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Interferon-stimulated-gene-15 gene polymorphism as a risk factor in Major-Depressive-Disorder patients

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ISG-15 gene is affected directly by IFN-α which the later used widely as a therapy for several diseases. Studies showed MDD can be developed after IFN-α treatments which can affect patient's normal life style. This study aims to investigate polymorphism in ISG-15 gene in MDD patients compared to healthy volunteers Peripheral blood mononuclear cells (PBMCs) were collected from 200 volunteers. About 100 patients diagnosed with MDD and 100 healthy individuals used as control. DNA was extracted, and ISG-15 polymorphism was evaluated. Serum ISG-15 levels were evaluated between Healthy and MDD patients by ELISA. Levels of serum ISG-15 were significantly higher in MDD patients ($P<0.0001$). Homozygous genotype was higher in MDD patients compared with healthy control ($P<0.031$) and serum ISG-15 levels were higher as well than healthy control. In addition, allele (a) polymorphism is more frequent in MDD patients compared to healthy control ($P<0.025$). Our results found that serum ISG-15 levels are higher in MDD than healthy control. Homozygous genotype was higher in MDD patients compared with healthy control. Polymorphism in ISG-15 gene can be a risk factor in MDD patients.

Keywords: ISG-15 interferon-stimulated-gene 15, MDD major depressive disorder

INTRODUCTION

In 1957 type I IFNs were discovered, following that IFNs were widely introduced in the treatment of variety of infections and cancer (Darnell, Kerr and Stark, 1994). Interferon-stimulated-gene was discovered in 1979 in IFN-stimulated-murine tumor cell RNA (PAUL J. FARRELL, 1979). ISG-15 protein was detected in human and bovine cell lines and plays essential role in innate immunity due their induction by IFN (Korant et al., 1984). ISG15 is an ubiquitin-like protein which can be found in several mammals and only in vertebrates with various conservation between those specious (Ritchie et al., 2004). Induction of ISG15 is promoted by type-I-IFN treatment, upregulation of ISG-15 expression occur due to internal or external stress on cells by infection such as bacterial and viral infection (Nicholl, Robinson and Preston, 2000; Mossman et al., 2001). ISG-15 can be detected in the intracellular and extracellular environment unconjugated or conjugated to a protein in a process called ISGylation, which is similar to protein ubiquitination (Cunha et al., 1996; Pickart, 2001). Free form of ISG15 is detected in blood and urine after cells excretion, this can bind to T-cells and increase their release of IFN-γ which can induce proliferation and cytotoxicity of NK cells (Cunha et al., 1996; Zhang and Zhang, 2011b). Moreover, a study on the effect of ISG-15 showed that increase ISG15-protein levels have been associated with tackling the effect of IFN-treatment in HCV-infected hepatocytes, this has led to failure of IFN\ribavirin therapy (Chen et al., 2010). In B-cells depleted

cells culture the effect of ISG15 protein both natural or recombinant have shown to induce the production of IFN-γ (Recht, Borden and Knight, 1991). ISG15 activity can also suppress lung cancer growth (Feng et al., 2008), nevertheless, elevated levels of ISG15 mRNA have been detected in cancerous mammary epithelial cell lines and in breast cancer (Bektas et al., 2008).

Depression is a common disorder that reflects essentially on the mood of patients. Depressed patients suffer from mood fluctuations that affect their life in several ways including how they feel, think and how they behave and engage society. Depressed-patients hormones levels are different from healthy individuals. Major depressive disorder (MDD) is developed during the few months after IFN-α treatment. IFN-α induced level has been associated with the incidence of depression (Udina et al., 2014). Also, patients suffer from MDD show symptoms include irritability, anxiety, poor concentration, sleep problems and suicidal attempts (Lotrich, 2009). Studies have shown that IFN-α treatment in MDD patients lead to increase levels of inflammatory cytokines (Maes, 2001). IFN-α can be produced inside the brain and peripheral and has the ability to access CNS via several methods (Licinio and Wong, 1997; Katafuchi et al., 2003). Overall, IFN induced depression is a type of disorder defined as substance induced mood disorder (SIMD) (V. et al., 1999). SIMD has been thoroughly reviewed science 1950 and in the current time the term has changed to be under the category of substance/medication-induced mental disorders (Udina et al., 2014).

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 MDD patients aged between 18-52 years following written informed consent, all those participants were diagnosed in the outpatient's departments of Taif Psychiatry Health hospital according to Diagnostic and Statistical Manual of Mental Axis I disorder (DSM-IV). All participants have developed MDD due to IFN-α treatment. While the control group were healthy individuals aged between 18-52 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 analysed for the 200 samples from both healthy and MDD 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 MDD patients.

Determination of *HaeIII* genotypes:

The PCR mix 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 $P < 0.05$. ISG-15 serum levels compared using t-test between healthy donors and MDD 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 MDD, the MDD patients have been diagnosed in Taif Psychiatry Health hospital. In table 3.1 the demographic of participants is illustrated. The objective was to evaluate the presence of gene polymorphism in ISG-15 gene and compare serum ISG-15 levels.

Genotyping:

Genotypes of ISG-15 gene results from *HaeIII* restriction enzyme in all subjects are shown in table 3.2. Homozygous genotypes showed a significant statistical difference between healthy and patient groups. Homozygous genotype was higher in MDD patients compared with healthy control (P<0.031). Detecting high polymorphism in the case of heterozygous and allele (A) no significant difference detected when MDD patients compared to healthy donors.

The levels of serum ISG-15 were assayed by using ELISA. MDD patients have higher ISG-15 protein expression than healthy controls. Moreover, heterozygous have shown significantly higher serum protein levels than healthy control (<0.0001). Homozygous have shown significantly higher serum protein levels than healthy control (<0.0173).

Table 3.1: Demographic analysis of the participants.

	Healthy (100)		MDD (100)	
	Males	**Females**	**Males**	**Females**
Age (years)	33 ± 5.5	34± 7	37 ± 6.25	35 ± 5
Weight (kg)	64 ± 10	56.5 ±9	63 ± 8	57 ± 9.25
BMI	22.9 ± 4.5	25.1 ± 4	22.6 ± 3.5	25.3 ± 4.5

Table.3.2: Single nucleotide polymorphism (SNP) detected within MDD patients. Homozygote genotype showed significant result when compared with the control. Allele (a) also, showed significant result when compared with the control

Genotypes *HaeIII*	Control (100) %	MDD patient (100) %	*P* value
Wild type	72 (72%)	64 (64%)	0.711
Heterozygous	26 (26%)	16 (16%)	0.567
Homozygous	3 (3%)	19 (19%)	0.031*
Allele A	170 (85%)	144 (72%)	0.622
Allele a	30 (15%)	56 (28%)	0.025*

i. Serum ISG-15 levels:

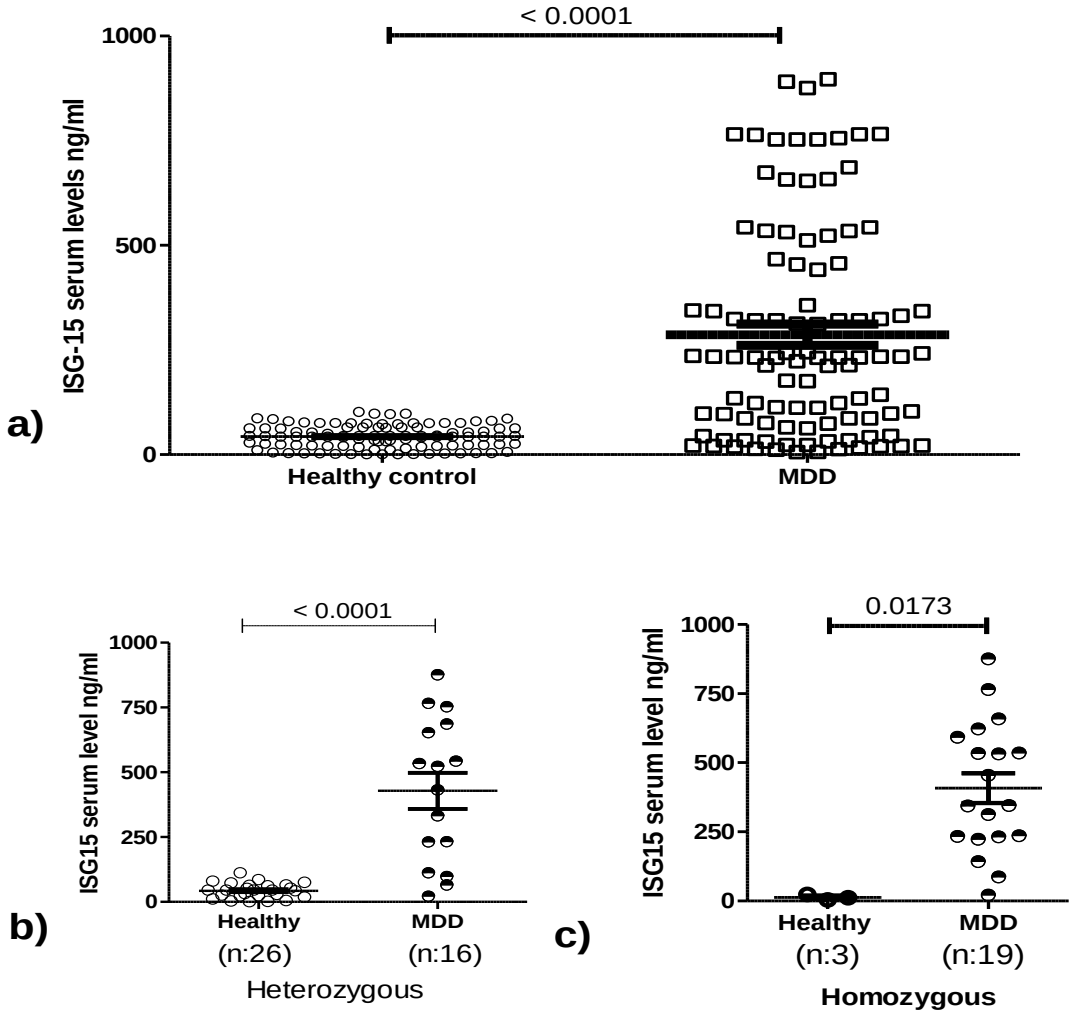

Figure 1: serum ISG-15 in all the 200 donors has been assayed and levels were calculated in nanogram per milliliter (ng/ml). In (a) the levels of ISG-15 are significantly higher in MDD patients than healthy control (<0.0001). In (b) the heterozygous group showed significantly higher in MDD than healthy group (<0.0001). In (b) the homozygous group MDD patients are significantly higher than healthy control (P. value =0.0173).

DISCUSSION

The study groups were 100 control and 100 MDD patients. Genotyping analysis revealed 19% of MDD patients who have homozygous genotype polymorphism in ISG-15 gene. Allele (a) has also revealed high genotype polymorphism of ISG-15 gene in MDD patients than healthy control. Heterozygous and allele (A) have not shown any significant difference when MDD patients results were compared to the healthy donors. The percentage of wild type from both MDD and healthy control was 68% (n=136), heterozygous genotype was 21% (n=42) and homozygous genotype was 11% (n=22).

Serum ISG-15 levels were assayed by using ELISA. Serum ISG-15 was higher significantly in MDD patients' groups than healthy donors. Also, heterozygous have significantly higher serum ISG-15 than healthy control even though genotyping of ISG-15 gene polymorphism show insignificant difference. Moreover, Homozygous have significantly higher ISG-15 than healthy control. ISG-15 is known to be a major player in ISGylation, the study of ISG-15 gene polymorphism in MDD was rarely studied previously. A trail has studied ISG-15 gene polymorphism in association with HIV-1 viral load.

They did not confirm any association between viral load and ISG-15 gene polymorphism (Chang et al., 2013).

In this study we detected that serum ISG-15 was elevated in all of MDD patients, ISG-15 has been proven effective in many of the immune system defenses in the body especially due to cells stress (Zhang and Zhang, 2011a), the ISG-15 protein expression is elevated in response to viral and parasitic infection (MacQuillan et al., 2003; Narasimhan et al., 2005), HIV replication tackled by ISG-15 (Mémet et al., 1991; Narasimhan et al., 2005), and effective against Influenza B (Narasimhan et al., 2005; Zhang et al., 2014).

The ISG-15 stimulation affected by type-I-interferon, which is widely used as a therapy of different disorders (Zhang and Zhang, 2011a). It is also affected by type-III-IFN because they both affected by JAK-STAT signaling pathway (Chang et al., 2004; Zhang and Zhang, 2011b). Moreover, even in absences of Type-I IFN induction, ISG-15 can be affected by interferon-regulatory-factors (Okumura, Lenschow and Zhang, 2008; Zhang and Zhang, 2011b). IFN-α was detected to be produced in the brain and has the ability to access CNS (Licinio and Wong, 1997; Hansen et al., 1998; Katafuchi et al., 2003).

MDD can be developed after IFN-α treatment and the levels have been connected to depression (Udina et al., 2014) and IFN-α has huge effect on ISG-15 expression (Nicholl, Robinson and Preston, 2000; Mossman et al., 2001) Inflammatory cytokines induced by this type of treatment promote anxiety, poor-concentration, sleep problems and suicidal attempts (Maes, 2001; Franzen et al., 2010). Psychological stress can also induce inflammatory cytokines (Miller et al., 2005; Pace et al., 2006). Cancer patients undergoing IFN-α treatment who developed MDD have also suffered sense of guilt, sense of failure, self-dislike and dissatisfaction (Pasquini et al., 2008). After MDD patients are diagnosed, they undergo treatment by using selective-serotonin reuptake inhibitors and tricyclic anti-depressants therapy (Hauser et al., 2002; Lotrich, 2009).

CONCLUSION

The number of donors in this study was not abundant. Our results found homozygous genotype and allele (a) was higher in MDD patients compared with healthy control. This finding can be further studied by involving other depression patients in this investigation. What is more, involving USP18 gene polymorphism. Moreover, this finding can be further investigated by studying levels of USP-18 proteins expression in MDD patients compared to healthy volunteers. The study can conclude that ISG-15-polymorphism and ISG-15 protein expression can have some type of effect in MDD patients and this point must be evaluated as no other study has focused in this matter.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest,

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AUTHOR CONTRIBUTIONS

Dr. MA designed the study and wrote the manuscript, Dr. AA analyzed PCR results, Mr. AA collected samples, Mr. AA, Dr. AS and Dr. WA performed PCR experiment, Dr. MA and Dr. MH performed and analyzed ELISA. All authors read and approved the final version.

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