

Anti- *Toxoplasma gondii* IgG, Ig M, and IgA among Type 2 diabetic Patients in Benghazi, Libya. A Comparison Study

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Abstract: Context: *Toxoplasma gondii* is an opportunistic parasite in immunocompromised people. On other hand, diabetes is disease which affects the immune system.

Aims: To explore the prevalence of *Toxoplasma gondii* in Type 2 diabetic Patients using ELISA methods in Benghazi, Libya. Design: A case control study of type 2 diabetic patients was conducted at Benghazi diabetic and endocrine centre between August to November 2016. Methods and Materials: Two hundred serum samples from diabetic Type2 patients and fifty from healthy non-diabetics from Alhya laboratory were used as a control group. All individuals aged 18–<51 years. Cases and controls were matched for detection of toxoplasma (Ab) with demographic parameter such as gender, age, abortion and the presence of wild animals especially cats. Levels of IgG, IgM, and IgA antibodies against *Toxoplasma gondii* in the patients' sera, using the Enzyme-Linked Immunosorbent Assay, were determined. Descriptive statistics and Pearson chi square used to check the difference. Results: The overall Prevalence of infection with *Toxoplasma gondii* among diabetic patients [Ig M+ (10.5%), IgG+ (41.5%) and IgA+ (3.5%)] was higher than non-diabetics individual group [IgM+ (4%), IgG+ (24%) and IgA+0(0%).

Conclusions: Anti-*Toxoplasma gondii* Immunoglobulin among Type 2 diabetic Patients was higher than non diabetic in Benghazi,

Keywords: *Toxoplasma gondii*, Immunoglobulin, Diabetes, Benghazi.

Key Messages: It's well known that both diabetes and toxoplasmosis can cause congenital malformation of the foetus, our study highlight that diabetic have higher prevalence of toxoplasmosis. Hence screening diabetic females for toxoplasmosis may be advisable.

I. INTRODUCTION

Toxoplasmosis is a zoonotic infection caused by intracellular protozoa parasite a *Toxoplasma gondii*¹. The protozoon infects different warm blooded organisms, including human,, as the intermediate hosts. Moreover, it infects domesticate and wild cats as the definite host^{2,3}

It causes asymptomatic disease, but is an important opportunistic parasite in immunocompromised people. Primary maternal infection during pregnancy can be transmitted to the foetus and result in serious sequelae³ During the acute infection *tachyzoites* rapidly proliferate Attacking the nucleated cell via active invasion. Destroying and kill the host cells and disseminate to the central nervous system, eyes, cardiac and skeletal muscles, and placenta.⁴

On the other hand, diabetes is the disease influences the cellular and humoral immunity predisposing the patient to opportunistic parasites one of them is *toxoplasma*. Its well known that Both diabetes and *toxoplasmosis* can cause congenital malformation of the foetus, Consequently, the aim of our study focused on the serologic detection of *Toxoplasma gondii* infection in diabetic patients and compared to non diabetic control.

II. SUBJECTS AND METHODS

2.1 Study design and Patients

A case control study was conducted in Benghazi city. The study has been done for 250 serum samples including 200 of them Type 2 diabetic patients (125 Female and 75 Male) recruited from government diabetes and endocrine centre and 50 cases of healthy non-diabetics (32 Female and 18 Male) control group has been recruited randomly from the, Alhaya laboratory during the period from August to November 2016. Cases and controls were matched with demographic parameters such as age, gender, abortion and the presence of wild animals especially cat's and history of diabetic patients was recorded

2.1 Methods

2.1.1 Antibody detection

The detection of *Toxoplasma*- specific antibodies is the primary diagnostic method to determine infection with *Toxoplasma*. *Toxoplasma*- specific antibodies are IgG , IgM and IgA

2.1.2 Blood samples and preparation of sera

Blood samples were collected from the venepuncture of the patient into plain sterile tubes, left to clot at room temperature for 3 h, and centrifuged subsequently at 3000 RPM for at least 20 minutes at room temperature. The separated sera were stored at -20°C or below until analysis of *T. gondii* by ELISA^{5,6,7}

2.1.3 Determination of antibodies to *T. gondii*

All 250 serum samples were tested using Euroimmun enzyme-linked immunosorbent assay (ELISA) kits (PerkinElmer Company Euroimmun, Lübeck, Germany) for the presence of *Toxoplasma* IgG, IgM and IgA antibodies and avidity of IgG antibodies. IgG and IgM indexes of <0.8 were regarded as the negative results, between 0.8 to 1.1 as the borderline, and ≥ 1.1 as the positive results. In the avidity kit, an avidity index of $<40\%$ indicated low avidity antibodies, between 40 and 60% represented the equivocal range, and $>60\%$ represented high-avidity antibodies. Positive IgG titers and negative IgM titers were considered a latent infection. Sera with concurrent positive IgG and IgM titers were further analysed by avidity testing.

Data were analysed with SPSS version 17 using analysis of chi-squared tests. Differences were considered statistically significant at P values of <0.05 .

III. RESULTS

In the present study out of 200 (38 positive and 162 negative) among Patients with type II diabetic subjects who were tested, 41.5% were sero-positive for *Toxoplasma* - IgG antibodies, 10.5% (21 /200) and 3.5% (7/200) were sero-positive for IgM and IgA antibodies, respectively, while in control group, 50 individuals 12 (24%) were positive for IgG and 2(4%) IgM antibody. IgA antibody was negative for all samples of control group (0 %). Table 1& Figure 1

The result shows no statistical difference in antibodies prevalence according to age either in diabetic or healthy non diabetic control except for IgG level in diabetics where significantly higher in younger diabetic's ($X^2 = 10.779a$ $P=.013$). Table 2 & 3. Similarly, no statistical difference in antibodies prevalence according to sex in either groups Table 4

Diabetic with domestic cat contact they have significantly higher antibodies (IgG, IgM, IgA) seropositivity compared to diabetic with no history of domestic cat contact. (IgM = $X^2= 20.231a$ $df=1$ $p= .00$, IgG= $X^2= 48.445a$ $df=1$ $p= .000$, IgA= $X^2= 11.991a$ $df=1$ $p= .001$) Table 5.

While in non diabetic only IgG was statistically different. (Ig M = $X^2= .136a$ $df=1$ $P= .712$ IgG= $X^2= 12.348a$ $df=1$ $p= .000$) .Table 6
The clinical inference of antibody status of all participant show 41.5% have probably previous exposure to *toxoplasmosis* and 56% seronegative and 10.5% of recent infection, 3.5%(only IgA positivity) of acute infection and others of probably of reactivated infection. Table 7

IV. DISCUSSION:

This is the first study aiming to determine anti *toxoplasma* antibodies in diabetic patients attending at government diabetes and endocrine centre in the city of Benghazi. In the present study out of 200 (38 positive and 162 negative) among Patients with type II diabetics who were tested, 41.5% were sero-positive for *Toxo-* IgG antibodies, 10.5% (21 /200) and 3.5% (7/200) were sero-positive for IgM and IgA antibodies,

respectively, while in control group, 50 individuals 12 (24%) were positive for IgG and 2(4%) IgM antibody. IgA antibody was negative for all samples of control group (0 %) therefore, these findings suggest that diabetics are more susceptible to *Toxoplasmosis* than those without.

This result in agreement of Shirbazou *et al* (2013) which they found the prevalence of IgG antibodies against *Toxoplasma gondii* in diabetic patients and healthy controls were 60.43% and 38% respectively. Also I agreement with ⁸

While in anon comparison study in Iran has shown that 70.3% among diabetic patients were seropositive for *Toxoplasma* antibodies ⁹ . Similarly, Gocke *et al.* study confirmed that 56% of diabetic patients were positive for *anti-T. gondii* IgG. ¹⁰

The value of 24% % a seroprevalence of *anti-T. gondii* IgG in control group is lower to a study by Akinbami *et al.*, (2010) in Nigeria 40.8 % in non diabetic pregnant women ¹¹

While the result of the current study is lower than 81.1% reported by Zemene *et al.*, (2012), Gebremedhin *et al.* (2013) in the South western and Central Ethiopia respectively. ^{12,13}

Similarly the results found in our study of *anti-T.gondii* IgM antibodies was 10.5% (21 /200) was in contrast to the result of the current study is higher compared to 5.3 % reported by Silva *et al.* (2015). ^{10,14}

IgA and IgM class antibodies are associated with recent infections (acute infection) while IgG class antibodies are associated with chronic infection. However, the IgA antibody test is not commonly

used, and mostly used to detect the disease in cases of congenital infection. Thus, the IgA antibody test is an important marker for *T. gondii* infection in new born babies. In our study the *anti-T. gondii* IgA was low 3.5% compared by to 69.4% reported by Fernando *et al.*, (2016) in high risk pregnant female. However, in the current study the *anti-T. gondii* IgA was 0% in healthy control group which is similar to finding of Carmen, *et al* 2006 among healthy individuals in Slovakia. ^{15,16}

The result shows no statistical difference in antibodies prevalence according to sex either in diabetic or non diabetics, this in contrast to finding of Entsar J. Saheb (2017) ¹⁷ but in agreement The same finding with age except for IgG level in diabetics where significantly higher in younger diabetic's ($X^2 = 10.779a$ $P=.013$) which in agreement with Entsar J. Saheb (2017). ¹⁷

Diabetic with history of cat contact they have significantly higher *Toxoplasma* antibodies (IgG, IgM, IgA) seropositivity compared to diabetic with no history of domestic cat contact. while in non diabetic only IgG was statistically different.

A Meta-Analysis published at 2016, show that cat contact can increase the risk of infection by *T. gondii* in various populations, including the general population, immune-suppressed/pregnant individuals, those with special occupations, and cat(s) owners. but while significant, cat contact may not be the most important risk factor for infection by *T. gondii* in cat owners. ¹⁸

Conclusions:

Anti-*Toxoplasma gondii* IgG, Ig M, and IgA levels among Type 2 diabetic Patients was higher than

non diabetic in Benghazi, Libya. Gender and age were not determining variable except for younger diabetic were the IgG was higher. History of domestic cat contact was risk variable for diabetics.

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VI. DISCLOSURES:

6.1 Authors contributions

Author A Contribute to most of the work including concepts, study design ,clinical and experimental testing and data acquisition, data analysis, Statistical analysis, and is the grantor of the research while A and B they share opinion in study design, literature search, revising it for important intellectual content, review of Statistical analysis and Manuscript preparation, Manuscript editing, Manuscript review.

6.2 Funding:

This work was funded by Department of Medical laboratory in higher institute of medical.

6.3 Conflict of interest NO conflict of interest

6.4 Compliance with ethical principles

As the risk is minimal so the study comply with ethical principles in general despite we don't have real IRB in Benghazi.

6.5 Approvals (Yes, No or Waived) By which IRB/Ethics committees Reference number .

NO because there is no real national active Ethical committee in Benghazi.

6.6 Consent

Verbal informed consent was taken from all participant after they read the form which consider the eight element of the consent .

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