Serological Study of Toxocariasis in Children in Mosul Province

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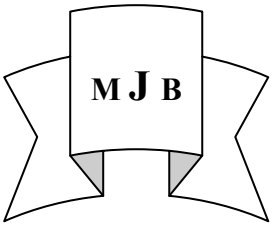

Abstract

This study was done to determine the seroprevalence of *Toxocara* infection in children by using ELISA test.

Blood samples were collected from 120 hospitalized children with age ranging from [1-15] years, and [25] children (apparently healthy) served as controls.

Serum samples of 120 children [66 males and 54 females] and 25 healthy controls were examined for anti-*Toxocara* IgG antibody by a commercial ELISA test. The seropositivity rates were 30.8% and 12% in diseased and healthy children respectively. No statistical differences were observed with regard to age and sex. Diagnosis of sera from children with geophagia in urban and rural areas revealed that 55.2% were seropositive for *Toxocara* IgG antibody. Haematological parameters in children showed that the highest ESR rate and lowest Hb% was frequent in 1-2 and 3-5 years old respectively. Eosinphilia was statistically frequent in 3-5 year old group.

Conclusion

Toxocara seroprevalence suggest that toxocariasis is frequent in children in this community.

دراسة مصلية لداء السهميات فى الاطفال فى محافظة نينوى

الخلاصة

اجريت هذة الدراسة لتحديد انتشار عدوى داء السهميات فى الاطفال بأستخدام فحص الربط الانزيمى الامتصاصى المناعى.

تم جمع نماذج الدم من ١٢٠ طفل داخل المستشفى يتراوح اعمارهم بين (١-١٥) سنة، وكذلك من ٢٥ طفل اصحاء على مايبدو كسيطرة .

أُخذت نماذج المصول لـ (١٢٠) طفل (٦٦ ذكور و ٥٤ إناث) وكذلك من (٢٥) طفل غير مريض ، أُختبر جميعهم لوجود أضداد الديدان السهمية نوع (الأضداد المناعية جي) باستخدام سيت اختبار الربط الأنزيمي الامتصاصي المناعي . إن نسبة الإيجابية المصلية كانت ٣٠,٨% و ١٢% فى ألاطفال المرضى والاصحاء على التوالى. وقد لوحظ عدم وجود فرق معنوي إحصائي بين مختلف الأعمار وكذلك بين كلا الجنسين .

إن التشخيص المصلي للأطفال الذين يأكلون التربة في المناطق الحضرية والريفية قد أُعطوا نتيجة مصلية موجبة لوجود الأجسام المناعية من نوع الأضداد جي ضد الديدان السهمية بنسبة ٥٥,٢% .وقد أظهرت القراءات الدموية في الأطفال أن أعلى نسبة لفحص ترسيب الدم كان غالباً في الفئة العمرية (١-٢) سنة وأقل نسبة للهيموغلوبين لوحظ في الفئة العمرية (٣-٥) سنوات. إن ارتفاع نسبة الخلايا الحمضية في الدم غالباً ما كان ضمن الفئة العمرية (٣-٥) سنوات وهي ذات أهمية معنوية إحصائياً .

الاستنتاج

الانتشار المصلي السهمي يوحي بأن داء السهميات غالباً ما ينتشر في الأطفال في مجتمعنا .

Introduction

Toxocariasis is an infectious disease caused by the accidental ingestion of infective egg of the dog roundworm Toxocara canis and less frequently cat roundworm Toxocara cati that usually live in the intestine of dogs and cats [1,2]. When they infect humans, the

illness is called toxocariasis, toxocarosis or visceral larva migrans VLM [3,4]. Toxocariasis usually affects children under the age of ten years [5]. The children at risk are those who like to put things in their mouths or those whose families own pet dogs or cats [6].

Young children (2-9 years) are most susceptible to infection with this organism, because they are more likely to ingest the eggs of the worm from contaminated soil or by direct contact with infected puppies [7].

There are three major clinical syndromes associated with human toxocariasis, visceral larva migrans (VLM), ocular larva migrans (OLM) and covert toxocariasis CT (2). The presentation of VLM includes eosinophilia (sometimes approaching 70%), leukocytosis, fever, hepatomegaly, anemia, cough, wheezing and bronchospasm resembling asthma [4,8].

The ELISA test has proved to be a sensitive and specific test in the diagnosis of VLM, Immunoglobulin mainly IgG, but to a lesser extent IgM and IgE are produced in response to the infection [9, 10, 11].

The present work is concerned with seroprevalence of human toxocariasis among children in Mosul area no previous work has ever been done in Mosul concerning this aspect of the disease.

Patients and Methods

Collection of Blood Samples:

Sera from 1-15 years old children were obtained from 120 blood samples submitted to Ibin- Al-Ather Hospital and Ibin Sina Teaching Hospital Pediatric Department. Collection started from January to May 2005.

In patient children (1-15 years old), specially those with pica, anemia, Two to three ml. of blood was drawn by venipuncture from 120 hepatomegally, and general weakness, blood was also drown from 25 control children (apparently healthy) of matched ages selected randomly from Mosul City [12].

Sera were collected and assayed for specific antibodies against T.canis and before that the sera were absorbed with extracts of Ascaris and Toxocara antigens and examined for C-reactive protein [13].

Calculation of Results

Samples were considered as positive if the absorbance value is higher than 10% over the cut-off value.

Samples with an absorbance value of less than 10% above or below the cut-off should not be considered as clearly positive or negative (gray zone).

Samples were considered as negative if the absorbance value is lower than 10% below the cut-off (NOVATEC IMMUNODIAGNOSTIC GMBH).

Statistical Analysis

Chi-square test, Anova test, and t-test were used for the analysis of data [14].

Results

Sera of children collected and analyzed by ELISA test showed that out of 120 sera collected from inpatients children aged (1-15) years old suffering from different pathological conditions with different signs and symptoms in addition to (25) sera collected from apparently healthy children of both sexes used as a control group, 37 (25.8%) of tested children and 3 (12.0%) of healthy controls were reactive for anti-Toxocara antibodies, including 20 (13.9%) of 66 and 17 (11.9%) of 54 sera of the male and female children respectively. Sera were also checked for C-reactive protein by using latex agglutination slide method (Biokit spanish comapny). The results showed negative reaction for the tested and control groups except two sera which gave positive reaction; one

serum was taken from child suffering from bronchitis and the other from child with arthritis.

Statistical analysis using t-test revealed no significant difference between the infection with toxocariasis in male and female groups (P= 0.508). Results also indicated no significant differences between different age groups(1-15years), as indicated in (Table 1). The number and distribution of toxocariasis among different age groups (from 1-15 years old), and in different clinical signs and symptoms is presented in (Table 2).

Statistical analysis by using ANOVA-F-test showed that the positive number of infected children with toxocariasis is not significant by comparing the different age groups (P= 0.837).

Diagnosis of sera from children with geophagia (eating soil and dirt), in urban and rural regions and among male and female subjects revealed that 20 (54.1%) out of 37 with geophagia in males and 7 (56.7%) out of 30 females, were seropositive for IgG (Table 4). Gender analysis showed, no statistical significance between children with geophagia and non-geophagia.

The mean age and standard deviation of the groups of 120 patients children was 5.86± years. About half of this number originated from villages surrounding Mosul area, geophagia was most frequently recorded in the 1-2 years old group.

Distinct hematological values were most frequently observed in the 1-2 years age group (Table 12), while the most common clinical symptoms were abdominal pain, bronchitis, hepatosplenomegaly, lymphadenopathy often accompanied by anorexia, vomiting and anemia (Table 11). Table 11 shows that the highest erythrocyte sedimentation rate was frequent in the 1-2 years old group (30%), while the lowest hemoglobin rate (15%) was seen in 3-5 year old group, with the most common clinical symptoms being, abdominal pain, hepatosplenomegaly, lymphadenopathy and less frequently anemia. As in all patients with larval toxocariasis, eosinophilia was also most frequent in the (3-5) year old group of children (22.5%) (Table 12), which was the most interesting point. Leucocytosis were most frequently detected in (11-15) year old children (25%), which was an indicator of inflammation.

Table 1 Seropositivity of toxocariasis according to age and sex of children.

Male				Female							
Age (year)	**Examined no.**	**Positive no.**	**%**	**Examined no.**	**Positive no.**	**%**	**Total no.**	**%**	**Healthy control**	**Control**	**%**
1-2	11	5	3.4	9	3	2.1	24	5.5	4	1	25
3-4	12	4	2.8	10	4	2.8	25	5.6	3	0	0
5-6	10	3	2.1	8	3	2.1	23	4.2	5	1	20
7-8	8	3	2.1	9	2	1.4	21	3.5	4	0	0
9-10	9	2	1.4	7	2	1.4	18	2.8	2	1	50
11-12	9	2	1.4	6	2	1.4	19	2.8	4	0	0
13-14	7	1	0.7	5	1	0.7	15	1.4	3	0	0
Total	**66**	**20**	**30.30**	**54**	**17**	**31.48**	**145**	**30.83**	**25**	**3**	**12**

Mean =	2.86	2.429
Standard deviation (st. D.) =	1.35	0.976
P.value =	0.508	

Table 2 Clinical symptoms of toxocariasis in hospitalized children

	Age in year												
	1-2			3-5			6-10			11-15			
Symptoms	N = 20	p.n.	%	n = 40	p.n	%	n = 33	p.n	%	n = 27	p.n	%	Healthy children (control)
Fever	5	1	20	12	2	16.7	7	2	28.6	5	1	20	0
Abdominal pain	4	2	50	14	3	21.4	9	2	22.2	8	2	25	0
Bronchitis	2	1	50	1	0	0.0	5	1	20.0	2	1	50.0	0
Pharyngitis	1	0	-	1	0	0.0	2	0	0.0	2	0	0.0	0
Hepatosplenomegaly	3	2	66.7	6	3	50	4	2	50	3	1	33.3	0
Anaemia	3	1	33.3	4	2	50	4	2	50	4	1	25	0
Arthritis	0	0	-	0	0	0.0	1	0	0.0	1	0	0.0	0
Lymphadenapathy	2	1	50	2	1	50	1	1	50	1	1	100	0
Macular lesion	0	0	-	0	0	0.0	0	0	0.0	1	1	100	0
Total =	20	8	40%	40	11	27.5%	33	10	30.30%	27	8	29.62%	3/25
Mean =		0.8889			1.222			1.111			0.8889		
Standard deviation =		0.7817			1.302			0.928			0.0601		

P.value = 0.837

Statistical analysis by (Avona table -F-test)

n = number examined
pn = positive number with ELISA test.

Table 3 Hematological parameters in children with clinical toxocariasis

Age group (years)	Variable	Increased ESR mm/h	Low Hemoglobin (gm/dl)	Leucocytes count $X10^3$	Eosinophilia %
1-2	No. examined positive no. increased suppressed * % Mean value + S.D.	20 8 6 30.0 39.5 + 8.76	20 8 5* 25.0 8.86 + 0.999	20 8 4 20.0 3.925 + 0.929	20 8 4 20.0 22.57 + 6.13
3-5	No. examined positive no. increased, suppressed* % Mean value + S.D.	40 11 7 17.5 37.14 + 6.54	40 11 6* 15.0 8.683 + 1.426	40 11 5 12.5 12.6 + 1.14	40 11 9 22.5 21.56 + 6.26
6-10	no. examined positive no. increased, suppressed* % mean value + S.D.	33 10 9 27.3 34.22 + 10.33	33 10 8* 24.2 8.25 + 1.018	33 10 7 21.2 4.071 + 0.745	33 10 6 18.1 25.33 + 4.5
11-15	No. examined positive no. increased, suppressed* % Mean + S.D	27 8 6 29.6 27.0 + 5.76	27 8 6* 29.6 8.233 + 0.898	27 8 7 25.9 3.671 + 0.559	27 8 4 14.8 21.0 + 4.47

P = 0.05
(S) = significant P = 0.76 (N.S) P = 0.965 (N.S) P = 0.572 (N.S) P = 0.012 (S)
(N.S) = Not significant
Statistical analysis by (ANOVA – F – test)

- Suppressed

Table 4 Relationship between toxocariasis, seropositivity of children from urban and rural places and geophagia in Mosul Governorate

Sex	Urban	Rural	No. with geophagia	No. with Toxocariasis	%
Male	30	36	37	20	54.1
Female	25	29	30	17	56.66
Overall	**55**	**65**	**67**	**37**	**55.2**

$(\chi^2) = 0.003$
P-value = 0.956
N.S = not significant between geophagia and Toxocariasis

Discussion

Human toxocariasis is still a poorly diagnostic disease, especially in places with conditions, which favors its development and its being largely unknown either to health professionals or the general population [13]. This is true in a country like Iraq. In this study, the frequency of *Toxocara* seropositivity in hospitalized children was found to be 30.8%. This rate is statistically higher than the rate identified in the control group. *Toxocara* seroprevalence may vary according to geographical region where the study is made and even to different populations inhabiting the same region [10,14,15]. When compared with seroprevalence rates ranging between 2.7% and 73% reported by different studies [10,13,14,15,16,17]. The rate of 30.8% which was found for children group, may not be considered very high. The observation of a high frequency of *Toxocara* seroprevalence in hospitalized children compared to the control group (12%) suggests that these cases in Mosul region may be considered under risk of *Toxocara* infection.

The present work reports for the first time serological proven human toxocariasis in Mosul area. The seropositivity reported in the present study shows that the disease is a zoonotic problem in the study area. Woodruff and colleagues (1981) reported that the prevalence of *Toxocara* antibodies (7.3%) in the sera of adult persons from Baghdad certainly indicates that the transmission of *Toxocara* is proceeding from dogs in Iraq.

The present result further suggests that toxocaral infection in the study area is acquired by the ingestion by soil containing infective *Toxocara* spp. eggs and confirms an earlier report by Woodruff and colleagues (1981) and Al-Dabbagh (1995) that there is a wide-spread contamination of the environment in Mosul with *Toxocara* spp. Eggs [18,19].

Toxocariasis is seen more frequently among children than among adults [2,4,20]. This may be due to such reasons as frequent contact with contaminated soil, poor hygiene, and consuming contaminated food [10,21, 22].

The rate of seropositivity found among children in the present study (30.8%) is consistent with reports conducted in children groups (26.6%) from Egypt (23), Nigeria 29.8% (24), Iran 25.6% (25), Spain 27.2% (15), Bolivia 24.8% (26). Turkey 25.9% (10) and Iran 25.6%. [17].

The seropositivity rates were higher among age groups between 1-2 and 7-8 year old children. This observation is in accordance with report by Caseiro (1996) who observed that 27.6% of prevalence rates ranging from 7-9 years old and 18.9% ranging 10-12 years old [27].

The high specificity and sensitivity of ELISA assay (92% and 78% resectively), are quite useful for serodiagnosis of human toxocariasis using IgG ELISA commercial kits [28]. *T. canis* IgG ELISA has been used in the current study, with its diagnostic specificity of 98% and diagnostic sensitivity of 98% as stated by the manufacturer (Nova Tec-Germany).

All infected children with toxocarasis showed the predominance of non-specific symptoms and disorders, which may result in the narrowing of the spectrum in the differential diagnosis [29]. However, the present study showed various symptoms among seropositive children of different ages. These symptoms include fever, abdominal pain, bronchitis, pharyngitis, hepatosplenomegaly, anemia, lymphadeno-pathy and macular lesion (Table 2).

Almost all cases of severe VLM are diagnosed in children up to 3 years of age [4]. The tendency of some children at this age to eat dirt places them at risk for ingesting many *Toxocara* spp. eggs. Children with geophagia pica (compulsion to eat dirt) a behavior disorder noted in 2-

10% of children at age 1-6 years are extremely vulnerable to infection if in a contaminated environment [3,4].
Despommier (2003) indicated that children with pica were at higher risk of ingesting embyronated eggs from soil than those not exhibiting this behavior[2]. A more recent publication stressed that VLM typically occurs in preschool-aged children with a history of eating dirt, emphasizing the importance of this habit in toxocariasis. In this regard, children should be discouraged from putting dirty fingers in their mouths and eating dirt, [20,30].

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