

## Evaluation of liver function, Vit D3, and some hematological parameters in pregnant women infected with *Toxocara*

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### Abstract :

In the past few years, toxocariasis has gained increasing global attention. It has been listed among the five most neglected parasitic infections, according to the US Center for Disease Control and Prevention. This study aims to investigate the liver function, Vit D3, and hematological parameters in pregnant women with *Toxocariasis*. The study was conducted in the Dhuluiya district / Salah Al-Din Governorate from October 2021 to July 2022, and the samples were collected from pregnant women with *Toxocariasis* and healthy women as a control group. The results showed the prevalence of toxocariasis in pregnant women with positive cases (14%), and the negative cases was 86 % ( $P < 0.05$ ). The results showed slightly elevated ALT enzyme ( $11.17 \text{ U/L} \pm 0.19$ ) in positive cases with no significant differences ( $P > 0.05$ ). While AST enzyme was low in positive infections ( $8.83 \text{ U/L} \pm 1.2$ ). Further, the level of calcium in the positive ( $8.917 \text{ mg/dl} \pm 0.16$ ) of toxocariasis was low. Also, the level of vitamin D3 in the positive cases ( $16.3 \text{ ng/dl} \pm 1.0$ ) was decreased ( $P > 0.05$ ). There was an increase in white blood cells in the infected samples ( $10.17 \times 10^9 \mu\text{l} \pm 0.73$ ). Also, red blood cells were ( $4.250 \times 10^9 \mu\text{l} \pm 0.095$ ) in infected samples. The number of platelets was ( $271.0 \text{ ml} \pm 4.7$ ) in infected samples. Haemoglobin in the infected samples was ( $11.6 \text{ gm/dl} \pm 0.31$ ). The number of lymphocytes in the infected samples was ( $2.320 \times 10^3 \mu\text{l} \pm 0.13$ ). The number of granulocytes in infected samples was ( $7.41 \times 10^3 \mu\text{l} \pm 0.67$ ). No significant differences were shown for blood variables in the current study ( $P > 0.05$ ). While the number of eosinophil cells in infected samples was ( $25.7 \times 10^3 \mu\text{l} \pm 0.67$ ) with significant differences ( $P < 0.05$ ). In conclusion, the levels of liver enzymes and eosinophil cells were high, while the levels of calcium, and vitamin D3, were low. This indicates the occurrence of physiological changes in pregnant during *Toxocara* infection.

**Keyword:** *Toxocariasis*, liver function, Vit D3, hematological parameters, pregnant women.

## تقييم وظائف الكبد وفيتامين D3 وبعض المتغيرات الدموية لدى النساء الحوامل المصابات بـ *Toxocara*

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### مستخلص

في السنوات القليلة الماضية، اكتسب داء السهميات اهتماماً عالمياً متزايداً. وقد تم إدراجه ضمن أكثر خمس حالات عدوى طفيلية مهملة، وفقاً للمركز الأمريكي لمكافحة الأمراض والوقاية منها. هدفت هذه الدراسة إلى التحري عن وظائف الكبد وفيتامين D3 والمتغيرات الدموية لدى النساء الحوامل المصابات بداء السهميات. أجريت الدراسة في قضاء الضلوعية / محافظة صلاح الدين خلال الفترة من تشرين الأول 2021 إلى تموز 2022، وتم جمع العينات من النساء الحوامل ونساء اصحاء كمجموعة سيطرة. أظهرت النتائج انتشار داء السهميات كانت الحالات الموجبة لدى النساء الحوامل (14%)، والحالات السلبية 86 % ( $P < 0.05$ ). أظهرت النتائج ارتفاع طفيف في إنزيم ALT ( $11.17 \pm 0.19$ ) وحدة/ لتر في الحالات الموجبة ولا توجد فروق معنوية ( $P > 0.05$ ) بينما كان إنزيم AST منخفضاً في الإصابات الموجبة ( $8.83 \pm 1.2$ ) وحدة/ لتر. كما أن مستوى الكالسيوم في العينات الموجبة ( $8.917 \pm 0.16$ ) ملغم/ ديسيلتر لداء السهميات كان منخفضاً. كان مستوى فيتامين D3 منخفضاً في الحالات الإيجابية ( $16.3 \pm 1.0$ ) نانوغرام / ديسيلتر ( $P > 0.05$ ) وكانت هناك زيادة في خلايا الدم البيضاء في العينات المصابة ( $10.17 \pm 0.73 \times 10^9$ ) ميكرو لتر. ، كما كانت خلايا الدم الحمراء ( $4.250 \pm 0.095 \times 10^9$ ) ميكرو لتر في العينات المصابة. أما بالنسبة لعدد الصفائح الدموية فقد بلغ ( $271.0 \pm 4.7$ ) مل في العينات المصابة. وكان الهيموكلوبين في العينات المصابة ( $11.6 \pm 0.31$ ) غم/ ديسيلتر. وكان عدد الخلايا الليمفاوية في العينات المصابة ( $2.320 \pm 0.13 \times 10^3$ ) ميكرو لتر. وكان عدد الخلايا المحببة في العينات المصابة ( $7.41 \pm 0.67 \times 10^3$ ) ميكرو لتر. لم تظهر فروق معنوية لمتغيرات الدم في الدراسة الحالية. بينما بلغ عدد الخلايا الحمضة في العينات المصابة ( $25.7 \pm 0.67 \times 10^3$ ) ميكرو لتر مع وجود فروق معنوية ( $P < 0.05$ ). نستنتج من ذلك أن مستويات إنزيمات الكبد والخلايا الحمضة كانت مرتفعة، بينما كانت مستويات الكالسيوم وفيتامين D3 منخفضة. وهذا يدل على حدوث تغيرات فسيولوجية لدى الحامل أثناء الإصابة بـ *Toxocara*.  
الكلمات المفتاحية: داء السهميات، وظائف الكبد، فيتامين D3، المتغيرات الدموية، النساء الحوامل.

## Introduction:

*Toxocara* is a nematode belong to phylum Nematoda, class Chromadorea. *Toxocara* infection in humans was first detected in 1950[1]. It has two species *T.canis* and *T.cati*, and its final hosts are dogs, cats, foxes, and wolves [2]. Humans are at risk of toxocariasis because they often come into direct contact with dogs and cats. Dogs and cats are among the most common pets worldwide[3]. Young dogs and cats appear to become infected via milk during lactation [4], and *Toxocara* has been found to be transplacental in dogs[5]. The eggs hatch when ingested by the host. The larvae are released and migrate to other tissues and organs, where they remain encapsulated in the third larval stage. In humans, larval migration results in ocular larval migrants (OLM) or visceral larval migrants (VLM) [6]. Helminthiasis infection during pregnancy may be associated with maternal anemia, susceptibility to inflammatory diseases, and the possibility of affecting the immune response of the fetus. Studies that specialized in studying the occurrence of toxocariasis during pregnancy have shown that it may be rare, as well as research on

the potential danger effects of these parasites on reproductive health. In addition, there is little information about the risk of infection to the mother and fetus during pregnancy[7]. In the past few years, toxocariasis has gained increasing global attention. It has been listed among the five most neglected parasitic infections, according to the US Center for Disease Control and Prevention [8]. The current study aims to investigate the liver function, Vit D3 and hematological parameters in pregnant women infected with *Toxocara* IgG.

## Material and Methods

### Sample collection

The study was conducted in the Dhuluiya district / Salah Al-Din Governorate from October 2021 to July 2022, and information was collected from pregnant women based on a questionnaire that included: name, age, sex, profession, residence location, date of sampling, and animal acquisition. As for the collection of samples, it was done in the laboratory of Al-Duluiya General Hospital and the modern medical laboratory. Blood samples were collected by drawing 5 ml of venous blood with a medical syringe, blood

was placed in laboratory tubes, serum was separated through centrifugation at 2000-3000 rpm for 20 minutes, then transferred to Eppendorf tubes and kept frozen at 20 °C.

### Detection of *Toxocara* IgG

It was conducted by ELISA device (Bio Tik /USA ) using SunLong /China ELISA kit.

### Measurement function of liver enzymes

Aspartate Amino Transferase (AST) and Alanine Amino Transfers Activity (ALT) were measured using Biolabo/ France / ELISA kit by ELISA device (Bio Tik /USA ).

### Measurement of Ca and Vit.D3

Calcium was measured using Assel/ Italia/ELISA kit by by ELISA device (Bio Tik /USA ) and Vit.D3 was measured using CL-900i device Mindary/ China.

### Examination of Complete blood picture(CBC)

CBC was examined by Autoanalyz-er swelab/ Sweden

### Statistical analysis

The results were analyzed statistically by applying the T test, the Chi-square test, and the analysis of variance test (F test), and the means were compared using the Duncan test, with a probability level of 0.05 and 0.01.

## Results:

### *Toxocara* IgG detection

The results of the ELISA test for the prevalence of toxocariasis in pregnant women from the city of Dhuluiya, as shown in Table (1) that the percentage of positive cases was 14%, and the percentage of negative cases was 86%, with significant differences at the level of probability  $P < 0.05$ .

**Table (1) Percentages of cases of toxocariasis in the study group**

Samples	Number	Percentage	Mean±SE
Positive samples	14	14%	0.441 ± 0.096
Negative samples	86	86%	0.231 ± 0.064
Total	100	100%	
T-Value = -1.98 P-Value = 0.04			

### Measurement function of liver enzymes

The results showed that the level of liver enzymes in toxocariasis-positive infections was slightly elevated in relation to the ALT enzyme ( $11.17 \text{ U/L} \pm 0.19$ ) with no significant differences compared with negative samples

( $10.39 \text{ U/L} \pm 0.43$ ) ( $P > 0.05$ ) as shown in Table (2). While there were significant differences for the AST enzyme, and its levels were low in positive infections ( $8.83 \text{ U/L} \pm 1.2$ ) compared with negative samples ( $17.9 \text{ U/L} \pm 2.2$ ) at a probability level ( $P < 0.05$ ) as in Table (3).

**Table (2) ALT level in toxocariasis patients compared with non-infected patients**

Samples	MeanU/L±SE
Positive samples	$11.7 \pm 1.9$
Negative samples	$10.39 \pm 0.43$
T-Value = -0.41 P-Value = 0.698	

**Table (3) AST level in toxocariasis patients compared with non-infected patients**

Samples	MeanU/L±SE
Positive samples	$8.83 \pm 1.2$
Negative samples	$17.9 \pm 2.2$
T-Value = 3.60 P-Value = 0.001	

### Measurement of Ca and Vit.D3

The results showed that the level of calcium in the positive cases ( $8.917 \text{ mg/dl} \pm 0.16$ ) of toxocariasis was slightly low with no significant differences compared with the negative samples ( $8.973 \text{ mg/dl} \pm 0.075$ ) of toxocariasis

( $P > 0.05$ ) as shown in Table (4). The results showed that the level of vitamin D3 in the positive cases ( $16.13 \text{ ng/dl} \pm 1.0$ ) of toxocariasis was low with no significant differences compared with the negative samples ( $19.5 \text{ ng/dl} \pm 1.7$ ) of toxocariasis ( $P > 0.05$ ) as in Table (5).

**Table (4) Calcium level in toxocariasis patients compared with non-infected patients**

Samples	Mean mg/dl±SE
Positive samples	$8.917 \pm 0.16$
Negative samples	$8.973 \pm 0.075$
T-Value = 0.31 P-Value = 0.763	

**Table (5) Vit.D3 level in toxocariasis patients compared with non-infected patients**

Samples	Mean ng/dl±SE
Positive samples	16.13 ± 1
Negative samples	19.5 ±1.7
T-Value = 0.98 P-Value = 0.358	

### Examination of Complete blood picture(CBC)

The results showed an increase in the number of white blood cells in the infected samples ( $10.17 \pm 0.37$ ) compared to the uninfected samples ( $8.81 \pm 0.73$ ), but there were no significant differences ( $P > 0.05$ ). Also, for red blood cells, it reached ( $4.250 \pm 0.095$ ) in infected samples compared to ( $4.089 \pm 0.061$ ) in uninfected samples. As for the number of platelets, it reached ( $271.0 \pm 4.7$ ) in infected samples compared to ( $239.1 \pm 3.7$ ) in uninfected samples. Haemoglobin in the infected samples

was ( $11.6 \pm 0.31$ ) compared to ( $11.3 \pm 0.29$ ) in the uninfected samples. The number of lymphocytes in the infected samples was ( $2.320 \pm 0.13$ ) compared to ( $2.205 \pm 0.095$ ) in the uninfected samples. The number of granulocytes in infected samples was ( $7.41 \pm 0.67$ ) compared to ( $6.19 \pm 0.32$ ) in uninfected samples. No significant differences were shown for blood variables in the current study ( $P > 0.05$ ). The number of eosinophil cells in infected samples was ( $25.7 \times 10^3 \mu\text{l} \pm 0.67$ ) compared to ( $15.1 \times 10^3 \mu\text{l} \pm 0.32$ ) in uninfected samples with significant differences  $P < 0.05$ .

**Table (6) The level of blood changes in patients with toxocariasis compared with non-infected patients**

Count cells	Samples	Mean±SE	P-Value
WBC×10 <sup>3</sup>	Positive samples	10.17± 0.73	0.140
	Negative samples	8.81± 0.37	
RBCs×10 <sup>9</sup>	Positive samples	4.250± 0.095	0.187
	Negative samples	4.089± 0.061	
Platelets /ml	Positive samples	271.0± 4.7	0.554
	Negative samples	239.1± 3.7	
Hemoglobin gm/dl	Positive samples	11.6± 0.31	0.497
	Negative samples	11.3± 0.29	
Lymphocyte ×10 <sup>3</sup>	Positive samples	2.320± 0.13	0.488
	Negative samples	2.205± 0.095	
Granulocyte×10 <sup>3</sup>	Positive samples	7.41± 0.67	0.144
	Negative samples	6.19± 0.32	
Eosinophil×10 <sup>3</sup>	Positive samples	25.7± 0.6	0.03
	Negative samples	15.1± 1.5	



### Discussion :

In global epidemiological surveys conducted for *T. canis* infection in humans in the past 36 years, the prevalence of anti-*T. canis* sero-antibody was estimated to be 1.6% in Japan, 2.4% in Denmark 6.3% in Austria, 7% in Sweden, and 14%. % in the USA, and 19.6% in Malaysia. The seroprevalence is more than 20% in some groups depending on the socioeconomic situation in Iran, for example, the seroprevalence rate of *Toxocara* infection in humans is 22%, while in Nepal 81% of the population is infected. It is worth noting that the seroprevalence rate in Indonesia was 85% [9]. The prevalence of toxocariasis varies greatly by geography and climate, ranging from 1.4% among nomadic people in Iran [10] to 92.4% of pregnant women in Nigeria [11].

A study conducted in Kuwait showed that the seroprevalence rate was lower than expected among Kuwaiti patients (0.7%) compared to non-Kuwaiti patients (14.0%) ( $p < 0.001$ ) from South/Southeast Asian countries. As seven out of eight patients (87.5%) were seropositive and had a history of direct contact with cats, dogs or both. The seroprevalence rate of toxocaria-

sis among allergic patients in Kuwait was 2.5%. Raising awareness, early treatment, and prevention of worms in small dogs and cats is still crucial for the prevention of toxocariasis [12]. Another study showed that the percentage of pregnant women positive for anti-*Toxocara* spp IgG was 12%, where it was 12%. The percentage of contacts with cats and dogs was 8% [13].

The results of the current study in terms of the prevalence of toxocariasis were similar to other studies conducted in Iraq, as a study conducted in the city of Kirkuk showed that the prevalence of *Toxocara canis* and *Toxocara cati* infection was high in stray dogs and cats by 25.98 and 39.58%, respectively [14].

A study of epidemiological data in Iran for the years (1969-2019) showed that there is a high prevalence rate of *Toxocara*, especially in cats and dogs in the northern parts of Iran. The presence of suitable animal hosts, optimal climate, and close contact between humans and animals was the reason for the high seroprevalence rates of human cases [15].

Liver infection is a major complication of toxocariasis and can lead to liver dysfunction and may eventually

alter the functioning enzymes of this organ[16]. Evaluation of common liver enzymes in the blood is a diagnostic guide for some liver-related diseases. The most common changes in the levels of liver enzymes are two types in hepatocytes and bile and in general, in the type of hepatocytes, ALT and AST levels may be elevated [17].

In some case studies of toxocariasis, blood biochemical tests were performed including measurement of liver enzymes. In most studies, the enzyme level was normal [18], but in some studies, an increased level of some liver enzymes, especially ALT and ALP, was observed [19].

As there are no previous studies showing the effect of toxocariasis on the level of calcium and vitamin D3, except for a study conducted by Murad *et al.* [20] in their study in the city of Dohuk showed out of 150 pregnant women, only 18 pregnant women (12%) had a positive serological result for anti-*Toxocara* antibodies. Also, 10 out of the 18 infected pregnant women (55.5%) had a vitamin D3 deficiency. While 132 pregnant women were negative for antibodies, 69 out of 132 (52.3%) pregnant women had vitamin D3 deficiency.

Vitamin D3 deficiency has been associated with an increased risk of pregnancy complications, including pre-eclampsia, impaired glucose tolerance, increased rate of cesarean section, neonatal complications including low birth weight, hypocalcemia in newborns, poor skeletal and lung growth, and decreased immunogenicity [21]. Where during pregnancy, a remarkable series of physiological changes occur, aimed at maintaining the physiological balance of the mother while simultaneously supporting the growth and development of the fetus. These include changes that have direct effects on calcium metabolism, increased kidney function, and calcium transport from the placenta. Maintaining calcium homeostasis is a complex process involving the availability of calcium and three hormones: parathyroid hormone, calcitonin, and 1,25-dihydroxyvitamin D3 [22].

Calcium homeostasis contributes to a number of cellular processes that determine the short- and long-term function of lymphocytes. In some studies, it was shown that calcium channels and transporters play a major role in balancing cytoplasmic calcium levels in cells. Therefore, modifying the calcium

balance in lymphocytes is a key factor in combating immune disorders [23]. Another study conducted in the city of Dohuk showed that hematological variables of white and red blood cells, hemoglobin, blood platelets, and lymphocytes were increased in pregnant women with toxocariasis compared to non-infected pregnant women [20].

Parasites can affect the efficiency of normal T regulatory lymphocytes by modulating the immune response of T lymphocytes at the site of infection, thus spreading the infection in the host for a longer period [24]. The immune response against the parasite leads to antibody production. Infection with helminths is associated with the production of IgG and IgM. Antibody-dependent cytotoxicity has been shown to play a role in infections caused by a number of parasites. Responder cells (macrophages, monocytes, neutrophils, eosinophils, and natural killer cells) bind to antibody-coated parasites via specific receptors [25]. Ultimately, toxocariasis may affect in the physiological parameters in pregnancy.

## Conclusions

Prevalence of toxocariasis was observed in pregnant women from Dulaiya City within the study group. A slight increase in the level of liver enzymes occurred in pregnant women positive for the *Toxocara* IgG test. While, there was a decrease in the hematological parameters, except, eosinophil cells were increased. So toxocariasis may affect the hematological and biochemical parameters of pregnant women during pregnancy.

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## Conflict interest

There is no Conflict of interest with other article.



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