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Original Research Article

Levels of Interleukin-6,17, Complement and Dopamine in Infected Women with Toxoplasmosis

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Abstract: The study was carried out to detection of *Toxoplasma gondii* in (135) women patients with repeated abortion with age ranged 18-43 years who attended two teaching hospitals in Baghdad. The diagnosis is done by Immunochromatography and ELISA methods. a blood samples was taken from each patients as well as other(30)healthy control matching in age. The study included measurement the concentration of Interleukin-6, Interleukin-17, Dopamine and Complement in sera of patients and control .The result indicated presence the of anti-Toxoplasma IgG in 26 cases a, Anti-toxoplasma IgM in 14 cases and 10 in both out of 135 cases of aborted women with Toxoplasma in immunochromatography methods while the level of IgM 1.57 IU/ml and IgG 142.73. Also, the result indicated significant increasing levels of IL-6 and IL-17, Complement and Dopamine in patients sera in comparison with healthy control.

Keywords: Toxoplasmosis, Interleukin -6, Interleukin -17, Dopamine.

INTRODUCTION

Intracellular parasites like *Toxoplasma gondii*, usually contaminate with an extensive level of infections to the eukaryotic cells. It is quite significant devious pathogenic organism in both humans as well as animals. Often this contamination comes with no symptoms, nevertheless, the two sets that threaten personally, the humanoid genes and the persons with immunosuppression, mainly to those who have weak immune system, which lead to lethal toxoplasmic encephalitis [1]. Parasites duplicate gradually and eventually form bradyzoite that remain in the cysts of the tissues of the neural hosts as well as the tissues of the muscles for the host life time [2].

The inherited contamination could lead to defects in birth which includes hydrocephalus, spontaneous abortion, chorioretinitis and intra cerebral calcification [3]. The innate immune system responds through monocytes, dendritic cells (DCs) and T. gondii which ends up infecting tissues. These types of cells are involved and are responsible for the microbial resistance [4-9] *T. gondii* is the ability to sense the pathogen and produce the cytokine IL-12, which stimulates natural killer (NK) cells and T cells to produce the cytokine Interferon-gamma (IFN-γ) [10–12]. IFN-γ is the major mediator of resistance to *T. gondii* and promotes multiple intracellular mechanisms to kill the parasite and inhibit its replication. This Th1 immune response, defined by the production of IL-12 and IFN-γ, is characteristic of infection with many intracellular pathogens [12, 13].

Neutrophils are another source of IL-12 during toxoplasmosis, as they contain pre-stored IL-12 and can secrete this cytokine in vitro and in vivo in response to T. gondii [14]. Additionally, there are reports that neutrophil depletion results in decreased levels of IL-12 and increased parasite replication [15]. Natural killer (NK) cells are another inmate population involved in immunity to *T. gondii*, and in mice that lack T cells they provide a limited mechanism of resistance through their ability to produce IFN-γ [16–18] NK cell activity peaks early during infection, and although their activity is elevated during chronic toxoplasmosis, they do not appear to be significant contributors to immunity during

the chronic stage of infection [19, 20]. NK cells can also act to promote adaptive immune responses. Thus, in the absence of CD4+ T cells, they can provide help to the CD8+ T cell response [21].

MATERIAL AND METHODS

Study groups

This research work was conducted in two teaching hospitals in Baghdad the patients' ages were between 18 to 43 years old. The patients were divided into two groups, which include suspected patients. Blood samples were collected from 135 women suspected as *T. gondii* infected which was tested and confirmed (clinically suspected cases) by specialist doctors and the other group was control including healthy individuals.

Blood Samples

The blood samples were taken from the participants' veins and were collected in pastoralized plastic tubes. Each sample was left for 30 minutes at room temperature in serum separator tubes in vertical position prior to centrifugation. The samples were centrifuged at 3000 rpm and kept for 5 min. The serum was collected from every sample using Eppendorf tube and was stored at –20°C. This sample was used to evaluate immunological and clinical biochemical aspects.

Immunochromatographic assay

About 100 µl of serum from each sample was added to the sample hole of the kit. The colour density is proportional to the antibody titer. The complexes (appears in colour band after 10 minutes) confirm that the test was performed correctly. This CerTest-Toxoplasma kit which is qualitatively determines the Toxoplasma in blood samples. Pre-coating was achieved to the membrane proceeding to test band region to the monoclonal antibodies of the mouse, it was achieved against Toxoplasma antigens. Through test, samples were reacted with conjugated colours (anti-Toxoplasma of monoclonal mouse microsphere (red antibodies)), the samples were dried before that, the combination then travelled to reach membranes via the act of capillaries. While samples move via the membranes tests, tinted particle were migrated. In positive results, certain antibodies that have existed on the membranes captured these particles which lead to appearance of red tinted line that can clearly observed while the other result appears in a green tinted line (the negative results that represent the control samples).

IgM Toxoplasma antibodies and IgG determination

To achieve the qualitative and quantitative investigation to IgG Toxoplasma gondii and IgM, two kinds of kits were used to detect the antibodies in infected women serums; these kits are (Toxoplasma IgM, EIA, enzyme immunoassay) and Toxoplasma IgG EIA enzyme immunoassay) (ACON Laboratories, Inc. San Diego, USA). Toxoplasma antibodies levels were assessed using ELISA technique. The level of IgM or IgG below 0.9 IU/ml was considered negative and from 0.9 to 0.99 IU/ml was equivocal limit and should be rechecked, while positive level was equal to 1.0 IU/ml or above.

Quantitative estimation of the complement:

Specimens for patients and healthy women for complement (C3,C4) were done by radioimmuno diffusion plate (LTA, Italy) according to method of Mancini, 1965 [22].

Estimation of the level of IL-6, IL-17 and Dopamine

The levels of IL-6, IL-17 and dopamine were estimated in 50 patients who were found anti-Toxoplasma antibodies positive according to manual procedure of Cusabio Biotech (China).

Statistical analyses

The results were analyzed using Statistical Package for the Social Sciences (SPSS) version-18 (T-test).

RESULTS

Diagnosis of *T. gondii*

The anti-Toxoplasma IgM present in 14 cases with a percent 10.37 %,also , the anti-Toxoplasma IgG present in 26 cases with a percent of 19.25 % while both IgM and IgG present in only 10 cases with a percent of 20% out of 135 cases in Immunochromatography method (Table-1).

Table-1: Distribution of anti-Toxoplasma gondii IgG and IgM antibodies using immunochromatography method in women with repeated abortion

Anti-Toxoplasma antibodies	Total	Positive		Negative	
		No.	%	No.	%
IgM	135	14	10. 37	124	89.63
IgG	135	26	19.25	109	80.75
IgM +IgG	135	10	7.40	125	92.60

The level of anti-Toxoplasma IgM present in 50 cases was 1.57 in comparison with healthy control 0.27 also, the level of anti-Toxoplasma IgG was 142.73 in comparison with healthy control 14.66 (Table-2).

Table-2: The Level of anti-Toxoplasma gondii IgM antibodies using ELISA method in infected women

Treatment	Total	IgM IU/ml	
		Mean	SD
Infected women	50	1.57	1.64
Healthy Control	30	0.27	0.09

Table-3: The Level of anti-*Toxoplasma gondii* IgG antibodies using ELISA method in Infected women

Treatment	Total	IgG IU/ml	
		Mean	SD
Infected women	50	142.73	94.66
Healthy Control	30	14.66	5.92

The level of C3 and C4 increased significantly in patients groups in comparison with healthy control (Tables 4 and 5).

Table-4: The Level of C3 Complement Infected women and healthy control

Treatment	Total	C3 mg/dl	
		Mean	SD
Infected women	50	148.6	55.7
Healthy Control	30	129.7	13.6

Table-5: The Level of C4 Complement Infected women and healthy control

Treatment	Total	C4 mg/dl	
		Mean	SD
Infected women	50	26.7	13.3
Healthy Control	30	23.2	4.8

The level of IL-6 pg/ml increased significantly in patients groups in comparison with healthy control (Table-6).

Table-6: The Level of Interleukin-6 in Infected women and healthy control

Treatment	Total	IL-6 Pg/ml	
		Mean	SD
Infected women	50	3.22	1.61
Healthy Control	30	1.78	0. 68

The level of IL-17 pg/ml increased significantly in patients groups in comparison with healthy control (Table-7).

Table-7: The Level of Interleukin-17 in Infected women and healthy control

Treatment	Total	IL-17 Pg/ml	
		Mean	SD
Infected women	50	3.26	1.69
Healthy Control	30	1.61	0. 69

The level of Dopamine ng/ml increased significantly in patients groups in comparison with healthy control (Table-8).

Table-8: The Level of Dopamine in Infected women and healthy control.

Treatment	Total	Dopamine ng/ml	
		Mean	SD
Infected women	50	1.95	0.86
healthy Control	30	1.03	0. 33

DISCUSSION

The majority of acquired infection in healthy individual are benign and either asymptomatic or with vague symptoms. About one-third of the world's population is estimated to carry Toxoplasma infection. There are large variations in prevalence and within different countries in humans [23]. The result indicated Anti –Toxoplasma IgG was 26 Cases while IgM was 14 cases in immunochromatography method (Table-1) while the level of anti-Toxoplasma IgG was (142.73) and IgM (1.57) by ELISA method (Table 2 & 3) in a general The prevalence of infection is related to several factors including nutritional habits, contact with soil, rural or urban settings and frequency of contact with domestic animals and climatic condition such as humidity and pregnancy [24]., The prevalence of anti- *T. gondii* antibody observed in the study was in agreement with seroprevalence data from previous studies conducted in our country 66 (29.2%) from a survey carried out in Salah– Adden government on 226 pregnant women had Toxoplasmosis. Anti-*Toxoplasma* IgG was 59 (26.1%) of cases while IgM antibody was 7 (3.1%) of cases [24]. The increasing in level of C3 and C4 (Table 4 & 5) may be contributed to the ability of the parasite to repair their membrane after the membrane attack complex of complement has been assembled on its surface and these factor may necessitate a high input of complement activity in order to cause cytolysis of the organism [25]. The increasing level of IL-6 (Table- 6) may be involved in early development of acute phase response, maintenance of hematopoi-esis [26] and immune barriers in ocular [27] as well as cerebral Toxoplasmosis [28]. It makes the NK cells to increment their cytotoxic activity and is also involved in the maturation of antibody secreting B lymphocytes and the differentiation of T lymphocytes [29]. Different immune cells such as MΦs, endothelial cells, monocytes, and fibroblasts are involved in the production of IL-6. This cytokine functions synergistically withmIL-1β and TNF-α. Hence, it is regarded as a remarkable pyrogenic factor mediating dominantly the production of hepatocyte based acute in- flammatory proteins [30]. The significant increasing level of IL-17 (Table-7) are secreted from Th17 cells. But a variety of immune cells including CD8+ and NK cells [31, 32]. also secrete IL17. It is an inflammatory cytokine that provides innate immunity from the recruitment of neutrophils [33] which make the host resistant against *T. gondii* infection [34]. A subset of CD4+ T cells has been identified which produces IL-6 , IL-17A, IL-17F, and TNF in response to IL -23 [35, 36]. The significant increasing level of dopamine (Table-8) may be due to ability of *T. gondii* infection as a risk factor for the development of schizophrenia and depression in humans. As *T. gondii* forms cysts that are located in various anatomical sites including the brain during a chronic infection, it is well placed anatomically to mediate these effects directly. The *T. gondii* genome is known to contain 2 aromatic amino acid hydroxylases that potentially could directly affect dopamine and/or serotonin biosynthesis [37].

CONCLUSION

The result indicated presence of the anti-Toxoplasma IgG in 26 cases , Anti-Toxoplasma IgM in 14 cases and 10 in both out of 135 cases of aborted women with Toxoplasma in immunochromatography methods while the level of IgM 1.57 IU/ml and IgG 142.73. also, the result indicated significant increasing levels of IL-6 and IL-17 ,Complement and dopamine in patients sera in comparison with healthy control.

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