Original Research Paper

Volume - 13 | Issue - 06 | June - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Transfusion Medicine

DISTRIBUTION OF HLA- B*27 ALLELE SUBTYPE IN PATIENTS WITH ANKYLOSING SPONDYLITIS AMONG NORTH INDIAN POPULATION

Dr Saroj Rajput*	MBBS, DNB Transfusion Medicine Indraprastha Apollo Hospitals, New Delhi, India *Corresponding Author
Mohit Chowdry	MBBS, MD Pathology Indraprastha Apollo Hospitals, New Delhi, India
Dr Raj Nath Makroo	MBBS, DIBT, MD Indraprastha Apollo Hospitals, New Delhi, India

ABSTRACT **Background** The association of Human Leukocyte Antigen-B27 (HLA-B27) with ankylosing spondylitis (AS) and spondyloarthropathies is well known. It varies among different racial and ethnic populations. **Aim:** The aim of this study is to perform an investigation regarding the distribution of the human leukocyte antigen HLA-B27 and its subtypes in Indian healthy controls and in patients with AS and to compare this with different reports from different populations. **Materials & methods:** One hundred and eighty seven patients with spondyloarthropathy were referred to our department for HLA-B27 typing. For all HLA-B*27 positive patients, sub typing was also done to find the specific HLA-B27 allele. The prevalence of HLA-B27 in the normal population was also studied which included 200 subjects of both genders with no family history of spondyloarthropathy. **Results:** An association between AS and HLA B27 was identified; HLA-B*27 allele was carried by 45 (24.1%) of patients and 13 (6.5%) subject from healthy controls was found to be positive . Three HLA- B27 alleles were observed in this study: B27: 05: 02 (n=30, 66.7%), B*27:04:01 (n=9,20.0%), B27:07:01 (n=6, 13.3%). **Conclusion:** HLA-B27 is strongly associated with AS in Indian population. Our results showed a restricted number of HLA-B27 subtypes with predominance of HLA-B27: 05 alleles. We found the HLA- B27 the prevalence is 6.3% in healthy groups, where in AS patients it is around 24.1%. This study showed that HLA-B27: 05, HLA- B27: 04, and HLA-: B27: 07 are the subtypes found in our population.

KEYWORDS : Ankylosing spondylitis, HLA-B27 subtypes, polymerase chain reaction- sequence specific primers

INTRODUCTION

Ankylosing spondylitis (AS) belongs to a group of disorders called seronegative spondyloarthropathies (SpA), that primarily affects the sacroiliac joints and the axial skeleton, and less frequently, peripheral joints and other extra-articular organs such as the eyes, skin, and the cardiovascular system [1].

Epidemiological have suggested that the prevalence of AS in a white population is 0.1% to 0.2% , in Chinese population 0.24% and about 0.86% in Caucasians. [2]. AS usually occurs in the second or third decade of life, it is more common in young men than women with a Male/Female ratio of roughly 2 to 1 [3]. The exact cause of AS is unknown, AS is thought to result as a consequence of abnormal immune response, which may be triggered by a complex of genetic ethnicity, and environmental factors [4].

HLA-B*27 is encoded by an allele of the major histocompatibility complex (MHC) class I HLA-B region, on the short arm of chromosome 6 [5]. Genetic studies have concluded that HLA B27 in the major histocompatibility complex (MHC) locus contributes to around 20.1% of AS heritability, with 4.3% associated with loci other than HLA B [5].

Currently, three major mechanistic hypotheses exist to describe the association between HLA B27 and AS. Firstly, aberrant peptide processing and presentation may be involved in the pathogenesis of AS due to the interaction between HLA B27 and endoplasmic reticulum aminopeptidase (ERAP) 1 [6,7,8,9] Secondly, misfolded HLA B27 molecules in the endoplasmic reticulum (ER) may trigger ER stress and provoke the *unfolded protein response (UPR)* . It has been previously demonstrated that this is followed by the subsequent up regulation of various cytokines, particularly IL 23 and IL 17, accompanied by the development of immune dysregulation. Furthermore, cell surface HLA B27 dimers may be important in AS pathogenesis, due to their role in binding to receptors on immune cells [6,7,8,9].

There are very few studies of HLA-B 27 association to Indian AS patients. This study sought to determine B27 subtypes of B27- positive patients with AS and healthy controls.

MATERIALS & METHODS

The study was undertaken in the Department of Molecular Biology and Transplant Immunology, Transfusion Medicine, Apollo new Delhi , from March 2015 To September 2015. Written and informed consent was taken from all the patients and the control group.

In this case control study, all the AS patients who visited rheumatology clinic during the study period with symptoms of inflammatory lower back pain for more than 3 months, sacroilitis on MRI were included as cases in the study.

Controls were all healthy individuals without any history of spondyloarthropathies and negative rheumatoid factor.

Patient Selection

One hundred and eighty seven patients with spondyloarthropathy were referred to our department for HLA-B*27 typing. A complete clinical evaluation was done for all patients. HLA typing was done and prevalence of HLA-B*27 was seen in this group. For all HLA- B*27 positive patients, sub typing was also done to find the specific HLA-B*27 allele.

Control Population

Two hundred control subjects of both genders with no family history of AS were studied for the prevalence of HLA-B*27 in healthy population.

Laboratory Analysis-

DNA was extracted using the commercially available, Life technology pure link ™Genomic DNA mini kit (Invitrogen), made in the USA. Six ml blood was collected in an ACD vial from peripheral veins. Molecular typing was performed by Polymerase Chain Reaction – Amplification (PCR - SSP) with specific primers of B loci. A positive sample for HLA-B*27, was further subtyped with life technology All set +™Gold SSP HLA - B*27 High resolution kit. PCR products were visualized in 2% agarose gel under ultraviolet illumination (Gel Doc Xr Bio Rad) following ethidium bromide staining.

Statistical Analysis:

Data is described in terms of frequencies (number of cases) and relative frequencies (percentages) as appropriate. For comparing categorical data, Chi square ($\chi 2$) test was performed and fisher exact test was used when the expected frequency is less than 5. A probability value (p value)less than 0.05 was considered statistically significant. All statistical calculations were done using (Statistical Package for the Social Science) SPSS 21version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

RESULTS

A total of 187 patients with AS and 200 controls were included in this study. Demographic characteristics of patients were given in (Table 1).

Out of total patients, 137 patients (73.26%) were males and 50 (26.74%) were females. Prevalence of AS was more common in the second and third decade of life (27% and 28% respectively).

Table 1 - Distribution Of Patient With As (n=187)

Age	Male		Female		Total		Chi-square value	P-value
0-10.0	2	1.5%	1	2.0%	3	1.6%	1.8327	0.766
11-20.0	11	8.0%	3	6.0%	14	7.5%		
21-30.0	37	27.0%	13	26.0%	50	26.7%		
31-40	41	29.9%	12	24.0%	53	28.3%		
41-50	22	16.1%	12	24.0%	34	18.2%		
51-60	18	13.1%	7	14.0%	25	13.4%		
60	6	4.4%	2	4.0%	8	4.3%		
Total	137	100.0%	50	100.0%	187	100.0%		

Table 2- Distribution Of HLA-b*27 Positive As Patients (n=45)

Age	Male		Female		Total		Chi-square value	P-value
0-10	1	2.7%	0	0.0%	1	2.2%	0.369	0.985
11-20	3	8.1%	1	12.5%	4	8.9%		
21-30	13	35.1%	2	25.0%	15	33.3%		
31-40	11	29.7%	3	37.5%	14	31.1%		
41-50	5	13.5%	1	12.5%	6	13.3%		
51-60	3	8.1%	1	12.5%	4	8.9%		
60	1	2.7%	0	0.0%	1	2.2%		
Total	37	100.0%	8	100.0%	45	100.0%		

Out of 187 patients, 45 patients showed the presence of HLA-B *27 allele (24.1%), comprising of 37 (82.2%) males and 8 females (17.8 %). (Table 2)

Table 3 - Distribution Of HLA-b*27 Subtypes In As Patients (n=45)

Subtypes	Male		Female		Total		Chi-square value	P-value
B*27:05:02	25	67.6%	5	62.5%	30	66.7%	0.152	0.927
B*27:04:01	7	18.9%	2	25.0%	9	20.0%		
B*27:07:01	5	13.5%	1	12.5%	6	13.3%		
Total	37	100.0%	8	100.0%	45	100.0%		

In these patients, HLA-B*27 subtyping was done using high-resolution kit. We found three allele i.e. HLA-B*27:05:02, HLA-B*27:04:01, HLA -B*27:07:01 prevailing in the study group. HLA-B*27:05:02 was found to be predominant subtype and was present in 30 patients (66.7%) followed HLA-B*27:04:01 present in 9 patients (20.0%) and HLA-B*27:07:01(13.3%) in 6 patients.
(Table 3)

Table 4 - Prevalence Of HLA-b *27 In Healthy Population (n=200)

	Negative		Positive		Total		Chi-square value	P-value
Male	89	92.7%	7	7.3%	96	48.0%	0.1904	0.663
Female	98	94.2%	6	5.8%	104	52.0%		
Total	187	93.5%	13	6.5%	200	100.0%		

Among the 200 healthy control group, 13 were positive for HLA-B*27 (6.5%). Out of which 7 (3.5%) were healthy males, 6 (3%) were females. (Table 4).

DISCUSSION

HLA-B*27 shows high genetic polymorphism, with increasing number of alleles. As per current database, there are about 161 subtypes of HLA B*27:01 to HLA B*27:16 [10]. A variable distribution of HLA-B27 gene is seen among world population, with the highest prevalence in northern territory of the earth [11]. HLA-B*27:05 is the ancestral subtype and is highly associated with AS worldwide. It was thought that HLA-B*27:05 all other subtypes could have evolved from HLAB*27:05 by point mutation (B*27:03), reciprocal recombination (B*27:07, B*27:09) and gene conversion (B*27:01, B*27:02, B*27:04, B*27:06) [12,13].

It is found that not all HLA-B*27 subtypes are associated with AS. In addition to B*27:05, alleles such as B*27:01,B*27:02, B*27:03, B*27:04, B*27:10, B*27:13, B*27:14, B*27:15 showed association with AS [14]. In Caucasians HLA-B*2701, *2702, *2705, *2708, and *2709 [15]. In Chinese, HLA-B*27:04 and in Mediterranean population it is HLA-B*27:02, association was found. Asians showed association with B*2704, *2706 and *2707. HLA-B*27: 09 is a rare subtype found primarily on the Italian island of Sardinia showed lack of association with typical AS [16-18]

In our study, analysis by gender and age brings out that the AS among males is than that found in females (73.26% vs. 26.74%, p<0.001), the male / female ratio was (2.74 %) and showed two peaks among aged 21-30 and 31-40 years. Lower prevalence in females can be explained because of disease predominance in males [3] and another cause may be lower presentation of Indian females at health care centers for the testing because of various socioeconomic reasons.

It is a well known that the prevalence of HLA-B*27 varies worldwide in the various ethnic and racial group. It shows the strongest association with AS among related spondyloarthropathies (SpA) . The prevalence of AS roughly correlates with the prevalence of HLA-B*27 but with certain exclusions, like HLA-B*27 is present in West African countries, such as Senegal and Gambia, but it is very difficult to find any cases of AS in these countries [19]. In this study, we evaluated 187 cases using Polymerase Chain Reaction – Amplification (PCR - SSP) with specific primers of B loci and we found 45 (24.06%) out of 187 were positive for HLA-B*27. The frequency of patients with HLA-B*27 in our study is lower than that documented in Middle Eastern and Western European population. The difference between frequencies could be due different sample size, ethnic background and the geographic variance. Prevalence of HLA-B*27 in healthy population was found to be 6.5%. Out of which 7 (3.7%) were males and 6 were females (3.2%).

In this study HLA-B*27:05 was the most frequent subtype (n=30; 66.6%) found. Twenty-five (83.33%) were males and 5 (16.66%) females. The frequencyr, in Western India the frequency was 70% [19]. Our results are in concordance with the previous reports, where HLA-B*27:05 was found most commonly associated with AS and related SpA around the world [12].

HLA B*27:04 was the second most frequent subtype (n=9; 20%) found in our study, seven (77.77%) were males and 2 (22.22%) were females. Liu et al. found subtype HLA B*27:04 [69%] was predominant in Han population in eastern china followed by HLA- B*27:05 (50%) [20]. Although this is not in concordance with our results. The reason could be due the differences in the HLA-B*27 sequence, or because of the linkage disequilibrium with other nearby AS-related genes or polymorphisms.

HLA-B*27:07 is a rare subtype that was first observed in Oriental and Jewish population and has been reported in association with AS. In the present study, HLA-B*27:07 subtype was found in 6 (13.3%) cases, 5 (83.33%) were males and 1in female (16.66%) and its prevalence was significantly higher in males than female patients. Similarly Chhaya SU also reported the frequency of B*2707 (12.86%) in western India [19]. In a study done in the western Indian population reported two new alleles HLA-B*27: 14 (2%) and HLA-B*27: 08 (12%), that have neither reported in previous studies nor in our present study done in the Indian population [19]. Our findings support the notion that HLA-B*27: 05 and HLA- B*27: 04 are the two most common subtypes found worldwide patients with AS.

CONCLUSIONS -

The present study provides interesting information on the HLA-B*27 association with AS in Indian patients. In this study, found the HLA-B*27 the prevalence is in healthy groups, where in AS patients it is around 24.1%. This study showed that HLA- B*27: 05, HLA- B*27: 04, and HLA-: B*27: 07 are the subtypes found in our population. More extensive typing of the Indian normal population is needed to resolve the evolutionary history of HLA diversity in the Indian population demography. The subtypes that show the strongest associations within the different ethnic groups and populations may indicate which peptides may contribute the disease susceptibility.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank all the patients for participating in this study.

REFERENCES

1. Bezerra Gouveia, E., Elmann, D. and Ávila Morales. M.S, "Ankylosing spondylitis and uveitis: overview," Rev Bras Reumatol, 52 (5). 742-756. Jun.2012.

2. Moschos, M.M., Gatzioufas, Z. and Margetis, I, "Intravitreal Triamcinolone Acetonide for Macular Edema in HLA-B27 Negative Ankylosing Spondylitis," Case Rep Ophthalmol, 1 (2). 105-109. Dec.2010.
3. Feldtkeller, E., Khan, M.A., Van der Heijde, D., Van der Linden, S. and Braun, J, "Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis," Rheumatol Int, 23 (2). 61-66. Mar.2003.
4. Akkoc N, Khan M A. Etiopatogenic role of HLA-B27 alleles in Ankylosing spondylitis. APLAR J Rheumatol 2005; 8:146-53.
5. International Genetics of Ankylosing Spondylitis Consortium (IGAS) Cortes A, Hadler J, Pointon JP, Robinson PC, Karaderi T, Leo P, Cremin K, Pryce K, Harris J, et al. Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nat Genet*. 2013;45:730–738. doi: 10.1038/ng.2667. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
6. Uncovering Etiologic Agent(S) of Autoimmune-Disease. *Curr. Top. Microbiol. Immunol.* **1989**, *145*, 127–135. [**Google Scholar**]
7. Grandon, B.; Rincheval-Arnold, A.; Jah, N.; Corsi, J.M.; Araujo, L.M.; Glatigny, S.; Prevost, E.; Roche, D.; Chiocchia, G.; Guenal, I.; et al. HLA-B27 alters BMP/TGFbeta signalling in Drosophila, revealing putative pathogenic mechanism for spondyloarthritis. *Ann. Rheum. Dis.* **2019**, *78*, 1653–1662. [**Google Scholar**] [**CrossRef**] [**PubMed**]
8. Pedersen, S.J.; Maksymowych, W.P. The Pathogenesis of Ankylosing Spondylitis: An Update. *Curr. Rheumatol. Rep.* **2019**, *21*, 58. [**Google Scholar**] [**CrossRef**] [**PubMed**]
9. Garcia-Montoya, L.; Gul, H.; Emery, P. Recent advances in ankylosing spondylitis: Understanding the disease and management. *F1000Res* **2018**, *7*, F1000. [**Google Scholar**] [**CrossRef**] [**PubMed**][**Green Version**]
10. IIPD-IMGT/HLA" Database. Available from: http://www.ebi.ac.uk/ipd/imgt/hla/docs/release.html.
11. Peschken CA, Esdaile JM. Rheumatic diseases in North America's indigenous peoples. Semin Arthritis Rheum 1999;28:368-91.
12. Khan MA. HLA-B27 and its subtypes in world populations. Curr Opin Rheumatol 1995;7:263-9.
13. Taurog JD, Dorris ML, Satumtira N, Tran TM, Sharma R, Dressel R, et al. Spondylarthritis in HLA-B27/human beta2-microglobulin-transgenic rats is not prevented by lack of CD8. Arthritis Rheum 2009;60:1977-84
14. Taurog JD, Dorris ML, Satumtira N, Tran TM, Sharma R, Dressel R, et al. Spondylarthritis in HLA-B27/human beta2-microglobulin-transgenic rats is not prevented by lack of CD8. Arthritis Rheum 2009;60:1977-84.
15. Reveille JD, Hirsch R, Dillon CF, Carroll MD, Weisman MH. The prevalence of HLA-B27 in the US: Data from the US National Health and Nutrition Examination Survey, 2009. Arthritis Rheum 2012;64:1407-11.
16. García-Fernández S, Gonzalez S, Miña Blanco A, Martinez-Borra J, Blanco-Gelaz M, López-Vazquez A, et al. New insights regarding HLA-B27 diversity in the Asian population. Tissue Antigens 2001;58:259-62.
17. Ma HJ, Hu FP. Diversity of human leukocyte antigen-B27 alleles in Han population of Hunan province, Southern China. Tissue Antigens 2006;68:163-6.
18. Hou TY, Chen HC, Chen CH, Chang DM, Liu FC, Lai JH, et al. Usefulness of human leucocyte antigen-B27 subtypes in predicting ankylosing spondylitis: Taiwan experience. Intern Med J 2007;37:749-52
19. Chhaya SU. HLA-B27 polymorphism in Mumbai, Western India. Tissue Antigens 2005;66:48-50.
20. Liu Y, Jiang L, Cai Q, Danoy P, Barnardo MC, Brown MA, et al. Predominant association of HLA-B*2704 with ankylosing spondylitis in Chinese Han patients. Tissue Antigens 2010;75:61-4.