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HLA-B27 antigen frequency among suspected Spondyloarthropathy patients attaining a tertiary level hospital of Bangladesh

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Abstract

Human leukocyte antigen B27 (HLA-B27), a class I molecules of the major histocompatibility complex has a strong disease association with different types of spondarthropathies (SpA). The strength of this disease association varies markedly among racial and ethnic populations. The present study aimed to identify the HLA-B27 antigen frequencies among suspected SpA patients as well as healthy Bangladeshi individuals. The frequency of HLA-B27 was determined in 1500 patients and 1000 healthy subjects attending the Bangabandhu Sheikh Mujib Medical University (BSMMU). HLA-B 27 typing was done by microlymphocytotoxicity test using commercial kit. A total of 738 (49.2%) suspected SpA patients and 107 (10.7%) healthy subjects tested positive for HLA-B27 antigen with higher frequency among younger age groups (54.9%, 52.4% and 56.2% in 0-14 years, 15-24 years and 25–34 years of age respectively). The male female positivity was almost same (11.4% and 9.6%) among control group, but in patient group it was 53.0% and 41.2% respectively. The findings of this hospital based study showed a high frequency of HLA-B27 among suspected SpA patients with male preponderance which is comparable with neighboring countries.

Introduction

Human leukocyte antigens (HLA) class I, are a group of glycoprotein found on the surface membrane of leukocytes and other nucleated cells. HLA plays a key role in immune responses such as antigen presentation and recognition of self-peptides and non-self peptides as these responses affect the T-cell receptors and coordination of cellular and humoral immunity. These are the inherited gene markers encoded by the major histocompatibility complex (MHC) molecules located on the short arm of chromosome 6. One of the strongest linkages known to date between the presence of HLA allele and disease susceptibility is that of HLA-B27 to inflammatory SpA which was first recognized in 1973.[1,2] SpAs are multifactorial diseases that constitute a cluster of interrelated overlapping chronic inflammatory rheumatic diseases, such as reactive arthritis (ReA), psoriatic arthritis (PsA), enteropathic arthritis, a subgroup of juvenile chronic arthritis, ankylosing spondylitis (AS), and undifferentiated spondyloarthropathy (USpA). They seem to have an immune-mediated pathogenesis that share a number of clinical, radiographic, and genetic characteristics with a well-defined group of rheumatic disorders.[3] Association of HLA-B27 with the entire group of spondyloarthropathies is well known and its association varies markedly among different forms of SpA and among different ethnic populations.[4]

Initial observations of the association of HLA-B27 with ankylosing spondylitis (AS), a prototype of SpA were made among Caucasians from Europe and North America.[2] Subsequent studies have established the presence of HLA-B27 in AS patients in almost every ethnic group, including Japanese, Chinese, Native Americans, Brazilians, Mexicans, African-Americans, Asian Indians, Iranians, Iraqis, Israelis, Lebanese, and Alaskan and Siberian Eskimos.[5-15] However, the prevalence of HLA-B27 varies among populations and ethnic groups worldwide. For example, about 8% of Caucasians, 4% of North Africans, 2-9% of Chinese and 0.1-0.5% of Japanese descent possess this gene.[16] Molecular studies have revealed at least 31 HLA-B27 subtypes with various distributions in different populations.[17-20] These subtypes differ by one or more amino acid substitutions in the antigenic peptide-binding groove.[21]

Population-specific distribution of HLA alleles is necessary both in population genetics and in HLA disease association studies. Anthropological studies show that the distribution of HLA alleles differ from one ethnic group to another. The genetic

background of Bangladeshi people appears to be a mixture of different populations, mainly including Indo-Aryan, Austro-Asiatic, Dravidian, Mughal, Arab, Persian, Turkic and British.[22] Although there has been a steady flow of publications describing the geographical prevalence of HLA-B27 and the frequency and distribution of its subtypes in different populations and ethnic groups, to our knowledge there is no published report on HLA-B27 distribution among the Bangladeshi populations. Therefore, the aim of this study was to investigate the frequency of HLA-B27 antigen among suspected SpAs patients as well as healthy people of Bangladesh.

Materials and Methods

A total of 2500 subjects-1500 suspected Spondyloarthropathies (SpA) patients and 1000 healthy organ donors referred to the Tissue Typing Laboratory of the Department of Virology, BSMMU for HLA-B27 typing and HLA- A, B and DR typing respectively, from January 2010 to June 2013 were enrolled in this prospective study as patient and control group. Relevant information was collected from the patient's record register. Owing to the lack of validation of modified New York criteria[23] or the new ASAS (Assessment of Spondylo Arthritis International Society) classification criteria[24] for the diagnosis of axial and peripheral SpA, subjects presenting with SpA related clinical manifestation, such as, low back pain with morning stiffness, buttock pain, enthesitis, peripheral arthritis, etc., were chosen as suspected SpA cases. Relevant data of control groups were collected from the laboratory record register of bone marrow and kidney donors tested at this laboratory at the same time frame. Donors who had history of chronic arthritis or SpA related complaints were excluded from the control group. All cases from both study and control groups were Bangladeshi in origin. The age distribution of the patients was not normal distribution. For data analysis, patients were divided into five age groups at 10 years of age interval. First and last age groups were considered as <14 yrs and >45 yrs as sample size was less among these two groups.

HLA Typing: The laboratory test was performed according to the microlymphocytotoxicity technique of Terasaki and McClelland.[25] The basis of this procedure is specific antibody mediated cytolysis in the presence of complement. This is a modified National Institute of Health (NIH) microlymphocytotoxicity method[26] that employs a sensitization step of cells (antigen) with serum (antibody) and the second stage is the specificity step achieved by addition of rabbit complement. In this method, freshly isolated lymphocytes from heparinised blood are incubated with a panel of known HLA-anti-sera present in Terasaki Typing Tray. After incubation, rabbit complement is added in each test well. Then the complement mediated cell lysis of the test positive well is visualized by dye exclusion method and interpreted under inverted microscope. In this study, Lymphocytes were isolated from 5 cc of fresh heparinized venous blood by Ficoll-Hypaque (Sigma diagnostic, USA) density gradient centrifugation technique and HLA-B27 Typing Tray with pre-dropped anti-sera and controls for HLA-B27 (ONE-LAMBDA INC, 21001 Kittridge st Canoga Park, CA 91303 U.S.A.) was used.

Statistical Analysis: Statistical analysis was carried out using SPSS 17.0 software. Chi-square test was used to compare HLA-B27 frequencies among patient and control groups. The confidence interval (CI) of the calculated odds ratio (OR) was estimated by approximate 95%. Results were considered significant when the *p*-value was less than 0.05.

Results

Demographic data of study and control groups are shown in Table I. The mean age of the patients was 30±10.2 years with lowest age 7 and highest 65 years of age. Maximum numbers (37.1%) of patients were within 25–34 years of age. HLA-B27 antigen was detected in 739 (49.3%) out of the 1500 patients, whereas, among the 1000 control subjects, only 107 (10.7%) had HLA-B27 antigen (Table II). Among the study group, 1014 (67.6%) were males and 486 (32.2%) were females. The distribution of HLA-B27 positivity among males and females were 539 (53.0%) and 200 (41.2%) among patients, and 69 (11.4%) and 38 (9.6%) respectively among control group. No significance of difference in HLA-B27 positivity was encountered among males and females of control group but in the patient group, it was significantly higher among males ($p<0.001$) (Table III). Among different age group of patients, the frequency was found to be 54.9%, 52.4% and 56.2% in the 0-14 years, 15-24 years and 25-34 years age groups respectively and then gradually decreased with the increase of age, and was 40.3% in 35-44 years and 30.0% among patients more than 45 years of age. There was no significant difference of B27 positivity within younger age groups, but when compared with older B27 positive subjects, this difference was highly significant ($p<0.001$) (Table IV).

Table I: Demographic data of study groups.

Characteristics	Patients Group (n-1500)	Control Group (n-1000)
Mean Age(±SD) (in years)	31 (30±10.2)	35 (34±9.5)
Sex (F/M)	486/1014 (1:2.09)	396/604 (1:1.53)

Table II: Frequency of HLA-B27 among suspected SpA patients and healthy controls.

HLA-B27	SpA Patients (n-1500)	Control (n-1000)	Total (n-2500)	Significance
Positive	739 (49.3%)	107 (10.7%)	846 (33.84%)	P <0.001
Negative	761 (50.7%)	893 (89.3%)	1654 (66.16%)	

Table III: HLA-B27 frequency among SpA Patients and control groups according to gender.

HLA-B27	SpA Patients (n- 1500)				Control (n-1000)			
	Male (%)	Female (%)	Total (%)	Signifi-cance	Male (%)	Female (%)	Total	Signifi-cance
Positive	539 (53.0)	200 (41.2)	739 (49.3)		69 (11.4)	38 (9.6)	107 (10.7)	
Negative	475 (47.0)	286 (58.8)	761 (50.7)		535 (88.6)	358 (87.9)	893 (89.3)	
Total	1014 (67.6)	486 (32.4)	1500 (100)	P<0.05	604 (60.4)	396 (39.6)	1000 (100)	P>0.10

Table IV: Distribution of HLA-B27 among different age groups of suspected SpA patients.

Age Group (yrs)	Positive (%)	Negative (%)	Total	Significance
0 – 14 (n-71)	39 (54.9)	32 (45.1)	71	
15 – 24 (n-385)	202 (52.4)	183 (47.6)	385	
25 – 34 (n-557)	313 (56.2)	244 (43.8)	557	P<0.001
35 – 44 (n-377)	152 (40.3)	225 (59.7)	377	
>45 (n-110)	33 (30.0)	77 (77.0)	110	
Total (n-1500)	739 (49.3)	761 (50.7)	1500	

Discussion

The human leukocyte antigen HLA-B27 is strongly associated with development of a group of inflammatory arthritis collectively known as the spondyloarthropathies that include ankylosing spondylitis (AS), psoriatic arthritis, reactive arthritis and arthritis with inflammatory bowel disease[1,2] and this association was first recognized in 1973. Since then, a great amount of scientific research has been performed on HLA-B27 worldwide, but in Bangladesh, little is known regarding HLA-B27 prevalence and its relation with SpAs. To the best of our knowledge, the present study is the first of its kind among Bangladeshi people.

The prevalence of HLA-B27 among healthy individuals vary greatly in different ethnicities ranging from 0% in African Bantu and Australian Aborigines to 50% in Native Americans.[27] It was found to be 18-50% in American Indians, 10-16% in Scandinavians, 6-9% in Western Europe, 2-6% in Southern Europe, 6-8% in Pakistanis, 2-6% in Indians, 1% in Japanese, and 1% in Africans.[27,28] Our study observed 10.7% frequency of B27 among healthy people with almost similar pattern of distribution among males (11.4%) and females (9.6%). This prevalence was similar to some prevalence reports from other Asian countries surrounding Bangladesh, e.g., 8.3% from West India[29] and 1.7% from Mumbai,[30] 12.5% from Punjab, 1.3% from Sindh and 7.2% from Urdu speaking people of Pakistan.[31] Low frequency in Mumbai of India and Sindh of Pakistan may be due to ethnic variations of their origin.

The degree of association between HLA-B27 and spondyloarthropathies (SpA) vary markedly among different diseases of this group and also between different populations. Ankylosing spondylitis (AS) is the most common subtype of SpA and exhibits the strongest association with HLA-B27. About 95% of the Caucasian AS patients express HLA-B27,[32] while only 50% of African American patients with ankylosing spondylitis possess HLA-B27 antigen.[33] In our study, out of 1500 patients 739 (49.3%) were HLA B-27-positive with the maximum numbers of patients (942 out of 1500) within 15 to 34 years of age. The HLA-B27 positivity was found to be high among this age range (52.4% in 15–24 years and 56.2% in 25–34 years of age). It then gradually decreased with the increase of age, and at >45 years age group, it decreased to 30.0%. This corresponds with the characteristics of AS and other SpAs, as these inflammatory disorders usually have an early age of onset while the degenerative arthritis are more likely to predominate at older age. Various studies from India show similar pattern of distribution. A study from South India reported the frequency of B27 as 46% in SpA patients,[34] while a study on 110 patients with Seronegative Spondyloarthropathy (SSA)- that is SpA patients who are negative for rheumatoid factor, the overall frequency was 43.6% with a higher positivity among children (68.75%) and in males (81.81%).[35] Similar findings were also reported from patients of Asian Indian origin, where frequency was 56% among SSA patients with male predominance.[36] The present study also revealed significantly higher prevalence of HLA-B27 among male patients (53.0%) than female (41.2%).

There is a marked variation in the general prevalence of HLA-B27 in various healthy populations as well as in AS and related SpA patients of different racial and ethnic groups. Therefore, the clinical usefulness of the HLA-B27

test in the diagnosis of AS and related SpAs differ appreciably among different populations. People with low general prevalence and strong disease association of HLA-B27 with AS and related SpAs have highest clinical usefulness of B27 test in disease diagnosis. For example, the Japanese show a strong disease association (>85%) of HLA-B27 with AS, but the frequency of this gene in their general population is less than 1%.[16] On the other hand, a positive test result will be relatively less useful among Eskimos, as because of high prevalence HLA-B27 (25% - 40%) among their general population.[16] Our study observed that, 49.3% of the suspected SpA patients were positive for HLA-B27 and in general population, it was 10.7%. However, findings of this study does not reflect the actual prevalence of HLA-B27 among confirmed AS and SpAs cases because all suspected cases presenting with any SpA related clinical manifestation tested for HLA-B27 at the Tissue Typing Laboratory of BSMMU were included without validation of ASAS classification criteria. Therefore, further study is needed to delineate the association of HLA-B27 antigen with confirmed SpA and AS cases and its clinical usefulness in disease diagnosis.

Conclusion: Despite the limitations our study, the findings revealed that the frequency of HLA-B27 is relatively high among Bangladeshi arthritis patients. However, many factors such as ethnic background, geographic variance, and overall environmental factors should be considered before making a definite conclusion. Further extensive studies on confirmed SpA patients are needed to assess the prevalence of HLA-B27 among different groups of spondyloarthropathies patients in Bangladesh.

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