

PREVALENCE OF HUMAN LEUKOCYTE ANTIGEN (HLA) B27 AMONG PATIENTS OF SPONDYLOARTHROPATHIES REFERRED TO A TERTIARY CARE CENTER

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ABSTRACT

Objective: To determine prevalence of HLA-B27 among patients of spondyloarthropathies.

Study Design: Cross sectional study.

Place and Duration of Study: Department of Immunology, Armed Forces Institute of Pathology Rawalpindi Jan 2015 to Aug 2016.

Material and Methods: All peripheral blood samples of spondyloarthropathy patients received for HLA-B27 typing were included in the study. Cells were stained with monoclonal antibodies against HLA-B27 and CD3 using lyse wash procedure. Cell acquisition and analysis was done on Cell Quest software in multi parameter flow cytometer. Data was entered in SPSS 20.0 to determine the frequency of HLA-B27 positive individuals.

Results: Over 20 months, 252 males and 77 females (total 329) were tested with age ranging from 5 to 80 years. Total 77 patients (23.4%) including 66 males (26.2%) and 11 females (14.3%) were positive for HLA-B27.

Conclusion: Nearly 23% patients of spondyloarthropathies carry HLA-B27 antigen, with male's predominance (26% vs 16%).

Keywords: Ankylosing spondylitis, Flow cytometry, Human leukocyte antigen, HLA-B27, Spondyloarthropathies.

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INTRODUCTION

Spondyloarthropathies (SpA) are a group of related but phenotypically distinct disorders that encompass psoriatic arthritis, reactive arthritis, juvenile idiopathic arthritis, arthritis related to inflammatory bowel disease (IBD) and ankylosing spondylitis (AS)¹. Most of these diseases particularly ankylosing spondylitis have strongly been associated with presence of human leukocyte antigen (HLA) B27². Individuals carrying HLA-B27 are about 90 times more likely to develop AS as compared to those not carrying this antigen³. Interestingly AS is a disorder that defies the custom of autoimmune diseases being more common in females. Males are affected about twice as often as females⁴. Although non MHC loci like IL23R, ERAP1 and KIR complex have also been implicated in pathogenesis^{5,6} actual mechanism of development of inflammatory process in SpA remains an enigma⁷. Several mechanisms have been proposed to explain HLA-B27 association with

disease process. These include presentation of an arthritogenic peptide of Yersinia and Chlamydia by particular HLA-B27 alleles⁸, aberrant/misfolded expression of HLA-B27 heavy chains and enhanced intracellular microbial survival⁹. Similarly various HLA-B27 alleles and haplotypes have been associated with susceptibility towards development of disease as well as its complications.

HLA-B27 prevalence in general population varies vastly, ranging from nil in Australian aborigines to 50% in Haida Indians¹⁰. HLA-B27 is increasingly being advised for diagnostically difficult cases, however, its diagnostic utility lays in particular clinical scenario. Populations that have low general population prevalence of HLA-B27 but strong SpA association like Japanese, can benefit from its diagnostic utility¹¹. In contrast, populations such as UK where 8% of general population carries HLA-B27 but only 1% develop the diseases, screening for HLA-B27 is a poor idea¹². The prevalence of AS in Pakistan has been estimated to be 4.7 per 10,000 which is less than most of the regions¹³. In Asia it is 16.7 per 10,000 with China, Taiwan and Malaysia ranking

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Received: 08 Nov 2016; revised received: 09 Feb 2017; accepted: 03 Mar 2017

highest. In sub-continent it ranges from 4.7 to 9.8 per 10,000¹⁴. In Pakistan, HLA-B27 frequency in general population is 5.5%¹⁵ while it's about 4.9% in renal/bone marrow transplant recipients and donors¹⁶. A study in Qatar including 12 Pakistani AS patients determined HLA-B27 prevalence 58% (7 patients out of 12)¹⁷. However, such prevalence has not been determined among large group of patients in Pakistan. Determining such figure will help predicting diagnostic utility of advising HLA-B27 test as we already know that in general population, its frequency is about 5%^{15,16}.

In our tertiary care center, SpA patients are referred to us from all over the country for testing HLA-B27. In this study we tested 329 SpA patients for presence/absence of HLA-B27 over a period of 20 months. Our objective was to determine prevalence of HLA-B27 positivity among such patients that would help improve diagnostic utility of the test.

MATERIAL AND METHODS

This cross sectional study was carried out from Jan 2015 to Aug 2016 in Immunology Department of Armed Forces Institute of Pathology, after approval by Institutional Review Board. All the peripheral blood samples of SpA patients sent to us for HLA-B27 testing by rheumatologists and orthopedic surgeons were included in the study, by non-probability consecutive sampling. This included 329 peripheral blood samples from 252 males and 77 females.

HLA-B27 testing was carried out using flow cytometry. Samples were received in EDTA bottles and were processed within 6 hours. Anti HLA-B27 fluorescein isothiocyanate (FITC) and anti cluster of differentiation (CD) 3 phycoerythrin (PE) monoclonal antibodies for staining of cells were procured from Becton-Dickinson (BD) Biosciences, San Jose, CA, USA. Isotype control used was mouse anti IgG1FITC/IgG2PE. These monoclonal antibodies were labeled with either of fluorescein isothiocyanate (FITC) or phycoerythrin (PE). Staining of cells was done with standard lyse wash procedure according to

manufacturer's instructions. The stained samples were analyzed on BD FACScalibur flowcytometer (FACScalibur, Becton Dickinson, United States of America) using CellQuest software provided by the manufacturer. At least 10,000 lymphocytes were selected for analysis through forward scatter side scatter (FSc / SSc) gating technique. The expression /absence of HLA-B27 was

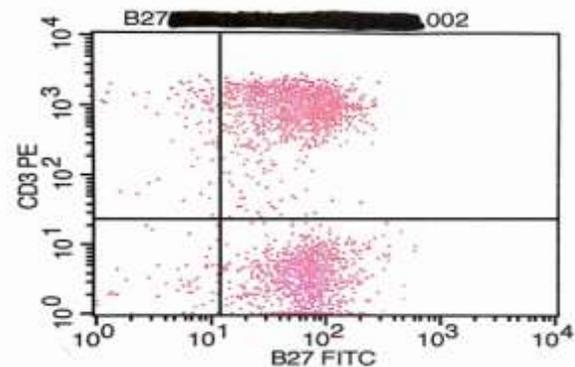


Figure-1: HLA-B27 positive sample as determined by flow cytometry.

determined by quadrant application using isotype control as quality control.

The data for lymphocytes with or without

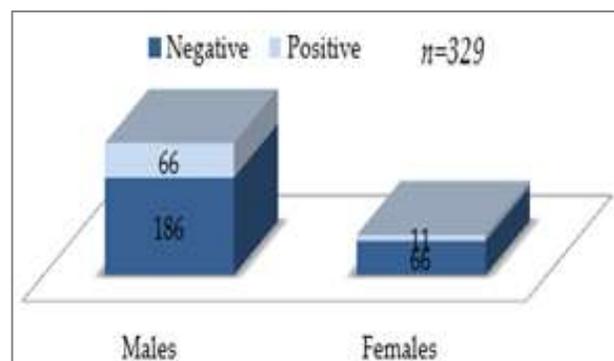


Figure-2: HLA-B27 positive and negative samples distribution in males and females.

expression of HLA-B27 was entered in Statistical package for Social Sciences (SPSS) version 20.0. These were then analyzed for frequencies. Mean and standard deviation were calculated for continuous variables.

RESULTS

Over a course of 20 months of study period, we received a total of 329 peripheral blood

samples for testing HLA-B27. These included 252 males (76.6%) and 77 females (23.4%) with age ranging from 5 to 80 years (mean 42 years \pm 10). Out of these 329, 77 (23.4%) samples were positive for HLA-B27. The positive samples included 66 males (26.2% of total males) and 11 females (14.3% of total females). Fig-2 shows distribution of males and females HLA-B27 positive and negative samples. Fig-1 shows an HLA-B27 positive sample as determined by flow cytometry.

DISCUSSION

The utility of HLA-B27 test in diagnosing SpA has always been debated. In presence of clinical features, diagnostic probability of AS increases from 50% to 90% if HLA-B27 is positive. Therefore, its inclusion in presence of symptoms has been advocated since radiological findings are late to appear¹⁸. It is an HLA class I antigen whose normal function is to present processed peptides to CD8 positive T lymphocytes¹⁹. So it is also found in normal population, though prevalence varies in different geographical regions and ethnicities. In Pakistan, its prevalence in general population is around 5% which is close to other countries of the region^{15,16}. However, we could not find any research conducted in Pakistan that determined HLA-B27 prevalence among patients of SpA particularly AS. We found that HLA-B27 is found in 23.4% patients suspected of having AS. Males having this particular HLA antigen were more prone to disease (26.2%) as compared to females (14.3%). Abdelrehman et al in 2012 had found that 58% of Pakistani patients of AS carried HLA-B27, however, they studied only 12 Pakistani AS patients (besides AS patients of other countries) while our sample size was much larger and included both AS and other SpA patients. In total they studied 119 AS patients of different countries and found 69% HLA-B27 prevalence. Esalat-Manesh et al determination of HLA-B27 prevalence in SpA patients was much close to our finding. They found that 26.5% patients of SpA carried HLA-B27 with 34% males and 16% females²⁰. Our study was limited in respect that

we studied all SpA patients instead of individual associations. Another important aspect of HLA-B27 and SpA associations is that there are more than 100 alleles and not all are associated with increased risk²¹. There remains a gap of knowledge about individual spondyloarthropathies association with over 100 HLA-B27 alleles. Filling of such gap will help not only determining disease risk but also understanding complex associations of amino acids in antigen binding pockets of disease susceptibility HLA-B27 alleles.

CONCLUSION

In the present study about 23% patients of SpA were found to be carrying HLA-B27 antigen, with males predominance (26% vs 16%).

ACKNOWLEDGMENT

We are obliged to Rheumatologists and orthopaedic surgeons for sending us patients for HLA B27 testing.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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