Interferon-Gamma +874 T/A Gene Polymorphism and Susceptibility to Toxoplasma Infection among Children with Type 1 Diabetes: A Possible Relationship

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Abstract | Interferon-gamma (IFNγ) is associated with a number of autoimmune diseases, in addition IFNγ is a key cytokine involved mainly in the defense against intracellular. We aimed to examine the role of IFNγ gene +874 T/A polymorphism in T. gondii infection susceptibility among children with type 1 diabetes. This study included 107 children and adolescents with T1DM in addition to 95 healthy controls. We evaluated levels of anti- T. gondii Ig-G and IFNγ in the participants’ sera. All participants were also genotyped for IFNγ gene polymorphism at position +874T/A. Among the type 1 diabetic patients, 51 cases were positive for anti-Toxoplasma gondii IgG , while in controls were 40 individuals. In addition, no significant difference was detected as regard serum levels of IFNγ between the two groups. Moreover, no statistically significant differences were detected as regard the IFNγ gene +874 T/A polymorphism genotypes frequencies among different Toxoplasma gondii Ig-G seropositivity subgroups (p >0.05). The serum IFNγ levels were significantly higher among diabetic patients with IFNγ +874 (A/T) genotype and T allele than controls (p=0.01). Our results do not support a role of IFN-γ gene +874T/A polymorphism in the development of T1DM or susceptibility to Toxoplasma infection in patients with type 1 diabetes.

Keywords | IFNγ Polymorphism, Toxoplasma, Type 1 Diabetes Mellitus

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite which can infect most warm-blooded vertebrates (Dubey and Beattie, 1988). This parasite is among the most prevalent chronic parasitic infection in humans estimated to be 30–50% of the world population (WHO, 2013).

Toxoplasmosis is a silent disease. During the acute phase of infection, almost 80% of infected individuals are asymptomatic. Following the acute phase, the T. gondii parasite enters the “dormant stage” as bradyzoites which multiply slowly within tissue cysts and persist for the life of the host without causing a host reaction. Immunocompromised individuals, especially those with deficient cellular immunity, are at risk of reactivation of the latent infection that can progress and lead to a broad spectrum of diseases (Dubey et al., 1998; Silveira et al., 2011).

Type 1 diabetes mellitus (T1DM) is a chronic, progressive immune-mediated destruction of pancreatic β-cells, leading to partial, or in most cases, absolute insulin deficiency. T1DM precipitates in genetically susceptible individuals...
γ is a pleiotrophic cytokine with immunomodulatory effects of various immunoregulatory systems (Cantor et al., 2005). Immuno-logic susceptibility which is characterized by an imbalance of pro- and antiinflammatory cytokines was speculated to influence immune complex disease. IFN-γ demonstrates that diabetic patients have an increased susceptibility to many opportunistic infections such as toxoplasmosis (Carruthers, 2002; Golke et al., 2008; Shirbazou et al., 2013).

Interferon-gamma (IFNγ) gene in human is located on chromosome 12, it contains four exons and intermediate introns (Trent et al., 1982). IFN-γ gene intron–polymorphisms was speculated to influence immune complex disease susceptibility which is characterized by an imbalance of various immunoregulatory systems (Cantor et al., 2005).

IFNγ is a pleiotropic cytokine with immunomodulatory effects where, IFNγ has long been believed to play a key role in driving the autoimmune pathogenesis of T1DM (Elinziki et al., 2009; Tang et al., 2010). On the other hand, IFNγ is the major mediator of the defense against intracellular pathogens such as T. gondii and is crucial for the activation of a variety of antimicrobial activities in haematopoietic and non-haematopoietic cells that limit parasite replication (Yap and Sher, 1999).

Therefore, we conducted the present study to examine the seroprevalence of Toxoplasma gondii and role of IFNγ gene −874 T/A polymorphism in T. gondii infection susceptibility among children with T1DM.

SUBJECTS AND METHODS

STUDY DESIGN

The study was designed to be a case–control study conducted during the period from the February 2014 to August 2015.

STUDY POPULATION

The current study included 107 patients with established type 1 diabetes for at least 2 years (50 females and 57 males); their mean age was 11.7±3.1 (years). They were recruited from outpatients of the endocrinology clinic of Mansoura University Children’s Hospital (MUCH), Mansoura, Egypt. The diagnosis of diabetes was based on the World Health Organisation criteria (Craig et al., 2014) (fasting plasma glucose ≥ 126mg/dl, two hours postprandial plasma glucose ≥ 200mg/dl, HbA1c ≥ 6.5%). The presence of one or more of diabetes-associated autoantibodies confirm the diagnosis of type 1 diabetes including: Glutamic acid decarboxylase 65 autoantibodies (GAD); tyrosine phosphatase-like islet cell autoantibody (IAA); and β-cell-specific zinc transporter 8 autoantibodies (ZnT8) (World Health Organization, 2006).

Patients who had other immune-related disease (e.g., Addison’s disease, autoimmune hepatitis, rheumatoid arthritis); patients who had diabetes-related complications (e.g., nephropathy, retinopathy, neuropathy) were excluded from the study.

We included ninety-five unrelated healthy control children from the same locality. They were recruited from the General Out-patient Clinic of MUCH, matched for age, sex and socioeconomic status. All participants (patients and controls) underwent the same research protocol, which included thorough medical history and clinical examination.

Data collection included: age, sex, weight, height, and body mass index (BMI). Diabetes-related variables recorded were: duration of diabetes, mean fasting blood glucose level (FBG), and HbA1c (%) levels.

The study patients were divided into 2 groups on the basis of IgG detection, into groups, with positive Toxoplasma IgG and those with negative test. All study participants provided written informed consent after receiving oral and written information about the study. The study protocol was approved by the Ethics Committee of Faculty of Medicine, Mansoura University, Egypt.

SAMPLING

Peripheral venous blood samples were withdrawn from all patients and control subjects in the morning after 12 hours of overnight fasting. Each blood sample was divided into 2 tubes. One tube was left for coagulation for 30 minutes and then centrifuged at 7000 rpm for 15 minutes to separate the plasma, devided into aliquots and stored at −80°C until being used for Toxoplasma IgG detection and determination of serum levels of interferon gamma and fasting blood glucose. The tube contained 2 ethylene-diamine-teta-acetic acid (EDTA)–K3 for whole blood collection. One part was used immediately for HB A1c determination and the rest of the EDTA anticoagulated blood samples were stored at −80°C until DNA extraction.

GENOMIC DNA EXTRACTION

Genomic DNA was extracted from EDTA–anticoagulated peripheral blood leukocytes using G-spin™ Total DNA Extraction Mini Kit (Cat: No. 17045, Intron Biotechnology, Sungnam-si, Kyeonggi-do, Korea) (Schur, 2001). The DNA concentration was measured from the absorbance at 260 nm (Jenway, Genova Model, UK) with average concentration at 0.138±0.017 µg/µl. All samples had a DNA concentration ≥ 0.5 µg/µl.

For each sample, 1.5 µL of DNA was added to 15 µL of the master mix solution (i-Taq™ Mix, Cat No. 25028-purchased from Bioline, Modular GmbH (5082 Grodig, Austria) for amplification using a specific single stranded oligonucleotide, synthesized to cover a 24-bp region for each allele (Bazzaz et al., 2011). An amplification refractory mutation system by polymerase chain reaction (ARMS-PCR) was performed. The ARMS-PCR method was applied for genotyping of IFN-γ −874 T/A polymorphism. The sequences of designed primers were as follows: Internal control primers (Human growth hormone; HGH-2 5′-AAATCAAATCA-3′, T allele primer; 5′-TTCTTACACAAACAAAACTCA-3′, 5′-CAACGCTGATACCTCA-3′, Common forward primer: 5′-TCACA-CAAACTGTGTTTC-3′, T allele primer; 5′-TTCTTACACAAACAAAACTCA-3′, 5′-CAACGCTGATACCTCA-3′). All samples were amplified for 30 cycles with 45 seconds at 95°C, 50 seconds at 62°C, 40 seconds at 72°C followed by 1 minute at 96°C followed by 10 cycles of 15 seconds at 96°C.

The thermal cycling program was adjusted as follows: 1 minute at 96°C followed by 10 cycles of 15 seconds at 95°C, 50 seconds at 62°C, 40 seconds at 72°C followed by 20 cycles of 20 seconds at 95°C, 50 seconds at 59°C and 50 seconds at 72°C.

ARMs-PCR amplified products were subjected to 2.0 % agarose gel electrophoresis, stained with ethidium bromide (0.5 mg/ml) and visualized on an ultraviolet transilluminator. In gel electrophoresis, according to the presence or absence of amplified targeted sequence, the type of alleles (genotype) was identified.

DETERMINATION OF SERUM INTERFERON GAMMA AND TOXOPLASMA IgG

Serum IFN-γ and Toxoplasma IgG were measured using a sandwich enzymelinked immunosorbent assay (ELISA) (ab46025 – IFN-γ Human ELISA Kit & ab108776 – Anti-Toxoplasma IgG Human ELISA Kit for IFN-γ and Toxoplasma IgG respectively). This assay was performed according to the manufacturer’s instructions using a plate ELISA reader (Sunrise Rimous/Touch Screen-Fcen Austria GmbH, 5082 Grodig, Austria) for reading the absorbance of each sample at 450 nm wavelength as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength. ELISA score of 1.4-fold higher than the ELISA cut-off value was considered positive by Coutinho et al. and Uchôa et al., respectively (Coutinho et al., 1970; Uchôa et al., 1999).

The fasting blood glucose level was determined using method of (Trender, 1969). Glycosylated Hemoglobin A1c (HbA1c) was measured using Stable Glycosylated Hemoglobin A1c (HbA1c) reagent kit by Enzymatic colorimetric method for biochemistry analyzer–Reference number: TMF420–Ningbo,China.

STATISTICAL ANALYSES

Data were analyzed using the Statistical Package of Social Sciences (SPSS) version 16 for Windows (SPSS, Inc., Chicago, IL, USA). Data were presented using mean and Standard deviation (SD) and number of cases (percentage) for qualitative values. The distribution of tested variables was examined with Kolmogorov-Smirnov test for normality. The significance of differences between continuous variables was determined with independent samples t-test. Chi- square or Fisher exact test was used for comparison between qualitative variables, as appropriate. The relationship between various interferon gamma genotypes and serum levels of interferon gamma was evaluated by analysis of variance (ANOVA), P values <.05 were considered significant.

RESULTS

One hundred and seven children and adolescents with T1DM (57 males and 50 females) with mean age 11.7 ± 3.1 years were included our study. Beside 95 apparently healthy children age matched for sex (50 males and 49 females) with mean age 11.4 ± 2.9 years as controls. The diabetic patients and control groups were quite similar regarding age, sex and BMI (P > 0.05). Demographic and laboratory characteristics of the study groups were presented in Table 1.

As regard T. gondii seroprevalence results among the study groups, 51/107 (47.7%) diabetic patients were seropositive compared to 40/95 (42.1%) seropositive controls with no statistical significance between both group (P > 0.05) (Table 1). In addition, no significant difference in serum IFNγ levels between different groups was detected (P=0.11) (Table 1).
The polymerase in IFN-γ at the +874 T/A position has been previously described to associate with several diseases. The A/A genotype has been shown to be associated with hepatitis B in China (Yu et al., 2006), Helicobacter pylori gastritis in Italy (Zamboni et al., 2003), tuberculosis in Spain (López-Maderuelo et al., 2003), type 2 diabetes mellitus in Greece (Tsiavou et al., 2005) and Wegener’s granulomatosis in Germany (Spriewald et al., 2005). The A/T and T/T genotypes have been associated with breast cancer in Iran (Karalami-Sarvestani et al., 2005), hepatitis C in Taiwan (Dai et al., 2006) and Hashimoto’s disease in Japan (Ito et al., 2006).

Interferon gamma (IFN-γ) is involved in the immunological response against intracellular parasites as T. gondii (Gazzinelli et al., 1994) and leishmaniasis (Karalami-Sarvestani et al., 2006). IFN-gamma also directly induces enterocyte resistance against Cryptosporidium parvum infection; this observation may have important consequences for our understanding of the mucosal immune system and autoimmune diseases (Biskos et al., 2001).

To the best of our knowledge, this is the first study to investigate the role of IFN-γ +874 T/A gene polymorphism in the susceptibility of patients with T1DM to Toxoplasma infection. High susceptibility to opportunistic infections such as toxoplasmosis has been described in diabetic patients, which may be caused by several defects of the immunological defense system (Shirbazou et al., 2013).

Moreover, no statistically significant differences were detected as regards the IFN-γ gene +874 T/A polymorphism genotypes frequencies among different Toxoplasma IgG-seropositivity subgroups (p>0.05) (Table 3).

In this study we found that the serum levels of IFN-γ were significantly higher among T/T genotype and T allele when compared with other genotypes and alleles in both diabetics and controls (p<0.001). Our results were similar to those previously reported by (Pravica et al., 2000; López-Maderuelo et al., 2003; Dai et al., 2006; Herno et al., 2006). They reported that IFN-γ gene polymorphism at position 874 T/A in intron-1 affects its gene expression and therefore plays a fundamental role in IFN-γ production: genotypes A/A, A/T and T/T are respectively linked to low-, medium- and high-IFNγ producers.

In addition, the serum IFN-γ levels were significantly higher among diabetic patients with IFN-γ A/T genotype and T allele than controls (p<0.01).

On the contrary, the first intron polymorphism of IFN-γ has been described and shown to influence gene transcription, leading to inter-individual variations in cytokine production (Doffinger et al., 2004).

Therefore, further studies are necessary to elucidate the role of IFN-γ +874 T/A polymorphism genotypes and alleles in the development of T1DM and other diseases such as toxoplasmosis.

Table 2: Interferon gamma +874 T/A polymorphism genotype and allele frequencies among children with T1DM compared to control subjects

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control subjects (n=107)</th>
<th>Diabetic patients (n=27,5)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>A/A</td>
<td>31 (11.9%)</td>
<td>22 (20.6%)</td>
<td>1.98 (0.90-4.33)</td>
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<td>A/T</td>
<td>52 (54.7%)</td>
<td>48 (54.5%)</td>
<td>0.67 (0.39-1.17)</td>
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<td>T/T</td>
<td>32 (33.8%)</td>
<td>37 (34.5%)</td>
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Alleles

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<tr>
<td>A</td>
<td>74 (39.8%)</td>
<td>92 (43%)</td>
<td>0.96 (0.54-1.72)</td>
</tr>
<tr>
<td>T</td>
<td>116 (61.1%)</td>
<td>122 (57%)</td>
<td>0.51 (0.23-1.11)</td>
</tr>
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Table 3: Type 1 diabetes mellitus patients with IFN-γ A/T polymorphism genotypes and alleles

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Table 4: Serum Levels of interferon gamma in patients with type 1 diabetes and controls in relation to different IFN-γ gene +874 T/A polymorphism genotypes and alleles

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In our study, type 1 diabetic patients with seropositive Toxoplasma IgG, were 47.7% compared to controls 42.1%, with no statistically significant difference between both groups (p>0.05), this results are similar to that reported by Albuquerque et al. (2009).
IFN-γ has repeatedly been implicated in the susceptibility, pathogenesis and progression of diabetes mellitus (Stallenhoef et al., 2003). It has been observed that the knocking out of the IFN-γ gene, IFN-γ neutralisation, IFN-γ blockade, or deletion of IFN-γ receptor (IFN-γR) positive cells in NOD mice and BB rats all led to delayed or decreased incidence of T1DM (Rubinovich, 1998). Moreover, the association between gene polymorphisms in the IFN-γ 5’UTR and T1DM have been reported in two independent studies (Awata et al., 1994; Jahromi et al., 2000).

In another report, Rafinejad et al. (2004) examined the relation between IFN-γ gene polymorphisms at position 5’UTR +5644 and T1DM in Iranian patients and found a negative association between IFN-γ gene and T1DM pointing to T allele as a protective factor against T1DM.

This apparent discrepancy may be due to the lack of linking this polymorphism with other important polymorphisms like that of TNF gene.

Although, the selected polymorphism had advantageous criteria (functionality, high frequency of both alleles), the number of patients were reasonably and well-defined diabetes patients. Hence, this difference was not statistically significant (13.21±2.13 vs. 12.83±4.1; p=0.11).

Foss-Freitas et al. (2007) observed that an increase in level of IFN-γ and TNF-α from PBMC of type 1 diabetic patient with adequate metabolic control, suggest that diabetic control improves the capacity of activation and maintenance of the immune response, reducing the susceptibility to infections.

Regarding the absence of similar studies on IFN-γ gene polymorphism in type 1 diabetes with toxoplasmosis, further studies involving gene polymorphisms in other cytokines and polymorphisms covering the entire length of the interferon gamma gene should be performed to understand the role of the immune system in the course of T. gondii infection among patients with type 1 diabetes.

Our results do not support a role for IFN-γ gene polymorphism +874 in the susceptibility to T1DM or increased susceptibility to Toxoplasma infection among T1DM.

ACKNOWLEDGEMENT

To all patients who were enrolled in this study.

CONFLICT OF INTEREST

No conflict of interest.

AUTHOR’S CONTRIBUTION

Doaa Salem presented the idea of the research, contributed in sample collection and processing, writing and correcting the manuscript. Nanees Salem helped in building idea of the research, contributed in clinical examination and selection of the cases, writing the manuscript, Abdel Hamid Fendos contributed in statistical analysis of the data and assisted in selection of cases and revised the manuscript. Ayman Elsamanoudy contributed in sample processing, geno typing and helped sharing in writing the manuscript.

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The Journal of Advances in Parasitology