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Impact of *Toxoplasmosis* in Immune Respons in Hemodialysis Patients

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Abstract

Background: *Toxoplasma gondii* is the most common infection of protozoa, affecting a wide range of hosts. *Toxoplasmosis* is usually asymptomatic in immunocompetent persons, but in immunocompromised persons (ex:patients on dialysis) significant problems can ensure, and may progress to a life-threatening infection.

Objective: To investigate the seroprevalence of anti-Toxoplasma antibodies in patients undergoing hemodialysis in Baquba / Iraq .

Patients and Methods: A group of blood samples, consisting of 75 dialysis patients and 50 healthy controls, were examined for the detection of anti-toxoplasma antibodies in Baquba Governorate from November 2020 to April 2021 using enzyme-linked immunosorbent assay (ELISA) for IgG and IgM.

Results: The total numbers of the 125 samples were examined, 32 (44.0%) and 2 (4.0%) were IgG-positive for both patients and healthy individual respectively, and the results of the current study showed only 1 (1.33%) was seropositive IgM for patient group, while no seropositive healthy subjects was found for IgM . The results showed that the percentage of seropositivity was higher in 17 males (40.47%) than in females 15 (45.45%) in the group of patients, but the differences were not statistically significant . As for the age groups, a positive increase was observed in the age group 11-30 years when comparing patients and healthy people, as the percentage reached 5 (29.41%). It was also observed that the highest seropositivity in the education levels was in the group of patients at the educational level 6-12 (45.45%) than in the healthy controls group, but the differences were not statistically significant between education levels. Patients living in urban areas had significantly higher serum (P = 0.0041) than patients in rural areas, and the odds ratio was 22,000. And those who had contact with cats showed a significant increase in seropositivity p = 0.0466 and odds ratio was 20.5789.

Conclusion: Dialysis patients are at risk for toxoplasmosis and should be screened on a regular basis to prevent the disease from spreading during hemodialysis.

Keywords: *Toxoplasma gondii, Hemodialysis, Antibodies ,IgG*

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Introduction

Toxoplasma gondii is an obligate intracellular parasite, a zoonotic disease, which causes the infectious disease toxoplasmosis[1]. *T. gondii* is found worldwide and can infect almost all warm-blooded animals[2]. It is different from

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almost all other members of the Apicomplexa family . This is because there are many types of host animals (almost all warm-blooded animals) in the middle life cycle (*T. gondii* infection can be acquired hosts by eating tissues from infected animals, ingesting food or drink contaminated with sporulated oocysts, may be by flies and cockroaches) [3]. However, felines such as domestic cats, are the only known end hosts (definitive host) of parasites that may reproduce sexually[4].Infection does not cause clinical disease in most animal species, were It is estimated that about 40% of cats in the United States are infected; Most do not show any symptoms, but they may develop jaundice or blindness and experience personality changes if the parasite spreads to the liver or nervous system, [5] and it can cause acute, life-threatening diseases[6].The parasite has three developmental stages which are the trophic phase (Tachyzoite) and therefore the refore the latent phase (Bradyzoite) which is found inside the tissue sacs (tissue cysts) and the egg sac (oocytes) that contain inside the (Sporozoite). These stages are associated with the parasite's complex life cycle and every one infective to humans and animals[7].*T.gondii* target autoimmune diseases in only one organ. like type 1 diabetes damages the pancreas. Other diseases, like systemic lupus erythematosus (SLE), affect the whole body. And also patients undergoing hemodialysis to be immu-nocompromised, mainly due to immune response dys-functions regarding phagocytosis, chemotaxis, and the complement system[8]. Studies have shown that dialysis patients are at a higher risk of infection, as the Sharif Maraghi study[9] proved that dialysis patients are a high-risk group for toxoplasmosis and should be tested periodically to prevent the spread of toxoplasmosis during dialysis. The study of Ebrahim Zadeh A [10] Correspond with it.

Toxoplasma gondii infection is asymptomatic in healthy humans and animals because the host's innate and adaptive immunity resists its initial proliferation and eliminates the majority of the parasites. *T. gondii* tachyzoite (a fast growing type of *T. gondii*) infection of monocytes substantially stimulates innate immune responses such as the generation of pro-inflammatory cytokines. As a result, adaptive immunological responses mediated by T and B cells are activated. Activation of adaptive immunity promotes cell-autonomous immune responses in infected cells to disrupt protozoa intracellular development and mediate its clearance, causing *T. gondii* conversion of a gondii stage into a bradyzoite (a form that grows slowly but evades host immune responses) This, in turn, leads to persistent infection. As a result, the immune balance between a healthy host and *T. gondii* is a key step *in T. gondii* immunobiology[11].

Patients and Methods

Study protocol

This study was a cross-sectional study included seventy-five confirmed cases of Kidney failure on dialysis at Baquba Teaching Hospital and and fifty healthy subjects who visited the hospital for examination were used to compare result.

Study population: Inclusion criteria in this study were the research sample is affected persons and control groups . no cases are excluded. depending on the blood sampling

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collected from the infected and control groups .

Study design: Blood samples were collected during (November 1, 2020) to (April 30, 2021). from a total of 75 patients and 50 healthy subjects.Three ml of brachial vein blood were drawn from each group . Sera were separated after centrifugation at 4000 rpm for 5 minutes, and stored at -20o C until required. Information were recorded from patients by using questionnaire paper.

Statistical analysis

The statistical analysis system- SPSS version 22 program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of odd ratio and CI. T-test was used to signify the comparison between means in this study.

Results

A serological prevalence study was conducted on 75 dialysis patients in Baquba General Hospital, for ages Less than 10 - more than 70. The results showed that that the total percentage of positive sera with *T.gondii* antibodies of class IgG in hemodialysis patients was significantly higher than that of the healthy group in the control group. When examining 75 serum samples obtained from hemodialysis patients, it was found that 32 samples (44.0%) were positive against of *T.gondii* IgG, while these antibodies were found in 2 samples (4.0%) of the total of 50 obtained from healthy persons as a control group. As for Toxoplasma antibodies of class IgM, they were diagnosed in only one sample from the dialysis group (1.33%) , while was diagnosed in healthy subjects (control group) as show in Table (1).

Table (1): The percentage of seropositive IgG and IgM antibodies for Toxoplasma gondii in healthy people and dialysis patients

Group	No. tested	No. positive IgG	IgM	Seropositivity% IgG	IgM	Dialysis patients versus healthy people (IgG only) Odds Ratio	95% CI	χ^2 (P-value)
Patients	75	32	1	44.0	1.33	19.6429	4.448-86.731	10.574 ** (0.0001)
Healthy	50	2	0	4.0	0			

** (P≤0.01)- Highly Significant.
NT= number tested; NP= number positive. 95% Confidence Interval..

Table (2) shows the effect of gender factor on the seropositivity of IgG against Toxoplasma gondii in the patient group in addition to the control group. The results showed that the percentage of seropositivity was higher in males than in females in the patient group, but the differences were not statistically significant. As for the control group, there were no differences in the percentage of seropositivity against IgG between males and females. But the males in patient group showed a significantly higher seropositivity (P = 0.0089) than the healthy males and the odds ratio was 16.32. Also, female patients showed significantly higher seropositivity (P = 0.0055) than their healthy female counterparts, with an odds ratio of 20.00. While the total sample showed differences between them, where the seropositivity of the patients was 0.0001 from their healthy counterparts, whereas the odds ratio was 17.8605.

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Table (2): The effect of gender factor on IgG seroprevalence of Toxoplasma gondii parasite in dialysis patients and healthy people

Gender	Seroprevalence of *T. gondii* IgG antibodies						Dialysis patients versus healthypeople Odds ratio	
	Patients			Healthy				
	NT*	NP* (%)	χ^2 (P-value)	NT	NP (%)	χ^2 (P-value)	(95% CI)**	χ^2 (P-value)
Male	42	17 (40.47)	Reference	25	1 (4.00)		16.32 (2.0122-132.36)	9.46 ** (0.0089)
Female	33	15 (45.45)	1.073 NS (0.724)	25	1 (4.00)	10.00 NS (1.00)	20.00 (2.414-165.712)	9.85 ** (0.0055)
Total	75	32 (32.0)		50	2 (4.00)	9.63 ** (0.0006)	17.8605 (4.038-78.986)	10.33 ** (0.0001)

** (P≤0.01)- Highly Significant., NS: Non-Significant.
NT= number tested; NP= number positive. 95% Confidence Interval

From the observations Table (3), it was found that the age factor has a significant effect on the percentage of seropositivity, as the results showed that the highest percentage of seropositivity in the group of dialysis patients was recorded in the age group whose ages ranged between 11-30 years, with a significant difference of 0.005.

Table (3): The effect of age on the seropositivity of IgG immunoglobin of Toxoplasma gondii parasite for dialysis patients compared with healthy subjects

Age (year)	Seroprevalence of T. gondii IgG antibodies						Dialysis patients versus healthypeople Odds ratio	
	Patients			Healthy				
	NT*	NP* (%)	χ^2 (P-value)	NT	NP (%)	χ^2 (P-value)	(95% CI)**	χ^2 (P-value)
11-30	17	5 (29.41)	9.362 ** (0.005)	44	2 (4.54)	0.883 NS (0.591)	8.750 (1.504-50.904)	6.127 * (0.0158)
31-50	17	10 (58.82)		5	0 (0.00)		15.40 (0.734-322.903)	3.196 NS (0.0782)
51-70	34	15 (44.12)		1	0 (0.00)		2.3864 (0.091-62.7061)	0.437 NS (0.6024)
71< More	4	2 (50.00)		0	0 (0.00)		1.000 (0.0136-73.269)	0.00 NS (1.000)
Total	72	32	1.613 (0.204)	50	0			

* (P≤0.05)- Significant.,** (P≤0.01)- Highly Significant., NS: Non-Significant.
NT= number tested; NP= number positive. 95% Confidence Interval

Search results are shown regarding the effect of the residue factor on the seropositiveness of IgG.Table (4) shows that there are no significant differences between rural and city housing between patients and both among the healthy, and patients, as the results showed that patients living in urban areas seropositive is significantly higher (P = 0.0041) than the healthy , the odds ratio was 22,000 . Likewise, patients living in rural areas showed a slightly higher seropositivity (P = 0.00984) than the healthy individual,

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and the odds ratio was 14,560 than their healthy counterparts . In healthy people, there were no seropositive differences between them.

Table (4): Effect of residue factor on IgG seroprevalence of Toxoplasma gondii parasite in dialysis patients and healthy people

Residue	Seroprevalence of *T. gondii* IgG antibodies						Dialysis patients versus healthy people Odds ratio	
	Patients			Healthy				
	NT*	NP* (%)	χ^2 (Pvalue)	NT	NP (%)	χ^2 (P-value)	(95% CI)**	χ^2 (P-value)
Urban	36	18 (50.0)	5.41 * (0.0317)	23	1 (4.35)	0.0761 NS (0.974)	22.000 (2.673-181.073)	10.38 ** (0.0041)
Rural	39	14 (35.89)		27	1 (3.70)		14.560 (1.779-119.109)	9.02 ** (0.00984)
Total	75	32 (42.67)		50	2 (4.00)		17.860 (4.0386-78.986)	11.52 ** (0.0001)

* (P≤0.05)- Significant.,** (P≤0.01)- Highly Significant., NS: Non-Significant.
NT= number tested; NP= number positive. 95% Confidence Interval

According to the effect of the socio-economic factor on the seropositive of the IgG antibody, the Table (5) shows the presence of significant differences between the socio-economic level among patients, but the patients with the average level showed significantly higher seropositivity (P = 0.0003) than their healthy peers, The odds are 16,000 and 95% CI are (3.505-73.025) .In healthy people , the average level showed seropositivity (4.76%) than the peers.

Table (5): Effect of educational level factor on IgG seroprevalence of Toxoplasma gondii parasite in dialysis patients and healthy people

Socioeconomic_ leve	Seroprevalence of *T. gondii* IgG antibodies						Dialysis patients versus healthypeople Odds ratio	
	Patients			Healthy				
	NT*	NP* (%)	χ^2 (P-value)	NT	NP (%)	χ^2 (P-value)	(95% CI)**	χ^2 (P-value)
Low	19	7 (36.84)	5.37 * (0.0394)	4	0 (0.00)	0782 NS (0.762)	5.40 (0.253-115.034)	9.84 ** (0.0006)
Medium	54	24 (44.44)		42	2 (4.76)		16.000 (3.505-73.025)	10.30 ** (0.0003)
High	2	1 (50.0)		4	0 (0.00)		9.000 (0.223-362.504)	11.93 ** (0;0001)
Total	75	32 (42.67)	1.613 (0.204)	50	2 (4.00)	P=0.000 (44.308)	17.860 (4.0386-78.986)	11.54 ** (0.0001)

* (P≤0.05)- Significant.,** (P≤0.01)- Highly Significant., NS: Non-Significant.
NT= number tested; NP= number positive. 95% Confidence Interval

Regarding the effect of the cat breeding factor on the seropositiveness of IgG, the Table (6) shows that there are no significant differences in the cat breeding factor among the patients, but the patients with the answer (yes) showed a significantly higher seropositivity p = 0.0466 than their healthy peers and the odds ratio was 20.5789 .As for the patients who answered (no), they showed significantly higher seropositivity (P = 0.0021) than their healthy peers, and the odds ratio was 25,323.

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Table (6): The effect of cat breeding factor on IgG seroprevalence of Toxoplasma gondii parasite in dialysis patients and healthy people

Cat_ breeder	Seroprevalence of *T. gondii* IgG antibodies						Dialysis patients versus healthypeople Odds ratio (95% CI)** χ^2 (P-value)	
	Patients			Healthy				
	NT*	NP* (%)	χ^2 (P-value)	NT	NP (%)	χ^2 (P-value)		
Yes	20	11 (55.0)	3.872 NS (0.0741)	8	0	0.367 NS (0.891)	20.5789 (1.046-404.691)	11.38 ** (0.0001)
N0	55	21 (38.18)		42	1 (2.381)		25.323 (3.237-198.091)	9.74 ** (0.0021)
Total	75	32 (42.66)	1.613 (0.204)	50	1 (2.0)		36.465 (79-278.229)	9.652 ** (0.0005)

** (P≤0.01)- Highly Significant., NS: Non-Significant.
NT= number tested; NP= number positive. 95% Confidence Interval

Discussion

Toxoplasmosis is one of the infectious causes of morbidity and mortality among hemodialysis patients and immune compromised individuals [12]. Patients with hemodialysis and persons with immune system abnormalities have high priority *T. gondii* infection [13]. Dialysis patients are at a greater risk of reactivating toxoplasmosis infection due to their immunodeficiency. An early diagnosis is therefore strongly recommended in these individuals [14].Since hemodialysis patients are at a higher risk of *T. gondii* infection ; thus, the aim of this study was to investigate seroprevelence of toxoplasmosis in hemodialysis patients of Baquba , in 2020-2021 . On the other hand, toxoplasmosis occurs mostly in tropical and subtropical regions [15]. The results of this study showed that about 44.0% and 1.33% of hemodialysis patients had elevated serum positivity for IgG and IgM antibody tests, respectively by ELISA, compared to healthy subjects whose serum positive for IgG and IgM was about 4.0% and 0%, respectively, It has been reported that sera positive for the anti-*T.gondii* IgG antibodies reveal a chronic pattern of infection and are frequently detected in individuals who have been infected in the distant past and usually endure for life, whereas sera positive for IgM antibodies reflect an acute pattern and are highly predictive of newly acquired infections, emerging earlier and fading quicker than IgG antibodies [16]. Our result conforms with results recorded by Shahrzad Soltani & [17] who mentioned that seroprevalence rate of *T. gondii* infection was higher in patients undergoing dialysis than control individuals (49.5%vs 23.0%). In addition the results of the current study are similar to the results of a study by Sarah Hussein [18], the study showed that the percentage of seropositive IgG antibody among chronic renal failure (CRF) patients was 54.1% while it was 38.2% among healthy volunteers, and this indicates a higher prevalence of toxoplasma infection in hemodialysis patients. A study

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Masoud Foroutan [19] revealed that patients on hemodialysis were more vulnerable to *T. gondii* ,due to their inability to guarantee adequate personal and dietary cleanliness. When the immune system is compromised, acute or active infections, such as encephalitis, can lead to life-threatening complications. The study of Forough Kazemi, [20] , Al-Saadawi and Alkhaled [21] , Ayser and Abdul-Aziz [22] Bayani,M [23] ,Al-Dulaimi [24], Sharif Maraghi [25], recorded similar results of our current study. On the other hand, there are studies that differ with it Seyyedpour [26]. Among the hemodialysis patients, the results showed that the percentage of seropositivity was higher in males 17(40.47) than in females 15 (45.45) but the differences were not statistically significant , Jones [27] . Did not find a significant difference between males and females regarding the prevalence of *T.gondii* in brazilian dialysis patients. In contrast, some researchers reported a significant link between *Toxoplsmosis* and gender in the Iranian hemodialysis patients [28], this result differed with Ayser I [22], where the prevalence of anti-Toxoplasma IgG antibodies were higher in females 72 (55.81%) than in males 57 (44.18%), with a significant difference between them ($p<0.05$).Our results are also different with the results recorded by Al-Shikly A.M.S [29]for females (38.45%) using ELISA-IgG and (42.1%) using mini VIDAS-IgG, while for males (12.83%) and (14.4%) respectively. The reason for this difference is due to different sampling methods, diverse life styles, or gender differences in different communities could explain this diversity. And the age groups (51-70) had the highest rate of positivity serum IgG in hemodialysis patients (Odds ratio: 2.3864) comparing with healthy individual , this study is similar to Ayser I. &[22] that showed that seroprevalence of *Toxoplasma gondii* specific IgG .antibodies. were higher in the age group (43-51) year, .the reason for this is that the total prevalence of antibodies has gradually increased with age and patients weakened immunity . With regard to the effect of educational level factor, this study no statistically significant differences between levels of education, both between patients and healthy people , were showed that educational level (6-12) showed significantly higher serum positivity ($P = 0.0068$) than their peers (Odds ratio:10,000).This result is identical to Saadat [30], a study were seroprevalence of *T. gondii* infection did not vary significantly with educational level. Despite this result, most children suffer from weak immune systems and are therefore most susceptible to toxoplasmosis at this stage, they can also contract this infection by accidentally swallowing soil or eating undercooked fruits or vegetables contaminated with cat feces from a cat with toxoplasmosis. Regarding rural and city residents shows that there are no significant differences between rural and city housing between patients and both among the healthy, and patients , Similar to the results of a study Bayani [23], did not observe significant differences in seropositivity among Iranian hemodialysis patients living in rural areas and those living in urban areas. It has been found that as *T. gondii* causes glomerular lesions and urinary abnormalities which lead to renal failure which can be detected by an increase in creatinine levels in the urine [31]. As for the effect of the cat breeding factor, it appears that there are no statistically

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significant differences in the cat breeding factor among patients, but the patients with the answer (yes) showed a significantly higher seropositivity p = 0.0466 than their healthy peers and the odds ratio was 20.5789. This study is similar to Ayser [22] study ,the results of this study indicate that there is a relationship between the spread of the disease and contact with cats, despite the differences in basic personal hygiene among the population surveyed in Diyala Governorate, and we found that Although not so many people own cats in Iraq, many stray cats can be seen in the streets and in the houses of people who do not have cats as they can move freely from house to house. Recently, Retmanasari [32] stated that " a small number of cats do not necessarily indicate there is no risk of infection in that location because a single cat can shed more than 100 million oocysts during the prepatent period (approximately 18 days) and these oocysts can survive outdoors for many months, remain viable for long periods of time in water, and resist freezing and moderately high water temperatures".

Conclusions

The results of the present study confirm a high prevalence of *T.gondii* infection among chronic kidney disease (CKD) patients undergoing hemodialysis in Iraq , since hemodialysis patients are immunocompromised and *T. gondii* can cause serious clinical complications.

Recommendations

Recommending the implementation of an awareness program for patients that shows them the available methods of infection so that they can take the necessary preventive steps, such as taking care of their health, avoiding consuming contaminated water or undercooked meat, and avoiding contact with loose cats and soil contaminated with feces.

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Conflict of interest: Nill

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تأثير داء المقوسات في الاستجابة المناعية لمرضى غسيل الكلى

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الملخص

خلفية الدراسة: توكسوبلازما كونداي هي العدوى الأكثر شيوعًا للبروتوزوا ، وتؤثر على مجموعة واسعة من العوائل. عادة ما يكون داء المقوسات بدون أعراض في الأشخاص المؤهلين مناعياً ، ولكن في الأشخاص الذين يعانون من نقص المناعة (على سبيل المثال: مرضى غسيل الكلى) يمكن أن تضمن المشاكل الكبيرة ، وقد تتطور إلى عدوى تهدد الحياة.

اهداف الدراسة: أجريت هذه الدراسة للتحقق من الانتشار المصلي للأجسام المضادة للتوكسوبلازما في المرضى الذين يخضعون لغسيل الكلى في بعقوبة / العراق.

المرضى والطرائق: تم فحص مجموعة من عينات الدم ، مكونة من ٧٥ مريض غسيل كلى و ٥٠ ضابط سليم ، للكشف عن الأجسام المضادة للتوكسوبلازما في محافظة بعقوبة من تشرين الثاني ٢٠٢٠ إلى نيسان ٢٠٢١ باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) لـ IgG و IgM.

النتائج: تم فحص إجمالي عدد ١٢٥ عينة ، ٣٢ (٤٤,٠٪) و ٢ (٤,٠٪) كانت موجبة IgG لكل من المرضى والأفراد الأصحاء على التوالي ، وأظهرت نتائج الدراسة الحالية أن ١ فقط (١,٣٣٪) كانت موجبة IgM موجب المصل لمجموعة المرضى ، بينما لم يتم العثور على أي مواد صحية إيجابية مصلية لـ IgM. أظهرت النتائج أن نسبة الإيجابية المصلية كانت أعلى عند ١٧ ذكر (٤٠,٤٧٪) منها عند الإناث ١٥ (٤٥,٤٥٪) في مجموعة المرضى ، لكن الفروق لم تكن ذات دلالة إحصائية. أما بالنسبة للفئات العمرية فقد لوحظ ارتفاع إيجابي في الفئة العمرية ١١-٣٠ سنة عند المقارنة بين المرضى والأشخاص الأصحاء حيث بلغت النسبة ٥ (٢٩,٤١٪). كما لوحظ أن أعلى إيجابية مصلية في مستويات التعليم كانت في مجموعة المرضى في المستوى التعليمي ٦-١٢ (٤٥,٤٥٪) منها في مجموعة الضوابط الصحية ، لكن الفروق لم تكن ذات دلالة إحصائية بين مستويات التعليم. كان المرضى الذين يعيشون في المناطق الحضرية لديهم مصل أعلى بكثير P=٠,٠٠٤١ من المرضى في المناطق الريفية ، وكانت نسبة الأرجحية ٢٢٠٠٠. وأولئك الذين تعاملوا مع القطط أظهروا زيادة معنوية في الإيجابية المصلية P= ٠,٠٤٦٦ ونسبة الأرجحية ٢٠,٥٧٨٩.

الاستنتاجات: مرضى غسيل الكلى معرضون لخطر الإصابة بداء المقوسات ويجب فحصهم بشكل منتظم لمنع المرض من الانتشار أثناء غسيل الكلى.

الكلمات المفتاحية: توكسوبلازما كونداي ، غسيل الكلى ، الأجسام المضادة ، IgG

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