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Socio-demographic, Clinical and Laboratory Predictors for the Diagnosis of Visceral Larva Migrans in Children - Upper Egypt

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LAG, AEM, RAHA, AE and DGM designed the study, and wrote the protocol. Author AE managed the patient examination and questionnaire. Authors LAG, AE and RAHA performed the laboratory examination. Author DGM did the statistical analysis. Authors AE and DGM managed the literature searches and authors AEM and RAHA wrote the first draft of the manuscript. Analysis of study and final draft was done by author LAG. All authors read and approved the final manuscript.

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ABSTRACT

Visceral larva migrans (VLM) is a worldwide neglected disease, prevalent among children from socio-economically disadvantaged populations in temperate and tropical regions. Infections may go undiagnosed as the required diagnostic tests; serological, molecular and/or imaging examinations are expensive, which may not be affordable or available. We aimed to establish predictors useful in the diagnosis of VLM in children in Upper Egypt. A one year cross-sectional study was conducted at Assiut University Children's Hospital and eighty-one children aged between 6 months to 13 years old (mean± SD 5.7 ± 3.2 years) were eligible to our inclusion criteria, 55.6% of

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them were males. Socio-demographic risk factors, clinical, laboratory and imaging tests were collected. ELISA (*anti-T. canis* IgG) results were positive in 60.5%. By using the bivariate analysis, a significant association was found between seropositive ELISA and younger age less than four years (*p*-value <0.0001), having underground water at their homes (*p*= 0.004), previous history of parasitic infection (*p*= 0.003) and positive liver ultrasonographic findings (*p*=0.001). In a multivariate logistic regression model with positive and negative ELISA results as a dependent factor, younger age (<4 years), history of parasitic infestation and positive liver ultrasonographic findings were found to be significant predictors, while no significant association with other factors was identified. Thus, clinicians should consider the positive liver ultrasonographic changes with the earlier history of parasitic infection in children under four years as predictors for VLM infection, according to which they should undergo ELISA or other tests to confirm their diagnosis.

Keywords: Children; eosinophilia; visceral larva migrans; predictors; ELISA.

1. INTRODUCTION

Visceral larva migrans (VLM) is a worldwide human neglected zoonosis, mainly occurring in temperate, tropical and sub-tropical areas and is also an important cause of morbidity in richer developed nations, mainly in children and socio-economically disadvantaged populations [1,2]. Infection in children occurs through ingestion of *T. canis* and to a lesser extent *T. cati* eggs either by direct hand contamination or indirectly by touching contaminated objects, and ingesting soil contaminated by larvae or infective eggs [3].

Clinical manifestation of toxocariasis and its syndromes is caused by the hematogenous 3rd stage larval migration to the internal organs, the duration of migration, the intensity of infection, host age, and immune-mediated host responses [4]. A wide variety of clinical patterns is exhibited including visceral larva migrans (VLM), ocular toxocariasis (OT), neuro toxocariasis (NT) and covert toxocariasis (CT) [1,5]. Most infections remain asymptomatic and clinically evident infections may go undiagnosed as diagnostic tools are expensive and can require serological, molecular and/or imaging tests, which may be unaffordable or unavailable [4]. To institute the definite diagnosis of VLM, making a direct parasitological diagnosis by using tissues biopsy is tremendously problematic and is the only way to check the existence of worms or larvae in tissues [6,7]. However, this method has serious constraints, since it is difficult to show intact larva in the eosinophilic granuloma [8]. Diagnostic tests that discover parasite DNA are uncertain to be reliable, except in biopsies known to contain a nematode larva [1], as it had been used to distinguish between the larval species [9]. Thus, serological methods are the diagnostic mainstay; the use of serodiagnostic tests had provided much-needed awareness about the prevalence of toxocariasis. Serological diagnosis based on ELISA using *Toxocara* excretory/secretory (TES) antigens are available and its specificity has increased over previously used tests in diagnosis and seroepidemiological studies /surveys. In areas where polyparasitism is endemic, ELISA tests stay tricky due to cross-reactivity which, in turn, declines its diagnostic value [10]. Moreover, in the lack of appropriate clinical data, ELISA tests complications can arise in defining whether an infection is mild or severe, current, recent, or long past [1].

The worldwide incidence of *Toxocara* spp. in humans is predisposed by many variables: at the population level; environmental, geographic, cultural and socioeconomic factors and at the individual level by the heterogeneity of vulnerability to infection predisposed by immunity, co-infection, genetics, age, gender, nutrition and behavior of the human hosts [4,11]. Children are the most susceptible due to their behavior (i.e contact with dogs, geophagia, the habit of putting objects in their mouths,etc.) and poor hygiene.These factors, along with the increasing human population and migration and interactions with definitive host (i.e. dogs and cats) suggest that toxocariasis is a public health hygiene problem [4]. In other parts of the world, spread and risk factors will vary: poverty, lack of education, unregulated numbers of cats and dogs and warm climatic coupled with poor hygiene and geophagia indeed provide an ideal setting to increase transmission opportunities worldwide [12].

To the best of our knowledge, there are no existing studies on the predictors of visceral larva migrans in Upper Egypt. We therefore aimed to establish useful predictors to determine the need

to undergo diagnostic tests that confirm VLM in children.

2. PATIENTS AND METHODS

A cross-sectional study was conducted over the one-year period (May 2014 - May 2015), in collaboration with the Department of Parasitology, Assiut Faculty of medicine. One hundred sixty-eight patients were presented to the outpatient clinic of Assiut University Children's Hospital (AUCH) with suspected general symptoms and signs of VLM infection [1]. Inclusion criteria comprised patients with eosinophilia > 5% and general symptoms and signs of VLM [1]. Exclusion criteria were children with chronic liver, heart, kidney and lung diseases or malignancy in addition to those who left the study and who didn't give any blood samples. Only 81 children were eligible for the study out of one hundred sixty-eight patients presented at that time.

A questionnaire was designed to collect the data in the outpatient clinic.The questionnaire was written in Arabic language and filled by a trained clinical nurse who interviewed the children's parents or guardians. It included questions relating socio-demographic data (i.e.history of previous parasitic infection, type of water supply, house flooring, geophagy). A full clinical history data related to toxocariasis (i.e. Contacts with pets (dogs or cats), history of allergy (skin rash, itching, sneezing, rhinorrhea), bleeding, abdominal pain, vomiting, cough, headache, convulsions, coma) and a last section for clinical examination data (i.e. Anthropometric measurements, radiological and laboratory investigations).

2.1 Detailed Physical Examination was Done Including

1. General appearance.
2. Vital signs: pulse, blood pressure, respiratory rate, temperature.
3. Anthropometric measurements: weight and height were taken to calculate the body mass index (BMI) which is expressed as weight in kilograms and was divided by the square of the height in meters (kg/m^2), and the results of the equation were applied separately for every child to the age and gender specific BMI curves [13]. BMI was categorized into underweight (less than the 5^{th} percentile), normal (5^{th} percentile to less than the 85^{th} percentile), overweight (85^{th} to less than the 95^{th} percentile),obese (Equal to or greater than the 95^{th} percentile).
4. Chest and heart examination for wheezes, rhonchi and heart failure signs.
5. Abdominal examination for organomegaly, ascites, and tenderness.

2.2 Radiological Examination

Chest X-ray for broncho-vascular markings and hyperinflation. Abdominal ultrasonography (US) for hepatomegaly associated with US echogenicity ± focal lesions.

2.3 Laboratory Investigations

- Blood samples were collected (2–2.5ml) to perform complete blood count (CBC) with differential count to confirm eosinophilia and serums were separated and stored at −20°C for the serological estimation including Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Serum Alkaline phosphatase, Total serum Protein, Serum Albumin and ELISA.
- Stool samples were collected from recruited children for parasitological examination (direct wet mount, formal-ethyl concentration technique and modified acid-fast stain for stool analysis) to exclude the presence of other parasites causing eosinophilia and/or cross-reacting with *Toxocara* larvae antigens.

2.4 ELISA Technique

Sera of the eighty-one patients were processed to detect Anti-*Toxocara* IgG antibody by *T. canis* excretory-secretory (TES) antigens using an ELISA kit (Bordier Affinity Products SA, Switzerland) according to the manufacturer's instructions. The results were read as Optical Density (OD) via an ELISA reader and the results were expressed as signal cut off ratio calculated by dividing OD of the sample / OD value of the assay cutoff for the run, the results ranging between 0.9-1 were estimated as positive and those under 0.9 were estimated as negative.

2.5 Statistical Analysis

Frequency tables were examined to explore missing data, data errors, (i.e. Outliers and incorrect data entry) and data consistency. All analysis was performed using SPSS Statistics

(version 16.0, IBM). Data were recorded and descriptive statistics were used to compare study variables.

2.6 Bivariate Analysis

The outcome (dependent) variable was based on the ELISA test results, patients were categorized into positive and negative groups. A Chi – square (χ^2) test and Fisher's Exact test was conducted in bivariate analysis to test the associations between ELISA test positivity or negativity and the independent variables prior to multivariate analysis. The independent variables included gender and age as demographic factors and some socioeconomic and environmental related factors as floor type and water supply (underground or piped), in addition to history of behavioral risk factors as geophagia history and having pets. Medical history was included in association analysis as a history of allergy, cough, rash, itching, bleeding, abdominal pain, vomiting and headache. Clinical and imaging findings were analyzed as lymph nodes, chest findings, heart problems, hepatomegaly, splenomegaly, convulsions, coma, stool parasites, abdominal ultrasonography findings and chest x-ray findings. Finally a bivariate analysis was done in relation to the laboratory findings as white blood cell count, haemoglobin, platelets count, eosinophilia, AST, ALT, alkaline phosphatase, total serum protein and serum bilirubin. The significance level was set at P <0.05.

2.7 Multivariate Model of Analysis

An initial multivariate logistic regression model was built containing a priori variable (age and gender) plus the most strongly associated variables from the bivariate screening analysis and some other non significant factors from the univariate analysis, which were near to the significance, including water supply, history of parasitic infection, itching, abdominal pain, chest findings, hepatomegaly, parasites in stools and abdominal ultrasonographic findings. Likelihood ratio test (LHR) and confidence intervals (CIs) was calculated to assess significance in the models. The final model fitting was tested for model robustness. Odds ratios and 95% CIs were based on these models. Finally, gender with the male as reference, age with age more than 8 years as a reference, water supply to the piped water as a reference, history of parasitic infection with no infection as a reference and liver ultrasonographic findings with negative findings as a reference were explored as prognostic factors for positive ELISA test in the multivariable logistic regression model. Therefore, we present the results with the final model.

2.8 Ethical Consideration

The non-interventional nature of the study, as well as the confidentiality of the information obtained, had been explained to children′s parents before participation in this work and verbal consent had been obtained from all of them. All the detected children with toxocariasis were treated accordingly. The Ethical Review Board (ERB) of the Assiut Faculty of Medicine had approved of the study protocol.

3. RESULTS

The Eighty-one children had a mean age of 5.7 ± 3.2 years with a range of 6 months to 13 years. Males were 55.6% and 44.4% were females. Positive ELISA test was observed in 60.5% (49 out of 81) of the patients and was then considered as a dependent factor. Sociodemographic data revealed that 80% of the participants have piped water supply at home and 65% have paved flooring. Pet cats and/or dogs were also found in 40.7% of their homes, 36% had a history of parasitic infection, while 21% of the children exhibit geophagia (Table 1).

Table 2 shows the clinical history and actual medical complaints of the seropositive patients (n=49), 41 (83%) of whom had clinical symptoms of VLM. Among them, 34.6% had a history of allergy, 38.3% had rashes, and 22.2% experienced itching. Bleeding and abdominal pain were present nearly in one-third of cases (38.3% and 33.3%, respectively) while vomiting and headache was manifested in only 18.5% and 17.3% of them respectively.

By using bivariate analysis in relation to positive ELISA result, there was highly significant percentage of cases among the younger ages (74.0% in <4 years, 64.5% in <8 years and 43.5% in > 8 years) (P-value<0.0001) and those who had underground water at their homes 87.5% (P= 0.004) (Table 2).

From the medical history of the participants, 75.9% of them had a history of parasitic infection and associated significantly with positive ELISA (P= 0.003), in spite, 70.4% of those with a history of abdominal pain had non-significant positive ELISA. Based on clinical examination findings, significant positive liver ultrasonographic findings were found in 76.5% of cases with positive

ELISA (*P*=0. 001). In spite of non-significant BMI findings, results, 76.5% of the underweight children had positive ELISA noting that, patients under two years old were excluded from the BMI calculation.

All the laboratory investigations, including serum levels of AST, ALT, alkaline phosphates, total serum protein and albumin and stool analysis were not significantly associated with positive ELISA. However, anemia was manifested in 60.5% of seropositive cases, while high eosinophilia (>10-30%) was found in 19.8% of cases (Table 3).

Table 4 and Fig. 1 represents the multivariable logistic regression model, with ELISA test results (positive and negative) as a dependent agent. The patients below 4 years old have a three-folds chance to have a positive ELISA (OR= 2.98 and CI 1.87 - 10.78, those with history of parasitic infection carries two-fold risk to be positive ELISA (OR = 2.35 and CI 1.81 -6.81) and liver ultrasonographic finding patients have 1.8 fold to be a predictor (OR= 1.84 and CI 1.63 -5.33). These findings are thus considered to be significant predictors of VLM infection in the current study.

Table 1. Relation of ELISA seropositivity and demographic related factors of the children patients in AUCH

	ELISA		Total	*P*- value*
	Negative No = 32	Positive No= 49	No= 81	
Gender				
Male (%)	16 (35.6)	29 (64.4)	45 (55.6)	0.495
Female (%)	16 (44.4)	20 (55.6)	36 (44.4)	
Age				
< 4 yrs (%)	7(29.6)	20 (70.4)	27 (33.3)	0.0001**
4 - 8 yrs (%)	11 (35.5)	20 (64.5)	31 (38.3)	
>8 years (%)	13 (56.5)	10 (43.5)	23 (28.4)	
Floor				0.585
Non-Paved (%)	11 (39.3)	17 (60.7)	28 (34.6)	
Paved (%)	21 (39.6)	32 (60.4)	53 (65.4)	
Water supply				0.004 **○
Underground (%)	2 (12.5)	14 (87.5)	16 (19.8)	
Piped (%)	29 (44.6)	36 (55.4)	65 (80.2)	
History parasitic infection				
Yes (%)	7 (24.1)	22 (75.9)	29 (35.8)	0.003**
No (%)	25 (48.1)	27 (51.9)	52 (64.2)	
Geophagia history				
Yes (%)	6 (35.3)	11 (64.7)	17 (21)	0.475
No (%)	26 (40.6)	38 (59.4)	64 (79)	
Pets animals				
Yes (%)	13 (39.4)	20 (60.6)	33 (40.7)	0.586
No (%)	19 (39.6)	29 (60.4)	48 (59.3)	

** χ2 test was used; ****significant P <0.05*
○ Fisher's exact test was used

Table 2. Relation between ELISA seropositivity and clinical data of the studied children at AUCH

Clinical data	ELISA results		Total	*P*- value*
	Negative No = 32	Positive No= 49	No= 81	
History of allergy				
Yes (%)	11 (39.3)	17 (60.7)	28 (34.6)	0.585
No (%)	21 (39.6)	32 (60.4)	53 (65.4)	
Cough				
Yes (%)	13 (41.9)	18 (58.1)	31 (38.3)	0.451
No (%)	19 (38.0)	31 (62.0)	50 (61.7)	

Clinical data	ELISA results		Total	*P*- value*
	Negative No = 32	Positive No= 49	No= 81	
Rash				
Yes (%)	12 (38.7)	19 (61.3)	31 (38.3)	0.549
No (%)	20 (40.0)	30 (60.0)	50 (61.7)	
Itching				
Yes (%)	10 (55.6)	8 (44.4)	18 (22.2)	0.097
No (%)	22 (34.9)	41 (65.1)	63 (77.8)	
Bleeding				
Yes (%)	13 (41.9)	18 (58.1)	31 (38.3)	0.451
No (%)	19 (38.0)	31 (62.0)	50 (61.7)	
Abdominal pain				
Yes (%)	8 (29.6)	19 (70.4)	27 (33.3)	0.148
No (%)	24 (44.4)	30 (55.6)	54 (66.4)	
Vomiting				
Yes (%)	7 (46.7)	8 (53.3)	15 (18.5)	0.365
No (%)	25 (37.9)	41 (62.1)	66 (81.5)	
Headache				
Yes (%)	6 (42.9)	8 (57.1)	14 (17.3)	0.502
No (%)	26 (38.8)	41 (61.2)	67 (82.7)	
Lymph nodes				
Yes (%)	10 (40.0)	15 (60.0)	25 (30.9)	0.571
No (%)	22 (39.3)	34 (60.7)	56 (69.1)	
Chest findings				
Yes (%)	8 (33.3)	16 (66.7)	24 (29.6)	0.315
No (%)	24 (42.1)	33 (57.9)	57 (70.4)	
Heart				
Yes (%)	0	1	1 (1.2)	-- •
No (%)	32 (40.0)	48 (60.0)	80 (98.8)	
Hepatomegaly				
Yes (%)	10 (32.3)	21 (67.7)	31 (42.9)	0.208
No (%)	22 (44.0)	28 (56.0)	50 (57.1)	
Spleenomegaly				
Yes (%)	4 (23.5)	13 (76.5)	17 (21.0)	0.107○
No **(%)**	28 (43.8)	36 (56.2)	64 (79.0)	
Convulsions				
Yes (%)	3 (27.3)	8 (72.7)	11 (13.6)	0.203○
No (%)	29 (41.4)	41 (58.6)	70 (86.4)	
Coma				
Yes (%)	1 (100.0)	0 (0.0)	1 (1.2)	--•
No (%)	31 (38.8)	49 (61.2)	80 (98.8)	
Stool				
Positive for parasites (%)	4 (66.7)	2 (33.3)	6 (7.4)	0.164○
Negative for parasites (%)	28 (37.3)	47 (62.7)	75 (92.6)	
Abdominal ultrasonography				
Yes (%)	8 (23.5)	26 (76.5)	34 (42.0)	0.001**
No (%)	22 (46.8)	25 (53.2)	47 (58.0)	
Chest X- ray findings				
Yes (%)	9 (37.5)	15 (62.5)	24 (29.6)	0.507
No (%)	23 (40.4)	34 (59.6)	57 (70.4)	
BMI[##]				
Underweight (%)	4 (23.5)	13 (76.5)	17 (22.1)	
Normal (%)	13 (44.8)	16 (55.2)	29 (37.7)	-- •
Overweight (%)	6 (54.5)	5 (45.5)	11 (14.3)	
Obese (%)	8 (40.0)	12 (60.0)	20 (26.0)	

** $\chi 2$ test was used; **significant P <0.05*

[##]Patients below two years old were excluded from BMI calculation

○ Fisher's exact test was used

-- • χ^2 test was not applicable in case of "variable= 0 " cells

Table 3. Relation between ELISA and laboratory investigations of the children cases studied at AUCH

	ELISA results		Total No= 81	*P*- value*
	Negative No = 32	Positive No= 49		
WBC				
Normal (%)	27 (38.6)	43 (61.4)	70 (86.4)	-- •
High (%)	0 (0.0)	2 (100.0)	2 (2.5)	
Low (%)	5 (55.6)	4 (44.4)	9 (11.1)	
Haemoglobin				
Normal (%)	4 (50.0)	4 (50.0)	8 (9.9)	0.391 ○
Low (%)	28 (38.4)	45 (61.6)	73 (90.1)	
Platelet				
Normal (%)	32 (41.0)	46 (59.0)	78 (96.3)	--•
High (%)	0 (0.0)	2 (100.0)	2 (2.5)	
Low (%)	0 (0.0)	1 (100.0)	1 (1.2)	
Eosinophilia				
Low (5-10%) (%)	27 (41.5)	38 (58.5)	65 (80.2)	0.572
High (>10-30%) (%)	5 (31.2)	11 (68.8)	16 (19.8)	
AST				
Normal (%)	28 (40.0)	42 (60.0)	70 (86.4)	0.547 ○
High (%)	4 (36.4)	7 (63.6)	11 (13.6)	
ALT				
Normal (%)	28 (40.0)	42 (60.0)	70 (86.4)	0.547 ○
High (%)	4 (36.4)	7 (63.6)	11 (13.6)	
Alkaline phosphatase				
Low (%)	2 (50.0)	2 (50.0)	4 (5.1)	0.541 ○
Normal (%)	29 (38.7)	46 (61.3)	75 (94.9)	
Total Serum protein				
Low (%)	21 (38.2)	34 (61.8)	55 (68.8)	0.401
Normal (%)	11 (44.0)	14 (56.0)	25 (31.2)	
Serum Albumin				
Low (%)	30 (38.5)	48 (61.5)	78 (96.3)	0.343 ○
Normal (%)	2 (66.7)	1 (33.3)	3 (3.7)	

** χ2 test was used; ****significant P <0.05*
○ Fisher's exact test was used
-- • χ^2 test was not applicable in case of "variable = 0 " cells

Table 4. Multivariable logistic regression analysis of the associated predictors with VLM in the studied participants

Variables	Category/increment	Odds ratio	95% confidence interval (CI)
Gender	Male	Baseline	-
	Female	1.75	(0.644 - 4.733)
Age	> 8 yrs.	Baseline	-
	4 -8 yrs.	1.28	(0.37 - 4.36)
	6 months – 4 yrs**	3.1	(1.87 - 10.78)
Water supply	Underground	1.53	(0.12 - 2.3)
	Piped	Baseline	
History of parasitic infection	Yes	2.35	(1.81 - 6.81)
	No	Baseline	
Liver ultrasonographic findings	Yes	1.84	(1.63 - 5.33)
	No	Baseline	

*** Significant predictors*
Note: The sample size no. = 81, this is the final model for multivariable logistic regression, explored predictors for positive ELISA test as the dependent variable

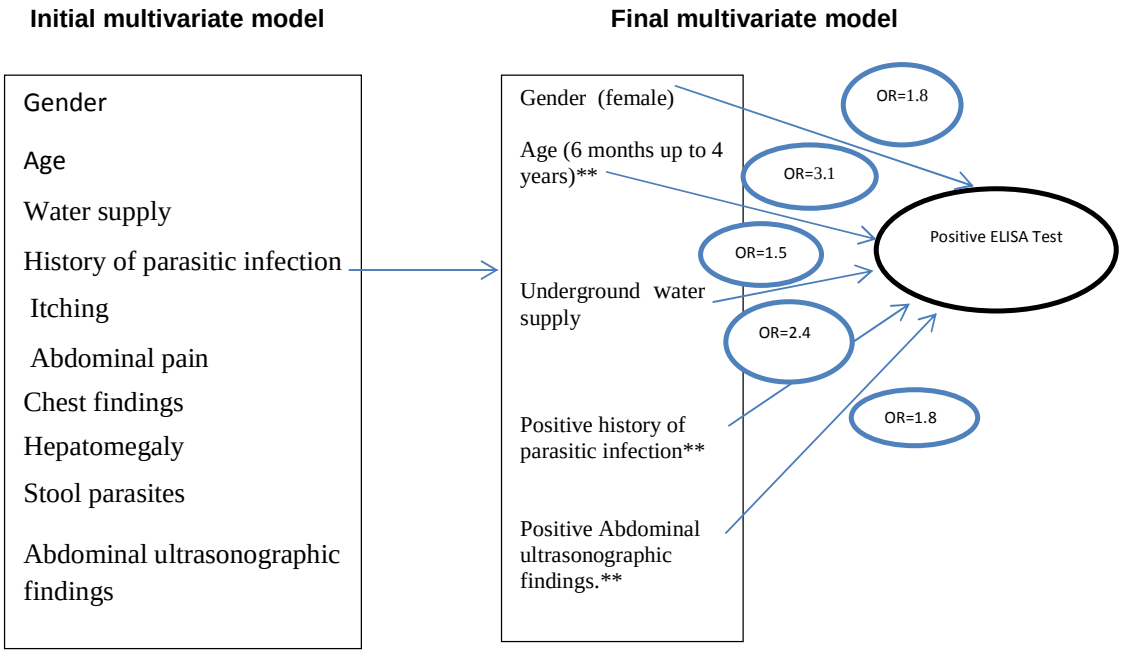

Fig. 1. Initial and final multivariate logistic regression models of analysis of the associated predictors with VLM in the studied participants

*** Significant predictors*

Note: Sample size n°= 81, OR = Odds Ratio. Model for multivariate analysis considered positive ELISA test as the dependent factor. The independent variables were investigated in the initial model, then the final model included the significant categories of the examined variables. Male was the baseline category, but the female was not a significant predictor. Age > 8 years was the baseline category, age 6 months up to 4 years was significant predictor and other age groups were not significant predictors. The piped water was the base line category, but the underground water was not a significant predictor

4. DISCUSSION

Human toxocariasis (VLM) is a zoonosis caused by migration of *Toxocara sp*. Larvae inside the human body. Clinical manifestations and complications are commonly dependent on the number and migration sites of *Toxocara* larvae.

In order to elucidate the magnitude of the problem, seroepidemiological studies were carried out in different countries. Karagöz et al. and Momeni et al. [2,14] have stated that the global distribution of the disease.Even though in developing countries other parasitic infections are more common, but reports show high rates of VLM. In developed countries, visceral larvae migrans is considered to be the second most common parasitic infection in human [15]. It is difficult to evaluate exactly how many cases of toxocariasis occur every year, as they are often misdiagnosed (or undiagnosed).The discrepancy between the high seroprevalence found in most countries and the low number of VLM cases had been recorded [16].

In the present study, we had used anti-*Toxocara* IgG ELISA test. ELISA is a simple, easy method, does not require complicated equipment. It has a commercially available kit with appropriate sensitivity and specificity and it is recommended as an effective and useful method in seroepidemiological studies of human toxocariasis [17,18]. The seropositivity may develop even when few eggs of *Toxocara* are ingested, without clinical signs and in chronic infection [19]. However, application of ELISA remains a challenge in areas of widespread poly-parasitism due to cross-reactivity which, in turn, declines its diagnostic value [10]. ELISA as serological diagnostic tests is still expensive. It may not be affordable or available especially among the targeted children from socio-economically deprived populations both in the tropics and sub-tropics and in developed residents [3].

The present study witnessed high seroprevalence rate, about, 60% of the studied children were seropositive. Momeni et al. [14] stated that the prevalence rate varies from one location to another even within the same country. The current rate is considerably high compared to previous studies done in Egypt. In the Egyptian Delta, the seroprevalence rate of 6.2% was detected in patients from Tanta [20] and were 12% among children recruited from Mansoura University Children's Hospital [21]. Similarly, in Upper Egypt, a rate of 7.7% was observed [22]. Low seroprevalence rate varying between 0.7% and 15% was also recorded among children in industrialized countries [5,23]. The high discrepancy in seroprevalence rate could be explained by the fact that the present study had restricted inclusion criteria, comprised patients with eosinophilia more than 5% and suspected general symptoms and signs of VLM.

Higher seroprevalence has been also reported from similar less industrialized and tropical countries (Africa, Asia, and South America) ranging between 30% and 93% [10,24–26]. Assiut Governorate, in which the study took place, about 63% of the population live in urban and semi-urban areas. The population mostly belong to the low socio-economic class in which the level of hygiene is questionable [27]. *Toxocara* is significantly more prevalent in rural than urban areas [4,28]. In rural or semi-urban populations, they are mostly living in conditions, to be confronted with multiple risk factors. About 65% of our patients with a history of geophagia were seropositive. In various parts of Egypt, the prevalence and intensity of geoparasites were studied; among areas representing the urban and rural areas of their respective Governorate .It was found by all the studies that *Toxocara eggs* had the highest prevalence of other parasitic infections. *Toxocara* egg was significantly higher in fields than streets and indoor-yards samples, due to defecation of man particularly children, domestic and wild animals [29,30].

Presently, thirty-eight out of forty-nine seropositive children were having low eosinophilia in contrast to only eleven with high eosinophilia. This indicates the strong relationship between *Toxocara* and eosinophilia. Toxocariasis is one of the most common reasons of eosinophilia [14,31,32]. In order to exclude other parasitic infection causing eosinophilia, we had performed stool examination and only two cases were found to have protozoal infection. This one of the reasons the present study had picked the patient with eosinophilia. In order to diminish the search for VLM infected patients.

Nearly similar percentage of eosinophilia was detected in 86.2% of seropositive children in Mansoura Children's Hospital, as both were more frequently detected among patients with allergy (bronchial asthma and urticaria) [21]. Eosinophilia can remain as a chronic feature after the disappearance of mild VLM as there is a positive correlation between eosinophilia and laboratory presentation of allergic threshold levels [33]. Fan et al. [5] has reviewed the great relation of toxocariasis with laboratory marker of allergy (eosinophilia, high level of IgE and others). In accordance with the previous statements, most of our patients have recorded clinical manifestations confirming the allergic nature of the disease. We recorded that 66.7% (16/24) of patients with chest finding (wheezes and rhonchi) and having cough 58.1% (18/31) were seropositive. *Toxocara* infection between asthmatic children and with chronic coughs was also related to the infection rate and was variable between countries [14]. The reviewed results of some epidemiological studies have shown an affirmative link between wheezing or asthma and *Toxocara* seropositivity .While other researchers were unsuccessful to expose any link [34]. Asthma-like symptoms have been attributed to parasite-induced atopy [35,36]. Other allergy related symptoms was present among the present study seropositive children in the form cutaneous manifestations (61.3% rash and 44.4% itching). Pure cutaneous symptoms have been reviewed by Piarroux et al. [37] and to be the only clinical manifestation in many reported cases and the most frequent systemic etiology of the pruritus [38]. The association of cutaneous signs and asthma in seropositive patients were found in studies done by Agin and Oteifa et al. [33,39]. We had similarly detected patients with cough ,wheezes and rhonchi in association with cutaneous manifestation (i.e.rash and pruritus) in 42.8% (21/49) of seropositive children.

The studied children had a mean age of 5.7 ± 3.2 years old. The study found that children below 4 years age are significantly at risk to contract *Toxocara* infection than older ages .This age was identified as a significant predictor for VLM. Higher prevalence among children between 1and 6 years old (10 to 30%) and being slightly more common in boys than in girls was recorded [37,40]. They have more chances to get contaminated ;when playing in the playground,

bad habits such as geophagia were practiced in 10-30% of children, contact with contaminated soil , poor hygiene, and consuming contaminated food [19,33]. Furthermore, the rate of infection would increase more if associated with factors related to the low socioeconomic level and poor environmental hygiene [41]. This is the case in the present study, besides the low age group risk factor, low and poor environmental conditions facing our patients: like the significant presence of underground water in 87.5% of the patients' homes who are seropositive. Most of our patients are from rural or semi-urban areas with low socioeconomic and low hygienic level; so these children are more prone to be in contact with the soil loaded by the infective stage in the fields or in the streets due to their outdoors activity [29,30]. The former activity renders the small age children (< 4 year) ,vulnerable to other parasitic infection, which was found to be one of the present predictors "history of parasitic infection". The significant predictors and association with human toxocariasis are different from one study and locality to others as the environmental pollution and lifestyle strongly affect the epidemiology of toxocariasis [19], and the significant effect of socioeconomic parameters on the rates of human infection [42].

Liver ultrasonographic changes are one of the clinical predictors assuming the higher risk of the patients to be seropositive for toxocariasis .The sonographic picture was nonspecific in the form of hypoechoic scattered focal lesions in the liver. Similar findings were found in studies done on adult patients [43,44]. It had to be mentioned that liver function test of our patient was also within the normal value (AST & ALT), therefore pediatrician examining little children with non-specific sonographic changes should be more aware to ask for eosinophilia's count and to suspect VLM.

Most of the cross-sectional community-based seroepidemiological surveys frequently recognize seropositive persons to have non-specific or mild symptoms. Consequently, there is a need for diffusion of information regarding each locality properties (environmental, socio-demographic) [3]. In our study, we recorded that (83.7%) 41/49 of the seropositive children were suffering from different clinical manifestations. The most frequent clinical manifestation among the seropositive patients was pulmonary manifestations (cough ,wheezes, and rhonchi) 69.4% (34/49). The most frequent clinical manifestation among the seropositive patients was the chest findings,in the form of cough,wheezes, and rhonchi in 69.4% (34/49). The most frequent abdominal symptoms were hepatosplenomegaly 69.4% (34/49) and abdominal pain 38% (19/49). Nearly equal values were documented by Bahnea et al. [45] among their studied children. A great difficulty is reached to establish the diagnosis of VLM, due to the miscellany of symptoms.

The clinical classification of the toxocariasis syndrome is still ambiguous when talking about covert and common toxocariasis. The combinations of abdominal pain, headache, cough, sleep disturbance, behavior disturbance, associated with high *Toxocara* titer signifies the clinical entity of covert toxocariasis [46]. Covert toxocariasis is the most clinical form of toxocariasis to be reported in children [47]. Piarroux et al. [37] have designed that was called 'covert toxocariasis' is the combination of abdominal pain, headache, and cough , more significantly associated with a high titer ($P < 0.05$) than were individual clinical features endorsed by previous results reached by Taylor et al. [48]. In the present study, only two of eosinophilia seropositive cases were having this exclusive association of abdominal pain, headache and cough and questioning to be diagnosed as "Covert toxocariasis" while the other 39/41 seropositive symptomatizing cases were suffering from various clinical manifestations as previously mentioned. This form of the disease was not yet recorded or studied in term of similarity of the clinical picture or even the prevalence in our community.

5. SUMMARY AND POLICY IMPLICATIONS

Study findings point that toxocariasis (VLM) is an important health problem in our community. However, insufficient attention is still being paid to this disease. Although toxocariasis cases are overlooked in clinical practice, serological tests that are useful tools in diagnosis are not routinely used due to their expensive cost. Therefore, the predictors detected in this study may help the pediatrician to suspect toxocariasis infection in children. Toxocariasis should be considered in children below 4-year-old with eosinophilia, and with the previous history of parasitic infection. Liver ultrasonography should be done to assess the associate positive changes.

Our study was limited by the cross-sectional nature of the data. The reached predictors were

identified as an association and not as a causation of VLM.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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