ENDOGENOUS INTERFERON GAMMA LEVELS IN PREGNANT AND ABORTED WOMEN INFECTED WITH TOXOPLASMA GONDII AND CYTOMEGALOVIRUS IN WASIT-IRAQ

Rahma A. A. Almansory[1] ,Altamemy A. A.[2]
1minstry of health public health laboratory,Wasit University/college of Science .IRAQ
rahma.abdalraheem.ameen@uowasit.edu.iq

Abstract
The study was conducted in different areas of Wasit province, there is a 180 venous blood samples were collected from pregnant women infected with toxoplasmosis, cytomegalovirus, and mixed infection.
By using ELISA there is 21 cases 13.12% were positive for toxoplasmosis, 85 cases 53.12% were positive for cytomegalovirus, and 54 cases 33.75% were positive for mixed infection with the parasite and the virus. The highest concentration of interferon-gamma in women with acute toxoplasmosis (IgM) was 135.69 pg./ml, and on the other hand, it was lower in chronic infection (IgG) 38.8.8 pg./ml, compared to the control group, 49.12 pg./ml. The levels of interferon-gamma in pregnant women with acute toxoplasmosis is statistically highly significant compare within healthy women. The highest concentration of interferon-gamma in women infected with cytomegalovirus in acute infection was 238.93 pg./ml, while in chronic infection its level was 55.99 pg./ml. As for the level of interferon-gamma in pregnant women who had a co-infection with the parasite and the virus, it was 238.9 pg./ml for acute infection and 49.12 pg./ml for chronic infection.
Keyword: Toxoplasma gondii, Cytomegalovirus, Interferon gamma

Introduction

Toxoplasma gondii is an obligate intracellular parasite and is the causative agent of toxoplasmosis in humans and animals. T. gondii has a worldwide distribution and infects a wide variety of animals, although only a fraction develop disease (Stelzer et al. 2019).Cytomegaloviruses (CMVs) are herpes family viruses that distributed everywhere, Contraceptives are the most frequent congenital infection is caused by cytomegaloviruses (CMVs), which are well known as one of the most common human illnesses. (Eremin and Sewell 2011). There is no evidence that humans are more likely to get T. gondii by having close contact with cats (Dubey et al., 2020). While there is no resistance at this stage, the tachyzoite is an important part of toxoplasmosis epidemiology if the main infection happens during pregnancy, when the chances of transmission to the fetus are high, thanks to a new the assay of western blot, it can identify anti T. gondii antibodie produced by oocyst infections. Humans and domestic animals, especially tiny ruminants, are at high risk for contracting toxoplasmosis by vertical transmission (Dubey et al., 2020). Among the type II interferons discovered in the last sixty years, only IFN-γ has been identified. White blood cells create IFN-γ after being activated, and it was initially described by E. Frederick Wheelock as a phytohemagglutinin-induced inhibitor of virus (Davidson JN et al., 2000). The IFN-γ is encoded by the IFNG gene encoding to Ifn-γ. It consists of two polypeptide chains that are connected in an antiparallel manner (Zaidi MR and Merline, 2011). There are three distinct fractions of IFN-γ that are found in human blood, each with a different molecular mass. The active free form of IFN-γ is represented by one fraction, whereas the other two fractions are believed to be mature molecules of IFN-γ at this stage. At the amino termini, the completely synthesized protein undergoes glycosylation, and the degree of glycosylation is what ultimately defines the weight of the designated fractions (Alspach et al., 2018; Likova et al., 2019) According to many lab reports, interferon inhibited the development of T. gondii in epithelial or fibroblast cells that were grown. When triggered by mitogens or antigens to which they were previously sensitized, T-lymphocytes release IFN-γ. Suppression of viral growth was the initial criterion for identifying each of these interferons. Aside from their well-known antiviral effects, crude interferons have on rare occasions been found to inhibit the development of additional intracellular infectious pathogens that are not viruses (Lieberman et al., 2004).

Materials and tools

The kits that used in the study listed on the table one and the procedures was applied according to company instruction Biotech (Hangzhou) and Beijing solarbio scince

Table 1: List of kits used in this study

N0.	Kits	Companies	Countries
1	Human Toxoplasma IgM ELASA Kit	Biotech (Hangzhou)	China
2	Human Toxoplasma IgG ELASA Kit	Biotech (Hangzhou)	China
3	Human Cmv IgM ELASA Kit	Biotech (Hangzhou)	China
4	Human Cmv IgG ELASA Kit	Biotech (Hangzhou)	China
5	Human ELASA IFN-γ immunoassay	Beijing solarbio scince	China

Specimens Collection

By using TORCH kit there is 160 blood samples were collected from pregnant women of different ages to identify IgM and IgG toxoplasmosis and CMV infection, the samples were subsequently tested by genuine ELISA kit for T. gondii and CMV infection in addition to 20 samples collected from healthy pregnant women as control.

Serological test

The method used in this test is the quantitative sandwich enzyme immunoassay according to company instructions. A microplate has been coated with a monoclonal antibody that is specific for interferon gamma. After incubation, the coated antibody captures any IFN-γ that may be present in the wells, which are pipetted with standards and samples. To detect the collected IFN-γ protein sample, a biotin-conjugated antibody specific for IFN-γ is applied after thorough washing. Next, the Tetramethy-benzidine (TMB) reagent is applied, followed by horseradish peroxidase (HRP)-conjugated streptavidin, for signal development. Enzyme- conjugate is applied to the wells after a wash to eliminate any

unbound mixture. The color intensity, which is directly proportional to the amount of bound protein measured at 450 nm, and a sulfuric acid solution is employed to halt the color development process.

Results and discussion

The ELISA findings the mean blood concentration of IFN-γ of all pregnant women infected with T. gondii was 135.69pg/ml. which is significantly different at ($P < 0.001$) from in healthy women (control group) 49.12 pg./ml. The mean concentration of IFN-γ was 193.62, 38.88 pg./ml of acute (IgM) and chronic (IgG) toxoplasmosis respectively. Compared to healthy pregnant women, the acute toxoplasmosis infection had considerably highly significant levels of IFN-γ. This results was agreement with a result of Ibtisam Al Aboosi, et al., (2023) in Thi-Qar province Iraq when recorded IFN-γ values of a patient with Toxoplasma gondii- IgM, T. gondii IgG equals to 38.64, 31.4 respectively which is significantly difference than the control 28.80 CD4 value in a women with IgM equals (3.045) that is statistically different than the control patient value 2.925. In addition, CD4 value of patients with T.gondii IgG 4.457 that significant higher than control with a significant value . Our study results agree with Diez et al., (2009) who reported that the activity of Toxoplasma-induced serum IFN-α/β and causes increasing concentrations gradually throughout early infection days, but the slight levels of interferon gamma was not detected in the blood circulation at any time during the course of infection; the authors also observed that IFN-α/β production levels and the ability to produce interferon gamma by spleen cells during infection were closely and inversely correlated. More IFN-α/β was produced as the infection progressed, and the capacity of spleen cells was decreased to produce IFN-γ; they also suggests that the production of IFN-α/β is an important factor correlated with acute Toxoplasma infection-induced immunosuppression.The mean serum concentration of IFN-γ of all pregnant women acute and chronic cases infected with CMV was 175.45pg\ml. it was a highly significant difference from the healthy women 49.12pg/ml. The mean serum concentration of IFN-γ in pregnant women with CMV infection was 238.93 and 55.99 pg./ml in acute and chronic infection respectively, it was a highly significant from the healthy women 49.12 pg./ml with IgM and IgG. several studies correlates about maternal immune protection of fetus after primary infection in pregnancy, and describe the kinetics ,magnitude of cytomegalovirus specific CMI in immunocompetent adults infected with CMV .A review about cell mediated immunity in pregnant primarily infected with CMV and its correlation to the risk of vertical virus transmission. Immunological parameters measurements after infection measured by ELISA in vitro stimulation with viral antigens to enumerating secreting IFN-γ from CMV-specific T cells, at a single cell level . In the patients with weak CMV-CMI there is observed a higher significant viral loads at the same time of post-infections point. (Chiereghin et al., 2021).The mean concentration of interferon gamma was 192.8 pg./ml in cases with mixed toxoplasmosis and CMV in aborted and non-aborted pregnant women, which had both positive IgM and IgG antibodies. However, there is a significant differences with healthy women 49.12pg/ml. The results of our study show that the levels of IFN-γ are low in aborted, in contrast, the high levels of IFN-γ in non-aborted pregnant women.

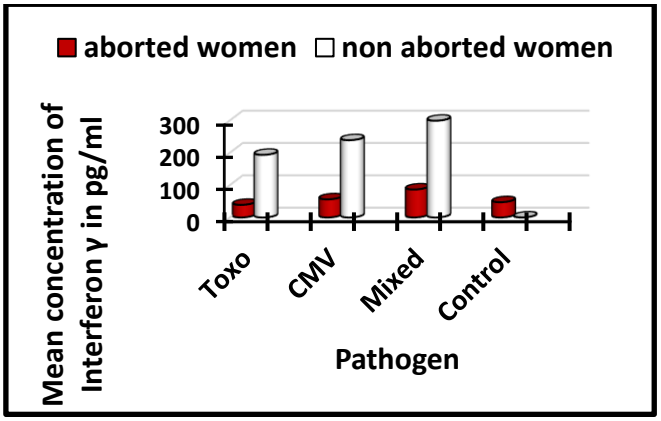

Figure 1 elucidate mean concentration (pg./ml) of IFN-γ in aborted and non-aborted women.

"In r "In response to cytomegalovirus (CMV) and Toxoplasma gondii antigens, T4+ cells from seropositive donors produce interferon-gamma (IFN-gamma) by different mechanisms; one (T. gondii) dependent upon and the other (CMV) largely independent of interleukin-2 (IL-2) and its receptor. To determine whether IFN-gamma-generating mechanisms unrelated to IL-2 also differ, we examined the requirement for accessory cells and their expressed or secreted products. In response to both specific antigens, IFN-gamma secretion was strictly dependent upon the presence of accessory cells (monocytes) and was largely inhibited by monoclonal antibodies to class II (HLA-DR and -DQ) but not class I MHC antigens. Both CMV and T. gondii antigens stimulated monocytes to release interleukin-1 (IL-1), and IFN-gamma production in response to both antigens was abolished by pretreatment with anti-IL-1 antibody. In contrast, the secretion of tumour necrosis factor (TNF) was not stimulated by either antigen, and IFN-gamma production was not diminished by antisera directed at TNF-alpha or TNF-beta. We conclude that CMV and T. gondii antigen-induced IFN-gamma production requires a similar accessory cell mechanism, and that soluble antigen-stimulated IFN-gamma secretion by human T4+ cells is dependent on monocytes, expression of class II MHC antigens, and the presence of IL-1"(Kelly et al.,1989).

Reference

Dubey, JP, FHA Murata, CK Cerqueira-Cézar, OCH Kwok, and YR Yang. 2020. 'Public Health Significance of Toxoplasma gondiiInfections in Cattle: 2009–2020', The Journal of Parasitology, 106: 772-88.

Stelzer, S, W Basso, J Benavides Silván, Luis M Ortega-Mora, P Maksimov, J Gethmann, FJ Conraths, and G Schares. 2019. *'Toxoplasma gondii infection and toxoplasmosis in farm animals: Risk factors and economic impact', Food and Waterborne Parasitology, 15: e00037.*

Zaidi MR, Merlino G (2011). *The two faces of interferon-g in cancer. Clin Cancer Res.;17(19):1–7.*

Alspach, E., Lussier, D. M., & Schreiber, R. D. (2019). *Interferon γ and its important roles in promoting and inhibiting spontaneous and therapeutic cancer immunity. Cold Spring Harbor perspectives in biology, 11(3), a028480.*

Lieberman LA, Banica M, Reiner SL, Hunter CA(2004). *STAT1 plays a critical role in the regulation of antimicrobial effector mechanisms, but not in the development of Th1-type responses during toxoplasmosis. J Immunol. 172:457–63. doi: 10.4049/jimmunol.172.1.457.*

Likova, L. T., Mei, M., Mineff, K. N., & Nicholas, S. C. (2019). *Learning face perception without vision: Rebound learning effect and hemispheric differences in congenital vs late-onset blindness.*

In IS&T International Symposium on Electronic Imaging (Vol. 2019, pp. 237-1). NIH Public Access.

Angela Chiereghin, Gabriella Verucchi and Tiziana Lazzarotto (2021). *CMV-Specific Cell-Mediated Immunity in Immunocompetent Adults with Primary CMV Infection: A Case Series and Review of the Literature. National Center for Biotechnology Information, 13(5): 816. Doi : 10.3390/v13050816.*

Ibtisam Al Aboosi1, Amall Y.AL-Mulla Mustafa mudhafar, *Qais R. Lahhob , Moamin Ibraheem Kate4, Mohammed Hassib* ***Ali 4 Murtadha A Al-khegane4 Mustafa Jawad Kadham7 Abdulrahman Abbas Jasim (2023).*** *Toxoplasmosis and Relation with Some Immunological Markers for Aborted Women in Thi-Qar Province. Journal of Population Therapeutics & Clinical Pharmacology, 309-316*

Likova, L. T., Mei, M., Mineff, K. N., & Nicholas, S. C. (2019). *Learning face perception without vision: Rebound learning effect and hemispheric differences in congenital vs late-onset blindness. In IS&T International Symposium on Electronic Imaging (Vol. 2019, pp. 237-1). NIH Public Access.*

Eremin, O., & Sewell, H. (Eds.). (2011)*. Essential immunology for Surgeons. Oxford University Press.Experimental Parasitology, Volume 172, January 2017, Pages 39-43.*

Kelly, C. D., Russo, C. M., Rubin, B. Y., & Murray, H. W. (1989). *Antigen-stimulated human interferon-gamma generation: role of accessory cells and their expressed or secreted products. Clinical and experimental immunology, 77(3), 397–402.*