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Toxoplasmosis and Its Potential Role to Change the Levels of C - reactive protein and Vitamin D3 in Atherosclerosis Patients

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Abstract

Atherosclerosis is a condition of the hardening of a blood vessel via the development of plaques around the artery wall which causes the artery to narrow, leading to severe complications. Toxoplasmosis is an opportunistic parasitic infection that causes pathological complications in immunocompromised patients, which lead to increase the burden on the immune system in these patients. This study aims to assess the incidence rate of toxoplasmosis in atherosclerosis patients and its potential to change C - reactive protein (C-RP) and vitamin D3 levels. Serum samples (150) were tested for the positivity of anti-*Toxoplasma* IgG and IgM antibodies by means of Enzyme-linked immunosorbent assay (ELISA). In addition, C-RP was assessed in all serum samples by means of Latex Fixation Test, while VtD3 was estimated by MiniVidas device. The results revealed that the seroprevalence of toxoplasmosis in atherosclerotic patients was comparatively higher as compared to that in the control group, with significant differences in C-RP and VtD3 levels. These results suggest that the decreased levels of VD3 lead to increase the incidence of *T. gondii* infection in atherosclerosis patients.

Keywords: Atherosclerosis; *Toxoplasma gondii*; Vitamin D3; C - reactive protein.

الدور المحتمل لداء المقوسات في تغيير مستويات البروتين التفاعلي C وفيتامين D3 في مرضى تصلب الشرايين

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الخلاصة

تصلب الشرايين هو حالة تصلب الأوعية الدموية الناتجة من تراكم اللويحات حول جدار الشريان مما يؤدي إلى تضييق الشريان ويؤدي إلى مضاعفات شديدة. داء المقوسات هو عدوى طفيلية انتهازية تسبب مضاعفات مرضية في المرضى الذين يعانون من نقص المناعة مما يؤدي إلى زيادة العبء على الجهاز المناعي لدى هؤلاء المرضى. تهدف هذه الدراسة إلى تقييم معدل الإصابة بداء المقوسات في مرضى تصلب الشرايين وقدرته على تغيير مستويات البروتين التفاعلي C وفيتامين D3. تم جمع 150عينة دم واختبارها لوجود الأجسام المضادة لـ IgG و IgM المضادة للمقوسات باستخدام مقايسة الممتص المناعي المرتبط بالإنزيم (ELISA). بالإضافة إلى ذلك ، تم تقدير C– البروتين التفاعلي سي (C–RP) في المصل في جميع المواد باستخدام اختبار تثبيت اللاتكس ، في حين تم تقدير VtD3 بواسطة جهاز MiniVidas. أظهرت

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النتائج فروق معنوية ($P \leq 0.05$) بين جميع المجموعات المدروسة. تشير هذه النتائج إلى أن انخفاض مستويات VD3 يؤدي إلى زيادة حدوث عدوى المقوسات الكوندية في مرضى تصلب الشرايين.

Introduction

A wide range of animals can be infected by the protozoan parasite, *Toxoplasma gondii*. Human infection with *T. gondii* occurs through contaminated soils [1], organ transplant or undercooked meat [2]. This parasitic infection includes an acute phase followed by a latent stage inside tissue cysts. Serum immunoglobulin IgM is the marker of the acute phase response of infection, while IgG is a marker of the response for the latent phase [3].

T. gondii-infected immunocompromised patients could develop severe neurological disease [4], myocarditis [5, 6, 7, 8], and severe heart damage [9]. Accordingly, *T. gondii* in myocarditis patients could exist with congestive heart failure and arrhythmias [10]. Inflammation plays an important role in the progress of heart illness. Atherosclerosis is an inflammatory disorder due to the existence of lipids in the vascular walls. This kind of inflammation includes the interaction of vascular wall components, inflammatory cells and lipoproteins, which causes the release of cytokines and eventually increase the acute phase reactants, including fibrinogen and C-RP [11].

C- reactive protein is one of the acute phase signs [12] that is produced by the liver. Moreover, CRP level is raised during pathogenesis and following the surgery. Studies suggest that CRP increases the production of adhesion molecules, thus decreasing the bioactivity of nitric oxide and changing the absorption of low-density lipoprotein by macrophages [13].

Hepatic 25-hydroxylase metabolize vitamin D into 25-hydroxyvitamin D (25(OH)D) while renal 1a-hydroxylase metabolize vitamin D into the vitamin D hormone calcitriol. Many other tissues also have 1a-hydroxylase enzyme, which is able to use circulating 25(OH)D as a substrate. Metabolite 25(OH)D level status is the best measurement for vitamin D [14]. The nutritional vitamin D supplies for most people are received either through exposure to cutaneous solar ultraviolet radiation or can be ingested orally [15], whereas foods naturally contain smaller amounts of this vitamin [16]. Vitamin D insufficiency is caused by numerous health consequences [17] such as non-skeletal complications including cardiovascular diseases [18].

Materials and methods

A total of 100 atherosclerosis patients and 50 toxoplasmosis patients were included in the present study, who attended Baghdad Teaching Hospital during the period from December 2018 to September 2019, as well as 50 healthy persons as a control group.

Blood sampling

Five milliliters of blood was collected by venipuncture from all patients and the control subjects. Each blood sample was placed in a tube, and then centrifuge was used to obtain serum for immunological and biochemical measurements. Vitamin D along with anti-*Toxoplasma* IgG and IgM antibodies were detected by Human ELISA Kit, Demeditec, Germany, while C-RP was detected by latex agglutination test (Montgat, SPAIN).

Results and discussion

Anti-*T. gondii* IgG antibodies were recorded in 73 (73%) atherosclerosis patients and 50 (40%) controls. While, anti-*T. gondii* IgM antibodies were recorded in 5 (5%) atherosclerosis patients and 2 (4%) controls. The seroprevalences of anti-*T. gondii* IgG antibodies and anti-*T. gondii* IgM antibodies were significantly higher in the patients than in the controls ($P < 0.01$) (Tables- 1, 2).

Table 1-Comparison between studying groups in results of anti-*Toxoplasma* IgG using ELISA test

Studying groups	**IgG (-)**		**IgG (+)**		P-value
	No.	**%**	**No.**	**%**	
Control	30	60.00	20	40.00	0.0073 **
Atherosclerosis patients	27	27.00	73	73.00	0.0001 **
P-value	0.0056 **		0.0056 **		---
** (P<0.01)					

Table 2-Comparison between studying groups in results of anti-*Toxoplasma* IgM using ELISA test

Studying groups	IgM (-)		IgM (+)		P-value
	No.	%	No.	%	
Control	48	96.00	2	4.00	0.0001 **
Atherosclerosis patients	95	95.00	5	5.00	0.0001 **
P-value	0.873 NS		0.873 NS		---
** (P<0.01), NS: Non-Significant					

Also, the results revealed that the seroprevalences of anti-*T. gondii* IgG antibodies and anti-*T. gondii* IgM antibodies were significantly higher in atherosclerosis patients than in the control group (P < 0.01), as demonstrated in Table-3.

Table 3- Comparison between anti-*Toxoplasma* IgG and IgM antibodies in atherosclerosis and the control groups

Antibody	Control		Atherosclerosis		P-value
	No.	%	No.	%	
Anti- *T. gondii* IgG (+)	20	90.91	73	93.59	0.736 NS
Anti- *T. gondii* IgM (+)	2	9.09	5	6.41	0.736 NS
P-value	0.0001 **		0.0001 **		---
** (P<0.01), NS: Non-Significant					

In relation to the age group, the results showed a high percentage of **seropositive** anti- *Toxoplasma* IgG patients, which was 44 (6.27%), in the age group of 61 -70 year. The statistical analysis also exhibited highly significant age differences (P<0.01) between atherosclerosis patients (Table-4).

Table 4-Distribution of the atherosclerosis patients according to the age groups in control and anti-*Toxoplasma* IgG

Age group (year)	Atherosclerosis patients (No. =100)	Atherosclerosis patients (IgG +) (No. =73)
40-50	10 (10.00%)	8 (10.96%)
51-60	37 (37.00%)	21 (28.77%)
61-70	53 (53.00%)	44 (6.27%)
Chi-square (χ^2)	9.936 **	7.608 **
** (P<0.01)		

According to gender, the overall percentage of toxoplasmosis incidence was higher in the male group, which was, 53 (72.60%), in the form of chronic infection, with significant differences (P<0.01), as shown in Table-5.

Table 5- Distribution of atherosclerosis patients according to the gender and anti- *Toxoplasma* IgG

Gender	Atherosclerosis patients	Atherosclerosis patients IgG (+)
Male	60 (60.00%)	53 (72.60%)
Female	40 (40.00%)	20 (27.40%)
Total	100	73
Chi-square (χ^2)	7.250 **	123.564 **
** (P<0.01)		

In the current study, there is a highly significant difference (P<0.01) in the level of VD3 between patients with toxoplasmosis and atherosclerosis) (12.3µg/ml and 10.5µg/ml, respectively) in comparison with the control group (32.3µg/ml), as shown in (Table-6).

Table 6-The mean concentration of VD3 in the serum of the studied groups

Studying groups	VD3 mean concentration (µg/ml)	P-value
Healthy control	32.3 ± 1.75 a	0.0001 **
Patients with toxoplasmosis	12.3 ± 0.52 b	
Atherosclerosis patients infected with toxoplasmosis	10.5 ± 0.44 b	
** (P<0.01)		

In addition, there is a high significant difference (P<0.01) regarding CRP levels between toxoplasmosis and atherosclerosis patients (26.90 ± 1.07 and 32.75 ± 1.29 µg/ml, respectively) in comparison with a control group which was 8.22 ± 0.37 µg/ml (Table- 7).

Table 7-The mean concentration of CRP in the serum of the studied groups

Studying groups	CRP mean concentration (µg/ml)	P-value
Healthy control	8.22 ± 0.37 c	0.0001 **
Patients with toxoplasmosis	26.90 ± 1.07 b	
Atherosclerosis patients infected with toxoplasmosis	32.75 ± 1.29 a	
** (P<0.01)		

Prevalence of toxoplasmosis in patients with heart atherosclerosis is unidentified yet. The results of this study showed that patients with atherosclerosis signify a risk group for toxoplasmosis infection. Levels of anti-*T. gondii* IgM antibodies were significantly lower than those of anti-*T. gondii* IgG antibodies, with the difference being higher in the patients compared with controls; such a result indicates that atherosclerosis could mostly be associated with the chronic than the acute infection with toxoplasmosis. This could be due to the chronic infection that stimulates the pathophysiological chronic inflammation. The infections implicate the starting, development, and rupture of atherosclerotic plaque. It was hypothesized that atherosclerosis is a chronic inflammation with lipid accumulation due to endothelial damage [19, 20]. Several studies suggest that infectious agents contribute to atherogenesis through two mechanisms; the first one is through the attack of cells which cause enhanced plaque growth by local effects, whereas the second is by producing systemic inflammatory cytokines which stimulate progression of plaque [21].

The presence of IgG in the sera indicates the presence of a chronic infection. Thus, the individual has been exposed to the parasite, but there is no evidence of acute infections. The presence of IgG antibodies in patients' sera may also indicate latent infections, therefore signifying the potential recurrence of the disease condition among the older patients population [22].

Parasites affect the host endocrine system, which is responsible for altering the host behaviors. Host modifications due to *T. gondii* infection is significantly related to the gender [23]; men have higher testosterone levels and consequently have more susceptibility to *Toxoplasma* infection, either due to a weakened immune response or personal hygiene which increases the sources of infection [24]. Vitamin D_3 synthesis is stimulated by cutaneous exposure to UVB radiation [25] and plays a part in calcium metabolism and immune regulating functions [26]. Several cardiovascular risk factors have been documented and VtD_3 deficiency is one of them. In addition, VtD_3 is recognized as having an important influence in cardiovascular condition [27].

Toxoplasmosis is a risk factor in immunocompromised individuals [28] and the immune systems is involved in this parasitic infection [29]. VtD is an immune-modulator [30] which can inhibit IL2 production. Populations have a high incidence of VtD deficiency [31]. In addition, *T. gondii* causes one of the widespread infectious diseases [3]. Concerning the current study's results, *T. gondii* infection was associated with vitamin D deficiency. More studies are suggested to be conducted for understanding the relationship between VtD and parasitic infections.

Inflammation plays a key role in the pathogenesis of atherosclerosis [32] and promotes the expression of CRP as an acute-phase proteins [33]. CRP concentration in the serum can increase to more than 1000-fold within 19 hours upon inflammation [34]. CRP is a sign of the inflammatory route, thus its levels in the plasma have a predictive value in the pathogenesis [35]. CRP could promote the formation and progression of atherosclerotic plaques [36]. On the other hand, there is an association between toxoplasmosis and CRP levels [37], which elevate during inflammation [38, 39].

Conclusions

The seroprevalence of toxoplasmosis in atherosclerotic patients was found to be comparatively higher than the healthy people. Since atherosclerosis weakens the immune response, it could lead the way to toxoplasmosis. Thus, *T. gondii* should be diagnosed and treated to decrease the burden on the immune system and reduce the consequences when both diseases are present.

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