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Correlation between the ankylosing spondyloarthritis disease activity score (ASDAS) and status of HLA-B27 and its subtype associated, to assess severity of spondyloarthritis

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Abstract
Introduction: Ankylosing Spondylitis is a chronic and progressive inflammatory disease of the spine and enthesis affecting skeletal and extra skeletal tissues and is largely associated with HLA B27. However, more than 95% of individuals in the general population with this gene never develop Ankylosing Spondylitis. A lot of studies have shown that HLA B27 reveals remarkable polymorphism with an ever-increasing number of alleles and atleast 105 subtypes of HLA B27 is now known. Correlating the severity of Ankylosing Spondylitis using ASDAS score with the presence of a particular subtype of HLA B27 can help in segregating a high-risk population and subjecting them to an aggressive and early onset treatment for greater therapeutic benefits and thus reducing the disability burden on the society.
Objective: To study the correlation between the disease severity and the HLA-B27 subtype associated. Thus, studying the influence of a particular HLA-B27 subtype on the disease severity that would be quantified using ASDAS.
Method: This is retrospective as well as a prospective study in which fifty consecutive patients of Ankylosing spondylitis of 16 years of age and older were recruited into the study along with Fifty healthy controls. HLA B27 typing and subtyping of all patients and controls was done by PCR Sequence specific primer method. Ankylosing Spondylitis Disease Activity Score (ASDAS) was determined using Clinical data of the patients and Erythrocyte Sedimentation Rate (ESR).
The frequencies of different HLAB27 subtype and its comparison with disease severity (ASDAS) were done using Chi-square test and one way Analysis of Variance test. Further, Bonferroni post hoc test was applied for multiple comparisons of each subtype. All statistical analyses were performed using SPSS for windows. Statistical tests were two sided and significant level with $p< 0.05$ was considered significant.
Result: Out of 50 patients of Ankylosing spondylitis, HLA-B27 was positive in 46 individuals (92%) in which the frequency of each subtype was: HLA B2705 (67.4%), HLAB2704 (17.4%), HLAB2702 (8.7%) and HLAB2707 (6.5%). Among the 2 healthy controls who were found to be HLAB27 positive, both of them were of HLAB2705 subtype. Further, One way Analysis of Variance and Post HOC test was used to determine statistical significant difference between the means of Ankylosing Spondylitis Disease Activity Score (ASDAS) for the each HLAB27 subtypes revealed that HLAB2704 is associated with a higher disease severity scores among its natives as compared to the other commonly found subtypes. Although, HLAB2705 by virtue of it being the commonest subtype found in our study was found in all the three severity groups, while HLAB2702 and HLAB2707 was associated with less severe disease states.
Conclusion: HLAB27 association with Ankylosing Spondylitis has been emphasized with HLA B2705 being more prevalent in Indian population. The correlation between the disease severity and the HLA-B27 subtype associated has been established with respect to HLA B2704 subtype .The study highlights that it is possible to predict the course of Ankylosing Spondylitis with the knowledge of the HLAB27 subtype of the patient.

Keywords: HLA B27, ankylosing spondylitis

Introduction
Ankylosing Spondylitis is a chronic and progressive inflammatory disease of the spine and enthesis affecting skeletal and extra skeletal tissues [1, 2]. Leading to deformities particularly affecting young men in the workforce, which leads to a significant health burden to the community [1].

Therefore, the need to diagnose the disease at an early stage and predicting its future and prognosis is of utmost importance.

Ankylosing Spondylitis Disease Activity Score (ASDAS) [3] is used to assess disease activity in Ankylosing Spondylitis based on clinical assessment and levels of an acute phase reactant, C reactive protein (CRP) or Erythrocyte Sedimentation Rate (ESR) [3, 4]

Association of HLA B27 with Ankylosing Spondylitis is known ever since its discovery in 1969. However, more than 95% of individuals in the general population with this gene never develop Ankylosing Spondylitis [5]. In last decade, a lot of studies have shown that HLA B27 reveals remarkable polymorphism with an ever-increasing number of alleles. There are at least one hundred and five known subtypes of HLA B27 that can be encompassed by the numbering system HLA B27 -01 to HLA B27 -106 (B2722 was deleted on the discovery that it had the same sequence as B2706). [5, 6] HLA B27 -05 is most widely distributed disease associated subtype and has been subject of many studies [5]. Correlating the severity of Ankylosing Spondylitis with the presence of a particular subtype of HLA B27 can provide us with a greater insight into the disease and also help in segregating a high-risk population and subjecting them to an aggressive and early onset treatment giving them greater therapeutic benefits and thus reducing the disability burden on the society.

Moreover, since the various subtypes of HLA B27 show variation in frequency among different racial and ethnic groups and due to paucity of such studies in the Indian population, it is imperative for conducting more studies in our Indian scenario.

Material and Method

This is retrospective as well as a prospective study was conducted in a tertiary care hospital, in which fifty consecutive patients of Ankylosing spondylitis of 16 years of age and older were recruited into the cross sectional study. HLA-B27 screening and sub-typing were also performed in the Department of Pathology and Molecular biology of this tertiary care hospital.

Clinical data were gathered from our patients and pathology databases and by review of medical records. Erythrocyte sedimentation rate (ESR) was done for all patients. Based on the clinical data and ESR levels ASDAS score for each patient was calculated. Fifty normal individuals recruited as controls (with no autoimmune diseases and no family history of AS) were also evaluated for HLA-B*27 status and its subtypes

Inclusion criteria

1. Patients of age 16 years and above and both the sexes were included.
2. All patients fulfilled the modified New York 1984 criteria for AS.
3. These patients were managed on NSAIDs only at the time of conducting the study

Exclusion criteria

1) Patients below 16years of age.

2) Patients on DMARDs and other long duration drug therapy

HLA typing

DNA extraction was done from EDTA anti coagulated whole blood sample using QUIGEN DNA isolation kit HISTO TYPE B27 SSP HLA typing was done using (Low resolution) kit and further subjected to gel electrophoresis to identify the presence of HLAB27 gene.

Whole blood sample of patients who were HLAB27 positive were retrieved and HLA B27 subtyping was done using Invitrogen Allset $^{+TM}$ Gold SSP HLA B*27 High Resolution kit. Results of HLA B27 low resolution as well as that of high resolution were entered into the master chart for further analysis.

The frequencies of HLAB27 positivity and also different HLAB27 subtypes were compared using Chi-square test and where needed, Fisher's exact test. Severity of the disease, as assessed by ASDAS groups of the patients was compared to the HLAB27 subtype in the HLAB27 positive group and their significance assessed by Chi square test. One way Analysis of Variance test was used to find significant correlation between each of the HLAB27 subtype and Ankylosing Spondylitis. Disease Activity Score (ASDAS). Further, Bonferroni post hoc test was applied for multiple comparisons of each subtype. All statistical analyses were performed using SPSS for windows. Statistical tests were two sided and significant level with $p< 0.05$ was considered significant.

Result and Analysis

Out of 50 patients of ankylosing spondylitis, there were 47 males (94%) and 3 (6%) females. The median age was 32 years (range 22-60). HLA-B27 was positive in 46 individuals (92%). Among the controls, two individuals (4%) were HLAB27 positive.

Frequency of HLA B27

The frequencies of HLA-B27 between patient and normal population was compared and tested using the chi-square test. There was a significant difference in HLA-B27 frequency between the patients and normal population ($p < 0.001$). (Table 1, 2)

Table 1: Comparison of frequency of HLAB27 in Patients and controls

Crosstab					
			GROUP		Total
			Cases	Controls	
HLA B27	Negative	Count	4	48	52
		% within HLA B27	7.7%	92.3%	100.0%
		% within GROUP	8.0%	96.0%	52.0%
	Positive	Count	46	2	48
		% within HLA B27	95.8%	4.2%	100.0%
		% within GROUP	92.0%	4.0%	48.0%
Total		Count	50	50	100
		% within HLA B27	50.0%	50.0%	100.0%
		% within GROUP	100.0%	100.0%	100.0%

Table 2: Chi Square test showing significant difference in HLA B27 frequency in case and control

		Group		Total	Chi-square value	p-value
		Case	Control			
HLA B27	Positive	46	2	48	77.56	<0.001
	Negative	4	48	52		
Total		50	50	100		

Ankylosing Spondylitis Disease Activity Score (ASDAS) and Disease severity

On the basis of Ankylosing Spondylitis Disease Activity Score (ASDAS), patients with Ankylosing Spondylitis were grouped according to the disease activity.(Table 3)

Table 3: Grouping of Patients into severity categories based on ASDAS and their frequency VAR00001

	Frequency	Percent	Valid Percent	Cumulative Percent
Valid high	23	46.0	46.0	46.0
moderate	17	34.0	34.0	80.0
Very high	10	20.0	20.0	100.0
Total	50	100.0	100.0	

Out of the 50 Ankylosing spondylitis patients, 17 (34%) had moderate disease activity (1.3≤ ASDAS <2.1), 23 (46%) had high disease activity (ASDAS 2.1≤ ASDAS ≤3.5) and 10 (20%) had very high disease activity (ASDAS >3.5).

HLA B27 Subtype

Among the 48 HLAB27 positive group, the frequency of HLA B27 subtypes was compared. (Table 4). 33 (68.8%) were found to have HLA B2705 subtype, 8(16.7%) had HLAB2704 subtype, 4(8.3%) had HLAB2702 subtype and 3 (6.3%) had HLAB2707 Subtype. Among the Ankylosing spondylitis patients the frequency of each subtype was: HLA B2705 (67.4%), HLAB2704 (17.4%), HLAB2702 (8.7%) and HLAB2707 (6.5%).

Table 4: Frequency of various HLAB27 subtype in the HLA B27 positive group

Subtype * Group Cross tabulation					
			GROUP		Total
			Ankylosing Spondylitis	Normal Controls	
Subtype	2702	Count	4	0	4
		% within SUBTYPE	100.0%	.0%	100.0%
		% within GROUP	8.7%	.0%	8.3%
	2704	Count	8	0	8
		% within SUBTYPE	100.0%	.0%	100.0%
		% within GROUP	17.4%	.0%	16.7%
	2705	Count	31	2	33
		% within SUBTYPE	93.9%	6.1%	100.0%
		% within GROUP	67.4%	100.0%	68.8%
	2707	Count	3	0	3
		% within SUBTYPE	100.0%	.0%	100.0%
		% within GROUP	6.5%	.0%	6.3%
Total		Count	46	2	48
		% within SUBTYPE	95.8%	4.2%	100.0%
		% within GROUP	100.0%	100.0%	100.0%

Among the 2 healthy controls who were found to be HLAB27 positive, both of them were of HLAB2705 subtype. Frequencies of the HLA- B27 subtypes between the patients and the normal population were compared and tested separately for each subtype using the Fisher's exact test. The frequency of none of the subtypes was found to be significantly different between the patients and healthy individuals (p>0.05).

Ankylosing spondylitis disease activity score (ASDAS) and HLA B27 subtype

Severity of the disease, as assessed by Ankylosing Spondylitis Disease Activity Score of the patients was compared to the HLAB27 subtype in the HLAB27 positive group, which is summarized in the Table 5 and Chi Square test applied as in Table 6.

Table 5: Comparison of severity of disease activity with associated HLA B27subtype

Disease Activity	2702	2704	2705	2707	Total
Moderate	2(15.38%)	0	10(76.92%)	1(7.6%)	13
High	2(8.69%)	2(8.69%)	17(73.91%)	2(8.69%)	23
Very high	0	6 (60%)	4(40%)	0	10

Among the very high disease activity group, 60% belonged to HLAB2704 subtype while the remaining (40%) belonged to HLAB2705 subtype. HLAB2702 and HLAB2707 subtypes are largely found among the moderate and high disease activity groups. While HLAB2704 subtype was majorly found among the high and very high disease groups. No such trend was noted among the HLAB2705 subtypes.

1. **Chi Square Test**

Table 6: Chi Square test

	2702	2704	2705	2707	Row Totals
Moderate	2(1.13) [0.67]	0(2.26) [2.26]	10(8.76) [0.18]	1(0.85) [0.03]	13
High	2 (2.00) [0.00]	2(4.00) [1.00]	17(15.50) [0.15]	2(1.50) [0.17]	23
Very high	0 (0.87) [0.87]	6(1.74) [10.44]	4(6.74) [1.11]	0(0.65) [0.65]	10
Totals	4	8	31	3	46

The chi-square statistic is 17.5184. The p-value is .007556. The result is significant at p< .05.

2. **One Way ANOVA test**:

One way Analysis of Variance test was used to find significant correlation between each of the HLAB27 subtype and Ankylosing Spondylitis Disease Activity Score (ASDAS)

Table 7: Frequencies of various HLAB27 subtype

Statistics[a]		
ASDAS score		
N	Valid	4
Missing		0
Mean		1.8750
Median		1.9500
Std. Deviation		.35940
Range		.80
Minimum		1.40
Maximum		2.20

a. Subtype =2702
HLA B2702

Statistics[a]		
ASDAS score		
N	Valid	8
Missing		0
Mean		4.0212
Median		3.8900
Std. Deviation		.82791
Range		2.30
Minimum		3.00
Maximum		5.30

a. Subtype =2704
HLA B2704

Statistics[a]		
ASDAS score		
N	Valid	31
Missing		0
Mean		2.5548
Median		2.4000
Std. Deviation		.81969
Range		4.04
Minimum		1.36
Maximum		5.40

a. Subtype =2705
HLA B2705

Statistics[a]		
ASDAS score		
N	Valid	3
Missing		0
Mean		2.3900
Median		2.2900
Std. Deviation		.44844
Range		.88
Minimum		2.00
Maximum		2.88

a. Subtype =2707
HLA B2707

Table 8: One Way ANOVA test

ASDAS score	Sum of squares	Df	Mean squares	F	Sig.
Between groups	17.556	3	5.852	9.547	0001
Within groups	25.745	42	.613		
Total	43.301	45			

One way Analysis of Variance shows that there is a statistical significant difference between the means of Ankylosing Spondylitis Disease Activity Score (ASDAS) for the HLAB27 subtypes. (Table 8)
Further, Bonferroni post hoc test was applied for multiple comparisons. (Table 9)

Post Hoc Tests

Table 9: Post Hoc Test for multiple comparisons.

Dependent Variable: ASDAS Bonferro						
(I)	(J)	Mea deference (1-	Std.	P-	95% Confidence	
					Loer	Upper
270	270	-	4794	.000	-	-
	270	-	.4159	.65	-	.471
	270	-	.5979	1.00	-	1.140
270	270	1.466*	.3104	.000	.606	2.326
	270	1.631*	.5300	.02	.163	3.098
270	270	.164	.4733	1.00	-	1.475

***** The mean difference is significant at the .05

Post Hoc test shows that there is statistically significant difference in the means of ASDAS scores between
1. HLAB2704 and HLAB2702 (p<0.05)
2. HLAB2704 and HLAB2705 (p<0.05)
3. HLAB2704 and HLAB2707 (p<0.05)

This highlights the fact that HLAB2704 is associated with a higher disease severity scores among its natives as compared to the other commonly found subtypes.
Since, there is no significant difference in the t values of the other pairs of HLAB27 subtypes (p>0.05), there does not seem to be statistically significant association between the other HLAB27 subtypes like HLAB2702,HLAB2707 and HLAB2705.
Although, HLAB2705 by virtue of it being the commonest subtype found in our study was found in all the three severity groups, while HLAB2702 and HLAB2707 was associated with less severe disease states.

Discussion

The aim of this study is to understand the correlation between the disease severity and the HLA-B27 subtype associated. Thus, studying the influence of a particular HLA-B27 subtype on the disease severity. In this study, out of fifty patients with established diagnosis of Ankylosing Spondylitis, 46 (92%) of them and 2 (4%) from Control group were found to be HLA B27 positive. This is in accordance with the first study of its kind by Pearson *et al*. [7] conducted at University of California in 1970s, in which 35 of 40 patients with Ankylosing spondylitis showed presence of HLA-B27, suggesting a high association of HLA-B27 with Ankylosing Spondylitis. Similarly, Van der Linden *et al*. in a European population study had shown HLA-B27 was present in 90–95% of patients with Ankylosing spondylitis [8]. This study is also in accordance with the Indian based study by Kankonkar *et al*. which highlighted association of HLA B27 antigen amongst one thousand three hundred and forty clinically suspected patients of Ankylosing Spondylitis (AS) [9] Out of the 50 Ankylosing spondylitis patients, 17 (34%) had moderate disease activity (1.3≤ ASDAS <2.1), 23 (46%) had high disease activity (ASDAS 2.1≤ ASDAS ≤3.5) and 10 (20%) had very high disease activity (ASDAS >3.5). Major result of our study was defining the HLAB27 subtypes in patients with Ankylosing Spondylitis. The two most common HLAB27 subtypes were B2705 (67.4%)and then B2704 (17.4%). The two less frequent HLAB27 alleles in our patients were B2702 (8.7%) and B2707 (6.4%). This result was partially in concurrence with an earlier study by MacLean *et al*. on 133 British patients, which revealed majority (95%)were B2705 and the remainder B2702 [10]. A similar study was conducted in Western India in 2005,wherein,HLA-B27 sub typing identified B2702 (1.43%), B2704 (14.29%), B2705 (70%), B2707 (12.86%)

and B2718 (1.43%), respectively[11] .This study by Chhaya *et al*. based on indian population showed HLAB2705 (70%) and B2704 (14.29%) to be the predominant in our population similar to our present study. Similar study on Indian population by Shankarkumar *et al*. in 2003 showed HLA-B27 subtyping identified B2704 (34.48%), B2705 (36.2%), B2707 (15.51%), B2708 (10.34%) [12]. However, it stands contrary to the study by Taurog JD *et al*. which suggests that HLAB2704 is the most comman subtype among Asians [13], even though it may not truly represent indian scenario.

Our study also demonstrated that severity of the disease, assessed by Ankylosing Spondylitis Disease Activity Score (ASDAS) was controlled by HLAB27 subtype to some extend. Severity marker (ASDAS) had tendency towards higher score in patients with HLAB2704 subtype. A trend toward lesser disease severity in patients was noted with B2702 and B2707 polymorphism. However, the study showed no significant difference between the subtypes B2705, B2707 and B2702 in Indian Ankylosing Spondylitis population for clinical severity of the disease. This is in concurrence with a study by Chavan *et al*. which showed no significant difference between two major subtypes (B2705 and B2704) in Indian Ankylosing Spondylitis population for clinical features. However, a trend in Ankylosing Spondylitis -associated uveitis was found in B2704 positive patients compared with B2705 positive ones (34.78% versus 16.36%, respectively) [14]. Wu *et al*. in their study noted similar findings that the average onset age of disease in patients with the B2704 was significantly lower than B2705 ones (20.45 ± 4.5 versus 26.67 ± 9.95, respectively). They also found that periarticular involvement was more common in B2704 positive patients compared with B2705 ones but without significant differences (83.72% versus 11.63%) [15]. However, Park *et al*. study on Korean patients with Ankylosing Spondylitis revealed no correlation between B27 subtypes (B2704 or B2705) and clinical features or disease severity [16]. Similarly a study in Iranian population by Sassan Falahi *et al*., too demonstrated no significant differences were for severity markers and clinical manifestations between HLAB27 subtypes; although trend toward lower values of severity markers, less intense dorsal kyphosis and less decrease of cervical slope were observed in B2704 and B2707 versus other polymorphisms [17]. Current study demonstrated the relationship of HLA-B27 statuses with severity of disease (confirmed by Ankylosing Spondylitis Disease Activity Score in our study) to some extent. A trend toward higher markers of activity in patients with the HLAB2704 as compared to the other subtypes indicated probably a more severe disease in this group Indian Ankylosing Spondylitis patients. Even though, this relationship is partly dissimilar in different ethnic and geographic populations. This result was in concurrence with Wu [15] and Chavan studies [14]. Genetic factors responsible for clinical features and disease severity of Ankylosing Spondylitis patients may be at least partly dissimilar between different races. That might be the cause of disparities between results of this study with Park [16] and Sassan Fallahi [17] studies in Korea and Iran, respectively.

Conclusion

HLAB27 association with Ankylosing Spondylitis has been emphasized, in accordance with previous studies. With regard to the frequencies of the subtypes, HLA B2705 has been found to be more prevalent in Indian population. The correlation between the disease severity and the HLA-B27 subtype associated has been established with respect to HLA B2704 subtype. However, there does not seem to be statistically significant association between the other HLAB27 subtypes and disease severity. In conclusion, our study highlights that it is possible to predict the course of Ankylosing Spondylitis with the knowledge of the HLAB27 subtype of the patient.

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