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Human Leukocyte Antigen B27 (HLA-B27)

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FREQUENCY OF HUMAN LEUKOCYTE ANTIGEN B27 (HLA-B27) IN CLINICALLY DIAGNOSED ANKYLOSING SPONDYLITIS; A STUDY OF 100 CASES

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ABSTRACT

Objective: To determine the frequency of Human leukocyte antigen B-27 patients with clinically diagnosed ankylosing spondylitis and frequency of peripheral joints' involvement and serum inflammatory markers (raised serum C-reactive protein & erythrocyte sedimentation rate).

Study Design: Descriptive, cross-sectional study.

Place and Duration of Study: Department of Rheumatology Pak Emirates Military Hospital, Rawalpindi for duration of 10 months from Jan 2018 to Oct 2018.

Methodology: We included 100 cases of Ankylosing Spondylitis. Sample was selected by consecutive sampling technique and used "(Modified) New York Classification Criteria for Ankylosing Spondylitis". Blood samples for C-reactive protein & erythrocyte sedimentation rate and Human leukocyte antigen B-27 were analysed by the same pathologist. We analyzed the data by using SPSS version 20.

Results: Mean age of cases was 25 ± 4.5 years. Seventy-seven patients were positive for Human leukocyte antigen B27 and 23 were negative. In 69% patients erythrocyte sedimentation rate was high and in 64% patients C-reactive protein was raised. In 33% patient there was only axial involvement while peripheral arthritis was present in 67% of patients and enthesitis in 60%. Only 5% patients have the uveitis (All positive for Human leukocyte antigen B-27). Only one patient had cardiac involvement.

Conclusion: Ankylosing Spondylitis was found a common disease and study results showed similar trends of positive Human leukocyte antigen B-27 cases as in the rest of the world. Human leukocyte antigen B-27 has a strong statistically significant association with peripheral joint involvement and acute anterior uveitis.

Keywords: Ankylosing spondylitis, Axial spondylitis, Extra articular manifestation, Human leukocyte antigen B-27 (HLA B-27), Inflammatory markers, Peripheral arthritis, Spondyloarthritis, Uveitis.

INTRODUCTION

A group of seronegative arthropathies which includes a number of chronic, progressive, rheumatic disorders and characteristically involves sacroiliac joint and, or peripheral joint (s) is termed as Spondyloarthritis (SpA). Along with articular involvement the patients suffering from these diseases may manifest with extra articular target organ involvement i.e. eye, bowel, heart, lungs and skin[1]. The most commonly recognized prototype of spondyloarthritis is Ankylosing spondylitis (AS) which presents with features of inflammatory lower backache[2]. Its incidence and prevalence vary demographically. Based on "(Modified) New York criteria of classification", 0.44-7.3/100.000 are the incidence rates and 0.007-1.7% are the prevalence rates reported in literature[3]. In 95% of Caucasians with AS have strong association with Human Leukocyte Antigen (HLA) B-27 have been found. Also, Caucasians have higher prevalence of AS[4]. In general population individuals positive for HLA B-27 have 90% more chances to develop AS in their life than those who are negative for HLA B-275. Thus, testing for HLA B-27 in a suspected patient of AS is of utility and gives a diagnostic probability of 90%, if it comes positive.

Moatter *et al*, In his study at Agha Khan University Karachi (South of Pakistan) reported the frequency of HLA in Pakistani population to

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be 1.3%[6]. Mohyuddin *et al*. Conducted a study in Northern Pakistan and found the prevalence of HLA B-27 relatively higher in Kalash and Pathans (4.2 and 9.4% respectively)[7]. Raza *et al*, Reported that frequency of HLA B-27 was 2.1% in Punjabi population; specially in Gujjar cast[8].

The etiology of the disease is not known but the frequency of AS in general population is directly related to HLA B27's prevalence in that population, suggesting a strong association with its pathogenesis[9].

We had the objective to determine the frequency of HLA B-27 in patients with clinically diagnosed ankylosing spondylitis and correlation of peripheral joints' involvement with serum inflammatory markers (raised serum CRP & ESR) and gender.

METHODOLOGY

We conducted this study in Rheumatology department, Pak Emirates Military Hospital (PEMH) Rawalpindi for a duration of ten months from January 2018 to October 2018. This was a cross sectional study in which we included 100 cases of Ankylosing Spondylitis. Sample size was calculated by WHO Sample size calculator with confidence interval of 95% and margin of error 5% and reference prevalence of 1.3%[6]. Minimum sample size calculated from this prevalence was 25 but we took 100 patients of AS. These cases were selected by using consecutive sampling technique and were diagnosed by using '(Modified) New York classification criteria'[10] for AS. The patients who presented with features of inflammatory lower back pain but were not fulfilling the '(Modified) New-York classification criteria' were excluded from this study. Initial clinical review of all the cases involved age, gender, patients' clinical history, duration of symptoms (site and duration of onset of back pain), associated illnesses and detailed physical examination including chest expansion by a rheumatologist. Chest expansion was measured unclothed, in a standing upright, face front posture by a measuring tape in fourth intercostal space and caliper method and a mean of three readings was recorded in each case (normal range: 5.8-7.6 cm). To correctly identify the sacroiliac joint involvement, rheumatologist carefully performed Modified Schober's test and Patrick's test. Spinal Range of movement was measured by using goniometer. Venous blood samples were taken from non-fasting cases at the time of first visit. We measured levels of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) by immunonephelometric method and capillary photometry respectively and were used as inflammatory markers of Ankylosing Spondylitis. CRP was expressed as mg/L while ESR as mm/hr. For diagnostic accuracy and to avoid the bias a single consultant radiologist performed the reporting of digital X-rays (prone position) of the sacroiliac joint (SI). MRI SI joint was performed in doubtful cases. We obtained written informed consent from all the participants.

We documented peripheral arthritis, enthesitis by clinical examination and musculoskeletal ultrasound. Cardiac examination, ECG and 2D echocardiogram were utilized to look for cardiac involvement. A consultant ophthalmologist documented eye features of the disease by performing slit lamp examination.

Institutional ethical review committee (IRB approval # A/28/2019) approved this study. SPSS version 20 software was used to analyze the data. Frequencies and percentages were calculated to express quantitative variables.

RESULTS

Out of total 100 patients there were 84 males and 16 females (Mean age 25 ± 4.5 years). Most frequent encountered patients were from 25-35 years age group. HLA-B27 was positive in 77 patients and negative in 23. In 69 patients, erythrocyte sedimentation rate (ESR) was elevated and C-reactive protein (CRP) was raised in 64 patients. Two third of the AS cases were having elevated inflammatory markers (i.e. ESR & CRP). In 67% of patients, peripheral arthritis was present while 33% patient only had axial involvement. Enthesitis was evident in 60%. Only 5 of patients had acute anterior uveitis (all of them

were positive for HLA B-27). Cardiac manifestation was evident on 2D echocardiogram in only one patient without peripheral arthritis or eye involvement (HLA B-27 was negative). No patient had apical fibrosis. Table showed the frequencies of examination findings and inflammatory markers.

Table: Frequencies of examination findings and inflammatory markers.

Exam and Lab findings	Positive/ Negative	Percentage
Human Leukocyte Antigen B27	Positive	77
	Negative	23
Peripheral Arthritis	Present	67
	Absent	33
Erythrocyte Sedimentation Rate	Raised	69
	Normal	31
C-Reactive Protein	Raised	64
	Normal	36
Enthesitis	Present	60
	Absent	40
Cardiac Manifestation	Present	01
	Absent	99
Apical Fibrosis	Present	Nil
	Absent	100
Uveitis	Present	5
	Absent	95

DISCUSSION

Ankylosing spondylitis (AS) is a part of disease group which share common characteristics and described as an umbrella term Spondyloarthritis (SpA)[11]. AS most commonly involves axial skeleton but may also present with extra-articular manifestations like psoriasis, acute anterior uveitis (AAU), and inflammatory bowel diseases (IBD). SpA are seronegative arthropathies and most of the patients in this disease group test positive for HLA B-27 which also suggests its role in the pathophysiology of disease[12].

AS is a disease of young age group. NA Lodhi et al, in their study on Pakistani population showed a similar trend of male predominance (45 men, 4 women) and involvement of younger age group (mean age 32.5 ± 9 years)[13]. Another large study conducted at AFIP Rawalpindi reported male predominance of 4:1 and mean age of patients being 42 ± 10 years. In Pakistani population the overall prevalence of HLA B-27 is around 5%, like other regional countries[14]. We reported mean age of Ankylosing Spondylitis to be 25 ± 4.5 years and most common age group of 25-35 years with a male preponderance of 5:1 (84% males, 16% females).

Mathilde *et al*, in a large clinical trial by Pfizer evaluated the relevance of CRP in AS. Their study confirmed higher frequency of CRP i.e. 61% in patients with AS and their results also statistically correlated increased CRP with severity of AS. In our study, CRP was positive in 64% patients, results comparable to this large randomized control trial[15]. ESR was raised in 69% cases and only a quarter of patients were clinically diagnosed cases of AS with normal inflammatory markers (CRP and ESR). A poster presented at 2017 ACR/ARHP Annual Meeting shared that 37.1% of AS cases had elevated CRP from USA and Europe each while ESR was elevated in 37.3% (AS) cases of US and 25.4% cases of Europe[16].

Ahsan *et al*, conducted a study at JPMC Karachi, and reported that in 84.4% of AS cases HLA B-27 was positive[17]. Hamid *et al*, reported a slight lesser incidence of positive HLA B-27 in his cross-sectional study i.e. 14 (23.4%). Abdel-rahman *et al*, in a study done at Qatar found out the prevalence of HLA B-27 positive cases amongst clinical AS overall to be 69%. On further categorization of the demographic data, it was reported that 58% of the Pakistanis in that sample were positive for HLA B-27. Amongst the Indians in this study sample, 73% were positive for HLA B-27 and the frequency was also comparable to another Indian study[18]. In our study at 77% of clinically diagnosed AS cases were positive for HLA B-27.

The prevalence of HLA B-27 positive cases varies with ethnicity and races. A systematic review shared that almost 90% of Germanic and Northern Europeans cases of AS were positive for

HLA B-27. Contrary to this Africans has a much higher proportion of HLA B-27 negative cases[2].

Noor *et al*, reported the distribution of HLA B-27 alleles in his paper and discovered that HLA B-27: 07 was the commonest allele in AS cases while HLA B-27: 06 was frequently found in controls. On ethnic classification, HLA B-27: 07 and 03 were predominant subtypes in Pathans and Punjabis respectively[13].

Göksal *et al*, reported 60% patients of AS with axial involvement while 40% cases with peripheral joint involvement and found out that raised Interleukin (IL) 20 along with raised inflammatory markers were found in the confirmed cases of AS and these levels were significantly higher than in cases with axial involvement[3]. Peripheral arthritis was evident in our study in 67% of the patients while only 33% had axial skeleton involvement. In cases with peripheral joint involvement positive correlation was found with HLA B-27 (p<0.05) while most of them had elevated inflammatory markers.

One of the most frequent extra-articular manifestation (EAM) of AS is ophthalmological involvement. Uvea is the middle layer of eye which includes iris, ciliary body and choroid. Uveitis means inflammation of uvea, which is clinically characterized by pain, redness of eye, photosensitivity, blepharospasm, watery eyes, blurring of vision or reduced vision, myoisis and oozed out cells in anterior chamber. Acute anterior uveitis (AAU) is commonest EAM of AS. In 90% cases with eye involvement AAU occurs while posterior uveitis occurs only in 10% cases[4]. A few studies suggest that Anterior uveitis is of autoimmune nature and AS with peripheral arthritis being one a few risk factors for Anterior uveitis. We however, only found 5% cases of AAU with raised serum inflammatory markers (CRP and ESR), positive HLA B-27 and peripheral arthritis. In literature the prevalence of AAU in AS varies from 20 to 30%. One explanation to such a low prevalence in our study is that we found out AAU incidentally on detailed ocular examination of AS cases and secondly AAU lasts only for a shorter duration of time but with a tendency to reoccur in contralateral eye[5].

Cardiac involvement is another important extraarticular manifestation of AS due to high morbidity and mortality associated with it[19]. The prevalence of cardiac involvement in AS varies from 2 (10%) in literature and may present as aortic insufficiency, atrioventricular (AV) block, bundle branch block or aortitis at ascending aorta. The first being the most common[20]. Mitral valve insufficiency is rare but can cause serious complication like heart failure. It is suggested that aortic valve insufficiency may occur because of inflammatory process of cells that ultimately leads to endarteritis around aortic root and valve[21]. It further leads to tissue insufficiency by platelet aggregation and hyperactivity of fibroblasts[22]. In our study only 1 case (1%) had cardiac involvement i.e. aortic valve insufficiency diagnosed at 2D echocardiogram. This case had no ocular involvement, was negative for HLA B-27 and had AS with axial skeleton involvement.

HLA B-27 has 106 alleles in total, moreover these alleles show demographic distribution too. A few of them may have a role in the pathophysiology of the disease while most of them are protective to[23]. The limitation of our study is that we didn't study the further subtypes of HLA B-27 alleles and that's probably the cause of such high prevalence of HLA B-27 positive cases reported in our study. The other limitation to our study is that we couldn't follow up the cases for the incidence of Uveitis in HLA B-27 positive cases which have otherwise higher risk of developing it.

CONCLUSION

Ankylosing Spondylitis was found a common disease and study results showed similar trends of positive HLA B-27 cases as in the rest of the world. AS has a positive correlation with rise in serum inflammatory markers (ESR & CRP). HLA B-27 has a strong statistically significant association with peripheral joint involvement AS and Acute anterior uveitis.

CONFLICT OF INTEREST

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

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