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Interferon-stimulated-gene 15 polymorphism in Type 1 diabetic patients

Mazen Almehmadi

Collage of Applied Medical Sciences, Taif University, Taif city, **Saudi Arabia**.

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***Correspondence:** Dr.mazen.ma@gmail.com **Accepted: 09** Mar. 2019 **Published online:** 16 Apr. 2018

Type-1-diabetes (T1D) or juvenile diabetes develops due to destruction of insulin-producing pancreatic β-cells leading to external replenishment of insulin. Saudi Arabia has been ranked as 8th of the world in number of T1D cases in children. Innate immune response inside the pancreatic cells can lead to destruction of islets of Langerhans. ISG-15 play an important role in innate immunity and ISG-15 mutation has been associated with T1D. Peripheral blood mononuclear cells (PBMCs) were collected from 200 volunteers. 100 patients with T1D and 100 healthy participants used as control. DNA was extracted, and ISG-15 polymorphism was evaluated. Serum ISG-15 levels were evaluated between Healthy and T1D patients by ELISA. Genotypic analysis has not detected significant polymorphism difference, serum ISG-15 were significantly higher in T1D adults and children than healthy control. Also, serum ISG-15 levels are higher in T1D adults more than T1D children. This study has demonstrated elevated levels of serum ISG-15 levels in T1D patients even though no significant polymorphism difference were detected. Overexpression of ISG-15 indicates an essential role but further investigation is required.

Keywords: ISG-15 interferon-stimulated-gene 15, T1D type 1 diabetic.

INTRODUCTION

Type-1-diabetes (T1D) or juvenile diabetes develops in patients due to destruction of insulin-producing pancreatic β-cells leading to external replenishment of insulin (Atkinson and Gianani, 2009), symptoms appear in patients leading to T1D diagnosis include hyperglycemia, polydipsia, polyuria, polyphagia, blurred vision, fatigue and weight loss (Gregory, Moore and Simmons, 2013; Atkinson, 2016). Imbalance in insulin levels and deficiency due to T1D will affect the metabolism leading to instability, malnourishment, ketoacidosis leading to mortality of the patients therefore external alternative to insulin is a necessity (Gregory, Moore and Simmons, 2013). Saudi Arabia has been ranked as 8th of the world in number of T1D cases in children and adolescent who are less than 20 years old, and ranked 4th globally for patients more than 20 years old (Robert et al., 2018).

T1D is inheritable disorder and children with a relative who suffer from this disease are at risk of inheriting T1D, however, some incidents have occurred in patients without family history of T1D (Gregory, Moore and Simmons, 2013). Therefore, it is hypothesized that environmental factors can induce the immune system to produce autoantibodies that target pancreatic β-cells, the destruction of these cells takes years but autoantibodies can be detect in patients serum (Gregory, Moore and Simmons, 2013). When the patient start suffering from T1D symptoms, then the criteria of T1D diagnosis is followed. These include evaluating HbA1c ≥ 6.5%, fasting plasma

glucose ≥ 126 mg/dL, 2 hours glucose tolerance test ≥ 200 mg/dL and random plasma glucose ≥ 200 mg/dL (American Diabetes Association, 2011).

Interferon-stimulated-gene-15 (ISG-15) plays an essential role in human innate immune response (Ritchie et al., 2004). Studies have shown that innate immune response induction inside islets of Langerhans can lead to T1D (von Herrath, Fujinami and Whitton, 2003; Eizirik, Colli and Ortis, 2009; Skog et al., 2013).

In a recent study, ISG-15 were over expressed in T1D patients (Lundberg et al., 2016). Another study stated that ISG-15 has a protective function that helps mouse-β-cell-line to resist apoptosis induced by cytokines such as IFN-γ, TNF-α, and IL-1β (Yoshikawa et al., 2014). We have previously showed that ISG-15 polymorphism plays a role in major-depressive-disorder patients and elevated serum ISG-15 levels were detected (Almehmadi et al., 2018). Also, we have detected elevated levels of serum ISG-15 in influenza patients in high-altitude cities (Almehmadi, 2019).

MATERIALS AND METHODS

Subjects:

This study was approved by Taif University Medical Ethics committee and done in communication with outpatient department of Taif Psychiatry Health hospital. The number of participants was 100 T1D patients aged between 12-42 years following written informed consent, all those participants were diagnosed in the outpatient's departments of Taif Psychiatry Health hospital to have T1D. The control group were healthy individuals aged between 12-45 years and they are free from autoimmune disease, infection and cancer.

Methods:

Genotyping:

The DNA was extracted from PBMCs collected in EDTA tube by using the Thermo SCIENTIFIC DNA isolation kit (Thermo SCIENTIFIC). Genomic DNA was amplified and analyzed for ISG-15 gene genotype by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) for BsuRI (HaeIII).

ISG-15 serum levels:

ISG-15 serum levels will be analyzed for the 200 samples from both healthy and T1D patients. ELISA kit for assay of ISG-15 levels was purchased from BT-laboratory cat number E1988Hu and the detection sensitivity between 10 ng to 3000 ng. The results have been compared between healthy control and T1D patients, the analysis was performed on Bio-Rad xMarkTM micro plate spectrophotometer.

Determination of *HaeIII* genotypes:

The PCR mix was contained 5 µL of each primer; the forward 5'- CAG TGC CTT GTG TGT GGT GG -3 and the reverse 5'- GAT GCT GGT GGA GGC CCT TAG -3' (10 pmol), 5 µL buffer, 1.5 µL MgCl2 (50 mM), 5 µL template DNA (50–100 ng), 5 µL dNTPs (2 mmol/L), Taq polymerase (MBI) 2 µL and H2O 26.5 µL. The DNA template was denatured at 95°C for 5 min. A total of 35 cycles of PCR were performed, consisting of denaturation step for 30 sec at 94°C, an annealing step for 30 sec at optimum temperature 7°C and an extension reaction for 30 sec at 72°C. A final extension step at 72°C for 7 min was added after the last PCR cycle. After amplification, the PCR products were digested by incubation with ApaI restriction enzyme in 37°C for 5 minutes to get its three genotypes on 1.5% agarose gel designated AA, Aa and aa.

Statistical analysis: -

SPSS software version 16 (SPSS Inc., Chicago, IL, USA) was used in the performance of statistical analysis. The correlations were tested by using Spearman`s test. The t-test was used in comparisons performance. Both comparisons and correlations were considered statistically significant when P value >0.05. ISG-15 serum levels will be compared using t-test between healthy donors and T1D patients and the results were compared by t-test via GraphPad prism 5.03.

RESULTS

Demographic study:

This study included 200 Saudi persons classified as 100 healthy subjects and 100 T1D patients, those patients have been diagnosed in Taif Psychiatry Health hospital. In table 1 the demographic of participants is illustrated. The objective was to evaluate the presence of gene polymorphism in ISG-15 gene and serum ISG-15 levels.

Genotyping:

Genotypes of ISG-15 gene results from *HaeIII* table 2. Wild type, homozygous genotype,

heterozygous and allele A and a have shown no significant difference between healthy and T1D patients.

Table 1: Demographic study of healthy and T1D participants.

	Healthy (100)		T1D (100)	
	Males	Females	Males	Females
Age (years)	29±9	34± 7	28 ±9.25	27 ±9.8
Weight (kg)	53 ± 10	56.5 ±9	57 ± 8	53 ± 9.25
BMI	22.9±4.5	25.1 ± 4	22.6±3.5	25.3 ±4.5

Table 2: Single nucleotide polymorphism (SNP) within T1D patients. No significant difference was detected between healthy and T1D patients

Genotype of *HaeIII*	Control100 (%)	T1D 100 (%)	P value
Wild type	78 (78)	71 (71)	0.139
Heterozygous	15 (15)	20 (20)	0.105
Homozygous	7 (7)	9 (9)	0.128
Allele A	171 (85.5)	162 (81)	0.102
Allele a	29 (14.5)	38 (19)	0.148

Serum ISG-15 levels:

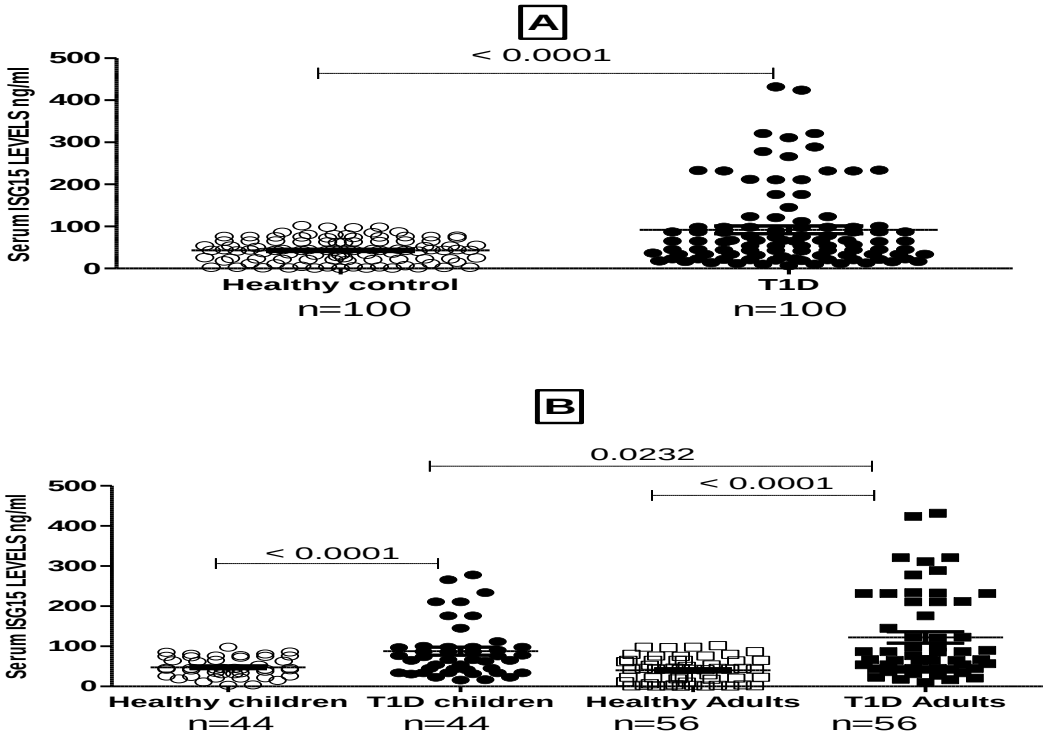

Figure 1: Serum ISG-15 levels compared between healthy and T1D patients. In scatter blot (A) serum ISG-15 were significantly higher in T1D patients than healthy individuals P value <0.0001. In scatter blot (B) both T1D children and adults have shown elevated levels of serum ISG-15 than healthy individuals P value <0.0001 and T1D adults have higher levels than T1D children P value 0.0232.

Serum ISG-15 levels were compared between healthy control and T1D patients. When the levels were compared between the two genders no significant levels were detected. After that, healthy and patients' groups were divided to two groups according to their age. Children between 12 years to 17 years, and adults from 18 and above. Serum ISG-15 was measured between those groups by using ELISA. In figure 1 the results are illustrated.

DISCUSSION

In the current study the two groups were 100 healthy controls and 100 T1D patients. Genotyping analysis revealed no significant difference between those two groups in term of ISG-15 gene polymorphism, it is difficult to draw a definitive conclusion if ISG-15 polymorphism has relation with T1D pathogenesis and this is due to the limited number of participants. However, mutation in ISG-15 gene has been correlated with the development of T1D (Crow and Manel, 2015; Lee-Kirsch et al., 2015). Moreover, serum ISG-15 levels were assayed by using ELISA. Serum ISG-15 was higher significantly in T1D patients' group than healthy donors which indicates an immunological role this protein perform in T1D patients however this role is unclear, when serum ISG-15 levels were compared between males and females, also, between BMI groups no significant levels were detected. Moreover, T1D children have higher levels of ISG-15 than healthy group and this was also detected in the adult groups. Moreover, elevated serum ISG-15 levels in adults were detected more than children. These results are consistence with other finding indicating elevated levels of ISG-15 in T1D (Yoshikawa et al., 2014). Also, another study revealed ISGs genes such as GBP1, TLR3, OAS1, EIF2AK2, HLA-E, IFI6 and STAT1 are overexpressed in the islet of patients with T1D (Lundberg et al., 2016). ISG-15 is greatly affected by IFN-α levels, a study has found that IFN-α is significantly increased in adults with T1D than children with T1D (Chehadeh et al., 2000) which can describe why serum ISG-15 was higher in adults than children in our study.

The elevated levels of ISG-15 can induce immune cells such as T-cells, macrophages, dendritic cells. And it has been established that T1D pathogenesis is due to autoimmunity and inflammation due to immune effector cells infiltration of the pancreatic cells and inflicting damage to islets of Langerhans (Arvan et al., 2012; Allam et al., 2014; Li, Song and Qin, 2014). ISG-15 levels also increase due to viral infection (Schneider, Chevillotte and Rice, 2014; Poynter and DeWitte-Orr, 2016) and it has been hypothesized that viral infection can lead to the development of T1D such as cytomegalovirus (Pak et al., 1988), mumps virus (Hyöty et al., 1988) and rota virus (Honeyman et al., 2000).

CONCLUSION

In conclusion, this study has demonstrated elevated levels of serum ISG-15 levels in T1D patients even though no significant polymorphism difference were detected. A negative point of this study is the small number of participants, increasing the number of donors can reveal more results. The overexpression of ISG-15 can indicate an essential role of this protein in T1D patients, however, to understand this role further studies are required as this study is only descriptive of ISG15 in T1D. It is unclear whether those high levels are due to pancreatic cells damage or was released due to other factors related to this disease. USP18 plays an essential role in the ISGylation process and study this gene in T1D patients can give some answers of the definitive role of ISG15 in T1D.

CONFLICT OF INTEREST

The author declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All research work like both PCR and ELISA and write-up was performed by single authors by Dr. MA.

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