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Association between the serotonin- transporter- linked polymorphic region (5-HTTLPR) and pemphigous disease in Iranian patients

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ABSTRACT

Introduction: Pemphigus vulgaris (PV) is a relatively rare autoimmune disease characterized by blistering of the skin and mucosa. The main objective of this study was to investigate the possible association between the 5-HTTLPR polymorphism and PV in Iranian patients. For this, 112 PV and 100 controls were enrolled in this study.

Materials and Methods: In this case-control study, Genomic DNA was extracted from whole blood and genotyping of all participants for the 5-HTTLPR polymorphism was carried out using the polymerase chain reaction (PCR) technique. The genotypes were grouped into three classes: homozygous for the short allele (SS), heterozygous for the short and long allele (LS) and homozygous for the long allele (LL).

Results: Our results showed no significant association between the 5-HTTLPR polymorphism genotypes, LL, SS and LS in PV patients compared to controls. Also, our finding did not reveal any evidence of an association between this disease and the allele frequency of S and L. Taken together, our findings suggested that the 5-HTTLPR polymorphism is unlikely to be a factor contributing to the risk of developing pemphigus vulgaris.

Conclusion: It can be concluded that although 5-HTTLPR polymorphism seems to be associated with some of auto-immune and stress-related disease.

Key words: Iranian Population, PCR, Pemphigus vulgaris, Polymorphism, Risk Factor.

Introduction

Pemphigus vulgaris (PV) as a rare and autoimmune disease causes very serious blisters on skin as well as inside the mouth, nose and throat [1]. So far, several types of this disorder have been reported such as mucosal pemphigus vulgaris, muco-cutaneous pemphigus vulgaris, paraneoplastic pemphigus, drug-induced pemphigus, pemphigus foliaceus, and IgA pemphigus [2]. The most prevalent variant is pemphigus vulgaris (PV) [3], which its annual incidence is about 1/100000 in Iran, with a mean age of onset of 42 years, a female to male ratio of

1.5/1 and a death rate of about 6% [4].

In PV, autoantibodies are produced against such desmosome components of epithelium as cadherin and desmoglein (Dsg), especially Dsg3 [2]. These glyco-protein molecules are found in a membrane in desmosome and strengthen intercellular connection. The lack of these connections due to the antibody-antigen reactions leads to weakness of intercellular connection, and finally separation of the cells, which in turn results in desquamation and blister formation [5]. While Dsg1 and Dsg3 both are expressed in skin, oral epithelium expresses predominantly only Dsg3 [6]. Thus, the first manifestation of the disease is mucosal involvement among 74% of the cases [7]. Moreover, up to 90%of the patients present oral lesion as the first sign of the disease development [5].

Previous studies have reported that various etiological factors such as, cosmetic materials [15], estrogen hormone [16] diet [8], such drugs as thiol [9] and non-thiol drugs [10], phenol drugs [11], rifampicin [12], diclofenac [13], and other angiotensin-converting-enzyme inhibitors [14] contribute to the pathogenesis of PV. In addition, it is well known that psychological stress may exacerbate skin diseases [17,18].

There is a body of evidences showing that psychological status is affected by serotonergic mechanisms especially serotonin-transporter mechanisms (5-HTT) [19]. Since 5-HTT regulates serotonergic neurotransmission, polymorphism in this gene is associated with anxiety-related behaviours [20, 21]. 5-HTT (SLC6A4) gene is located on chromosome 14q12 and contains promoter and 14 Kb31 spanning exons [22, 23]. Two polymorphic regions, a 44-bp insertion/deletion polymorphism within the promoter region (5-HTTLPR) and a 17-bp variable number of tandem repeat polymorphism (VNTR) in intron 2 [22, 24] in SLC6A4 have been demonstrated. These allelic forms, the long (L) and the short (S) variants are found in the first polymorphic region (5-HTTLPR) [22]. It has been reported that the two alleles differently regulate SLC6A4: S allele reduces the transcriptional efficiency of the 5-HTT, and consequently decreases uptake of serotonin [20], whereas L allele has the opposite effect of S allele.

In spite of the fact that several studies have been conducted on the association between various types of polymorphisms with PV, to our knowledge, none of them has investigated the association of 5-HTT polymorphism with PV.

The aim of the present study was to investigate 5-HTTLPR polymorphism in 5-HTT gene in the Iranian patients with PV.

Materials and Methods

In this case-control study, we investigated a total of 212 Iranian subjects comprising 112 PV cases and 100 healthy controls. All patients were seen at Skin Disease Ward, Razi Hospital, Tehran, Iran. The participants gave their written informed consent, and the study was approved by the TUMS ethics review board as part of the reviewing process of TUMS research projects. The study was conducted according to the principles of the Helsinki Declaration.

All individuals underwent a complete clinical examination. The diagnosis of PV was made by a dermatologist, further confirmed by biopsy. The size of the lesion was measured, and the duration of the disease as reported by the patient was recorded.

Genotyping

Five ml blood sample of all participants were collected, and DNA genome was extracted using a Pure Nucleic Acid Isolation Kit (Roche Applied Science, Mannheim, Germany). Genotyping of all individuals for the 5-HTTLPR polymorphisms were conducted using the PCR technique. PCR amplification was performed using specific primers: 5HTTF: 5'-TGAATGCCAGCACCTAACCC-3'; 5HTTR: 5'-TTCTGGTGCCACCTAGACGC-3') 1U Taq DNA polymerase (Roche, Mannheim, Germany), 10 pM of each primer, 200 μM of each dNTPs, 0.67 μl of 50 mM MgCl2, 60 ng DNA and 2.5μl of 10X PCR buffer in 25 μl PCR reactions. The PCR was performed based on the following conditions: initial denaturation at 94°C for 5 min; followed by 35 cycles including denaturation at 94°C for 35 s, annealing at 58°C for 35 s, and extension at 72°C for 1.5 min; and a final extension at 72°C for 5 min. In order to identify S and L alleles in 5-HTTLPR polymorphism, PCR products (400bp and 450 bp, respectively) were visualized by agarose gel electrophoresis. All the subjects were divided into three genotype groups: homozygote for S alleles (SS), heterozygote for S and L alleles (LS), and homozygote for L alleles (LL).

Statistical analyses

SPSS version 11.2 served for statistical analyses. Association between 5-HTTLPR and PV was tested by Fisher's exact test at 0.05 significance level.

Results

The mean duration of illness among PV patients was 7.9 years (95%CI: 6.8-8.9) and the mean size of their lesions was 10 mm (95% CI: 9.6-12.1). The principal characteristics of the subjects in case and control groups are shown in the Table 1.

Genotypes in our study were identified using PCR . The most frequent genotype among both groups was SL (about 60%), followed by LL (20%), and SS (less than 20%). The distribution of the genotypes between the two groups showed no statistically significant difference (P=.791). The frequency of three genotypes of 5-HTTLPR in case and control groups is shown in Table 2.

No statistically significant difference was found between the two groups in this regard (P=.553). Table 3 shows the frequency of L and S alleles of 5-HTTLPR in case and control groups.

Discussion

In the present study identified the genotypes of 112 patients with PV and 100 healthy subjects (SS homozygote, LS heterozygote, and LL homozygote) using specific primers of 5-HTTLPR in a PCR technique. According to the results no statistically significant differences existed between the case and control groups regarding the frequency of the three genotypes, and also the frequency of S and L alleles. These findings suggest neither that frequency of neither genotypes nor alleles of 5-HTTLPR could be a risk factor for PV.

Association between serotonin transporter promoter polymorphism (5-HTTLPR) and autoimmune diseases has been frequently reported, though it has been a matter of controversy in the last decade. The main problem in genetic studies on PV as a multi-factorial disease is probably contribution of various factors such as psychological stress to disease development. Moreover, clinical diagnoses may be multi-factorial constructs that combine elements with distinct genetic influences.

A study on stressful life events, 5-HTTLPR, and major depression found no evidence of either genetic support for the disease, or interaction between genetic and environmental factors [25]. Another study could not relate signs of mental disorders and stress factors to 5-HTTLPR among children exposed to urban violence [26]. On the other hand many studies have related anxiety and major depression disorders to 5-HTTLPR genotype [27-29]. For example, a meta-analysis by Srijan Sen et al. reported significant association between 5-HTTLPR and personality-related anxiety [30]. A systematic review in US investigated association of polymorphism in 5-HTTLPR with anxiety-related behaviours. Meta-analysis was done on 23 studies and the results indicated a strong relationship between 5-HTTLPR polymorphism and neuroticism-related behaviours [31].

Victoria et al studied serotonin transporter gene polymorphism among 69 patients with recurrent aphthous stomatitis (RAS) and 70 healthy subjects and found remarkable increase in frequency of SS genotype, S alleles, and 5-HTTLPR polymorphism among RAS patients compared to healthy controls [32].

In conclusion, our data suggest that the 5-HTTLPR polymorphism is not a genetic risk for the development of PV within the Iranian population. Although the 5-HTTLPR variant (L) is associated with an increased expression of serotonin levels in the disease with psychological stress, other molecular genetics could be implicated in the initiation, progression and development of PV.

Conclusion

It can be concluded that although 5-HTTLPR polymorphism seems to be associated with some of auto-immune and stress-related disease, we could not find such an association for PV among Iranian population. Future studies can be done among various ethnic or age groups, with larger sample sizes.

Table 1. Distribution of background characteristics of patients with pemphigus volgaris (case group, n=112) and healthy subjects (control group, n=100).

Characteristics	*PV* (*n=112*)	*Control* (*n=100*)
Age (*year*): *Mean* (*95% CI*)	*41.5* (*18 - 65*)	*38* (*20 - 56*)
Minimum	*18*	*20*
Maximum	*65*	*56*
Lesion Size: *Mean* (*95% CI*) (*cm2*)		
Gender: *Female , Male*		

Table 2. The frequency of three genotypes of serotonin-transporter-linked polymorphic region (5-HTTLPR) in patients with pemphigus vulgaris (case group), and healthy subjects (control group).

	LL *n* (%)	*SL* *n* (%)	*SS* *n* (%)
Case group (*n=112*)	*25* (*23.3*)	*69* (*61.6*)	*18* (*16.0*)
Control group (*n=100*)	*26* (*26.0*)	*60* (*60.0*)	*14* (*14.0*)
Total	*51*	*129*	*32*

Table 3. The frequency of L and S alleles of serotonin-transporter-linked polymorphic region (5-HTTLPR) in case (patients with pemphigus vulgaris) and control (healthy subjects) groups.

	S allele	*L allele*
Case group (*n=112*)	*105* (*46.9%*)	*119* (*53.1%*)
Control group (n=100)	88 (44.0%)	112 (56.0%)

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