

Investigation of heat shock protein HSP70 and measurement of some Immunomodulatory parameters in people infected with toxocariasis in Dhuluiya district / Salah al-Din Governorate / Iraq

Alhan Jassim Hamash Ashraf Jamal Mahmoud Zangana*

Coll. Of Edu. For women

Biology Dep./ Tikrit univ./Iraq

dr.ashraf_bio@tu.edu.iq*

dr.ashraf2004@yahoo.com*

Summary: 181 blood samples were collected from Dhuluiya General Hospital auditors in Dhuluiya District / Salah al-Din Governorate / Iraq, from both gender, and their ages between 5-58 years, to investigate the incidence of toxocariasis using IgG TES-ELISA technique for period from November to July. 2020, and the results showed that the percentage of serum positive samples for ELISA test reached 20.99%.

The results showed a relationship between the incidence of toxoplasmosis and age, gender, possession of dogs and cats. In female highest percentage was within age group 16-26 years and it reached 31.57%, while for males, highest percentage was within age group 48-58 years, reaching 36.36%. The highest infection rate was recorded for those who owned dogs and cats, reached 26.47%, while group that did not own dogs and cats had an infection rate of 18.58%.

level of heat shock protein HSP70 was measured, and its concentration in serum of injured reached 339 ± 210 compared to control group, 264.2 ± 45.3 .

As for the examination of interleukins, an increase in levels of interleukin-4 and interleukin-5 was recorded in serum samples for ELISA test compared with control group. For IL-4 test, percentage of infected levels was 0.864 ± 0.913 compared with levels of control group 0.2167 ± 0.0727 . As for IL_5 test, percentage of infected levels was 1.118 ± 0.990 compared with control group 0.2420 ± 0.0620 .

Key words: heat shock protein (HSP70), , IL4, IL5, Toxocariasis, epidemiology, Iraq.

Introduction: The parasitic helminthes *T.cati* and *T.canis* are classified according to (Myers *et al.*, 2020) to Kingdom: Animals, Phylum: Nematoda, Class: Secernentea, Order: Ascaridida.

Studies indicate that exposure to *Toxocara* is common, especially in children who live in tropical and subtropical regions (Rostami *et al.*,2020). (Mahmoud, 2014) conducted a local epidemiological study in Salah al-Din Governorate, where infection rate was 6.98%. Tests for seroprevalence indicated that approximately 5% of children and 50% of children in contact with animals and soil contaminated with *Toxocara* eggs or children suffering from chronic respiratory diseases and carrying antibodies to *T.* (Ban, 2019).

The general prevalence of *T.* infection was 5.14% in primary school children in Henan Province, and the prevalence was lower than other provinces in China such as Sichuan Province, 10.96% and 11.49%. (Yang *et al.*, 2017; Lu *et al.*, 2016)%

Studies have shown that the geographical location is an important factor, and the difference in rates of positive serum in different regions may be attributed to dietary habits, population density, climatic conditions, number of stray cats and dogs, and value of *T.* serum prevalence for males is approximately equal to level of females, which indicates that Exposure levels for both gender were broadly similar (Crottes *et al.*, 2015); Rostami *et al.*, 2019).

Studies have shown that prevalence of *T.* is associated with an increase in children's lifespan, and we hypothesized that this phenomenon results from an increase in number of years of exposure as children grow in a generally highly polluted environment (Zangana&Erdeni,2015; Meriguetti *et al.*, 2017; Bakhshani *et al.*, 2019)

type II immune response Th2 plays an important role in expelling the parasites that includes increased intestinal motility, fluid secretion, antibodies, mucus production. In order to achieve type II immune cytokines, intestinal epithelial cells appear to be the main source of required cytokines (Harris, 2016). Studies have revealed the ability of *T. canis* to stimulate the type II immune response, Th2, and suppress type I response, Th1 (Dlugosz *et al.*, 2015).

Th2 helper cells and eosinophils link together with protective immune response of type II parasitic worms, and when infection becomes chronic, weakening this response reduces the damage to host, and the secretion products of parasitic worms are responsible for regulating host's immunity by stimulating the regulatory B cells and regulatory T cells, which it also produces IL₁₀ (Nutman, 2015).

Italian geneticist Ferruccio Ritossa discovered the so-called Heat Shock Protein (HSP70) or Stress protein in 1962 (Wang, 2003). It was discovered when heat shock was used as a stress inducer, and this protein is found in all types of cells of living organisms in humans, animals and plants, but in different proportions from one place to another and from one cell to another, and it increases when exposed to high temperatures, and is few in cells that are not subject to stress (Morano, 2007; Mustafa, M.A & AL-Samarraie, 2020). HSP70, which has a number of similar branches and shapes, plays an important role in protecting cells from heat stress (Sung *et al.*, 2011). Its protective function on heart has been studied during heat stress (Yang *et al.*, 2017; Yin *et al.*, 2018; Mustafa *et al.*, 2020).

Materials and methods:

1- Epidemiological study:

A: location and time study: 181 blood samples were collected from Dhuluiya General Hospital auditors in Dhuluiya district / Salah al-Din governorate for period from October to December. Information was collected from people according to a questionnaire that included name, age, gender, profession, location of residence, date of samples collection, and acquisition of animals.

B: Collection of Blood Samples: Blood samples were collected by drawing 5 ml of venous blood using a Tourniquet using a medical syringe with a capacity of 5 ml. The blood was placed in laboratory tubes free of any substance and left for 15 minutes at room temperature for blood to coagulate and then placed in a centrifuge device 3500 rpm. Accurate to isolate Serum, transfer it to a bendorf tubes and keep it frozen at -20 °C until testing.

2: Tests Serological

A: The Toxocara_IgG Test: using diagnostic kit produced by China -Sunlong Biotech company to diagnosis Toxocariasis.

B: HSP70 heat shock protein assay: The concentration of heat shock protein HSP70 was measured in serum samples using ELISA technology using diagnostic kit produced by China -Sunlong Bio tech company.

C: Interleukin 4 test: The diagnostic kit produced by the manufacturer (China – Sunlong Biotech) was used to measure level of IL₄.

D: Interleukin-5 test: The diagnostic kit produced by the manufacturer (China – Sunlong Biotech) was used to measure level of IL₅.

Statistical Analysis: The results were analyzed statistically by applying T test, Chi-Squar and test of variance analysis. The arithmetic averages were compared using polynomial Duncan test with a probability level of 0.05 and 0.01 (Morgan *et al.*, 2013).

Results and discussion: The study was conducted to detect the prevalence of toxocariasis for period from November to July 2020 in Dhuluiya District / Iraq, and showed that the infection rate was 20.99% of serums of people who were examined of different age groups ranging from 5 to 58 years of age, Both gender, using ELISA-IgG test (Table 1).

Comparing the total incidence rate of 20.99% at different ages in Dhuluiya district in Salah al-Din governorate with other studies that have the same climatic conditions (hot and dry). This percentage is considered a great percentage for the prevalence of toxocariasis in Iraq and different countries. The incidence of toxocariasis in

adults is 7.3% in Iraq, and in a study conducted in Egypt in adults, the infection rate was 6.6% (Safar *et al.*, 1995), and (Mahmoud, 2014) an infection rate of 6.98% was recorded. The difference in the prevalence of *Toxocara* infection between different regions to local dietary habits, population density, climatic conditions, and the number of stray dogs and cats (Cortes *et al.*, 2015).

The highest rate of injury was found in age group 15_5 and 58_48 years, which was 29.62% for both groups, and the lowest percentage of injury was found in age group 16-25, reaching 16.07%, Table (1). When comparing the average values in units to the results of the ELISA test, the concentration of TEST-IgG antibodies against toxocariasis was found, and highest percentage was in age group 15_5 and 58-48 years, and this indicates that this age group continues to be exposed to *Toxocara* infection, which leads to presence of large numbers of live larvae that They secrete their antigens in body, which means that toxocariasis remains widespread. This may be due to the recurrence of infection, which in turn leads to the survival of antibodies, while (Bundy *et al.*, 1987) showed that there is a relationship between the elderly and young, and that the reason for this difference may be due to behavioral difference that results in many chances of exposure to infection. While playing in a polluted environment, eating raw meat, unsterilized fruits and vegetables.

This study also agrees with another study conducted in Netherlands where the incidence rate was 30% in age groups older than 30 years, while in age groups younger than 30 years the incidence rate ranged between% (Overgaauw, 1997). In Mexico, the incidence rate was 12.2% (Cortes *et al.*, 2015), in Serbia the incidence rate was 10.0% (Gabiell *et al.*, 2017), and explanation of high percentages compared to low percentages is due to thickness of the egg shell of *Toxocara*, which enables them to survive. In the environment for several years, it gives it resistance to environmental conditions in soil (Zhan *et al.*, 2015).

Table (1): percentage of people with toxoplasmosis according to age group under study.

Age group	Samples no.	Positive samples	Percentage %
5-15	27	8	29.62
16-25	56	9	16.07
26-36	41	8	19.51
37-47	30	5	16.66
48-58	27	8	29.62
total	181	38	20.99
Statistical Analysis: ns Pearson Chi-Square = 0.431 P-Value = 0.806			

The results showed the relationship of toxoplasmosis with the gender of infected persons. The highest rate of infection was recorded in males within age group 58-48 years, reaching 36.36%, and lowest rate in age group of 26-36 years was 9.09%. Injury within age group 26-36 years, reaching 31.57%, and the lowest percentage within age group 16-25 years, reaching 17.24%, and the results of the statistical analysis indicated that there are significant differences at probability level $p < 0.05$ between males and females, Table (2).

The differences in infection percentages between males and females may be due to differences in terms of playing in contaminated environment, as well as playing with animals and their relationship to the possibility of infection. On the other hand, some studies have shown that gender was not a critical risk factor associated with *Toxocara* infection. Males have the same values as females, indicating that exposure levels for both gender are similar (Cortes *et al.*, 2015), and the incidence of toxoplasmosis in males was higher than that of females aged 14 and over (Rai *et al.*, 1996).

This supports the findings of (Mahmoud, 2014) that the rate of infection in females was higher compared to the rate of infection in males, and the reason for this difference is due to the behavior of playing in polluted environment between the two genders, and that both sexes are vulnerable to infection, because they live in the same conditions. On the similarity of the studied areas and the pollutants that surround them, and this is what makes both sexes susceptible to developing toxicity, and in close proportions (Holland *et al.*, 1995) stated that males are more susceptible to infection than females. This may be due to continuous play with dogs and cats.

Table (2): relationship between age groups, gender, and people with toxoplasmosis under study.

Age group	female			male		
	Samples examined	Positive no.	%	Samples examined	Positive no.	%
15_5	11	3	27.27	16	5	31.25
25_16	29	5	17.24	27	4	14.81
36_26	19	6	31.57	22	2	9.09
47_37	17	3	17.64	13	2	15.38
58-48	16	4	25	11	4	36.36
total	92	21	22.82	89	17	19.10
Pearson Chi-Square = 4.123 P-Value = 0.047						
Statistical analysis						

The results showed the relationship between the acquisition of animals (dogs and cats) and age by using the ELISA test to detect Toxocariasis. The highest infection rate was recorded in the group that owns animals in age group 5-15 with a rate of 45.45%. The age group 26-36, when it reached 21.05%, and the results of the statistical analysis showed the presence of significant differences at a likelihood level of $P < 0.01$, Table (3).

Table (3) The relationship between age group and the possession of animals among infected with Toxocariasis under study.

	Age group	Samples examend	Positive samples	%
Ecquisition of dogs and cats	5_15	11	5	45.45
	16_25	15	3	20
	26_36	12	4	33.33
	47_37	14	3	21.42
	58-48	16	3	18.75
	total	68	18	26.47
Not ecquisition	5_15	30	6	5_15
	16_25	20	3	16_25
	36_26	19	4	36_26
	47-37	11	2	47-37
	58_48	33	5	58_48
	total	113	21	

Statistical analysis	Pearson Chi-Square = 38.253 P-Value = 0.0009**
----------------------	--

The results proved that there is a relationship between getting toxocariasis and contact with dogs and cats, and it was found that people who had a history of contact with dogs and cats showed higher tendencies to infection with *Toxocara* than others, and also the presence of eggs on animal hair may be a possible route of infection, as in some In homes, dogs and cats are acquired for various purposes and are not given full treatments and vaccinations, so when contacted frequently, the likelihood of infection with *Toxocara* increases (Shokouhi and Abdi, 2018; Bakhshani *et al.*, 2019). The results do not agree with the results of (Mahmoud, 2014), which showed through her study that there is no relationship between infection and the acquisition of dogs and cats, and the highest infection rate for those who owned dogs and cats was 7.55%, and 6.12% for those who did not possess them.

Examination of 96 blood samples of people with toxoplasmosis was performed and it was found that the concentration of heat shock protein HSP70 reached 48.27 ng / ml, and the results of the statistical analysis showed significant differences at a significant level of $P \geq 0.05$, Table (4).

The results of the current study showed that the rate of heat shock protein HSP70 concentration in the serum of people with the toxoplasmosis parasite is higher than the rate of its concentration in the serum of uninfected people, and the results of this study are in agreement with the findings of (Hassan, 2019), as it showed a high rate of heat shock protein in women. Women who have been aborted and infected with *toxoplasma gondii*.

An increase in the levels of interleukin-4 and interleukin-5 was recorded in the serum samples for the ELISA test compared with the control group. $p < 0.01$, Table (4). As for the IL_5 test, the percentage of infected levels was 1.118 ± 0.990 compared with the control group 0.2420 ± 0.0620 . The results of the statistical analysis revealed significant differences at the level of probability $P < 0.01$, as shown in Table (4).

Toxocariasis leads to elevated concentrations of IL-4 and IL_5 in these patients, this result is consistent with its findings (Zhan,2015), as it showed that toxoplasmosis leads to elevated concentrations of IL_6 and IL_10 in epilepsy patients. *Toxocara* with a high concentration of IL_10 has a low level of IL_6 which is consistent with the fact that IL_10 may have a role in reducing the production of nonspecific inflammatory cytokines.

Table (4) : the concentration of HSP70 and IL4,IL5 among infected with Toxocariosis under study.

group	IL_4	IL_5	HSP70
infected	0.864±0.913	1.118±0.990	339±210
control	0.2167±0.0727	0.2420±0.0620	264.2±45.3
T-test	4.35	5.43	1.97
P_Value	0.01	0.01	0.05

References:

- Bakhshani, A.; Maleki, M.; Haghparast ,A.;Parande Shirvan, S.; Borji ,H.(2019).** A survey on *Toxocara cati* eggs on the hair of stray cats :a potential risk factor for human toxocariasis in Northeastern Iran. *Comparative Immunology, Microbiology and Infectious Diseases*, 64, 10 _13. [CrossRef] [PubMed] [Google Scholar].
- Ban,A.S.;Assim,K.A.;Nadham,M.(2019).**Seroprevalence of *Toxocara* spp Among Epileptic Patients in Iraq/Basra.*International Journal of Academic Health and Medical Research(IJAHMR)*ISSN:2643-9824 Vol.3 Issue 8, August- 2019,Pages: 18-23.

- Bundy, D. A. P.; Tompson, D. E.; Robertson, B. D. & Coope, E. S. (1987).** Age relationship of *Toxocara canis* seropositivity and geohelminth infection prevalence in two communities in St. Lucia, west Indies. *Trop. Med . Parasitol.*, 38: 309 – 312.
- Cortes, N.N.; Nunez, C.R.; Guiliana, B.; Garcia, P.A.; Cardenas, R.H.(2015).** Presence of anti_ toxocara canis antibodies and risk factors in children from the Amecameca and Chalco regions of Mexico. *BMC Pediatrics*, 15, 65. [CrossRef] [PubMed] [Google Scholar] .
- Dlugosz, E.; Wasyl, K.; Klockiewicz, M.; Wisniewski, M.,(2015).** *Toxocara canis* micins among other excretory-secretory antigens induce in vitro secretion
- Gabrielli,S.;Tasic-Otasevic,S.;Ignjatovic,A.;Fraulo,M.;Trenkic-Bozinovic, M,Momcilovic,S.;Cancrini,G.(2017).**Seroprevalence and risk factors for *Toxocara canis* Infection in Serbia during 2015.*Foodborne Pathogens and Disease*.14(1),43-49.
- Hanson, J.; Hossain, A.; Charunwatthana, P.; Hassan, M.U.; Davis, T.M.; Lam, S.W.; Chubb,S.A.; Maude, R.J.; Yunus, E.B.; Haque, G.; White, N.J.; Day, N.P.; Dondorp, A.M. (2009).** Hyponatremia in severe malaria: evidence for an appropriate antidiuretic hormone response to hypovolemia. *Am J Trop Med Hyg*, 80 (1): 141–145.
- Harris, N., (2016).** The enigmatic tuft cell in immunity, an intestinal cell stimulates the immune response to parasitic infections. *Science* 351, 1264–1265.
- Holland , C . V .; O'orcain , P .; Taylor , M . R .& Kelly , A . (1995)** Seroepidemiology of toxocariasis in school children . *parasitology* , 110 : 535 – 545 .
- Lu, H.; Haragopal, K. G.; Slepchenko, C.; Stork, and Y. V. Li.,(2016).** “Intracellular zinc distribution in mitochondria, ER and the Golgi apparatus, ” *International Journal of Physiology, Pathophysiology and Pharmacology*, vol. 8, no. 1, pp. 35–43.
- Mahmoud,A.J.(2014).** The prevalence of Toxocariasis in Salah al-Din province with an immune attempt in laboratory mice Balb/c. Thesis, Tikrit Univ., Iraq.
- Meriguetti, Y.; Sihtirem, V.A.; Ramires, L.M.; da Silveira Batista, A.; da Costi Beserra, L.V.;Nuci ,A.L.; de Piula Ecposte, T.M.(2017) .** Protective and risk factors associated with the Presence of *Toxocara* SPP. eggs in dog hiar. *Veterinary Parasitology*, 244,39_43. [CrossRef] [PubMed] [Google Scholar].
- Morano , KA .(2007) .** New tricks for an old dog: the evolving world HSP70. *Ann. N.Y. Acad. Sci.* 1113:1-4.
- Morgan , T. M. and Case , L . D. (2013) .** Conservative sample size Determination for Repeated Measures Analysis of Covariance .*Ann Biom Biostat* , 1 .
- Mustafa, M.A., AL-Samarraie M.Q., Ahmed M. T. (2020).** Molecular techniques of viral diagnosis, *Science Archives*, 1(3), 89-92 <http://dx.doi.org/10.47587/SA.2020.1303>.
- Mustafa, M.A & AL-Samarraie, M.Q . (2020) .**SECONDARY MENOPAUSE and its RELATIONSHIP to HORMONAL LEVELS AMONG WOMEN at SALAH AL-DIN HOSPITAL . *European Journal of Molecular & Clinical Medicine* . Volume 7, Issue 09, PP 96-104.
- Myers,P.R.;Espinosa,C.S.;Parr,T.;Jones,G.S.;Hammond,and Dewey,T.A. (2020).**The Animal Diversity Web (online) . Accessed at <https:// animaldiversity. Org>.
- Nutman ,T.B.(2015).** Looking beyond the induction of Th2 responses to explain immunomodulation by helminths. *Parasite Immunol.*;37(6):304-313 on human and animal dimensions. *Iran J Vet Res* 18(4):233–242.
- Overgaauw, w. P. A.; & Nederland, V. (1997).** Aspects of *Toxocara* epidemiology: toxocarosis in dogs and cats. *Critical reviews in microbiology*, 23(3), 233-251.
- Rai, S.K.; Uga, S.; Ono, K.; Nakanishi, M.; Shrestha, H.G. and Matsumura T. (1996) .** Seroepidemiological study of *Toxocara* infection in Nepal. *Southeast Asian Journal of Tropical Medicine and Public Health* 27, 286–290.Retrieved 2017 Edited.
- Rostami, A.; Ma, G.; Wang, T.;Koehler, A.V.; Hofmann, A.;Chang, B.C.H.;Macpherson, C.N.; Gasser,**

- R.B.(2019)** .Human toxocariasis_ a look at a neglected disease through an epidemiological „prism,, .Infection, Genetics and Evolution,74, 104002 [CrossRef] [Google Scholar].
- Rostami,A.;Riahi,S.M.;Hofmann,A.;Ma,G.;Wang,T.;Behniafar,H.;Taghipour,A.;Fakhri,Y.;Spotin,A.; Chang,B.C.H.; et al.(2020)**.Global prevalence of Toxocara infection in dogs.Adv.Parasitol.,109,561-583.
- Shokouhi ,S.and Abdi.(2018)**. Seroprevalence ofToxocara in children from urban and rural areas of Ilam Province,West Iran.Osogn Public Health and Research Perspectives,9(3), 101_104.[CrossRef] [PubMed] [Google Scholar].
- Sung, Y.Y.; MacRae, T.H., (2011)**. Heat shock proteins and disease control in aquatic organisms. J Aquacult. Res. Dev. S2, 006.
- Wang, SH.; Diller ,DR.; Aggrawal ,S.J. (2003)** . Kinetics studies of endogenous heat shock protein 70 expression .J.Biomech. Eng. (125)794-797.
- Yang, F. L.; Lee, C. C. ; Subeq, Y. M. ; Lee, C. J. ; Ke, C. Y. and Lee, R. P. (2017)** . Heat adaptation from regular hot water immersion decreases proinflammatory responses, HSP70 expression, and physical heat stress. J. Ma, G., Therm. Biol. 69:95–103.
- Yin, B.; Tang,S.; Sun, J. R. ; Zhang, X. H. ; Xu, J.; Di, L. J. ; Li, Z. H.; Hu, Y. R. and Bao E. D.(2018)** . Vitamin C and sodium bicarbonate enhance the antioxidant ability of H9C2 cells and induce HSPs to relieve heat stress.
- Zangana ,Ashraf J . Mahmoud & Erdeni , Abed .Ahmad (2015)** . Contamination of soil public places & children's play grounds by *Toxocara canis* and *Toxocara cati* eggs in Salad din e Province . Kirkukniv . J Ys Scientific Studies (KUJSS) 11(1) : 92 – 103 .
- Zhan, B.;Ajmera, R.;Geiger, SM. et al.(2015)**. Identifcation of immunodominant antigens for the laboratory diagnosis of toxocariasis. Trop Med Int Health; 20: 1787–96.
- Zhang, X. H.; Zhu,H.S.; Qian,Z.; Tang,S.; Wu,D.; Kemper,N.;Hartung ,J.; and Bao,E.D. (2016)**. The association of Hsp90 expression induced by aspirin with anti-stress damage in chicken.myocardial cells. J. Vet. Sci. 17:35–44.