CD14, CD16 and CD22 as Immunological markers for toxoplasmosis

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ABSTRACT

Backround: *Toxoplasma gondii* is an apicomplexan parasite that may infect any nucleated cell in any warm-blooded animal and cause toxoplasmosis. **Methods**: blood sample were collected from couples who attended in Al-Zahraa Hospital and Baqubah teaching hospital in Diyala Province during the period from March to August 2024 from both genders (males and females) whose ages ranged between 14 to ≥ 40 years. ELISA test was conducted for all samples (376) to detect the infection and later to determine the concentration of, CD14, CD16 and CD22. **Results:** there was significant differences in CD14 and CD16 levels between the patients and the control group, meanwhile CD22 registered no significant difference between the studies groups. Results showed significant differences in mean level of CD14, CD16 and CD22 in IgM positive compared with IgM negative, also results showed no significant differences in mean level of CD14, CD16 and CD22 in IgG positive compared with IgG negative. Sensitivity and specificity testes were measured for the studied CD, results indicated that both CD 16 and CD22 have a high sensitivity to the test. The study showed positive correlation of IgM with the studies CD markers however CD16 has the strongest positive correlation with IgM and significant negative correlation with IgG that may indicate that CD 16 marker have a role which increased in acute infection rather than in chronic infections. Further investigations are required to better understand the role of each CD (CD14, CD16 and CD22) in the acute and chronic toxoplasmosis.

Keywords- Toxoplasma gondii, ELISA, CD22, toxoplasmosis.

Introduction

Toxoplasma gondii is an apicomplexan parasite capable of infecting any nucleated cell in warm-blooded animals, resulting in toxoplasmosis [1]. Although only a small portion of those infected may experience severe illness, *Toxoplasma gondii* is one of the most harmful zoonotic infections in the world due to its widespread distribution, which affects about 2 billion people [2]. If toxoplasmosis is initially acquired during pregnancy, it can result in miscarriage or congenital problems in the fetus; even in immunocompetent individuals, it can cause major eye disease; and it can induce a deadly encephalitis in immunocompromised individuals [3].

T. gondii possesses three infectious stages: the rapidly dividing tachyzoite, the slowly developing bradyzoite within tissue cysts, and the environmentally resilient

sporozoite encapsulated within an oocyst [4]. Human infection typically arises from the ingestion of food or water contaminated with tissue cysts or oocysts [5]. Congenital "vertical" transmission occurs when *T. gondii* tachyzoites are transmitted from mother to fetus via the placenta. Alternative transmission pathways for *T. gondii* encompass organ transplantation and blood transfusion [6].

Humoral immunity plays an important role in acquired resistance against toxoplasmosis, as immunity develops as the infection progresses, and this leads to the formation of immunoglobulin antibodies [7]. The first antibody formed is IgA, which appears for a short period, and begins to appear when the parasite enters the intestinal lining. It is one of the most important antibodies found in the intestinal lining [8]. It is unstable at the beginning of infection with toxoplasmosis and is considered an indicator of acute infection. In the event that the parasite spreads to the rest of the body, immune globulin begins to appear about 1-2 weeks after infection, and after about 4-8 weeks of infection, IgM begins [9], it decreases and remains for 6 months while IgG appears after the fourth week of infection in people with complete immunity, IgG levels remain high for several months [10].

The cluster of differentiation (CD) designation pertains to cell surface proteins. Each distinct molecule is allocated a unique numerical designation, facilitating the identification of cell phenotypes. The surface expression of a certain CD molecule may not be exclusive to a single cell or cell lineage; however, many are valuable for characterizing cell phenotypes [11].

Pro-inflammatory cytokines are crucial in initiating and sustaining both innate and acquired immunity to inhibit the proliferation of Toxoplasma. A diverse array of cytokines is generated upon the activation of antigen-presenting cells and adaptive immune cells (B and T lymphocytes) [12]. Innate immunity serves as the initial defense against invading pathogens, and this response has been thoroughly researched. Despite the crucial function of innate immunity, the adaptive immune response is vital for the host's ultimate survival following infection. For example, a study indicated the establishment and preservation of a resilient CD8 T cell immunity, supported by helper CD4 T cell responses, is essential for maintaining the parasite in a chronic condition and averting the reactivation of latent infection [13].

CD14 was first identified as mediating the host cytokine response to sepsis, particularly TNF. Apart from macrophages, CD14 expression has been found in a number of other immune cell types, such as dendritic cells and human and mouse neutrophils [14]. It has been demonstrated that when the CD14 glycoprotein receptor binds to oxidized lipids or LPS, it stimulates macrophage activation. A membrane receptor for lipopolysaccharides (LPS) on macrophages [15]. Meanwhile, CD16 is a molecule present on the surface of natural killer cells, neutrophils, monocytes, macrophages, and specific T lymphocytes. CD16 is an IgG Fc receptor (FcγRIIIa) present on natural killer cells and phagocytes. The most recognised function of CD16 in NK cells is activation triggered by IgG binding, however an IgG-independent role in activation has also been established. The extracellular domains of CD16 have been delineated, in part, by specialised monoclonal antibodies that attach to particular areas of the protein [16]. Moreover, CD22 is a transmembrane glycoprotein present on the surface of mature cells. CD22 is a constituent of the SIGLEC family (sialic acid-binding immunoglobulin-like lectin), characterised by membrane proteins that feature an immunoglobulin variable-like shape, a solitary transmembrane domain, and abbreviated cytoplasmic tails. CD22 has a crucial function in regulating B-cell trafficking via blood arteries, apoptosis, T-cell modulation, and likely in the control of cell proliferation. CD22 is believed to be significant in the migration of lymphocytes. CD22 plays a role in the development of B-cell germinal centres and promotes the clustering of antigen

receptors. CD22 is crucial for starting B cell development from stem cells in the bone marrow [17]. The range of molecular pathways that may influence the effects of Monocyte-Toxoplasma interactions is increased by the fact that there are numerous techniques to achieve each of these infection outcomes. In addition, it is believed that only CD16+ monocytes, which phagocytose the parasite, are capable of eliciting an efficient host response to the parasite, as indicated by the generation of IL12. This is in contrast to the three recognised subsets of human monocytes: classical, intermediate, and non-classical [18].

The definitive method for the serological identification of anti-Toxoplasma IgG and IgM antibodies remains the Sabin-Feldman dye test. However, most laboratories cannot use live tachyzoites, which is required for this approach. The detection of particular IgG and IgM antibodies against *T. gondii* is frequently accomplished using an enzyme-linked immunosorbent test (ELISA). IgG titers are a good indicator of TE risk since they peak one to two months after infection and remain elevated for the rest of one's life [19]. The avidity of IgG is low during the acute phase and high during the chronic phase of toxoplasmosis; thus, the detection of low IgG avidity serves as a reliable diagnostic of recent infection, whereas high avidity indicates infection occurred 3-5 months prior. while IgG detection provides significant specificity and sensitivity, IgM detection remains comparatively low in sensitivity [20]. CD markers are leukocyte cell surface molecules and their corresponding ligands expressed by various organs. CD markers are utilised to detect, quantify, analyse, purify, eliminate, or otherwise manipulate leukocytes. The nomenclature originates from research with antibodies targeting leukocytes, and the term CD marker is employed in antibody-based studies, however the CD number pertains to the leukocyte molecule rather than the antibody utilized for detection [21]. Recently, many studies indicated the ability of CD to be a reliable marker for many diseases. Accordingly, the current study aimed to investigate the ability to use CD14, CD16 and CD22 as immunological markers for Toxoplasmosis.

Methods

Study Design

The study has been conducted in Diyala Province during the period from March 2024 to August 2024 from both genders (males and females) and from ages ranged between 14 to ≥ 40 years. Blood samples were collected randomly from couples intend marriage who attended in Al-Zahraa Hospital and Baqubah teaching hospital.

Blood samples and Immunological tests

Five ml of venous blood was drawn. The blood samples were placed in clean plastic plan tubes devoid of any substance, then left for 15-30 minutes (allow the blood to clot by leaving it undisturbed at room temperature), after which they were placed in a centrifuge for 5 mins/3000 rpm for the purpose of separating the blood serum and kept frozen (-20C) until laboratory tests were performed. ELISA test was conducted for all samples (376) to detect the infection and determine the concentration of IgG and IgM specific antibodies. After that 96 blood samples were classified to three categories, Toxoplasmosis IgG positive, Toxoplasmosis IgM positive and Control (no infection with Toxoplasmosis) to measure the levels of CD14, CD16 and CD22.

Statistical analysis

The used program is IBM SPSS version 27.0 for calculating the frequency and percentages, and drawing graphs.

Results

CD14, CD16 and CD22 levels in patients and control group

The result of this study showed significant differences mean level of CD14 between the patients and the control (Table 1). The results also indicated significant differences in

the mean value of CD16 in patient compared with control. Whereas, the mean level of CD22 in patients indicated no significant difference from the controls (Table 1).

Table 1: Comparative study of CD14, CD16 and CD22 between patient and control group calculated by t test

Groups		N	Mean	SD	SE	*P* value
CD14	Patients	65	3.89	4.72	0.58	< 0.01*
	Healthy	25	2.04	0.90	0.18	
CD16	Patients	65	0.562	0.497	0.061	< 0.01*
	Healthy	25	0.392	0.073	0.014	
CD22	Patients	65	3.87	6.69	0.83	> 0.05
	Healthy	25	2.74	3.13	0.62	

Comparative study of CD parameters based on IgM

The result of the study illustrated significant differences in mean level of CD14, CD16 and CD22 between blood samples that IgM positive compared with IgM negative (Table 2).

Table 2: Comparative study of CD parameters based on IgM calculated by t test

Groups		N	Mean	SD	SE	*P* value
CD14	IgM (+)	27	7.03	6.0	1.15	< 0.001**
	IgM (-)	63	1.81	0.97	0.12	
CD16	IgM (+)	27	0.987	0.538	0.103	< 0.001**
	IgM (-)	63	0.324	0.091	0.011	
CD22	IgM (+)	27	8.26	8.72	1.67	< 0.001**
	IgM (-)	63	1.53	2.19	0.27	

Comparative study of CD parameters based on IgG

The result of study showed no significant differences at 0.05 in the mean levels of CD14, CD16 and CD22 between IgG positive blood samples and IgG negative blood samples (Table 3).

Table 3: Comparative study of CD parameters based on IgG calculated by t test.

Groups		N	Mean	SD	SE	*P* value
CD14	IgG (+)	52	3.40	4.42	0.61	> 0.05
	IgG (-)	38	3.33	3.73	0.60	
CD16	IgG (+)	52	0.504	0.479	0.06	> 0.05
	IgG (-)	38	0.548	0.335	0.057	

CD22	IgG (+)	52	3.94	7.23	1.0	> 0.05
	IgG (-)	38	3.02	3.46	0.56	

Study of receiver operating characteristic (ROC) for CD14, CD16 and CD22 based on IgM

Results of ROC test (Table 4, Figure 1) shows that the three examined variables (CD14, CD16, and CD22) could be considered important biomarkers in distinguishing between toxoplasmosis patients and toxoplasmosis free people. The results revealed both CD22 and CD16 were excellent biomarkers for toxoplasmosis, with sensitivity 92.6 %96.3 % respectively, whereas CD14 can be considered a good biomarker for toxoplasmosis (66.7 % sensitivity) Table 4.

Table 4: Sensitivity and specificity of CD14, CD16 and CD22 based on IgM

Variables	AUC	Std. Error[a]	*P* value	Sensitivity %	Specificity %
CD14	0.890	0.039	< 0.001**	66.7 %	97.4 %
CD16	0.976	0.017	< 0.001**	92.6 %	94.7 %
CD22	0.975	0.017	< 0.001**	96.3 %	94.7 %

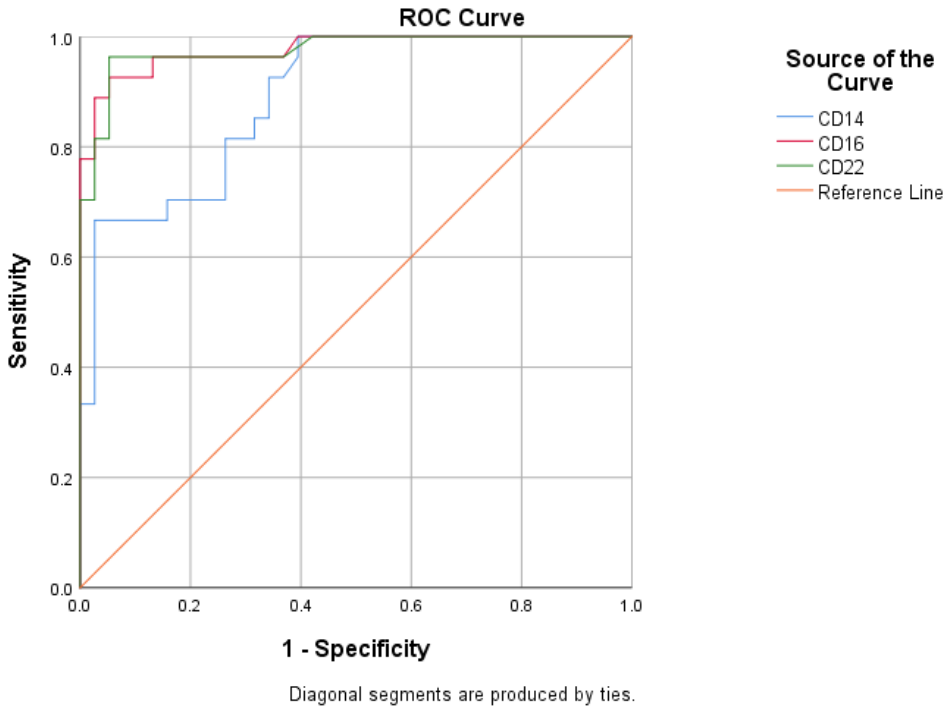

Figure 1: Roc curve of CD14, CD16 and CD22

Correlation between studied parameters

The study demonstrated a substantial positive link between IgM levels and CD14, CD16, and CD22, while

revealing a significant negative correlation between IgM and IgG. Additionally, a substantial positive association was observed between CD14 and both CD16 and CD22, although a non-significant negative correlation existed between CD14 and IgG. CD16 exhibits a positive connection with CD22 and a negative correlation with IgG. CD22 exhibited a favourable connection with IgG (Table 5).

Table 5. Correlation between studied immune parameters

		IgM	CD14	CD16	CD22	IgG
IgM	Pearson Correlation	1	.553**	.711**	.558**	-.593**
	Sig. (2-tailed)		.000	.000	.000	.000
	N	65	65	65	65	65
CD14	Pearson Correlation	.553**	1	.822**	.698**	-.238
	Sig. (2-tailed)	.000		.000	.000	.056
	N	65	65	65	65	65
CD16	Pearson Correlation	.711**	.822**	1	.765**	-.280*
	Sig. (2-tailed)	.000	.000		.000	.024
	N	65	65	65	65	65
CD22	Pearson Correlation	.558**	.698**	.765**	1	.022
	Sig. (2-tailed)	.000	.000	.000		.859
	N	65	65	65	65	65
IgG	Pearson Correlation	-.593**	-.238	-.280*	.022	1
	Sig. (2-tailed)	.000	.056	.024	.859	
	N	65	65	65	65	65

Discussion

The infectious disease toxoplasmosis, caused by the obligatory intracellular protozoan parasite, can cause mild symptoms in healthy people and potentially fatal complications in those with impaired immune systems [22].

The study result indicated that the present of both IgG and IgM in the serum of the study participants. IgM antibodies are initially created in response to toxoplasmosis, with their levels increasing temporarily before subsequently declining. Specific IgM levels may decline to below the detection threshold within three months' post-infection [23].

CD markers are helpful in the diagnosis and treatment of several illnesses. The abundance of a given cell type, detected using high-throughput flow cytometry of a biopsy sample, may suggest that the patient is experiencing one particular condition rather than another. CD14 has a pivotal function in innate immune responses [24]. A study illustrated that CD14 identifies bacterial lipopolysaccharides, pathogen-associated molecular patterns, and damage-associated molecular patterns, therefore it promoting inflammatory immune responses [25]. Additional markers for neutrophils include CD16; mature neutrophils have high levels of CD16, whereas eosinophils and monocytes have low levels of CD16. Differentiating between these two subsets of granulocytes is made possible by CD16 [26]. Matsushita *et al.*, [27] discovered that CD22, a phosphoglycoprotein adhesion molecule on the surface of B cells, is one of the most frequently expressed antigens in haematologic malignancies, including human B-cell lymphomas and leukaemias.

Since CD14 is involved in immune responses to bacterial challenges -a lipopolysaccharide (LPS) receptor- it likely plays a smaller role in parasite-specific responses elicited by parasite antigens [25], which may help to explain why there was no strong correlation between monocyte CD14 expression levels and parasitic infection in the current study

(Table 5).

While Landmann *et al.* [29] found a correlation between CD16 and infection status, they did not find any association between infection intensity and CD14 or CD16 expression. This suggests that the presence of infection in the host, rather than the burden of infection, was the key factor in this relationship and this could be also the case in our study (Table 1). Additionally, it has been observed that there is a significant correlation between monocyte CD16 expression level and protection against infection in healthy persons. This could be due to an active monocyte phenotype and protective IgG antibodies (total IgG and IgG1) [30, 31]. Furthermore, a similar mechanism may mediate protection in parasitic infections, particularly involving CD16-IgG1 interactions [32].

Multiple lines of evidence point to Toxoplasma inducing inflammatory responses, which could contribute to the acute infection symptoms and signs. For instance, together with granulocytes, the CD4+ portion of T-lymphocytes aids in the immunological response. In certain conditions, the parasite can set off a devastating cascade of inflammatory cytokines, killing the animals. In humans, infections manifest as the development of T-lymphocytes exhibiting cytolytic activity specific to parasites. Human donors who tested positive for the antibody have had their cytolytic CD4+ and CD8+ cells harvested. It is unclear if the cytolytic function is significant during infection due to the fact that certain T-cell subsets can simultaneously produce the protective cytokine IFN-γ and show cytolytic activity [33].

The study showed a significant difference in mean level of CD14 and CD16 while showed no differences in mean level of CD22 between studied group (Table 1). Meanwhile our result agreed with the study of Al-Qadhi *et al.,* [34] that showed there was a negligible rise in CD22 levels in toxoplasma patients compared to the control group, the proportion of CD14 cells in toxoplasma patients was considerably lower than that in the control group which contrast with our study. Again this may due to the control group in Al-Qadhi *et al.,* study [34] have different kind of common bacterial infections since studies proved that CD14 is involved in immune responses to bacterial infections [25].

The study of Ehmen *et al.,* [35] demonstrated that monocytes from individuals with chronic toxoplasmosis had reduced CD16 and CD14 expression and a lower frequency of CD62L+ cells, while showing an increased frequency of CD64+ cells compared to monocytes from sero-negative controls. Our study indicated significant differences in CD16 and CD14 levels between IgM positive and negative groups (Table 2) and at the same time results registered no considerable differences in their levels between IgG positive and negative groups (Table 3). This can be explained by that most toxoplasma infections in our study was chronic.

CD22 (B-lymphocytes) exhibited a relatively increased, albeit not significant, presence in B-lymphocytes compared to the control group which agree with Salas-Lais et al., [36]. The elevated quantity of B-cells may stem from polyclonal activation of these cells [37].

Conclusion

The qualitative and quantitative ELISA technique used in the current study has high sensitivity and specificity and is a good method for detecting and measuring the concentration of specific antibodies to toxoplasmosis. The study showed appositive significant correlation of IgM with the studied CD markers (CD14, CD16 and CD22). However, CD16 has the strongest positive correlation with IgM and a significant negative correlation with IgG. This indicated that CD16 marker have a role which increased in acute infection rather than in chronic infections. Meanwhile CD14 and CD22 are more relevant to chronic infections. Further investigation

is required to better understand the role and mechanisms of CD14, CD16 and CD22 in the acute and chronic toxoplasmosis.

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