Original Article

MHC Gene Polymorphism in Bangladeshi Patients with Vitiligo- a laboratory based cross-sectional observational study

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Abstract

Background: Vitiligo is a common depigmenting and disfiguring skin disease. Association of Human Leukocyte Antigen (HLA) and vitiligo is well known and several HLA studies across the population worldwide are done and found to be important in prediction of disease susceptibility.

Aims: To investigate whether any HLA alleles were associated with Vitiligo among Bangladeshi patients.

Methods: It was a cross sectional study, conducted in Bangabandhu Sheikh Mujib Medical University (BSMMU) among 32 clinically diagnosed patients of vitiligo and 30 healthy controls. With all aseptic precaution 2 ml of venous blood was collected in ethylene diaminetetraceticacid (EDTA) containing tube and stored at -20 c until testing. DNA extraction was carried out according to manufacturer's instructions and were investigated for their HLA genotype. In this study HLA class I and class II typing was done by amplification of extracted DNA by PCR based technique with Micro Sequence-Specific Primers (SSP). Then amplified DNA was run in 2% agarose gel and visualized by UV light. Presence of band of specific PCR product was interpreted with the worksheet supplied by manufacturer.

Results: Significantlyhigher frequency of HLA A*33(01) and HLA B*44(02) of MHC class-I alleles and DRB1*07(01) and DQB1*02(01) of class II alleles were reported from vitiligo patients.

Limitations: Small sample size.

Conclusions: Increased frequencies of some alleles of HLA class I and class ll antigens in patients showed a notable association of MHC genes with manifestation of vitiligo from Bangladeshi patients.

Keywords: vitiligo, HLA, Polymorphism

Introduction

Vitiligo is a common depigmenting disorder of the epidermis and hair follicle that affects 0.38-1.13% of the world population.[1] It is a disfiguring disease due to progressive and chronic loss of melanocytes from the cutaneous epidermis.[2]Vitiligo affects all races and both sexes almost equally worldwide with highest incidence recorded in Indian subcontinent followed by Mexico and Japan. [2,3] This cosmetically disfiguring lesion has a significant impact on quality of life because of its psychosocial effect and cause emotional trauma in both children and adults.

The pathogenesis of vitiligo is unknown but it has been made clear that genetic and immunological factors play a significant role in its development. Association of Human Leukocyte Antigen and diseases is well known and several HLA studies across the population worldwide are done and found to be important in prediction of disease susceptibility, resistance and of evolutionary maintenance of genetic diversity.HLA is the most polymorphic system of the human genome located in Major histocompatibility complex (MHC) region of short arm of chromosome 6.It is now recognized as a

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major contributing factor for susceptibility to a variety of autoimmune diseases like ankylosing spondylitis, rheumatoid arthritis, celiac disease and vitiligo.[4,5]Strong association of specific HLA haplotypes with family history of vitiligo, severity of disease, age of onset, and population geography has been reported from several international studies.[6-8]

The incidences of vitiligo ranges from 0.1 to over 8.8% wereshown in different studies [9-13] Like other countries,vitiligo is quite prevalent also in Bangladesh and constitutes a major psycho-social problem reflected in few socio-demographic study reports from Bangladesh. [14, 15]It is an ancient polygenic autoimmune disease of unknown etiology.[16,17]Though the disease pathogenesis is unknown, a large number of scientific studies had done worldwide to detect genetic association with different candidate genes and most of the studies in different populations' have consistently showed a significant association between HLA system and vitiligo predisposition. However, to the best of our knowledge, there is no published report of any genetic study on vitiligo patients from Bangladesh. Thus, the present study was aimed to investigate whether any HLA alleles were associated with Vitiligo among Bangladeshi patients.

Materials and methods

This hospital based case-control study was carried outfrom March 2017- February 2018, among thirty two vitiligo patients attaining at the Dermatology & Venereology out Patient Department (OPD) of Bangabandhu Sheikh Mujib Medical University (BSMMU). Prior to selection of participants, ethical clearance was obtained from Institutional Review Board (IRB) of BSMMU.The control group consisted of thirty healthy volunteers that did not have vitiligo or other autoimmune diseases and had a negative family history for vitiligo. Diagnosis of all localized or generalized Vitiligo cases were made on typical history of depigmented lesions with or without progression, then clinical examination was done and finally confirmed with Wood's lamp examination. All relevant data were recorded in pre-designed data collection sheet after taking informed written consent. All laboratory tests were performed at the Tissue Typing Laboratoryof the Department of Virology of the same university.

Laboratory methods

With all aseptic precaution 2 ml of venous blood was collected from patients and control groups in ethylene diaminetetracetic acid (EDTA) containing tubeand then storedat -20º c until testing. Briefly,DNA extraction was carried out by phenol-chloroform method from EDTA anti-coagulated blood samples according to manufacturer's instructions.HLA class I (A and B locus) and class II (DRB1 andDQB1locus) typing was done by amplification of extracted DNA by PCR based technique with Micro Sequence-Specific Primers (SSP) Generic HLA class I and class II DNA Typing Trays (One Lambda, U.S.A.). Amplified DNA was run in 2% agarosegel and visualized by UV light. Presence of band of specific PCR product was interpreted with the worksheet supplied by One Lambda.

Statistical Analysis

The antigenic frequencies of Class I and Class II from vitiligo patients and controls were compared. Z-test was done to calculate the p values along with the 95% confidence interval (95% CI). P-value less than 0.05 were considered as statistically significant. The strength of the association was determined by relative risk. A relative risk of 1 indicates no association and greater than 1 is considered to be clinically significant.

Results

The demographic profile of the study participants is shown in Table 1.

Table 1: Demographic data of cases and control

Indicators		Cases (n=32)		Control (n=32)	
		Frequency	Percentage	Frequency	Percentage
Sex	Male	21	65.62	22	73.33
	Female	11	34.37	8	26.66
Age groups in years	< 20	10	31.25	4	13.33
	21-40	19	59.37	18	60
	>40	3	9.37	8	
Age of disease onset in years	< 10	2	6.25		
	20-Nov	8	25		
	21-40	16	50		
	>40	6	18.75		

Among 32 vitiligo patients, males (65.6%) outnumbered females (34.4%). Most of them were above 20 years of age with lowest 5 years and highest 44 years and belonged to middle income group.50% of them had their onset of disease at 20

to 30 years of age. The distribution of patients according to marital status represented that the majority is unmarried (62.5%).Majority of patients was presented with generalized vitiligo was found in 29 (90.6%) patients while only 9.4% presented with localized vitiligo. Only 25% vitiligo patients had positive family history and the rest 75% did not.

Table-2: Frequency of HLA Class I antigens in vitiligo patients and controls.

HLA Allele	Vitiligo patients (n=32)	Control (n=32)	Relative Risk	Z	P value
A*01:01	5	9	0.62	0.78	>0.05
A*02:01	4	10	0.48	1.82	<0.05*
A*03:01	6	3	1.34	1.08	>0.05
A*11:01	9	18	0.50	2.30	<0.05*
A*24:02	10	8	1.10	0.56	>0.05
A*32:01	1	3	0.47	1.03	>0.05
A*33:01	16	6	1.80	2.65	<0.05*
A*68:01	3	2	1.20	0.41	>0.05
B*44:02	14	6	1.66	2.17	<0.05*
B*15:02	11	9	1.66	0.54	>0.05
B*35:01	7	6	1.03	0.31	>0.05
B*07:02	3	1	1.50	1.03	>0.05
B*51:01	3	3	0.98	0	>0.05
B*52:01	3	1	1.50	1.03	>0.05
B*58:01	3	2	1.20	0.46	>0.05
B*15:01	3	3	0.98	0	>0.05

*P value is significant

In this study, among HLA Class I allele, 18 alleles of HLA-A locus, 28 alleles of HLA-B locus were detected. Among HLA Class II allele, 14 alleles of HLA-DR and 6 alleles of HLA-DQ locus were typed for all participants. Of these, 13 alleles of HLA-A, 16 of HLA-B, 10 of HLA-DR, 6 of HLA-DQ and 8 of HLA-A, 21 of HLA-B, 12 of HLA-DR and 6 of HLA-DQ locus were detected among vitiligo patients and healthy control respectively.

Allele frequencies of HLA Class I and Class II for vitiligo patients and control group were shown in Table-2 and Table-3. When all the vitiligo patients were compared to controls a significantly increased positive association of HLA A*33:01 and HLA B*44:02found and HLA A*11:01 and HLA A*02:01 showed significantly negative association with vitiligo in HLA Class I. Moreover, in HLA class II, DRB*07:01 and DQB*02:01 showed significantly positive association while DQB*07:01 had a significantly negative association.

Table-3: Frequency of HLA Class II antigens in vitiligo patients and controls.

HLA Allele	Vitiligop atients (n=32)	Control (n=30)	Relative Risk	Z	P value
DRB*07: 01	25	9	2.92	2.3	<0.05*
DRB*15: 01	11	16	0.66	1.28	>0.05
DRB*12: 01	9	10	0.88	0.27	>0.05
DRB*04: 01	4	9	0.52	1.56	>0.05
DRB1*1 3:01	4	5	0.84	0.36	>0.05
DRB*10: 01	4	3	1.14	0.4	>0.05
DQB*02: 01	22	10	2.06	3.05	<0.05*
DQB*05: 01	11	10	1.01	0.26	>0.05
DQB*06: 01	11	11	0.96	0	>0.05
DQB*07: 01	9	18	0.5	2.3	<0.05*
DQB*08: 01	2	4	0.62	0.86	>0.05
DQB*09: 01	4	5	0.84	0.36	>0.05

*P value is significant

Discussion

Vitiligo is a disfiguring disease with psychosocial stigma due to chronic and progressive loss of melanocytes from the skin, hair, and mucous membranes.[15,18] Strong evidence of vitiligo as an autoimmune disease was provided by singh et. al.[19] A hallmark of autoimmune disease is the association of susceptibility with certain alleles of the major histocompatibility complex gene, in particular, the subset of genes that encode antigen-presenting proteins of the cell surface, that is the HLA antigens. The association of multiple HLA class I and II alleles with vitiligo have been suggested from different population. Several studies have reported the association of vitiligo with different alleles of HLA locus. To the best of our knowledge, this is the first study on HLA association with vitiligo patients from Bangladesh.

A total of 32 vitiligo patients were investigated for their HLA genotype in this study. According to their demographic profile, the mean age of onset was found to be 20 to 30 years. An earlier age of onset of

vitiligo was reported in a previous study by Rahman et al., from Bangladesh.[15] In their study, the highest age of onset was found to be 11-20 years of age. In most of the studies from India, age of onset was mainly in second and third decade.[8,20] However, all these findings indicate that vitiligo predominantly affects the younger population. In our study, 25% participants had positive family history of vitiligo. Similar findings also reported by Rahman et al and Babar et. al., where they reported 36.8% and 32% of positive family history among study participants.[15,7] The earliest formal consideration of genetic basis of vitiligo was considered from this frequent familial clustering of patients.[21-24]

HLA antigens are encoded by MHC (major histocompatibility complex) gene, the highest polymorphic gene of human genome and mainly regulate the immune system. This region has been associated with almost all diseases involving autoimmunity.[25-26] To date, the main generalized vitiligo genome wide association studies (GWASs) in Caucasian andChinese populations have identified the HLA region as the strongestdeterminator of genetic risk.[27, 28] In our study, significantly increased frequency of HLA A*33:01 and HLA B*44:02 of MHC class-I alleles and DRB1*07:01 and DQB*02:01 of class II alleles were reported from vitiligo patients. This findings are in agreement with Singh et al.who reported an increase frequencies of HLA-A*33:01, HLA-B*44:03 and DRB1*07 in patients from North India and Gujarat.[19] Another study from India by Misri et al., reported higher frequency of HLA A33 and B44 along with other antigens like HLA A2, A 11, A31, B17, B35 and B40.[29] Findings from a meta-analysis of 11 case-control studies by Liu et al.,strongly suggested an association between HLA-A2 and vitiligo. [28]HLA associations with vitiligo from Kuwaiti population was HLA-B21, Cw6; DR53, DR4 and DQw3 from African-Americans; A30, B27 Cw6, DR07 and DQw3 from Italians; B13 in Jewish Moroccans and Bw35 in Jewish Yemenites; A2, Bw60 and DRw12 in Northern Germans, Cw7 DQB1, DR06 and DRB4*010 in the Dutch population; a Bw6 and DR07 in native Omanis.[30-35,9,36]In our study, HLA A*02, A*11 was observed less frequently among patients and may have a negative association as because these two alleles reported as most common HLA-A locus alleles among Bangladeshi in a previous population based study.[37]Negative associations of A19, DR52 and DR01, DR03 Antigens were observed in the Kuwaiti population and Italians, respectively. [30-35]In the Slovak population,a positive association of DRB1*0701, DQB1*0201, HLA-A2 and HLA-Dw7 DPB1*1601genotyping of HLA complex was showed.[38]DRB1*07, which was observed more frequent among vitiligo patients in this study, was also reported significantly higher among vitiligo patients from South India, Gujarat,Slovakia and China.[19,38,39] Our study showed a significant increase in HLA DQB*02 in vitiligo patients.However, in case of vitiligo, the diversity of the different allelesamong different ethnic groups and geographical areas was observed.

Limitations

Sample size of the study is small.

Conclusion

The exact etiology of vitiligo is still on evaluation. In current study, increased frequency of HLA A*33 and HLA B*44, DRB1*07 and DQB1*02 are observed in vitiligo patients. Under the light of our study we like to recommend a large scale study that might support our inference.

Conflict of interest

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