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Research Article

Assessment of Genetic Markers for Coeliac Disease in Iraqi Recurrent Aphthous Stomatitis Patients

Dr. Batool Hassan Al-Ghurabi *; Dr. Ahmed Abdulhassan Abbas; Dr. Mustafa M. Salah***; Dr. Fatin M. Jarallah******

[*] Medical Microbiology \ Clinical Immunology, Dept. of basic Science, College of Dentistry/Univ. of Baghdad. Iraq.
[**] Medical Microbiology\ Clinical Immunology, Department of Microbiology\ Medical College/ Al-Nahrain University. Baghdad. Iraq.
[***] Oral Microbiology \Forensic Medicine Institute, Baghdad, Iraq.
[****] Iraqi Ministry of HealthBaghdad, Iraq.
*Corresponding author

Abstract

Background: Recurrent aphthous stomatitis is an inflammatory condition characterized by painful recurrent, single or multiple ulcerations of the oral mucosa. Coeliac disease is an autoimmune enteropathy triggered by the ingestion of gluten-containing grains in susceptible individuals.The association between two diseases has been evaluated in several studies. ***Aims of Study*:** The main objective of this study was to evaluate the frequency of genetic markers (HLA-DQ2 and HLA-DQ8) of coeliac disease in patients with recurrent aphthous stomatitis. ***Subjects and Methods:*** Thirty patients with recurrent aphthous stomatitis and twenty five healthy age- and sex- matched controls were enrolled in this study. Polymerase chain reaction-specific sequence primers (PCR- SSP) assay was conducted to assess HLA-DQ2 and DQ8 typing. ***Results:*** Among the 30 cases, 14 (48.3%) had the genotype DQ2, 4 (13.3%) had DQ8, and 12 (40%) did not have any of these genotypes. DQB1*02 was the most frequent allele in recurrent aphthous stomatitisas compared to healthy control. ***Conclussion:*** These results showed that that genetic predisposition at the HLA-DQ2 locus could become a fundamental test for recognising underlying celiac disease in recurrent aphthous stomatitis. Further genetic markers evaluation of recurrent aphthous stomatitis patients for celiac disease must be performed.

Keywords: recurrent aphthous stomatitis, coeliac disease, HLA allele.

Introduction

Recurrent aphthous stomatitis (RAS) is an inflammatory condition characterized by painful recurrent, single or multiple ulcerations of the oral mucosa. It is estimated that up to 20% of individuals have been afflicted at least once with aphthous ulcers and about 5–20% have had an episode of RAS (1). The disease occurs in men and women of all ages, races and in all geographic regions.

Although RAS is a common disease of oral mucous membranes, its aetiology and pathogenesis remains unknown. It was shown that genetic, immunological and microbial factors may play a role in the pathogenesis. Attacks may be precipitated by local trauma, stress, food intake, drugs, hormonal changes or vitamin and trace element deficiencies. However, no principal cause has been discovered (2, 3). Currently a hypothesis is being discussed that it might be pathogenetically related to coeliac disease (CD).

Celiac disease is an autoimmune enteropathy triggered by the ingestion of gluten-containing grains in susceptible individuals (4). It is the result of the interplay between environmental and genetic factors.

The gliadin and glutenin fractions of wheat gluten and similar alcohol-soluble proteins in other grains are the environmental factors responsible for the development of the intestinal damage. The genetic predisposition is related to HLA (human leukocyte antigen) class II genes: most of CD patients are HLA-DQ2 positive, and the remaining patients are usually HLA-DQ8 positive (5).These genes are estimated to explain some 40% of the disease heritability; the remaining 60% of the genetic susceptibility to CD is shared between an unknown number of non-HLA genes, each of which is estimated to contribute only a small risk (6). The typical intestinal damage is characterized by loss of absorptive villi and hyperplasia of the crypts and it completely resolves upon elimination of gluten-containing grains from the patient's diet (7).

The association between CD and RAS has been evaluated in several studies but conflicting results have been reported (8, 9). RAS and CD are common diseases in Iraq, yet no available data on the association between two diseases, as well as not much has so far been done to study genetics aspects of both diseases in details. This prompted us to carry out this study in order to evaluate the frequency of genetic markers (HLA-DQ2 and HLA-DQ8) of coeliac disease in patients with recurrent aphthous stomatitis.

Subjects and Methods

A total of thirty Iraqi patients with RAS (16 females and 14 males) were included in this study. They were among patients attending to the teaching hospital dentistry college in Baghdad. Their age ranged from 18-40 years. Diagnosis was made by specialized dentists in the hospital. All the cases had received no treatment with no complain of chronic or systemic diseases. Apparently healthy volunteers their ethnic, ages, and gender were matched, consisted of 25 individuals who were considered as control. All of them have no history or clinic evidence of RAS lesions. Their age ranged from 18-31years.

Two ml of venous blood with EDTA as anticoagulant were collected from each subject. Extraction of DNA from peripheral blood was done according to the modified method of Miller (10), using the EXTRA-GENE-I kit (BAG-Germany).

HLA-DQ genotyping was performed by PCR-SSP according to a method presented by Olerup and Zetterquist (11, 12), using low resolution typing kits (HISTO TYPE / DNA-SSP Kits-BAG- Germany).

Statistical analysis were presented in terms of percentage frequencies, and alleles showing variations between patients and controls were further presented in terms of odds ratio (OR) and etiological fraction (EF). The significance of these differences was assessed by fisher's exact probability (P). P values of $P<0.001$and $P<0.05$ were considered significant (13).

Results

The current results showed that the age of patients ranged between 16-40 years with a mean age of 23.1±0.26 years. There was female's predominance among patients, table (1). Among the 30 RAS cases, 14 (48.3%) had the genotype DQ2 while among controls 5 (20.8%) had DQ2 with significant differences ($P<0.05$). 4 (13.3%) of patients had DQ8 but in control was 6 (25%), ($P>0.05$), and the rest 12 (40%) of patients and 14 (55%) of controls did not have any of these genotypes, table (2). Furthermore, DQB1*02 was the most frequent allele in RAS patients as compared to controls ($P<0.05$), table (3).

Table 1: Ages and sex distribution of the studied groups

		RAS cases		***Healthy control***	
Age in year	Minimum	16		18	
	Maximum	40		31	
	Mean	23.1±0.26		21.4±0.85	
	Total	30		25	
Gender					
	Female	20	66%	15	60%
	Male	10	34%	10	40%
	Total	30	100	25	100

Table 2: Frequencies of genotypes DQ2 and DQ8 in patients and control relatives.

HLA	*Control* (%) n (%)	*Patients* (%) n (%)	OR	P (Fisher's exact)
DQ2	5 (20.8%)	14 (48%)	3.55	P<0.05
DQ8	6 (25%)	4 (12%)	4.45	NS
Absent	14 (55%)	12 (40%)	1.12	NS
Total	25 (100%)	30 (100%)	-	-

OR: odds ratio

Table 4: HLA-DQ2 (DQB1*02:01:0) and DQ8 (DQB1*03:01:0 and DQB1*03:02:0) alleles genotyping in RAS cases in comparison to healthy control group.

	Control		*Patients* (RAS)				P (Fisher's exact)	
	N	%	N	%	OR	EF		
DQB1*02:01:0	**5**	**20.8**	**14**	**48.3**	**3.55**	**0.347**	**0.028**	**P<0.05**
DQB1*03:01:0	**3**	**12.5**	**2**	**6.9**	**0.52**	**-**	**NS**	
DQB1*03:02:0	**3**	**12.5**	**2**	**6.9**	**0.52**	**-**	**NS**	

EF: etiological fraction

Discussion

Recurrent aphthous stomatitis was found in 10-40% of untreated CD patients. The prevalence of RAS in the general population is approximately 20% (15, 16). As RAS is frequently seen in CD patients, evaluation of individuals with this symptom may reveal the patients with undiagnosed CD. Although the exact cause for aphthous stomatitis is still unknown, nutritional factors play a well defined role, and contribute to the relationship between CD and RAS (17).

Among the known genetic markers, the HLA genes have the greatest impact on the development of the CD and RAS (18). In this study 48.3% of patients had the genotype DQ2 wheras only 13.3% had DQ8, and 40% did not have any of these genotypes. Moreover, the DQB1*02 allel was the most frequent allele in RAS. Intrestingly numerous studies showed that the presence of genes coding for DQ2 and DQ8 molecules explains up to 40% of the occurrence of CD (19, 20). So the present study may support the immuogentic association between CD and RAS. Similarly other studies have demonstrated that DQB1*02 expression with confer a genetic risk for CD (21, 22). About 90%–95% of patients with CD carry the DQB1*02 allele compared to a prevalence of 20%–30% in the general population (20).At variance with present study Majorana and colleagues were analyzed the frequency of HLA class II (DQ) antigens in 113 subjects affected by CD, nineteen of them suffering from RAS, they noticed that a significant association was found between DQ1 HLA antigens and the two diseases in the 19 subjects suffering from both diseases (23). In conclusion the results showed that that genetic predisposition at the HLA-DQ2 locus could become a fundamental test for recognising underlying CD in RAS. Further genetic and serologic markers evaluation of RAS patients for CD must be performed.

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