Effect of interleukin-10 polymorphism on susceptibility to type I diabetes in children with latent toxoplasmosis

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Objective
The aim of this work was to investigate the effect of interleukin (IL)-10-1082 polymorphisms on susceptibility to type I diabetes in children with latent toxoplasmosis.

Materials and methods
IL-10 (–1082) polymorphisms were assessed by PCR in a small sample size of 75 patients with diabetes mellitus (DM) type I. Moreover, serum levels of C peptide and toxoplasma were determined by enzyme-linked immunosorbent assay. Glycosylated hemoglobin in blood was determined by colorimetry.

Results
Distribution of AA was higher in the case group (38.7%) compared with (18.7%) in the control group, whereas GG and AG were higher in the control group (45.3 and 36.0%, respectively) compared with (32.0 and 29.3%, respectively) in the case group. Regarding toxoplasma, it was more frequent in the case group (49.3%) compared with (30.7%) in the control group. The main risk factors of DM type I were high HbA1c [odds ratio (OR)= 10.7], positive toxoplasma finding (OR=4.7), high blood glucose (OR= 1.04), GG IL-10 polymorphism (OR=0.31), and low level of C peptide (OR=0.7). On the contrary, C peptide level and blood glucose were statistically significantly higher in positive toxoplasma cases (P<0.05). GG distribution was statistically significantly higher (P<0.001) in positive toxoplasma cases (48.6%) compared with (15.8%) in negative toxoplasma cases. The most significant predictors for G allele were positive toxoplasma result (OR=3.5) and high HbA1c level (OR=1.22).

Conclusion
GG genotype can no longer be viewed as a protective allele for DM type I, as higher IL-10 production with the ubiquitous nature of toxoplasma infection can lead to more pancreatic necrosis. The use of IL-10 as therapeutic cytokine for treatment of DM type I should be revised.

Keywords:
-1082 polymorphisms, diabetes mellitus type I, interleukin-10, toxoplasma

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Introduction

Toxoplasma gondii is an intracellular protozoan that infects ~33% of the world's population (Robert-Gangneux and Dardé, 2012). Different conditions, such as number of parasites, genetic background, virulence of the organism, immunological status, and sex, seem to affect the course of infection (Dupont *et al.*, 2012). Systemic dissemination to all tissues such as central nervous system, eye, kidney and pancreas is reported in acute toxoplasmosis (Weiss and Dubey, 2009). Acute phase is mostly resolved with T helper 1 immunity in immunocompetent patients with residual latent tissue cysts (Lüder and Rahman, 2017). Most common form of the infections in humans is latent (asymptomatic) toxoplasmosis (Dalimi and Abdoli, 2012). There is a serological linkage between toxoplasmosis and diabetes; the global protozoan parasite, *T. gondii*, infects many warm-blooded animals and humans by employing different transmission routes, leading to the probable relevance between infectious agents and diabetes (Majidiani *et al.*, 2016). Immune suppression in diabetes mellitus (DM) also confers an increased risk of various pathogens including toxoplasma infections, making a vicious cycle between toxoplasma and diabetes (Hassanain *et al.*, 2014). Interleukin (IL)-10 works as an anti-inflammatory and immunosuppressive cytokine, which is implicated in the pathogenesis and complication of DM (Barry *et al.*, 2016). Additionally, IL-10 inhibits the killing of *T. gondii* by human macrophages and leads to suppression of cell-mediated immunity to this parasite and decreases the pathology linked to its infection (Vouldoukis *et al.*, 2011). Serum level of IL-10 is genetically controlled, which is associated with single nucleotide polymorphisms in the IL-10

promoter gene and can influence the outcome and degree of severity of diabetes (Xie *et al.*, 2013).

The aim of this work was to investigate effect of IL-10-1082 polymorphisms on susceptibility to type I diabetes in children with latent toxoplasmosis.

Materials and methods

A total of 75 patients with DM type I, diagnosed according to WHO criteria for DM, were recruited from Mansoura University Children Hospital during the period between December 2017 and December 2018.

The patients presenting with other congenital metabolic diseases, cardiomyopathy, known hypertension with or without treatment, valvular heart disease, ischemic heart disease, severe anemia, heart failure, chronic pulmonary illness, undernutrition, hemoglobinopathies, HIV infection, other parasitic disease, and obese patients were excluded. In addition, 75 healthy control children without the symptoms of an acute or chronic infection were included in the study.

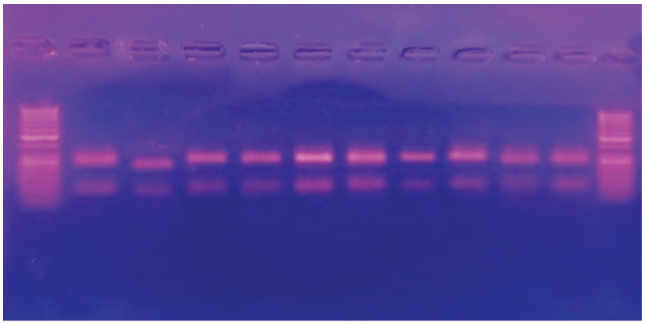
Stool examination was done, to exclude parasitic diseases. Toxo IgG was estimated using the Diapro Diagnostic Bioprobes Srl (Milano, Italy) 'Capture' Enzyme Immuno Assay. Glycosylated hemoglobin in blood was determined by quantitative colorimetric kit from STANBIO laboratory company procedure NO: 0350 (North Main Street, Texas, USA) (Goldstein *et al.*, 2004). Serum levels of C peptide were determined by immunosorbent assay kit from Calbiotech company catalog no: CP179s (Bonger and Garcia-Webb, 1984).

For IL-10 polymorphism, 5 ml of venous blood was drawn from each individual, and GFX blood DNA purification kit (Amersham Biosciences Bucking hamshire United Kingdom Limited) was used for the isolation of genomic DNA. IL-10 (−1082) polymorphism assessments were done by polymerase chain reaction amplification in a thermal cycler (Techne Genius, England) followed by digestion with a specific restriction enzyme. The sequences of PCR primers are 5'-CCAAGACAACACTACTAAGGCTCCTTT-3'and 5'-GCTTCTTATATGCTAGTCAGGTA-3'. Overall, 5 units of XagI enzyme (MBIFermentas, Vilnius, Lithuania) were used for digesting the products. Digestion products of 280 + 97 and 253 + 27 bp were obtained for A and G alleles, respectively (Fig. 1). The visualization was performed by gel electrophoresis (10% polyacrylamide) (Cordeiro *et al.*, 2008).

Ethical consideration

Approval of Institutional Review Board (Faculty of Medicine, Mansoura University) was obtained. An informed verbal and written consent from children guardians to participate in the study was obtained with assurance of anonymity and confidentiality of the data.

Figure 1



1 2 3 4 5 6 7 8 9 10 11 12

Lane 1 and 12: DNA size marker (50-1000 bp); lanes 4, 5, 8 and 9: (wild-type AA was found, which appeared at 280 + 97 bp); lanes 1, 6 and 7: (heterozygous-genotype AG which had 280, 253, 97 and 27 bp); lanes 3, 10 and11: (homozygous-genotype GG was found, which had 253 +27 bp).

Statistical analysis

Data were analyzed with SPSS version 21 (Armonk, NY: IBM Corp.). The normality of data was tested first with one-sample Kolmogorov–Smirnov test. Qualitative data were described using number and percentage. χ^2-test was used to associate between categorical variables. Continuous variables were presented as mean ± SD for parametric data and median for nonparametric. The two groups were compared with Student *t* test for the parametric data and Mann–Whitney test for the nonparametric. Analysis of variance test was used for comparison of means of more than two groups concerning parametric data and Kruskal–Wallis test was used for comparison of medians of more than two groups concerning nonparametric data.

The most significant determinants were predicted using the forward Wald statistical technique (logistic regression model) (Sham and Purcell 2014).

Results

Comparison between cases and control groups regarding the studied parameters (Table 1) shows that there were significant differences between cases and control groups regarding C peptide, HbA1c, and blood glucose (P<0.001). Regarding IL-10 polymorphism, distribution of AA was higher in the case group (38.7%) compared with (18.7%) in control group, whereas GG and AG were higher in control group (45.3 and 36.0%) compared with (32.0 and 29.3%, respectively) in the case group. Regarding toxoplasma, it was more frequent

in the case group (49.3%) compared with (30.7%) in the control group.

In Table 2, the following were independently associated with diabetes: high HbA1c [odds ratio (OR)=7.66], high blood glucose (OR=1.08), positive toxoplasma (OR=2.2), low level of C peptide (OR=0.603), GG IL-10 polymorphism (OR= 0.341), and AG IL-10 polymorphism (OR=0.393).

After multivariate regression analysis and adjusting the confounding factors, the highest risk of cases were high HbA1c (OR=10.7), positive toxoplasma (OR=4.7), high blood glucose (OR=1.04), GG IL-10 polymorphism (OR=0.31), and low level of C peptide (OR =0.7).

On the contrary, the comparison between positive and negative toxoplasma cases in diabetic group regarding the studied parameters shows that C peptide and blood glucose level were statistically significantly higher in positive toxoplasma cases (P<0.05). Moreover, GG distribution was statistically significantly higher (P < 0.001) in positive toxoplasma cases (48.6%) compared with 15.8% in negative toxoplasma cases.

Table 3 shows that there was a significant relation between IL-10 polymorphism, C peptide, and HbA1c. HbA1c was higher in GG and AG compared with AA, whereas C peptide was higher in AA group compared with GG and AG. On the contrary, low C peptide level, high blood glucose level, high HbA1c level, and positive toxoplasma finding were associated with G allele.

Table 4 shows that low C peptide level, high blood glucose and HbA1c level, and positive toxoplasma finding were associated with G allele.

Table 1 Comparison between cases and control groups regarding the studied parameters

Variables	Cases (n=75)	Control (n=75)	Test of significance	P
Age (years)				
Mean±SD	7.24±4.66	7.03±4.25	t=0.284	0.777
Sex [n (%)]				
Male	35 (46.7)	44 (58.7)		0.141
Female	40 (53.3)	31 (41.3)	χ^2=2.16	
C peptide (ng/ml)				
Median (range)	0.2 (0.09-10.26)	1.70 (0.10-3)	Z=6.276	<0.001**
HbA1c (%)				
Mean±SD	7.18±2.09	4.91±1.26	t=8.034	<0.001**
Blood glucose (mg/dl)				
Median (range)	198 (101-440)	101 (90-195)	Z=8.445	<0.001**
IL-10 poly [n (%)]				
AA	29 (38.7)	14 (18.7)	χ^2=7.46	0.024*
GG	24 (32.0)	34 (45.3)		
AG	22 (29.3)	27 (36.0)		
Toxoplasma [n (%)]				
Positive	37 (49.3)	23 (30.7)	χ^2=5.44	0.020*
Negative	38 (50.7)	52 (69.3)		

*Significant; **Highly significant.

Table 2 Logistic regression analysis of independent predictors of diabetes in the studied group

Independent predictors	Univariate regression			Multivariate regression	
	β	P	OR (95%CI)	P	OR (95% CI)
C peptide (ng/ml)	–0.506	0.005*	0.603 (0.424-0.859)	0.009*	0.7 (0.41-0.92)
HbA1c (%)	2.036	<0.001**	7.66 (3.8-15.1)	<0.001**	10.7 (3.1-36)
Blood glucose (mg/dl)	0.082	<0.001**	1.08 (1.061.11)	0.002*	1.04 (1.01-1.06)
IL-10 poly					
AA(r)	-	-	(1)	0.013*	0.31 (0.12-0.78)
GG	–1.077	0.01*	0.341 (0.15-0.78)	0.111	0.48 (0.19-1.18)
AG	–0.933	0.032*	0.393 (0.17-0.92)		
Toxoplasma					
Positive	0.789	0.021*	2.2 (1.12-4.3)	0.029*	4.7 (1.17-19)
Negative (r)					
Constant		0.788			
Model χ^2% correctly predicted		22.94, P<0.001 72.7%		-	-

*Significant; **Highly significant. CI, confidence interval; HbA1c, glycosylated hemoglobin; OR, odds ratio.

Regression analysis (Table 5) showed that the following were independently associated with G allele: positive toxoplasma finding (OR=3.016), low C peptide level (OR=0.78), high HbA1c level (OR=1.91), and high blood glucose level (OR=1.18). After multivariate regression analysis and adjusting the confounding factors, the most significant predictors for G allele were positive toxoplasma finding (OR=3.5) and high HbA1c level (OR=1.22).

Hardy–Weinberg equilibrium shows that the observed genotype frequencies are consistent with expected ones (Table 6).

Discussion

In the current work, we studied for the first time the relation of IL-10 gene polymorphism with the susceptibility to type I diabetes in children with latent toxoplasmosis. The results of the present study, on a small sample size, showed that the highest risk of diabetes was associated with GG genotype of IL-10 promotor gene −1082.

Serum level of IL-10 is under genetic control and the presence of adenine at position −1082 is associated with lower production of IL-10, whereas higher production occurs when guanine is present at the same site (Turner *et al.*, 1997).

Table 3 Relation between IL-10 polymorphism and other parameters in diabetic group

Variables	AA (*n*=29)	GG (*n*=24)	AG (*n*=22)	Test of significance	P/P_c
C peptide (ng/ml)					
Median (range)	0.70 (0.12-10.26)	0.12 (0.09-0.19)	0.24 (0.10-10.26)	KW=28.65	<0.001/<0.001
HbA1c (%)					
Mean±SD	6.0±2.34	8.25±2.3	7.49±2.08	*F*=4.21	0.019/0.057
Blood glucose (mg/dl)					
Median (range)	156 (131-420)	198 (131-466)	178 (131-420)	KW=3.31	0.191

IL, interleukin; HbA1c, glycosylated hemoglobin; P_c=Bonferroni corrected *P* (number of comparison × *P*).

Table 4 Relation between IL-10 polymorphism alleles and other parameters in diabetic group

Variables	A allele (*n*=80)	G allele (*n*=70)	Test of significance	P/P^c
C peptide (ng/ml)				
Median	0.50	0.13	*Z*=5.06	<0.001/<0.001
Range	0.10-10.26	0.09-10.26		
HbA1c (%)				
Mean±SD	6.84±2.01	8.01±2.48	*t*=3.12	0.002/0.004
Blood glucose (mg/dl)				
Median	150	178	*Z*=2.71	0.007/0.014
Range	131-420	131-466		
Toxoplasma [*n* (%)]				
Negative	51 (63.8)	25 (35.7)	χ^2=11.7	0.001/0.001
Positive	29 (36.2)	45 (64.3)		

IL, interleukin; HbA1c, glycosylated hemoglobin; Pc=Bonferroni corrected *P* (number of comparison × *P*).

Table 5 Logistic regression analysis of independent predictors of G allele

Independent predictors	Univariate regression			Multivariate regression	
	B	*P*	OR (95%CI)	*P*	OR (95%CI)
C peptide (ng/ml)					
Median	−0.250	0.029*	0.78	-	-
Range			0.62-0.97		
HbA1c (%)			1.91		
Mean±SD	0.112	0.003**	1.02-2.99	0.039*	1.22 (1.06-3.86)
Blood glucose (mg/dl)					
Median	0.004	0.018*	1.18	-	-
Range			1.07-1.4		
Toxoplasma					
Negative (*r*)			3.16		
Positive	1.15	0.001**	1.6-6.2	0.001**	3.5 (1.7-7.1)
Constant model χ^2% correctly predicted			1.08 23.5, *P*<0.001* 60.7%		

*Significant; **Highly significant. CI, confidence interval; HbA1c, glycosylated hemoglobin; OR, odds ratio.

Table 6 Hardy-Weinberg equilibrium to determine whether observed genotype frequencies are consistent

	Observed	Expected
Homozygote AA	29	21
Heterozygote AG	22	37
Homozygote GG	24	16
χ^2	0.466	
P	0.533 (consistent with HWE)	

This study agrees with several studies that stated Th2 cytokines, namely, IL-10, can lead to β-cell destruction (Moore *et al*., 2001; Watanabe *et al*., 2002; Zhang *et al*., 2002). Moreover, IL-10 causes changes in vascular address in expression on endothelium, resulting in vascular occlusion and hypoxic necrosis in islets of pancreas (Wogensen *et al*., 1994; Brunicardi *et al*. 1996). On the contrary, a study in Turkey stated that G allele is a protective allele for type I DM (Mohebbatikaljahi *et al*., 2009). However, a study in Japan stated that there is no association between IL-10 polymorphism and genetic susceptibility to type I DM (Ide *et al*., 2002).

This discrepancy can be explained by different number of patients and control in each study, or may be ethnic difference in genetic control of DM. Moreover, the effect of IL-10 on type I DM in nonobese diabetic mice is paradoxical as it is dependent on mode and time of administration (Balasa *et al*., 2000). Early systemic administration inhibits type I DM, whereas local expression accelerates the onset of disease (Moritani *et al*., 1994; Pennline *et al*., 1994; Smith *et al*., 1997; Nitta *et al*., 1998). In this study, the highest risk of diabetes is associated with toxoplasmosis, and toxoplasmosis is associated with GG genotypes. Acute toxoplasmosis causes pancreatic necrosis (Waree, 2008). Strong Th1 immune response is needed to convert tachyzoites to dormant bradyzoites to maintain chronic asymptomatic toxoplasmosis (Gigley *et al*., 2009).

IL-10 as immune modulator cytokine acts in an antagonistic manner to Th 1 cytokines, which is necessary to maintain the dormant bradyzoites stage (Denkers and Gazzinelli, 1998), which may lead to reactivation of bradyzoites to tachyzoites producing more pancreatic necrosis in GG genotypes (high IL-10 production). IL-10 is an anti-inflammatory cytokine. During infection, it inhibits the activity of Th1 cells, NK cells, and macrophages, all of which are required for optimal pathogen clearance but also contribute to tissue damage. In consequence, IL-10 can both impede pathogen clearance and ameliorate immunopathology (Couper *et al*. 2008).

In the present study, C peptide level and blood glucose were statistically significantly higher in positive toxoplasma cases. Oz (2014) shows that insulin has a stimulatory effect on the *in vitro* replication of *T. gondii*. Moreover, Zhu *et al*. (2006) show that insulin and d-glucose have a synergistic dose-responsive stimulating effect on the *in vitro* replication of *T. gondii* tachyzoites.

Conclusion

In conclusion, GG genotype can no longer be protective allele for type I DM as higher IL-10 production with the ubiquitous nature of Toxoplasma infection can lead to more pancreatic necrosis. The use of IL-10 as a therapeutic cytokine for treatment of type I DM should be revised. Moreover, the results need to be confirmed by a study of a larger sample size.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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