DOI : 10.35124/bca.2019.19.2.2919

Biochem. Cell. Arch. Vol. 19, No. 2, pp. 2919-2922, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

THE ROLE OF INTERLEUKINES- 6, 12 AND TUMOR NECROSIS FACTOR - ALFA IN THE IMMUNE RESPONSE TO TYPE 2 DIABETIC PATIENTS INFECTED WITH TOXOPLASMOSIS

Nadia Ahmed Hadi Al-Ubaydi, Fadhil Abbas Manshad AL-Abady and Ali Naeem Salman

Department of Biology, College of education for pure sciences, Unuiversity of Thi-Qar, Iraq
e-mail : na0103760@Gmail.com

(Received 12 November 2018, Revised 12 January 2019, Accepted 22 January 2019)

ABSTRACT : This study was performed on Type 2 diabetic patients infected with Toxoplasmosis to estimate the role of interleukine-6,interleukine-12 and Tumor necrosis factor –alfa in immune response against *Toxoplasma gondii* parasite .Study groups includes also patients infected with type 2 diabetes only 30samples, patients infected with Toxoplasmosis only 30 samples and controls 30 samples. We collected samples from Thi-Qar hospitals and private laboratories in the period from 1st of November 2017 to the end of July 2018. Toxoplasmosis infection diagnosed by ELISA test by using kits from (Elabscience Company, U.S.A.). Atotal of 120 sample was infected of chronic Toxoplasmosis among 520 samples of type 2 diabetes mellitus. This study founded high concentration of interleukine-6 in type 2 diabetes infected with Toxoplasmosis then agroupe infected with Toxoplasmosis only, then agroupe of type 2 diabetes only compared with control which recorded lowerst concentration. The study founded increased concentration of interleukine 12 and TNF-α in type 2 diabetes infected with Toxoplasmosis, then agroup of patients infected with Toxoplasmosis only, then a group of patients infected with type 2 diabetes only compared with control which recorded lowest concentration.

Key words **:** IL-6, IL-12, TNF-α, toxoplasmosis, type 2 diabetes.

INTRODUCTION

Toxoplasmosis is a parasitic infection result of *Toxoplasma gondii* an obligate intercellular parasite that infected animals from warm-blooded group. Cats and all fileade family is definitive hosts while human, rats birds and some other animals is an intermediate hosts (Dubey, 2009). Infection with Toxoplasma happened by ingestion oocysts of parasite contaminated food or drinks, infection rout also includes eating raw or undercooked meats or by contacts with soil has oocysts(Dubey,2008). Type 2diabetes mellitus is a chronic disease results of disturbances of carbohydrates, fats and proteins metabolism caused Hyperglycemia, this disease results of abnormalities in insulin action, secretion or both (Shaw and Tanamas,2016; Karlove,2013). Type 2diabetes infected more than 90% of poeple from all other types of diabetes. It's called maturity onset because it's infected people in maturity age and above (Defronzo, 2004). Interlukeukinse play acritical role in both innate and adaptive immune response to Toxoplasmosis. interleukines are small soluble proteins weight between (5-20) Kd, secreted by many cells like Macrophages, T and B lymphocytes, Mast cells,Endothelial cells and Fibroblasts (John, 2010). Interleukine-6 is aproinflammatory cytokine induce immune response against infectious agents like parasite and injury, causing inflammation which has a critical role to resistance infection (Boulauger *et al*, 2003). IL-6 intermediate fever and acute phase proteins, IL-6 induce activity of cytotoxicity of Nk cells and Cytotoxic T-lymphocytes, IL-6 also inhibit insulin secretion from pancreas and institute in insulin resistance (Luigi *et al*, 2007).

Interleukine-12 induce the induction of interferone-gamma (INF-γ) and Tumor Necrosis Factor –alfa (TNF-α) from Nk cells. Also IL-12 intermediate differentiation of Naïve T cells to T helper 1 and reduce the induction of IL-4 which inhibit INF-d. IL-12 induce the activity of Cytotoxicity of Nk cells and CD8+ T lymphocytes, so all those responses play much important role in resistance of Toxoplasmosis (Newport *et al*, 2007). Tumor Necrosis Factor –alfa has acritical role in *Toxoplasma gondii* resistance. TNF-α institute in fever, Apoptic cells death and Inflammation and inhibit cancer and viruses prolifration (Swardfager *et al*, 2010). TNF-α induce cytotoxic activity of Macrophage and play as Co-signaling with INF-d to activate Macrophages to inhibit parasite

proliferation (Johnson, 1992). There are relationship between TNF-α and insuline resistance (Popko *et al*, 2010). This study aims to Estimate the role of interleukines 6, 12 and Tumor necrosis factor –alfa in immune response to Toxoplasmosis in Type 2 diabetic patients by calculate the concentration of each one in study groups, and Find the relationship between Toxoplasmosis and Type 2 diabetes mellitus.

MATERIALS AND METHODS

Study Groups

Study groups divided to four (a group of Type 2 diabetes mellitus infected with Toxoplasmosis, a group of Type 2 diabetic patients only, a group of Toxoplasmosis patients only and control group).

Collection of samples

We collected study samples from Thi-Qar hospitals and private laboratories for the periods started from 1st of November 2017 to the end of July 2018. A total of 120 samples of type 2 diabetes were diagnosed infected with Toxoplasmosis from 520 samples, while 400 samples were infected with type 2 diabetes only, 30 samples collected from patients infected with Toxoplasmosis only and 30 samples as acontrol. We collected blood samples from each patients then separated serum by using gel tubes and centrifuge for (5-10) minutes on (2500-3000)c/min, Then freeze samples under -20c°.

Using Enzyme-linkedImmuno Sorbent Assay (ELISA) to calculate concentration of Cytokines (IL-6,IL-12,TNF-α)

Add 100 µL standard or sample to each well. Incubate for 90 min at 37^{0}C, Remove the liquid, Add 100 µL HRP Conjugate. Incubate for 30 min at 37^{0}C. Aspirate and wash 5 times, Add 90 µL of Substrate Reagent. Incubate for 15 min at 37^{0}C, Add 50 µL Stop Solution and Read at 450 nm immediately. Calculation of results.

Table 1 : Interleukine-6 concentration in study groups.

Samples	Interleukins IL-6
DM+Toxo	344.01 ± 59.29
DM	289.58 ± 52.91
Toxo	198.87 ± 54.97
Control	86.11 ± 17.54
L.S.D	**35.67**

Table 2 : Interleukine -12 concentration in study groups.

Samples	Interleukins IL-12
DM+Toxo	610.53 ± 82.61
DM	506.54 ± 103.20
Toxo	765.58 ± 121.01
Control	194.63 ± 38.46
L.S.D	**106.82**

Table 3 : The concentration of TNF-α in study groups.

Samples	Interleukins TNF
DM+Toxo	427.09 ± 22.59
DM	367.47 ± 33.77
Toxo	406.21 ± 44.65
Control	299.08 ± 18.36
L.S.D	**23.03**

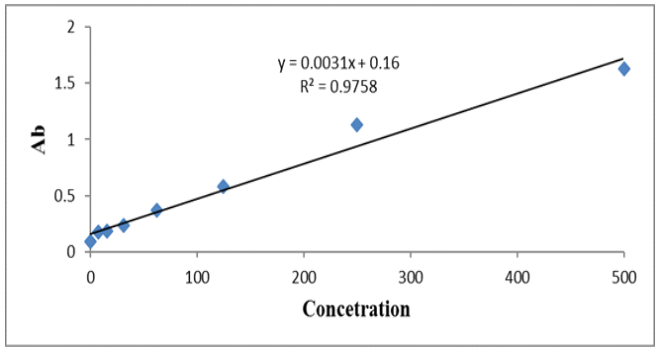

Figure 1 : Standard curve of Interleukine -6 concentration in study groups.

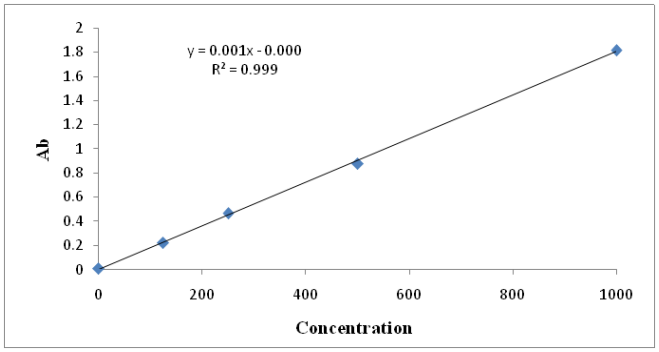

Figure 2 : Standard curve of Interleukine -12 concentration in study groups.

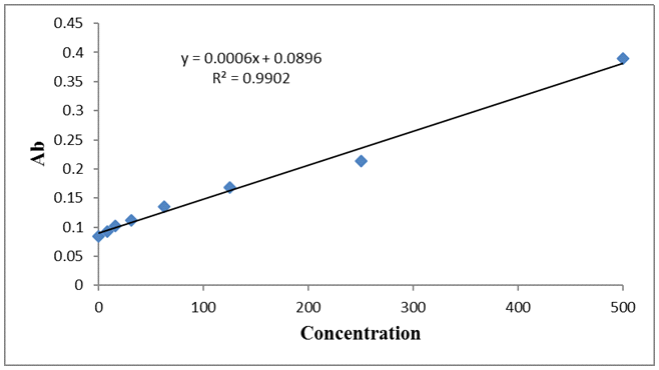

Figure 3 : Standard curve of TNF-α concentration in study groups.

RESULTS AND DISCUSSION

Interleukin- 6 result

This study recorded highest concentration of IL-6 in type 2 diabetes mellitus infected with Toxoplasmosis group which recorded (344.01 ± 59.29)pg/ml, then type 2 diabetes mellitus only group which recorded (289.58 ± 52.91)pg/ml, then Toxoplasmosis infected group which recorded (198.87 ± 54.97)pg/ml, compared with control group which recorded the lowest concentration of IL-6 which recorded (86.11 ± 17.54)pg/ml.

Interleukine -12 result

This study recorded highest concentration of IL-12 in Toxoplasmosis infected group which recorded (765.58 ± 121.01)pg/ml, then type 2 diabbetes mellitus infected with Toxoplasmosis group which recorded (610.53 ± 82.6)pg/ml, then type 2 diabetes mellitus only group (506.54 ± 103.20)pg/ml, compared with control group which recorded the lowerst concentration of IL-12 (194.63 ± 38.46)pg/ml

Tumor Necrosis Factor –alfa result

This study recorded highest concentration of TNF-α in type 2 diabbetes mellitus infected with Toxoplasmosis group which recorded (427.09 ± 22.59)pg/ml, then Toxoplasmosis infected group which recorded (406.21 ± 44.65)pg/ml, then type 2 diabetes mellitus only group which recorded (367.47 ± 33.77), compared with control group which recorded the lowerst concentration of TNF-α (299.08 ± 18.36)pg/ml.

This study founded significant increasing of interleukin-6 in the group of type 2 diabetes infected with Toxoplasmosis, then the group of type 2 diabetic only, then the group of Toxoplasmosis only, compared with control, which recorded lowest concentration. IL-6 has an acritical role in immune response against *Toxoplasma gondii* because it is aproinflammatory cytokine leads to increase the cytotoxic activities of Natural killer cells and induce the differentiation of B lymphocytes and Cytotoxic T-lymphocytes IL-6 also mediated acute phase proteins and play as multifunction in regulation and response, so IL-6 associated with the inflammatory response (Hermes *et al*, 2008; Melo *et al*, 2011). IL-6 one of the cytokines that institute in development pathology of type 2 diabetes by its role in less mass of pancreas cells (Luigi *et al,* 2007). IL-6 contribute to the dysfunction and death of β cells during progression of T2DM (Ramadan *et al, 2011).*

This study agrees with Saeed (2018) which founded increasing of IL-6 level in type 2 diabetes infected with Toxoplasmosis, then group of type 2 diabetic only compared with control. This study also agrees with Nuhair (2018), Al-hefadhi (2013) and Hammed *et al* (2012) which recorded increasing of IL-6 concentration in T2DM compared with control.

The present study founded significant increasing in IL-12 concentration in the group of Toxoplasmosis only, which recorded the highest concentration, then the group of type 2 diabetes infected with Toxoplasmosis, then the group of type 2 diabetes only, compared with control, which recorded lowest concentration.

The high level of IL-12 in first two groups belongs to the important role of IL-12 in immune response against Toxoplasmosis IL-12 induce the induction of Interferon -d and TNF-α from Natural Killer cells which is also central in host resistance to *T. gondii* and mediated the cytotoxic activity of CD8+ cytotoxic T-lymphocytes. *T.gondii* elicits avery strong T-helper 1(Th1) cell-mediated inflammatory response (Filisetti and Candolfi, 2004). The Th1 response, defined by the production of IL-12 and INF-d,is characteristic of infection with the intercellular pathogens, including *T. gondii* (Dupont *et al*, 2012; Tait and Hunter, 2009).

The IL-12 also, has role in pathology of type 2 diabetes it is one of the pro-inflammatory cytokines that institute in dysfunction and death of pancreatic beta cells also reactive oxygen and nitric oxides, which induced by IL-12 from Macrophages can destroy beta cells (Ramadan *et al,* 2011).

This study recorded significant increasing in TNF-α concentration in the group of type 2 diabetes infected with Toxoplasmosis, then the group of Toxoplasmosis only, then the group of type 2 diabetes only, compared with control, which recorded the lowest concentration. The high level of TNF-α in these groups belong to the role of TNF-α Which trigger the Cytotoxic activity by Stimulates macrophages and Phagocytosis, TNF-α, also one of the pro-inflammatory cytokines which mediated inflammation and stimulates acute phase proteins, TNF-α PLAY with INF-d to inhibit the proliferation of parasite *et al, 2010;* Swavoop *et al,* 2012).

Many reports suggest that insulin resistance is associated with elevated levels of inflammatory cytokines such as IL-6, IL-12 and TNF-α,increasing the possibility that metabolic abnormalities in diabetic patients may cause by exacerbated by cytokine's overproduction. A possible contributor to this prolonged low-grade inflammation that subsequently leads to clinical expression of type 2 diabetes is the parasites *T. gondii.*

REFERENCES

Al-Hefadhy R E A (2013) Detection of Heat Shock Protein 70 and some Immunological & Biochemical parameters in patients with Diabetes Mellitus in Thi-Qar province, a thesis. collage of

Education for pure science, *University of Thi- Qar*, 116.

Boulanger M J, Chow D C, Brevnova E E and Garcia K C (2003) Hexameric structure and assembly of the interleukin- 6/IL-6 alpha – receptor r/gp 130 complex. *Science*, **300**(5628), 2101–210.

De-Fronzo R A (2004) "Pathogenesis of type 2 diabetes mellitus," *Med Clin North Am* **88**, 787–835.

Dubey J (2008) The history of Toxoplasma gondii. The first 100 years. *J. Euk. Microbiol*. **55**, 467-475.

Dubey J P (2009) Toxoplasmosis in sheep-The last 20 years. *Veterinary Parasitology.* **163**, 1-14.

Dupont C D, Christian D A, Hunter C A (2012) Immune response and immunopathology during toxoplasmosis. *Semen Immunopathology*. **34**(6), 793–813.

Filisetti D and Canolfi E (2004) Immune response to Toxoplasma gondii. *Ann. Ist. Super. Sanita*. **40**(1), 71 – 80 .

Hammed I K, Rashid N F and Abed B A (2012) Serum Interleukin-6 level in children with type 1 diabetes mellitus, *Fac Med Baghdad*. **54**(3), 228-230.

Hermes G, Ajioka J W, Kelly K A, Mui E, Roberts F, Kasza K (2008) Neurological and behavioral abnormalities, ventricular dilatation, altered cellular functions, inflammation, and neuronal injury in brains of mice due to common, persistent, parasitic infection. *J Neuroinflammation.* **5**, 48.

John Lackie (2010) "Cytokine" A Dictionary of Biomedicine. Oxford University Press. ISBN 9780199549351.

Johnson L (1992) A Protective role for endogenous tumor necrosis factor in Toxoplasma gondii infection. *Infect Immun.* **60**(5), 1979-83.

Kralove H (2013) Diabetes mellitus. Diploma Thesis.

Luigi F, Christopher J, Maria E, Philipp E and Samuel K (2007) Visceral fat adipokine secretion is associated with systemic inflammation in obese humans", *Diabetes*; **56**, 1010–1013.

Melo M B, Jensen K D and Saeij J (2011) Toxoplasma gondii effectors are master regulators of the inflammatory response. *Trends. Parasitol*. **27**(11), 487-495.

Newport M J, Holland S M, Levin M, Casanova J L (2007) "Inherited disorders of the interleukin-12/23-interferon gamma axis". In Ochs HD, Smith CI, Puck J. Primary immunodeficiency diseases: a molecular and genetic approach. New York: Oxford University Press. 390–401.

Nuhair R S (2018) The role of TGFb1 Polymorphism in Diabetes Mellitus patients in Thi-Qar Province and Ameliorative Effects of Vit-D3 on Beta cells,thesis, college of education, Thi-Qar university, 147.

Popko K, Gorska E, Stelmaszczyk-Emmel A (2010) Proinflammatory cytokines Il-6 and TNF-a and the development of inflammation in obese subjects. *European Journal of Medical Research*. **15**, 120–122.

Ramadan J W, Steiner S R, O'Neill C M, Nunemaker C S (2011) The central role of calcium in the effects of cytokines on beta-cell function: implications for type 1 and type 2 diabetes. *Cell Calcium.* **50**(6), 481–90.

Saeed S A (2018) Serological and Immunological study of Iraqi diabetic patients infected with Toxoplasmosis, thesis, College of education for pure science, *Baghdad university*. 130.

Shaw J, Tanamas S (2016) Diabetes: the silent pandemic and its impact on Australia. Diabetes Australia Available from: www.healthinfonet.ecu.edu.au/keyresources bibliography/lid=22970

Swardfager W, Lanctot K, Rothenburg L, Wong A, Cappell J, Herrmann N (2010) "A meta-analysis of cytokines in Alzheimer's disease". *Biol Psychiatry.* **68**(10), 930–941.

Swaroop J J, Duggirala Rajarajeswari D and Naidu J N (2012) Association of TNF-a with insulin resistance in type 2 diabetes Mellitus. *Indian. J. Med. Res*. **135**, 127-130.

Tait E D and Hunter C A (2009) Advances in understanding immunity to Toxoplasma gondii. *Memorias Do Instituto Oswaldo Cruz*, **104**(2), 201–210.