



Study of Epidemiology of Toxoplasmosis in Hemodialysis Patients in **Baghdad Hospitals**

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Abstract:

This study aims to assess some seroprevalence of toxoplasmosis in chronic renal failure (CRF) patients undergoing hemodialysis in dialysis centers in some Baghdad hospitals, by testing the blood samples with enzyme linked Immunosorbant assay (ELISA) IgG and IgM, to determine the incidence of toxoplasmosis in hemodialysis patients. Hemodialysis patients infected with Toxoplasma. gondii were appeared with 129(32.25%) seropositive anti-Toxoplasma IgG. The age groups (43-51) year and (52-60) years of hemodialysis patients showed the highest percentage of anti-Toxoplasma gondii antibodies. An increase of the seropositivity rate was detected with increasing length of time on hemodialysis treatment. Females had the highest significant percentage of anti-Toxoplasma IgG 72 (55.81%) than males 57 (44.18%). Domestic poultry breeder had high percentage 71(55.03%) and 8 (57.14%) of anti-Toxoplasma (IgG⁺&IgM⁺) antibodies respectively in comparison with patients who do not breed domestic poultry.

Keywords: Toxoplasmosis, Hemodialysis, immunocompromised patients.

دراسة في الوبائيه المصليه لداء المقوسات الكونديه لمرضى غسل الكلى في مستشفيات بغداد

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الخلاصة:

تهدف هذه الدراسة لتقييم الانتشار المصلى لداء المقوسات الكوندية في مرضى CRF الذين يخضعون لغسل الكلى في مراكز غسل الكلي في مستشفيات بغداد . أستخدم اختبار الامتزاز المناعي المرتبط بالانزيم (ELISA) نوع IgG و IgM لتحديد نسبة حدوث داء المقوسات في مرضى غسل الكلى . أظهرت النتائج النسبة المئوية (32.25 %) 129 لمرضى غسل الكلى المصابين بطغيلي T. gondii والحاملين للضد المناعي IgG. أظهرت المجاميع العمرية (43-51) و (52-60) سنة نسبة الاصابة الاعلى بالاضداد المناعية المضادة للمقوسات. تم الكشف عن زيادة في معدل إيجابية المصل مع زيادة طول وقت العلاج بغسل الكلى. أظهرت الاناث اعلى نسبة اصابة مئوية بالاضداد المناعية للمقوسات72 (55.81%) منها للذكور 57 (44.18%). كما اظهر الاشخاص المربين للدواجن المحلية نسبة مئوية عالية للاصابة الموجبة بالاضداد المناعية †IgG للمقوسات وللاصابة الم وجبة لكلا الاضداد المناعية (†IgM و +IgM) وكانت النسبة 71 (55.03%) و 8 (57.14%) على التوالي بالمقارنه مع الاشخاص غير المربين للدواجن الكلمات المفتاحيه: داء المقوسات الكونديه، غسل الكلي، مرضى نقص المناعه.

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Introducton

Toxoplasma gondii, the causal agent of toxoplasmosis, is an important water and food borne protozoan parasite ubiquitous throughout the world [1]. It belongs to the phylum Apicomplexa along with other well-known member's like- Plasmodium, Sarcosystis, Eimeria, Cryptosporidia and Neospora [2]. Although cats are often blamed for spreading toxoplasmosis, but contact with raw meat is a more significant source of human infections in many countries. Also undercooked meat containing tissue cysts and fecal contamination of hands is a greater risk factor [3]. The infection can also be transmitted vertically from an infected woman to her fetus during pregnancy via tachyzoites contained in blood products, [4]. Acute T. gondii infections are asymptomatic and self –limiting, especially among healthy immunocompetent hosts. However, the infection may cause severe complications in pregnant women and immunocompromised patients [5], such as human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) patients [6], cancer patients [7], end stage renal disease undergoing hemodialysis patients and those having organ transplantation [8]. In the latter setting, the disease can result from T. gondii transmission either within the allograft from a seropositive donor into a seronegative recipient [9].

Such patients have deficient cellular immunity and this makes them susceptible to the infection [9]. The infection most often involves the nervous system, with diffuse encephalopathy; meningoencephalitis or cerebral mass lesions [10]. Toxoplasmosis in these patients can be due to recently acquired infection or more commonly due to reactivation of latent infection [11].

Many studies have been carried out on the immune response in patients with chronic renal failure (CRF), and proved there was impairment of cell- mediated immunity [11]. CRF patients are under risk from a variety of infection [12], either due to their depressed immune statue or through hemodialysis [13].

This study was aimed to assess the seroprevalence of toxoplasmosis in CRF patients undergoing hemodialysis in some dialysis centers in Baghdad hospitals, by testing blood samples with (ELISA) anti-*T. gondii* IgG and IgM specific antibodies and to determine the incidence of clinically confirmed toxoplasmosis in renal patients.

Materials and Methods:

Blood samples were collected during November 2012 till May 2013 from a total of 400 patients undergoing hemodialysis treatment because of chronic renal failure. They were attended dialysis centers in Medical Center Hospital, Al- Kadmeea Hospital, Al- Yarmouk Hospital and Al-Karama Hospital. Also 50 healthy subjects were used as a control group. Five ml of brachial vein blood were drawn from each subject. Sera were separated after centrifugation at 3000 rpm for 10 minutes, and stored at -20° C until required. Informations were recorded from patients by using questionnaire paper. **Serological technique:** ELISA kits were used (Humen Gesellscaft für Biochemica und Diagnostica-Germany), for detecting IgG and IgM anti-*T.gondii* antibodies, which were performed following the manufacturer's instructions.

Statistical analyses: Chi-square test was used and P < 0.05 were considered as significant and P < 0.01 as highly significant.

Results and Discussion

In this study ELISA test recorded14 (3.5%) for both seropositive anti-Toxoplasma IgG and IgM antibodies. 129 (32.25%) for anti-Toxoplasma IgG seropositive antibodies and IgM seronegative comparing with healthy control group (24.0%) with a statistical difference between them (P<0.05), table-1.

Table 1- The percentage distribution of hemodialysis patients from Baghdad hospitals infected with toxoplasmosis according to ELISA IgG and IgM tests.

Test Subject	ELISA									
	IgG ⁺ &IgM ⁻		IGg ⁺ &IgM ⁺		IgG ⁻ &IgM ⁺		IgG ⁻ &IgM ⁻		Total	
	No	%	No	%	No	%	No	%	No	%
Hemodialysis patients	1 2 9	32.25	1 4	3.50	0	0	2 5 7	64.25	4 0 0	88.88
Healthy control	1 2	24.00	2	4.00	0	0	36	72.00	5 0	11.11
Total	1 4 1	31.33	1 6	3 . 5 5	0	0	2 9 3	65.11	4 5 0	100
Chi-square – χ²		4.533		0 . 8 4 1 NS		0.00 NS		4.624		12.683
* (P<0.05), ** (P<0.01), NS: Non-significant.										

It is generally assumed that approximately 25 to 30% of the world's human population is infected by *T.gondii* [14]. Toxoplasmosis can vary from an asymptomatic, self-limiting infection, to a fetal disease, as seen in patients with congenital infections, or in immunocompromised patients [8]. This result differed from the results recorded by [12] (2008) in a study carried out on hemodialysis patients in Al-Najf province 41.4% by ELISA test. It is mostly agree with the result recorded by [14] (1996) which was 23(38.3%) in patients undergoing hemodialysis in Egypt. But, differs from other results recorded in different countries. In Turkey [8] detected anti-*Toxoplasma* IgM and IgG antibodies in 173 hemodialysis patient which were 1.73% and 56.06% respectively. In the same country [15] recorded 0.8% and 76.5% for IgM and IgG seropositive respectively in the same category of patients.

In a study carried out in Jahrom, Iran, anti-*Toxoplasma* IgM and IgG antibodies were found (6.80%) and (59.10%) respectively [10] while it was (7.9%) and (29.3%) in southwest of Iran for IgM and IgG respectively [16] Maraghi *et al.* (2013) [17] recorded (8.67%) IgM and (40.67%) IgG antibodies in the same country. In Malaysia, IgM and IgG seropositive were (5.7%) and (46.6%) respectively [18].

This differences may be due to the seroprevalence estimated, may vary widely between countries (from 10 to 80%) and often within a given country or between different communities in the same region [19]. Higher prevalence was classically observed for tropical countries with a humid and warm climate, and conversely, lower prevalence was found for arid countries or for colder countries, but anthropogenic factors explain a large part of the variations in human seroprevalence, including dietary habits (method of cooking meat, hand washing, kinds of meat or vegetables consumed, and vegetable cleaning, etc.); economic, social, or cultural habits; quality of water; and sanitation coverage [20].

1. Toxoplasmosis seroprevalence according to age groups:

The seroprevalence of T. gondii-specific IgG antibodies — were higher in the age group (43-51) year and (52-60) years, it was 35 (27.13%), while the lowest percentage was noticed in the age group of \geq 70 years. The anti-T. gondii IgM antibodies were recorded in two age groups only 34-42 and 43-51 which reached the same percentage 7 (50%). There was highly significant between them (P<0.01), table-2.

This result agree with the results recorded by [21] who mentioned that, the overall prevalence of antibodies was gradually increased with age reaching 23.7% in the age group 35-45 years among pregnant women and is consistent with other study mentioned by [22] in Tanzania recorded 41.7% in the age group 35-44 years.

Table 2- The percentage distribution of hemodialysis patients from some Baghdad hospitals infected with

toxoplasmosis by ELISA IgG and IgM tests according to age groups.

Test	IgG ⁺ &IgM ⁻		IgG ⁺ &IgM ⁺		IgM ⁻ &IgG ⁻		Total			
Age group	No	%	No	%	No	%	No	%		
16-24	13	10.07	0	0	28	10.89	41	10.25		
25-33	11	8.52	0	0	49	19.06	60	15.00		
34-42	10	7.75	7	50	35	13.61	52	13.00		
43-51	35	27.13	7	50	63	24.51	105	26.25		
52-60	35	27.13	0	0	56	21.78	91	22.75		
61-69	18	13.95	0	0	14	5.44	32	8.00		
> 70	7	5.42	0	0	12	4.66	19	4.75		
Total	129	32.25	14	3.5	257	64.25	400	100		
Chi –square		9.426 **		12.785 **		10.034 **		10.169 **		
** (P<0.01)	** (P<0.01)									

2. Toxoplasmosis seroprevalence according to dialysis length time:

This study also investigated the relationship between length time of hemodialysis treatment and anti- *T.gondii* IgG and IgM antibodies seropositivity. The highest percentage of IgG seropositive antibodies recorded in the period 25-36 months of dialysis and the lowest percentage was in the period 1-6 month of dialysis. The statistical analysis showed highly significant differences between them

(P<0.01), table (3). Our results came in line with the results recorded by [14] in Egypt, [8 and 15] in Turkey and with [10] in India.

Table 3- The percentage distribution of hemodialysis patients from some Baghdad hospitals infected with

toxoplasmosis according to comparison between dialysis length time and ELISA IgG and IgM tests.

Test Dialysis	IgG ⁺ &IgM ⁻		IgG ⁺ &IgM ⁺		IgG & IgM		Total			
time(month)	No	%	No	%	No	%	No	%		
1-6	7	5.42	3	21.42	49	19.06	59	14.75		
7-12	25	19.37	3	21.42	63	24.51	91	22.75		
13-24	22	17.05	4	28.57	56	21.78	82	20.5		
25-36	39	30.23	3	21.42	35	13.61	77	19.25		
≥37	36	27.90	1	7.14	54	21.01	91	22.75		
Total	129	32.25	14	3.5	257	64.25	400	100		
Chi –square		10.74 **		8.774 **		4.133 *		4.026 **		
* (P<0.05), ** (P<0.01).										

Patients with CRF gradually being immunocompromised. In uremic renal failure patients, humoral and cellular immunity are suppressed and in such patients weakened cell functions have been reported. Absolute number of circulating T- cells will reduce and suppressor cells increase, so that hemodialysis is unable to restore the impairment of the immune system [23]. *Toxoplasma gondii* is an opportunistic protozoan parasite, reactivates with impairing of the immunity and emerges as a life-threatening risk in immunocompromised individuals. In other hand, the frequency of blood transfusion may lead to impairing immunity and increasing the chance of reactivation of the parasite.

3. Toxoplasmosis seroprevalence according to the gender:

In relation to the gender, the prevalence of anti-*Toxoplasma* IgG antibodies were higher in females 72 (55.81%) than in males 57 (44.18%), with significant difference between them (p<0.05). While anti-*Toxoplasma* IgM antibodies were equal in both of them 7 (50%) with no significant difference, table-4.

These results came in line with the results recorded by [24] for females (38.45%) using ELISA-IgG and (42.1%) using mini VIDAS-IgG, while for males (12.83%) and (14.4%) respectively. [25] recorded that anti-*T. gondii* IgG seroprevalence was (30.7%) in females and (13.1%) in males by ELISA test.

Table 4- The percentage distribution of hemodialysis patients from some Baghdad hospitals infected with toxoplasmosis by ELISA IgG and IgM test according to the gender.

Test Subject	IgG ⁺ &	IgG ⁺ &IgM ⁻		IgG ⁺ &IgM ⁺		:IgM ⁻	Total	Total	
	No	%	No	%	No	%	No	%	
Male	57	44.18	7	50.0	173	67.31	237	59.25	
Female	72	55.81	7	50.0	84	32.68	163	40.75	
Total	129	32.25	14	3.5	257	64.25	400	100	
Chi –square		4.832 *		0.00 NS		10.648		7.025 **	
* (P<0.05), ** (P<0.01).									

[26] recorded (64.1%) and (53.8%) of *T.gondii* seroprevalence in Ethiopian females and males respectively. In China it was (10.5%) for males versus (14.3%) in females [27]. [18] recorded different result in Malaysia, it was (50.5%) for females and (51.4%) for males undergoing hemodialysis. Several studies showed higher seroprevalence of Toxoplasmosis in females than males. One of the reasons for this high prevalence may be related to women handling raw meat more frequently than men. Or, maybe because, males are less susceptible than females to several parasites including *T. gondii*, *Schistosoma mansoni*, *Babesia microti* and *Taenia crassiceps* [28]. The cause of reversed sex differences in response to certain parasites is not well understood but may involve differences in host–pathogen interactions that were affected by the endocrine system [29].

4. Toxoplasmosis seroprevalence according to domestic poultry breeder:

The present study showed hemodialysis patients who breed domestic poultry had high percentage of anti- *Toxoplasma* IgG antibodies 71 (55.03%) while patients who do not breed had 58 (44.96%). There was a significant difference between them (P<0.05). Also, with anti- *Toxoplasma* IgM antibodies there were 8 (57.14%) and 6 (42.85%) in patients who breed and don't breed domestic poultry respectively, table-5.

These results coincided with other results recorded by [30] among 44 pregnant women of them 27(61.36%) with seropositive anti- *Toxoplasma* IgG and [31] who recorded (52.59%) seroprevalence of anti- *Toxoplasma* IgG antibodies. In contrast [32] recorded low seropositivity of anti- *Toxoplasma* IgG antibodies (9.0%) among domestic poultry breeder. The presence of cats at home and raising animals may significantly associate with *T. gondii* infection [33].

Table 5- The percentage distribution of hemodialysis patients from Baghdad hospitals infected by toxoplasmosis according to domestic poultry breeder by ELISA IgG and IgM tests.

Test	IgG ⁺ &IgM ⁻		IgG ⁺ &:	IgG ⁺ &IgM ⁺		IgG & IgM		Total	
Subject	No	%	No	%	No	%	No	%	
Domestic poultry breeder	71	55.03	8	57.14	109	42.41	188	47.00	
Non- domestic poultry breeder	58	44.96	6	42.85	148	57.58	212	53.00	
Total	129	32.25	14	3.5	257	64.25	400	100	
Chi –square		4.843 *		6.548 **		6.428 **		1.025 NS	
* (P<0.05), ** (P<0.01).									

The seroprevalence of *Toxoplasma* in free-range or backyards chickens is usually high, up to 100%. Due to their habit of feeding close to the ground, free-range chickens are indeed considered a good indicator of environmental contamination by *Toxoplasma* oocysts [34]. The prevalence in other meat-producing animals, such as sheep, goats, and cattle is varying from 2-92% depending on the age and kind of the animal [35].

It concluded that seroprevalence of anti-Toxoplasma antibodies increased in CRF patients undergoing hemodialysis in some Baghdad hospitals according to their age and duration of hemodialysis treatment.

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