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Evaluation of some biochemical and immunological parameters changes in Iraq male with Toxoplasmosis

Ahlam Jassim Taher
Department of Biology, College of Education for Pure Science (Ibn Al-Haitham),Baghdad University

Abstract

A total of 33 Iraq male positive for Toxoplasmosis and Iraq male negative for Toxoplasmosis (controls) were studies to Evaluation of some biochemical and immunological parameters changes .The parameters included lipid profile such as (Cholesterol(C), Triglycerides(TG), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL) and very Low -Density Lipoprotein (VLDL) and complement component C3 and C4. The results revealed significant decrease in the total cholesterol, Triglycerides, LDL and non-significant in vLDL (129.96±1.63, 130.69± 2.80, 87.19±1.97 , 29.24± 0.83 mg/dl respectively) and non-significant increase in HDL(24.22 ±0.62) mg/dl compared with control group(152.07± 1.63, 156.48± 6.55, 99.26 ±1.39, 31.49± 1.30 and 21.31± 0.36 mg/dl).The immunological tests recorded a significant increase in C3, C4 (150.60± 9.67 , 31.47± 1.71 mg/dl respectively) compared with control group (52.86 ± 3.46 , 15.15± 0.47 mg/dl respectively). There for these results reveal that the infection with *Toxoplasma gondii* may have an essential role in alterations of lipid profile levels and complement components in infected men.

Keywords: *Toxoplasma gondii*, lipid profile, Toxoplasmosis, C3, C4 .

INTRODUCTION

Toxoplasmosis, one of the most important common parasitic zoonosis world-wide and the obligate intracellular protozoa *T. gondii* is the causative agent of this disease[1] [(2)] this parasite has a complex life cycle involving sexual replication in members including both domestic and wild felids as definitive host and asexual proliferation in a wide variety of warm blooded hosts included human as intermediate host [(3)(4).] There are three infective stages of *T. gondii*: a rapidly dividing invasive tachyzoite, a slowly dividing bradyzoite in tissue cysts, and an environmental stage, the sporozoite, protected inside an oocyst [(4) (5) (6).] Human infection is mainly developed by either oral ingestion of water and foods contaminated with parasite oocysts excreted by cat feces as final host, or eating raw and undercooked meat of intermediate hosts containing tissue cysts. Moreover, the infection can be transmitted through placenta, milk, organ transplantation, and blood transfusion [(6) (7).] *T. gondii* infection is widespread among humans and its prevalence varies widely from place to place approximately one-third of all humanity has been exposed to this parasite [(3).] Infections are usually asymptomatic in healthy individuals. But can cause severe disease in fetuses who cannot develop an effective immune response against the parasite and in immunocompromised individuals, such as AIDS patients or patients undergoing immunosuppressive therapy, can result in life-threatening disease[(5)(8) (9).]

Parasite can enter and infect any nucleated cells, then being to growth and replicate inside a parasitophorous vacuole (PV), way out, and then infect neighboring cells. This parasites activate a potent host immune response that eliminates most of the parasite and convert back into dormant cysts witch contain bradyzoites[(4) (9) (10).]

Lipids such as Total cholesterol, Triglycerides ,High-Density Lipoprotein, Low-Density Lipoprotein and very Low-Density Lipoprotein have been shown to play an important role in defending against parasitic infections [(11,12)] also consider important mediators of host defense during the acute phase of innate immunity. Infection and inflammation typically lower blood total cholesterol and high density lipoprotein cholesterol but increase triglycerides [(13) (14)].

Many studies observation alteration in the levels of serum lipid during infection with intracellular parasites such as malaria [(15) (16)], *Leishmania* [(17) (18)] The complement system consists of more than 30 proteins that are either present as soluble proteins in the blood or are present as membrane-associated proteins [(19)] .Its play a major role in innate immunity where a robust and rapid response is mounted against invading pathogens. Also acting an important role in adaptive immunity involving T and B cells that help in elimination of pathogens. [(20) (21).]

MATERIALS AND METHODS:

Subject's collection: The study included 60 blood samples collected from voluntaries males (33 male infected with toxoplasmosis (patients group) and 27 healthy male witch negative to toxoplasmosis as control group) who had attended to Imamein Kadhimein Medical City in Baghdad at the period March to September 2014. The ranged age between 18-52 years old. Five ml of venous blood were collected from each subject. The blood was placed in a plain tube and left to stand for 30 minutes at room temperature to clot. Then, centrifuged (3000 rpm) for 10 minutes to collect serum, which was frozen at -20°C till they were analyzed.

Biochemical Tests

Level of lipid profile included Total Cholesterol(C), Triglycerides (TGS) and High-Density Lipoprotein (HDL) were determined using a standard enzymatic assay (Linear chemicals, Montgat-Barcelona, Spain). While Low Density Lipoprotein (LDL) and very Low- Density Lipoprotein) vLDL) was calculated according to Friedewald formula [(17)]

LDLmg/dl = C – HDL – TGS/5

vLDLmg/dl = TGS/5

C= Cholesterol

TGS= Triglycerides

The immunological tests

The test was carried out by using the onsite Toxo IgG / IgM (Rapid Test Kit, USA), which was a lateral flow chromatographic immunoassay for the stimultaneous detection and differentiation of IgG and IgM anti-*Toxoplasma gondii* in human sera or plasma. Complement component test is performed by using Radial immune diffusion (RID) kit (Human-Germany) for determination C3 and C4 in serum .The plate was removed from Its envelope and leaved to stand at room temperature for few minutes so that Any condensed water in the wells was evaporated. Then the wells were filled with 5µl of samples and controls and waited they have been completely Adsorbing Before handling the plate. The plate was closed and waited the required incubation period 72 hour. Measured the precipitating ring around the well after incubation and compared with conversion table that provided with the kit.

Statistical analysis:

The Data analyzed by using the software statistical packages social sciences (SPSS) version 13 and the contrast between the patients and control were analyzed by student t- test. The $P \leq 0.05$, $P \leq 0.001$ were considered to be statistically significant and results were expressed as mean ± standard error (SE).

Table (1) prevalence of anti-toxoplasma antibodies in male volunteers

antibodies	Nos	Positive (%)	Negative (%)
IgG	60	33 (55 %)	27(45 %)
IgM	60	0	0

Table (2) Lipid profile values of prevalence in male infected with toxoplasmosis and control

Group	No	Mean±SEM				
		Cholesterol mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl	vLDL mg/dl
patients	33	129.96± 1.63*	130.69 ± 2.80*	24.22 ± 0.62	87.19± 1.97*	29.24± 0.83
control	27	152.07± 1.63	156.48± 6.55	21.31±0. 36	99.26± 1.39	31.49± 1.30

* Significant= $P \leq 0.05$

Table (3): C3 and C4 complement in male infected with toxoplasmosis and control

Group	Nos.	C3 mg/dI	C4 mg/dI
patients	33	150. 60 ± 9.67**	31.47± 1.71**
control	27	52.86± 3.46	15.15± 0.47

**Significant= $P \leq 0.001$

RESULTS

The Results in the Table (1)of this study show that 34 male was positive to anti-toxoplasma antibodies IgG and negative to anti-toxoplasma antibodies IgM also 26 male was negative to anti-toxoplasma antibodies IgG and IgM (control group).

DISCUSSION

The aim of this study was to assess and comparison of lipids profile and complement components in males with toxoplasmosis (patients group) and non-toxoplasmosis males (control group). Lipids are defined as organic compounds that are poorly soluble in water but miscible in organic solvents and they are play a critical role in almost all aspects of biological life they are structural components in cells and are involved in metabolic and hormonal pathways [23]. The results of the Biochemical tests showed that the mean of TGS, C, LDL are significantly decrease levels and non-significant in vLDL in patients, compared with control group, While the mean of HDL seemed increased but non significantly levels in patients, compared with control group, these results were agreement with study of [24 25]) who revealed that in changes of lipid profile values in infected women with *T. gondii*. On the other hand this results were don't agree with study of Flegr [26] which observed an increased level of cholesterol and LDL in men infected *with Toxoplasmosis* .

Several types of infections viral, bacterial, and parasitic have been linked to alteration in blood lipid levels Viral infection, as in human immunodeficiency virus (HIV) infections, are associated with lower blood levels of total-C and HDL-C [27 28] Experimental inflammation from bacterial endotoxin (lipopolysaccharide, LPS) induces similar dyslipidemias [29]). The hypocholesterolemia and remodeling of lipoproteins during acute phase responses of innate immunity increases clearance of LPS, particularly through increased binding of LPS to HDL particles [30 31] .Specific parasitic infections also cause dyslipidemias. A study of the Shipibo, another indigenous Amazonian group, showed an inverse correlation of HDL-C with the density of infection by three of five parasitic worm species [32]. Similar-sized samples from a city hospital in Chandigarh, India, showed lower HDL-C for patients with entamoebic and giardia parasites [33]. other studies have shown elevated levels of HDL, LDL and total cholesterol in patients suffering from parasitic infection [34 35] Another study were record lower in blood lipid values in patients infected with *Plasmodium falciparum* Malaria compared to control subjects, although the values were within the normal range also In the pretreatment, HDL , LDL and triglycerides were higher while total cholesterol was lower compared to post treatment [36 37] record abnormalities levels of lipids characterized by decreased levels of total cholesterol, LDL, and HDL and by the increased levels of VLDL and triglycerides in children infected with *Plasmodium vivax* .Also identified Plasma lipid profile alterations like hypocholesteraemia and increased triglyceridemia are reported in patients infected with visceral leishmaniasis [38 18 17.] .

Lipids are particular importance for pathogens, and some pathogens deliberately seek out lipid-rich host niches [39)] or enhance the availability of lipids by manipulating the host [40 41]. Intracellular pathogens have evolved sophisticated mechanisms to manipulate and tap into the lipid metabolism of their host cells. And Within cells of host it's often develop in specialized vacuoles and the flow of lipids between host and pathogen-controlled membranous compartments is key to the pathogen's ultimate success [42 43 44].

A numeral of previous studies have demonstrated the requirement of membrane cholesterol in host-pathogen interactions [45 46.] . Cholesterol(C) is an important component of higher eukaryotic cellular membranes and plays a crucial role in the function and the organization of membrane proteins and receptors [47 48] some of which being necessary for parasite entry [44] . *Toxoplasma* cannot synthesize cholesterol novo and depends upon acquisition of LDL-derived cholesterol from the host cell, via endocytosis mediated by the LDL receptor [49] or the LDL receptor-related protein[50]. A mechanism by which host and not parasite cholesterol controls the entry of *Toxoplasma* into cells has been proposed [51]. . These studies indicated that cholesterol does have an important role in pathogenesis of toxoplasmosis. However, data on parasite lipid sources are scarce and the molecular mechanisms by which *Toxoplasma* acquires host cell lipids are largely indefinite [47] .

The complement system plays an essential role as a first line of host immune defense promoting the recognition, opsonization, and lysis of invading pathogens ([52]; [53]) and its forms an important bridge between innate and adaptive immunity.([54]).

The present results indicated that there were an increase significantly in the levels of C3 and C4 in patients compare with control .these results was agree with the results by other researches, ([55]; [56]) revealed that the highest level of C3 and C4 in women with positive anti *Toxoplasma* but don't agree with Study of Al-Samarrae [57] which record highest level of C3 in patients compare with controls while lowest level of C4 in patient compare with controls but without reported significant differences. Study of Ad'hiah *et al*., [58] record highest level of C3 and C4 in patients compare with controls while with no significant differences, Al-kalaby *et al.,* [59] record highest level of C3 in aborted women infected with *T. gondii* compare with controls with significant

differences while the highest level of C4 in patient without reported significant differences. Also Schreiber & Feldman, [60] in vitro investigations showed that *T. gondii* tachyzoites are rapidly lysed by the activation of complement through the classical pathway in the presence of specific antibodies so that (Suzuki and Kobayashi, [61] prove that the presence of Ca++ is essential for the antibody-dependent cytolysis of *Toxoplasma* organisms, and confirm that the lytic reaction is mediated by an activation of the classical complement pathway. Hence, it is possible that the antibody-dependent killing mechanism by the activation of complement, which was observed *in vitro*, contributes to host defense for *Toxoplasma* infection *in vivo* by the activation of complement, which was observed *in vitro*, contributes to host defense for *Toxoplasma* infection *in vivo*. The collaboration between specific Antibody and presence of complement have been found capable of killing extracellular *T.gondii* [62]. All these studies indicated of an important of complement component in host defense against toxoplasma.

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