THE PROBABLE RELATION BETWEEN *TOXOPLASMA GONDII* AND DIABETES MELLITUS

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ABSTRACT: Present study aims to determine probable relation between *Toxoplasma gondii* infection and diabetes mellitus by investigating serum levels of insulin and anti-*Toxoplasma* antibodies through ELISA among diabetic and non-diabetic persons in Lahore, Pakistan. Results showed that insulin level was higher in subjects positive for *Toxoplasma* infection as compared to *Toxoplasma* free one's ($p<0.05$). The analysis revealed that *Toxoplasma* infection was more prevalent in diabetic patients ($p<0.05$) than non-diabetic individuals. Results also showed that insulin level was higher in persons with chronic infection. Gender-wise comparison revealed that in males, insulin level had significant association with *Toxoplasma* infection ($p<0.05$). It was concluded that participants who had *Toxoplasma* infection had higher insulin level that showed the probable relation of infection with diabetes.

Key words: Toxoplasmosis, Seroprevalence, Diabetes, Anti-*Toxoplasma* antibodies.

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INTRODUCTION

Toxoplasmosis is caused by a protozoan parasite *Toxoplasma gondii* that affects birds and mammals through various routes (Tenter *et al*., 2000). At early stages toxoplasmosis is asymptomatic, although sometimes it may cause fever and headache, etc. (Lindstrom *et al*., 2006). In immunocompromised individuals, *T. gondii* infection can cause severe abnormalities of various body organs including brain, heart and eyes, etc. (Alvarado-Esquivel and Estrada-Martínez, 2011). Exposure to cats, meager hygienic measures, consumption of oocyst contaminated water, fruits and vegetables are considered some common transmission risk factors for *T. gondii* (Montoya and Liesenfeld, 2004 and Cook *et al*., 2000;). In acute infection, tachyzoites proliferate rapidly and invade many tissues of the body. In immune-competent persons, tachyzoites are converted into bradyzoites and persist in host tissues for the whole life span (Montoya, 2002 and Mordue *et al*., 2001).

Beta cells of the pancreas produce insulin and carbohydrate metabolism is mainly dependent on it. Genetic factors or life styles are responsible for its impaired production in diabetic persons (Danaei, 2011). Moreover, patients with pancreatic problems may also have higher levels of circulating insulin (Joshi *et al*., 1999).

Disturbances in insulin levels may results finally in persistent hyperglycemia that characterize diabetes mellitus and affects metabolism of fat, carbohydrate and protein (*American Diabetes Association*, 2008). Diabetes is of considerable medical importance with wide spread distribution and is mainly affected by genetic elements, autoimmune processes, environmental factors and infectious agents too (Fernandes *et al*., 2009). The disease reduces cellular and humeral immune status through long hyperglycemic course and may also possibly stimulate latent opportunistic pathogens. It finally leads to increased susceptibility rate and host is then more vulnerable to various infections (Prandota, 2013). Diabetic patients are more vulnerable to opportunistic infections such as toxoplasmosis (Majidiani *et al*., 2016). Presence of *T. gondii* in the pancreas probably damages the beta pancreatic cells directly that reduces the levels of insulin in host body, and then may increase the risk for the development of diabetes (Shirbazou, 2013).

A lot of work has been done in Pakistan with reference to *Toxoplasma* prevalence among poultry birds (Mahmood *et al*., 2014) and domestic animals like sheep (Shah *et al*., 2013). Currently, there is no study available in Pakistan specifically that determines the relationship of *T. gondii* with diabetes and specifically in relation to insulin level. Based on the hypothesis that toxoplasmosis may possibly cause diabetes. The present research work is designed to determine serum level of insulin and to relate it with *T. gondii* infection in diabetic and non-diabetic persons.

MATERIALS AND METHODS

Questionnaire survey: Socio-demographic data (age, gender, education and residence) of study population was collected with the help of questionnaire. Questions were also asked about different health problems.

Collection of blood samples: Approval for present research was taken by Ethical Research and Review Committee, Zoology Department, Lahore College for Women University, Lahore, Pakistan. Participants included in this study were taken from general population of different localities of Lahore like Suk nehr, Mehmood Booti and Baghbanpura. Written consent was taken from the participants before blood sampling. Random collection of blood samples (n=360) was made only from those respondents who gave their consent. Selection of diabetic and non-diabetic individuals was made by a medical doctor. It was also noted that diabetic individuals that participated in present research study were mostly of type 2. Blood samples were taken from diabetic (n=200) and non-diabetic (n=160) individuals. Samples were brought to the central Research Laboratory of Zoology Department, LCWU, Lahore for further processing.

Serum sample analysis: Anti-*Toxoplasma* IgM and IgG antibodies (BiocheCk, USA) and serum insulin levels (Monobind, USA) were determined with the help of automated ELISA (Coda EIA Analyzer, Bio-Rad, USA) as per manufacturer's instructions. Inactivated *T. gondii* antigens were coated on microtiter plates. For IgG only, those serum samples were considered as positive whose titer was higher than 32 IU/ml. In contrast to those samples with serum titer less than 32IU/ml, were categorized as negative. Moreover, IgM was positive for serum samples that had O.D greater than 1. Serum insulin level was compared with the cut-off value (8µIU/ml) given by manufacturer. Chronic *Toxoplasma* infection was designated for those individuals who were positive only for *Toxoplasma* IgG. Further, individuals who were positive both for *Toxoplasma* IgG and IgM, were considered to have acute infection of *Toxoplasma*.

Statistical analysis: Tabulation of data collected through the questionnaire was done. Averages and percentages were calculated. Student's *t*-test was applied for the analysis of significant relationship of mean differences for insulin level in persons with acute and chronic *Toxoplasma* infection. Prevalence percentage among various categories was compared with each other using Chi-square test and also to find the relationship of insulin levels and *T. gondii* infection (Pal and Sarkar, 2008). Probability level for statistical significance was taken as <0.05.

RESULTS and DISCUSSION

It was found that overall 224/360 respondents showed higher levels of serum insulin with average concentration of 224µIU/ml. Gender-wise comparison showed that males had higher average concentration of insulin (102µIU/ml) as compared to females (24µIU/ml).

Results of present study showed that *Toxoplasma gondii* infection was prevalent among subjects (60%, 217/360). When comparison was made to find the association of insulin levels and *Toxoplasma gondii* infection, it showed that average insulin concentration was more in study respondents with *Toxoplasma* infection (132µIU/ml) as compared to those who had no *T. gondii* infection (93µIU/ml) (Fig-1). Difference was considered statistically significant ($p<0.05$).

Data analysis revealed that *Toxoplasma* infection was significantly higher among those individuals with diabetes as compared to non diabetic persons (Table 1).

When gender-wise comparison was made for the assessment of relationship of *T. gondii* infection and insulin, it was observed that in males *Toxoplasma* infection had significant association with high level of insulin in those males who had toxoplasmosis. Whereas, in case of females the results showed that the females who were negative for *Toxoplasma* infection they had higher levels of insulin (Table 2).

It was found that chronic *Toxoplasma* infection had significant association with insulin level as compared to acute infection indicating that longer exposure with parasite may affect the host metabolism in such a way that ultimately leads to severe metabolic diseases like diabetes (Table 3).

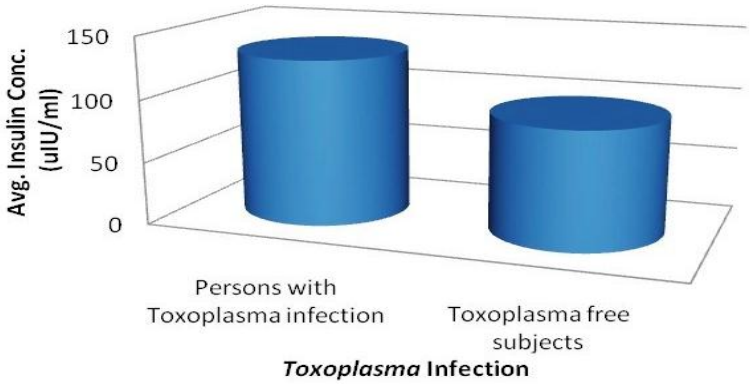

Figure-1. Comparison of average concentration of Insulin (µIU/ml) among persons with and without *Toxoplasma* infection.

Table 1. Association of *Toxoplasma gondii* infection in diabetic and non- diabetic groups among human population.

Risk factors	Number (n)	Infection				X^2 (D.F.=1)	P value
		Positive		Negative			
		n			%		
Diabetic individuals	200	140	70	60	30	5.08	<0.05
Non Diabetic individuals	160	77	48	83	51	6.03	

Table 2. Gender-wise relationship of higher insulin level in persons with and without *T. gondii* infection.

Gender		Infection				X^2 (D.F.=1)	P value
		Positive					
	Number (n)	n			%		
Male	102	83	5.08	<0.05	20	7.02	<0.05
Female	122	48	6.03		80	6.05	

Table 3. Relationship of higher insulin level with acute and chronic *T. gondii* infection.

Toxoplasma Infection	Number	Average insulin level µIU/ml	p value
Chronic infection	127	126	<0.05
Acute infection	90	20	

The probable reason for higher insulin level among males might be due to high resistance of female hosts in comparison to male individuals as literature review has showed that the level of various antibodies like IgA, IgM, IgG and IgE were more in females than in male individuals (Morales-Montor *et al*., 2004). Reports have revealed that due to gender associated variations in exposure to contaminated surfaces or because of high concentration of male reproductive hormones *i.e*. testosterone, that is immunosuppressive in nature, males were considered more susceptible to different parasitic infections as compared to females (Shirbazou, 2013 and Qureshi, 2004).

In present study high *Toxoplasma* prevalence was found. Similarly in Venezuela Chacin-Bonilla *et al.* (2003) also reported the higher prevalence rate (49.8%). In Brazil Avelino *et al.* (2003) also detected 65.8% prevalence rate for *Toxoplasma* infection among humans. In another study Sharif *et al.* (2007) found overall anti-*Toxo* IgG prevalence percentage of *T. gondii* infection as 77.4% among human population of Iran.

Findings of current study showed that *Toxoplasma* infection had significant association with diabetes. These results are strongly supported by Gokce *et al*. (2008) who determined in their study that *Toxoplasma gondii* infection had significant association with the prevalence of diabetes,that showed as to how *Toxoplasma* can impair the body metabolism by affecting the pancreatic function. In another study Siyadatpanah (2013) revealed the association between *Toxoplasma* infection and diabetes showed that chronic toxoplasmosis may significantly lead to diabetes mellitus.

In a study carried out by Modrek *et al*., (2015) pointed out that in 145 cases (70.3%) among 205 diabetic subjects were seropositive for *Toxoplasma* infection. Mousavi *et al*. (2016) also detected *T. gondii* in diabetic patients and showed significant by higher association between toxoplasmosis and diabetes.

Zandman-Goddard and Shoenfeld (2009) and Shapira *et al*. (2010) also supported the results of the present study and demonstrated that genetic individual susceptibility is the main factor responsible for geoepidemiology of autoimmune diseases in triggering the protective agents which depend on environmental conditions, lifestyle habits, eating practices, socioeconomic status, pollutants and various types of viral, bacterial and parasitic infections.

In a study Shapira *et al*. (2012) reporteded that pathogenic process can be initiated by *T. gondii* which caused clinically overt autoimmunity due to positive anti-*T. gondii* IgG antibodies (42%) in patients (n=1514) including various autoimmune diseases where as 29% was in control specimens ($p<0.0001$).

Conclusion: It was concluded that toxoplasmosis is significantly associated with serum insulin levels both in diabetic and non-diabetic persons. Higher Insulin levels were found in those individuals who had *Toxoplasma* infection that showed the probable relation of infection with diabetes. More detailed studies are strongly recommended regarding the relationship between *T. gondii* and diabetes to rule out that whether diabetes enhances the chances for parasitic infection or parasite itself play important role in the development of this metabolic syndrome..

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