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Toxoplasma Gondii Related Programmed Cell Death (Pd1 and Pd-L1) in Patients with Diabetes Mellitus

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Abstract :

Toxoplasma gondii is one of the most successful microorganisms on Earth due to its high compatibility with a wide range of host species, including mammals, Diabetes mellitus is a worldwide condition, however it is more prevalent in the industrialized nations. According to the International Diabetes Federation's most recent estimates for 2019, there are currently an estimated 537 million adults living with diabetes worldwide.in Iraq more than 13.9% of adult live with diabetes . The main objective of the current study is to verify the presence of immunosera in diabetic patients against Toxoplasma gondii infection and its relationship with role programmed death (PD-1) and programmed death ligand (PD-L1) in patients .One hundred twenty (120) serum sample take from diabetic patients match with 60 control group were obtained and tested using widely available enzyme immunoassay kits for anti-Toxolasma IgG. The total infection with toxoplasmosis among patients with DM was 42(35%). Sandwich ELISA technology were used to explain the level PD-1and PD-L1 and the result were found a high level of pd-1 and pd-l1 in patient compared with control .

Keyword: Toxoplasma gondii , Diabetes mellitus , ELISA , PD-1 ,PD-L1

Introduction

Toxoplasma gondii is neglected parasite belong to apicomplexan parasite that has infect several humans around the world and is capable of infecting both domestic and wild animals (1, 21, 22). People are primarily affected by eating raw or inadequately cooked meat (especially lamb and swine) that contains infectious tissue cysts or by consuming sporulated oocysts in produce, food, or water that has been contaminated with cat feces (2). Infections of Toxoplasma gondii in healthy

persons don't cause any symptoms; but, in immunocompromised people and neonates, it's thought to be an opportunistic parasite that can be fatal (3, 22). During pregnancy (21), retinochoroiditis, encephalitis of the central nervous system, abortion, deformity, microcephaly, hydrocephalus, and long-term injury to the eyes can all result from primary infection of toxoplasmosis (4). Once infected, T. gondii can spread to numerous organs in the host, including the pancreas (5). Diabetes mellitus is a broad category of illnesses marked by hyperglycemia brought on by a relative or absolute deficiency in the production or action of insulin. The chronic metabolic disease that is expected to impact over 300 million people globally by the year 2030 (6).PD-1is transmembrane group of protein consistence from 288 amino acid. (IgV) like shape extracellular N-terminal domain and tail consistence from cytoplasm found at end respectively with two tyrosine bases. PD-1 found on the surface of B-cells, T-cells, and natural killer (NK) cells 42, 43, principally regulating effector T-cell activity inside tissues and restricting their lytic activity in malignancies(7).PD-l1 is also type of transmembrane glycoproteins consisting of IgC and IgV domains . The activity of PD-1 and its ligands PD-L1 is responsible for stimulation of T cells, proliferation, and cytotoxic secretion, which leads to the degeneration of anti-tumor immune responses (8).

Material and Methods :

A total of 120 blood samples were collected from Thi-Qar specialize endocrine & metabolism center (TDEMC) during the period from July to December 2022 . The ages of the participants range from 15 to 85 years, and the control group were also included . 5 ml of blood sample were collected from patient and placed in test tubes for three minutes at room temperature to allow it to coagulate and then separated by using centrifuge. After completing the process, the sera is kept in the refrigerator to conduct tests on it. Enzyme linking immunosorbent assay (ELISA) were used to detect the level of anti-toxoplasmosis (IgG,IgM) antibodies in patients serum according to (DRG) Germany kite . The (PD-1, PD-L1) level in patients serum was read through enzyme linking immunosorbent assay (ELISA) technique using the available kits (Fine Test kit) which prepared according to chain company manufacture by using serological (ELISA) techniques.

Statistical Analysis :

The result of data detect by using the program (SPSS) .The findings of present study The mean and standard deviation were used to calculate the probability of significant differences. The categorical variables were compared using the Chi-square test and also used degree of freedom .

Result:

The current study were found the Seroprevlance of anti-Toxoplasma IgG antibodies in the study groups was 35% among patients with diabetes mellitus and 21.6% in the control, with a high significant difference between the groups as listed in the following table:

Table (1): T.gondii (IgG) among diabetic patients

Case	Toxo (Igg)	NO.(%)	Mean (IU/Ml)
Patient	Positive	42(35%)	231 IU/Ml
	Negative	78 (65%)	13.4 IU/Ml
Control	Negative	47(78.3%)	6.9 IU/Ml
	Positive	13(21.6%)	230 IU/Ml
Total NO Of Diabetic Patients= 120		Control =60	

X2=7.840 df=1 p-value=0.005 high Significant differences P≤ 0.05

The level of PD-1 in diabetic patients without Toxoplasmosis recorded (188.6 pg/mg) while in DM patients with T.gondii recorded (193.5 pg/mg) in compared with (595 , 95 pg/mg) of its level in control group that positive for Toxoplasmosis without DM as explain in table (2).

Table (2): level of PD-1 (pg/ml) in diabetic patients with and without T. gondii-IgG :

Case	Mean Of PD-1(Pg/Ml)
Diabetic Mellitus(-Toxo Igg)	188.6
DM+T.Gondii(Igg)	193.5
Control Positive (+Toxo Igg)	595
Control Negative (-Toxo Igg)	95

X2=553.867 df=3 p value = 0.00 significant differen P≤ 0.05

The level of PD-L1 in diabetic patients without Toxoplasmosis were recorded (67pg/mg) while diabetic patients infected with T.gondii records (83pg/mg) compared with (49 pg/mg, 38 pg/mg) among control positive and negative respectively , Table (3) explain the level of PD-L1 in DM patients with or without toxoplasmosis.

Table (3): Level of PD-L1 in diabetic patients with and without Toxoplasma gondii-IgG antibody

Case	Mean Of PD-L1(Pg/Ml)
Diabetic Mellitus (-Toxo Igg)	67
DM+T.Gondii(Igg)	83
Control Positive (+Toxo Igg)	49
Control Negative (-Toxo Igg)	38

X^2=9.817 df=3 p value =0.020 significant differen P≤ 0.05

Discussion:

The primary objectives of this study were to examine the seroprevalence of anti-Toxoplasmosis IgG antibodies in diabetic patients and to define the role of programmed death-1 (PD-1) and (PD-L1) in diabetic patients (DM) patients with chronic toxoplasmosis. According to the findings of the current investigation, 35% of diabetic people were infected with T.gondii compared with control one , it was achieved to identify seropositive for anti-T. gondii IgG antibodies. The present study were agreed with study done in Kirkuk Province which showed a high prevalence for ant-Toxoplasma IgG among diabetic patients by using ELISA technique (9).Also , it were agreed with study conducted in Iran which revealed that 37% of diabetic individuals infected with toxoplasmaosis (10). A recent study conducts in Baghdad, the overall anti-Toxoplasma IgG positive rate was 82% in diabetes individuals than in the control group and in compatible with the current study (11). It is also disagree with study done in Egypt and showed a highly sero-prevalence for anti-toxplasma IgG among diabetic patients with percentage (77%) in comparison with control (12). Despite the strength of the common relationship between toxoplasmosis and diabetes, as each of them leads to the other, but this depends on which of them affects the body first . Hosts with impaired immune systems, in particular those with insufficient cellular immunity, are susceptible to the reactivation and spread of chronic infections (toxoplasmosis). Tachyzoite present during infection in pancreas lead to destruction of beta- cell (13). programmed death receptor PD-1 is one of trans membrane glycoprotein with a molecular weight of (50–55 kDa) and 288 amino acids is known as CD279. This cell surface inhibitory receptor protein is a member of the Ig superfamily and is known as a programmed death ligand. The type I transmembrane protein receptor PD-L1A belongs to the B7 family, and PD-L1 (also known as B7-H1 or CD274)

is a 290-amino-acid protein. IgV- and IgC-like extracellular domains, a transmembrane domain, and a cytoplasmic domain are all present. This protein is produced by a variety of cell types, including antigen-presenting cells (APCs), T cells, B cells, monocytes, and epithelial cells. When activated in response to pro-inflammatory cytokines, these cells produce more PD-1 (14).

The current study display a high level of PD-1 (193.5 pg/ml) among diabetic patients with T.gondii than without its (188.6 pg/ml) in comparison with control positive and negative (595 pg/ml) and (95 pg/ml) respectively also in PD-L1 recorded a high level (83 pg/ml) among diabetic patients infected with toxoplasmosis than diabetic alone (67 pg/ml) in comparison with both type of control positive and negative (49 pg/ml) (38 pg/ml) respectively .

The results of the current investigation were in agreement with a study conducted in the Iraqi province of Baghdad to evaluate the serum levels of PD-1 and PDL-1 in 180 diabetic patients, which showed that soluble levels of PD-1 and PDL-1 were much greater in the diabetic patient than in the normal population [15]. Another study was conducted in Babil, Iraq, examined the level of PD-1 and PD-l1 in breast cancer patients with toxoplasmosis, and it discovered that these patients had higher levels than the control group [16]. In the Iraqi province of Baghdad The serum of aborted mothers with T.gondii who were investigated had elevated levels of PD-1 and PD-L1 [17].In USE conducted a study about the function of PD-1and PD-L1during the infection with toxoplsmosis the result showed the increase level(PD-1 ,PD-L1) in patients [18]. Because Toxoplasma gondii, which can trigger both innate and adaptive immunity and cause T-cells and CD8 exhaustion, in which these cells stop functioning or T cells produce less cytokines and cytotoxic chemicals, the rising levels of PD-1 and PD-L1 could be a return to the latent infection. Furthermore, PD-1 and other inhibitory receptors, like PD-L1, are expressed more frequently on worn-out T cells, preventing T-cell activation and subsequently stimulating the process of lymphocyte apoptosis. In addition, PD-1 signals change the function of T-cells by increasing lipolysis and lipid consumption[19,20].

Conclusion :

Chronic toxoplasmosis may be leading to pancreas dysfunction . Apoptosis is common among diabetic patients with and without toxoplasmosis by increasing the level of PD-1 and PD-L1.

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