental conditions and geographical areas even within the same country, and within the same city, the reason for the difference may be due to a number of factors related to the studied samples, such as cultural level, dietary habits, age, living, as well as diagnostic methods used, size and sample type, and resistance to

different strains of *T. gondii* that play an important role in differences in infection rates (Tenter et al. 2000).

The extracted DNA samples of the patients were examined using a real-time PCR (TaqMan probe) to detect B1 gene special of the *T. gondii* parasite in the patient's DNA, the results showed the presence of the gene in 15/200 (7.5%), were Fig. 1 shows the amplification plot of the real-time PCR reaction for product positive samples for the *T. gondii* parasite diagnosis by B1 gene, where the (y-axis) represents the dye measurement unit while the (x-axis) represents the thermal cycles of the reaction.

This result higher than what recorded by Hernández-Cortazar et al. (2016) a ratio (3.84%) by quantitative PCR and (6.92%) when using nested PCR at they examined 161 blood samples for aborted women in Mexico, while lower than Darweesh et al. (2018) in Baqubah city/where they recorded (15%) infection rate by used real-time PCR, and with study Badr et al. (2016) in Egypt when they used traditional Polymerase chain reaction to identify for *T. gondii* in aborted women and they recorded (12.5%), as well as with Al-nasrawi et al. (2014) when they detected of the parasite in blood samples from aborts woman reviews for Al-Mothana hospital laboratory and record (16%) by using real-time PCR based TaqMan prob. Molecular diagnosis of toxoplasmosis is the most accurate and sensitive because the ability of this methods to diagnose the modern infection of the parasite before the body begins to form antibodies, while the serological examination depends on the formation of antibodies after several weeks from infection of the host parasite (Al-Mosawi et al. 2015; Al-

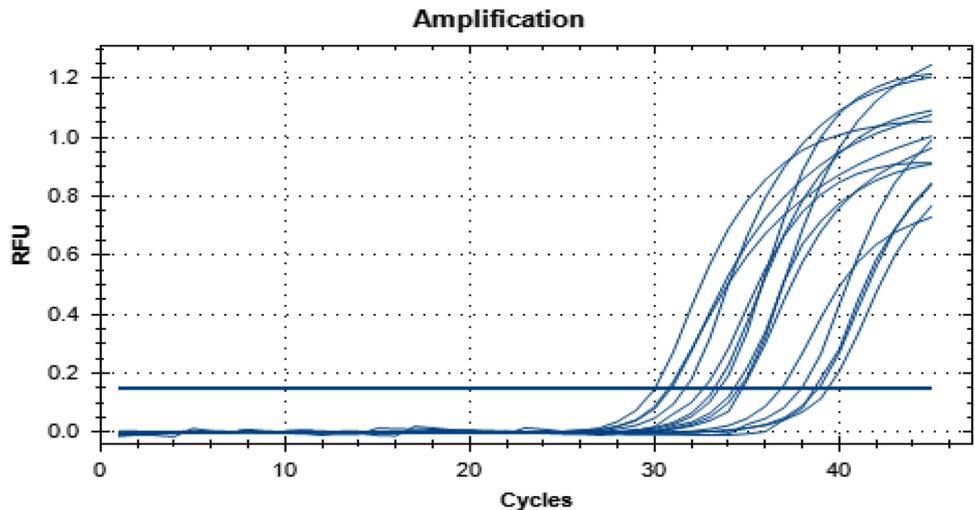
**Table 2** The percentage of distribution for males students blood donors according to ELISA IGg and IgM

Test	ELISA test					
	Positive		Negative		Total	
	N	%	N	%	N	%
<i>Subject</i>						
IgG	20	*10	180	90	200	100
IgM	12	*6	188	94	200	100

$$\chi^2 = 51.39 \text{ df} = 1 \text{ } P < 0.05$$

\*The results of the statistical analysis showed a significant difference between the percentage of IgG and IgM under the probability level  $P < 0.05$

**Fig. 1** The amplification plot of real-time PCR according B1 gene in *T.gondii* parasite from blood samples of infection men under study



abodi et al. 2015), the difference in the results of the B technique between the current study and the other studies is due to differences in methods of extraction of DNA and its methods of work and to the amount of parasite in the sample, in addition to this type of sample under study and population density and surrounding environmental conditions (Al-abodi et al. 2015).

The positive samples for *T. gondii* using molecular technique (15 samples) modern infection were subjected to the test for the concentration of the HSP70 using special kit and the results showed that the concentration of the HSP70 protein (28.21) ng/ml for infected males was higher compared with (6.96) ng/ml for the control group that consists of 10 males, the results of statistical analysis showed significant differences under level ( $P < 0.05$ ) between the concentration of HSP70 in the serum of infected males with toxoplasmosis compared with non-infected, as explain Table 3.

The results of the present study were agreed with a study (Al-Mosawi 2015) about patients with leishmaniasis as he observed rise concentration of HSP70 in serum for patients compared with healthy individuals, and with Al-Abady et al. (2011) when she showed a high HSP70 protein level in women with toxoplasmosis compared to control group,

**Table 3** The concentration of HSP70 in males with toxoplasmosis compared to the control groups

Groups	Number of samples	Mean Nanogram/ml	Standard deviation rate S.D
Infection group	15	28.21	15.11
Control group	10	6.96	4.53

T = 2.86 Df = 65 P = 0.07 A = 0.05

\*The results of the statistical analysis showed a significant difference between the concentration of HSP70 in infected and control groups under the probability level  $P < 0.05$

although HSPs are effective in living cells in normal condition, they play an essential role in difficult and emergency conditions such as severe cold, oxygen depletion, drought, and exposure to pathogens for example parasites, bacteria, viruses, and cancers, the role of these proteins appear in stimulating the body’s immune response to resist different emergency conditions (Pramod 2008), this explains high concentration of HSP70 in serum of infected males with toxoplasmosis in current study compared with control group.

The values of the protein concentration recorded in our current study ranged from (10.2 to 31.89) ng/ml, and the samples which possessed of IgG antibodies showed higher concentration of which possesses of IgM, this may be due to the fact that the samples which recorded the presence of IgG represented the chronic infection with parasite of when it does in stagnation conditions (bradyzoite phase) so that it cannot cause the disease, the HSP70 is responsible for the weak of a pathogen to prevent it from causing damage as long as the host’s immune system is healthy and effective (Dubey 2002).

HSP70 plays a large role in the resistance of *T. gondii* with the different immune cells as macrophage and T-lymphocyte cells to form the final outcome of the immune response and elimination of the parasite or transformation into the weakened state is done by associating with parasite antigens and presenting them to the antigen-presenting cells and with assistance with MHC conformational antigens are introduced to T-cells, which act as parasite resistance (William and Wetts 1995; Ajioka et al. 2002), this explains the increased HSP70 in males with toxoplasmosis currently under study due to its involvement with the immune system within the body to get rid of the parasite, it should be noted that the current study is also useful in practice in the community, where the advice was given to cases that showed positive infection and inform

them of the results and urged them to see a specialist doctor and take the necessary treatment to eliminate the disease.

## Conclusion

We conclude from the results of the study above the prevalence of toxoplasmosis among young males in recorded infection percentage (19.5%) for ancient and modern infections, and (7.5%) by using real-time PCR for modern infection only, the HPS70 was found closely with this infection and plays a large role in men body's resistance for this disease by effect on *T. gondii* parasite through increased concentration of HSP70 in the blood and playing important role in immune process within the body in cooperation with different types of immune cells in a way that kills the parasite or weakens, HPS70 in the positive samples of infection and showed a high concentration of HPS70 in the blood samples for infected males (28.21) ng/ml compared with control group (6.96) ng/ml, statistical analysis showed significant differences between them, it was noticed that the concentration HPS70 was higher in cases of chronic infection of males and may indicate that this protein is responsible for keeping the parasite in the weakened state and inactivity to curb its pathology with help immune system, as well as the current study is also useful in practice in the community, where the advice was given to cases that showed positive infection and inform them of the results and urged them to see a specialist and take the necessary treatment to eliminate the disease.

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## Compliance with ethical standards

**Conflict of interest** I announced that there is no difference in interests.

**Ethical approval** All ethical research criteria were complied with by minimizing damage and risks, increasing benefits, respecting the dignity, privacy and independence of donors, taking additional precautions with vulnerable groups.

**Informed consent** Informed consent was obtained from all individual participants included in the study, and the research was based on convincing the donors to study the personal benefit by ensuring their safety from diseases and the benefit of the community by providing data research.

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