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Research Article

Association of HLA-B*27, Sub Types with Ankylosing Spondylitis and Other Auto-Immune Diseases Using Flow Cytometry and DNA PCR Molecular Typing

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Abstract

Ankylosing Spondylitis (AS) is a disease which primarily affects spine along with other joints. The vertebrae in some cases fuse together and form a rigid structure.

500 clinically symptomatic suspected cases of AS and a few Auto-immune disorders/diseases such as, Psoriasis, Rheumatoid arthritis, Reiter's syndrome, Uveitis, diseases etc. were referred for detection and association of HLA-B27 antigens/alleles/genes/epitopes, including HLA-B7, B40, B 22, B 13, genes. 70 normal subjects, were also studied.

The present study, was carried out using Flow Cytometer (FCM) and PCR-molecular (sequence specific oligonucleotide) techniques. Our findings show a considerable and consistent association of HLA-B27 antigen with AS, irrespective of the community and hygiene or socio-economic conditions.

We found, the people in the age group of 14-40 years were most vulnerable, when severity of disease taken into consideration. Males members showed preponderance over females in positivity with HLA-B27.

Therefore, detection of HLA-B27 could help in diagnosis of Ankylosing Spondylitis.

AS patients and those suffering from other auto-immune diseases such as rheumatoid arthritis, psoriasis, Reiter's syndrome and uveitis. A few cases with inflammatory bowel diseases, colitis, eczema, bacillary or fungal infection, were also found to be positive.

Keywords: Major Histocompatibility Complex (MHC); Human Leukocyte Antigens (HLA)

Introduction

The Major Histocompatibility Complex (MHC), Human Leukocyte Antigens (HLA) system is a Cluster of genes complex (alleles/specificities/proteins) in Human and in Jawed vertebrates with its integral role in the "Immune System" [1].

HLA expression contains more than 2000 genes, and have essential role in the control of self-recognition and thus defends against micro-organism [2]. It is a one of most polymorphic genetic systems in human beings. Polymorphic proteins of HLA vary in peptides, can bind and thus determine the nature of the peptides, that body can amount an immune response [3,4].

This complex genes (proteins) are present on the short arm of Human Chromosome No. 6 at bp. 21 position.

Spondyloarthritis is a group of diseases characterized by inflammation in the spine and joints. It includes Ankylosing Spondylitis, Axial Spondyloarthritis, Enteropathy Spondyloarthris, Peripheral

Spondyloarthritis, Psoriatic arthritis, Reactive arthritis. Ankylosing Spondylitis is a variant of Spondyloarthritis that affects young adults. It is an inflammation in the spine and sacroiliac joints, causes chronic pain and stiffness in the back, peripheral joints and inflammation at the attachment site of tendons and ligaments of the bones.

Psoriatic Arthritis is an anther variant of Spondyloarthritis. The psoriasis of skin is paired with musculoskeletal features of arthritis, affects peripheral joints. Eye inflammation is common with Ankylosing Spondylitis/or psoriatic arthritis.

The Axial Spodyloarthritis (axSpA) is a variant with chronic inflammatory disease, effects sacroiliac joints, spine, TNF interleukon (UL) 17A are key factors.

Spondyloarthritis develops through the interaction of genetic and environmental factors. Patient with inflammatory Bowel (Crohn's) disease (Betty's) [5] may develop Spondyloarthritis suggesting that certain disease mechanisms are shared [5].

The diseases associated with HLA-B27 include Ankylosing Spondylitis (AS), Reactive arthritis, Reiter's syndrome (RS), Inflammatory Bowel disease and Psoriasis [3]. Ankylosing Spondylosis (AS) is characterised by Axial arthritis involving Sacroiliac joints and spine, peripheral arthritis, Entheses with extra-articular features, such as Uveitis, inflammatory Bowel disease and Psoriasis [6,7].

The prevalence of AS in Caucasians has been estimated at 5-100 per 1000 adults. It is important to note that form and degree of disease expressions are extremely variable [3]**.** The frequency of HLA-B27 ranges from 88-90% in AS patients, as compared to in Normal Controls. However, different races show different rates of association [3].

HLA molecules are Polymorphic proteins vary in peptides, they can bind and thus determine the nature of the peptides, the body can amount an immune response. HLA Cl I and Cl II genes are cell-surface 45-kd glycoproteins that bind intra-cellular and extra-cellular respectively. The HLA genes (leukocyte are Body's immune defence [2-4].

HLA - CL I and CL II genes are cell-surface 45-kd glycoproteins that bind intra-cellular and extra-cellular respectively. The HLA genes (Leukocyte antigens) are specialized in presentation of peptides to T-cells and play a key-role in the body's immune defence [2-4].

The most important function of HLA molecule is in the induction and regulation of Immune response. T-cells recognize foreign antigens in combination with HLA molecules. The foreign antigen is processed by and presented on the surface of cell (i.e. macrophages). HLA has peptide-binding cleft, in which antigen is inserted. T-cells interact with foreign antigen/HLA-molecules are activated [4,8]. The TCR react with the foreign antigen in the form of peptide from foreign material and bound to HLA. This foreign peptide fills the groove of HLA and TCR-lies above the two. This allows the loop at the end of TCR-α and β strands to connect both HLA and foreign peptide with complex mentarity dendritic cells. The activated T-Lymphocytes, antigens. CD^4-T-cells are presented by HLA-Cl II molecule Appearance of HLA-Cl II on B-lymphocyte which normally do not express them stimulated by cytokines like interferon G (Shrivastava., *et al.*) [8]. The role of HLA-Cl II molecule is to initiate general immune response. HLA – genes yield heterodimers of two monovalent associated glycosylated peptide chain which are composed of α&β antigens. HLA genotype perform diagnostic role of certain auto-immune diseases because the loci (alleles) controlling all HLA determinant are closely linked with HLA-area encoded by a 4-MP region of human chromosome 6 pb. 21 and it is the most variable region in the human genome (Hildebrand WH; 2013 [28] and Reveil JD; 1994 [40].

There are 3 regions on the human chromosome '6' viz. Cl I, Cl II and Cl III. MHC is a gene region-coding for cell-surface proteins called "CLUSTER" important for "Immune system. The HLA system in human and H2 in the mice located on Short arm of chromosome 6 bp.21 position, occupying genetic region of 4Mbps. HLA antigens are responsible for presentation of foreign-peptides to the immune competent cell - T-cells; HLA-genes complex contributes more than of 10% OF "Genetic Diversity" in human. Most of allelic variant in the HLA-genes are in Exon-2 and 3 for Cl I and Exon 2 for Cl II code for the antigen binding regions of proteins. Difference between different alleles are due to multiple nucleotide polymorphisms (SNPs).

This mechanism of alleles formation is via segmental exchange of alleles at some locus. It could be sequence motif variation, or could have be arisen from recombination (Ref Tx. Immunology).

The association of HLA-B27 with AS was first reported by Schlosstein L., *et al*. (1973), [4] and these complex genes (proteins) are present on the short arm of Human Chromosome No. 6 at bp.21 position [9]. Further, association between HLA antigen (specificities) and increase susceptibilities, to various diseases has been almost established, though the specific mechanism involved in has not been elucidated. However, currently it is believed that HLA specificities are genetic markers of linkage disequilibrium with disease related genes, most likely that regulates immune functions. The association of some of the diseases with HLA - Haplotypes supports this hypothesis [10]. This would mean that combination of specificity type results from genes.

In all auto-immune diseases, the first Human, Leukocyte Antigen (HLA) association with human inflammatory diseases was discovered in 1972, Betty., *et al*. (1978) [5] correlating HLA-B27 with Ankylosing Spondylitis. This remained one of the strongest known associations of the disease with HLA-B27. Since than more than 100 diseases association [5] have been made including among many exclusively occurring diseases and also systemic diseases with specific ocular manifestations of AS, Reiter's syndrome (RS), Reactive arthritis and inflammatory Bowel diseases which are associated with HLA-B27 alleles and other cross reactive alleles/epitopes viz. HLA - B7, B40 (60), B13, B22 etc. [11,12]. These diseases primarily effect sacroiliac joints and spine [11]. The prevalence of AS in Caucasians mentioned earlier, has been estimated 5-100 per 1000 adults [11,13]. It is also to be noted that the form and degree of disease expression are extremely variable Tiwari and Terasaki, (1985) [3].

The association of HLA-B27, with AS was reported by Brewerton., *et al*. in 1973 [9] and was later confirmed by other investigators. In all Auto-immune Diseases. tendency for disease conditions to run in a family is well documented and association of HLA-B 27 is consistent with a dominant or additive mode of inheritance on the same chromosome, that predict a predisposition of certain diseases. Thomson., *et al* (1983) [14] reported that the proportion of HLA-B27 positive individuals who actually develop clinical symptoms and signs of this disease has been estimated to be 1-7%. Further studies carried out by various researchers have shown that HLA-B27 positive relatives of AS patients are much more likely to be affected than unrelated HLA-B27 positive individual [50]. This predicts mode of inheritance reported by Khan., *et al*. 1997 [13,15,16].

Ankylosing Spondylitis (AS) and Reiter's syndrome (RS) have similar features suggesting that they might be auto-immune diseases having a common aetiology. It has also been reported that 95% of AS patients and more than 80% RS patients carry the HLA-B27 alleles, whereas occurrence of these alleles is less than 7% (Kankonkar., *et al*.) [12] in the general population. However, a few monozygotic twins either AS or RS, suggesting that additional environmental factors may also play a role in disease progression [15].

Research Studies carried out in western countries showed that the frequency of HLA-B27 changes with different races living there. As far as Indian population is concerned no such extensive work has been done though scattered reports are available. However, a few monozygotic twins having AS or RS, suggests that additional environmental factors may also play a role in disease progression (Kidd., *et al*. 1977) [17].

Uveitis: Iridocyclitis has been found to be frequently associated with systemic diseases, tuberculosis, Syphilis, Brucellosis etc. Brewerton., *et al*. [9] was the first to report the association between Uveitis and HLA-B27, and since then many other workers have independently confirmed Such an association and have even reported that the patients developing uveitis after Yersinia injection had 100%. association with HLA-B27. This disease has acute onset, unilateral, no granulomatous inflammation, involving the iris, tendency of recurrence. Environmental factor plays a critical role in pathogenesis [18,19]. Chang., *et al*. (2005) [18]. A person suffering from Arthritis can also suffer from AS and associated uveitis (Dashti) [16]. One patient having H/o back pain, neck pain, stiffness, pain in axial skeleton, poly arthritis, knee pain a swelling, R/E glaucoma with total atrophy, gastrointestinal problem, morning inflammation, headache and fever. Other patient has H/o tubectomy of R/E, conjunctivitis L/E and inflammation of iris and ciliary

body with tendency to recurrent attacks. Uveitis is common form of intra-ocular inflammation of the iris, ciliary body choroid which present predominately as Anterior Uveitis [18,20]. Approximately 50% of acute anterior Uveitis cases are associated with alleles of HLA-B27 environmental factors play critical role pathogenesis [19]. It's a granulomatous inflammatory disease [6,20-23].

Psoriasis: Is a skin disease, common symptoms thick skin, patchy typically seen on elbows, knees and lower back, joint stiffness peeling of skin, rashes. It is a long lasting, noncontagious patchy disease. Cardiovascular events as heart attacks and strokes.

Materials and Methods

Total 500 clinically suspected cases of Ankylosing Spondylitis, Polyarthritis, Uveitis, Psoriasis were referred to Histocompatibility and TX Immunology (HLA Lab), Goa Medical College and Hospital, Goa. For detection of HLA-B27 and other related and associated alleles during 2021-2024. These referred cases were tested for detection of HLA-B27 genes (alleles) and a few cases of other autoimmune disorders/diseases. 70 Normal Controls were included for parallel study. The present study was carried out using flowcytometer (FCM). However, inconclusive samples of FCM were confirmed by DNA-PCR, SSO technology on Luminex analyser.

Sample type and collection

Blood samples of patients and normal subjects were collected in EDTA bulb along with their clinical History viz. RA, AS, pain and stiffness of upper limbs, joints, knee, neck and fever, headache, and Shoulder pain, inflammation, gastrointestinal problem, glycoma, total optic atrophy, redness of eyes and nodular Episclaritis along with family history.

Techniques used

Flow cytometry (FCM) technology

Patient's blood samples were analysed using Flow Cytometer - BD FACS via Tm and BD, USA.

Kits and reagents

DNA-PCR SSO molecular typing: Using One Lambda - LAB Screen (Luminex Technology, of Thermo-Fisher Scientific Brand, USA and One Lambda kits, USA. This procedure was for inconclusive results obtained on FCM, to confirm the diagnosis and association with HLA-B27 and its sub-types (alleles).

Principles and procedures: Flow cytometry (FCM) technology

FCM is based on laser Tech. to detect/analyse chemical and physical characteristics of cells or particles, e.g. Bone marrow, peripheral blood and other fluids of body.

HLA-B27 test is a qualitative, two-colour direct Immuno-florescence method for detection of HLA-B27 from erythrocytes lysed blood (LWB). Reagents contains Anti- HLA - FITC and CD3, PE.

The test used to analyse characteristics of cells or particles - suspended in fluid and injected into a FCM Analyser (Becton Dickinson).

Method

- Daily Instrument QC. was determined before, acquisition of samples testing.
- HLA-B27 calibration beads were used to "Set up" Target.
- Decision-Marker was set-up in the BD- HLA-B27 reagent Lot - Suffix.

Software automatically "Gates" the CD3T-Lymphocytes population and displays it in HLA-B27-FITC-"Histogram – Plot".

Florescent intensity produced by fluorescent labelled antibodies specific to protein on/or in cell or ligands that binds molecules measured, such as propodeum iodine binding to DNA.

Procedure

When Antibody reagent is added, the fluorochrome labelled antibodies bind specifically to leukocytes surface antigens. The anti HLA-B27 binds and to HLA-B27 antigens and the CD3 monoclonal antibodies binds CD3 antigens.

The stained samples are then lysed to remove red cells. These cells are washed and fixed before analysis.

- Blood Samples each 3 ml in ADTA bulb were analysed using B.D. lysing solution.
- Samples were acquired on FCM by HLA- B27 acquisition software for 2200 events.
- Data were analysed using BD Software and recorded on the Log Median. Fluorescence (LMF) and displayed on Histogram along with Suffix value.

Interpretation of Results

Indicates: Positive/Negative or Detected/Not Detected.

A sample with Log median fluorescence (LMF) greater than the Decision Marker +10 channels are considered -HLA-B 27 Positive (+). Samples with Less than (<) the Decision Marker, considered HLA-B 27 Negative (-). Sample with LMP greater (>) than/or equal (=) to the Decision Marker and (<) or/ (=) equal to 10 channels above the Decision Marker are considered to be Inconclusive. Inconclusive Results: of B27 on (FCM) were confirmed by Molecular DNA-PCR SSO Sequence Specific Oligonucleotides, typing method of On Lambda, USA. This method confirms results by identifying two-Haplotypes" with alleles. Total 500 clinically referred cases and 70 normal subjects were analysed and studied in the present study.

Multiplex DNA PCR Tech:

- DNA of each pts sample was separated.
- DNA sample was processed for separation of HLA Cl B locus.
- Sample was Amplified and run on Luminex-3D analyser.
- Results of HLA-B-locus with alleles were interpreted.

Procedure

- DNA of test samples were separated using Hi geno MD kits of Hi MEDIA.
- SNA samples were amplified.

PCR: Set Up

- Enter PCR programme on Thermal Cycler.
- Turn on thermal cycler to warm up.
- Thaw DNA, Amplification Primers and D-mixer.
- Adjust concentration of DNA.
- Label microfuge tubes with HLA-B Locus
- Run HLA - Type SSO Programme.

Post-Amplification

- Turn on Thermal Cycler and start 600 C programme to preheat.
- Prepare separate tubes and add Denaturation buffer and Amplified DNA mixture, keep at room temp. for 10 minutes.
- Add Neutralization buffer and add to above solution, mix well and place at 600C for 15 min.
- Give 3 washing with buffer
- Prepare SAPE Conjugate for samples used and place at 600C on Veriti Thermal Cycler for 5 min.
- Wash again with buffer, add 70 ul buffer in each sample and shake
- Acquire the samples with beads on LAB-Scan 3D (Luminex machine) for analysis of HLA-B27 alleles (genes/specificities).

Results

Clinically suspected cases of Ankylosing Spondylitis (Spondylosis) and a few other - Auto-immune disorder or diseases were referred to Histocompatibility and Tx. Immunology- (HLA-Lab) for detection of HLA-B27 and related alleles/specificities and during 2021-2024.

The present study includes 500 clinically and suspected cases with various Complaints and 70 normal subjects were analyzed for detection of HLA-B27 genes/alleles or specificities, including a few other auto-immune diseases using Flow Cytometer (FCM) of BD and Multiplex DNA-PCR molecular SSO technique on Luminex Auto-Analyzer, One lambda – Themo Fisher.

Out of these 500 referred cases with clinical diagnosis of AS (482 cases) and Uveitis (18 cases).

The samples were analyzed on FCM using BD kits. 47 (9.4%) cases were positive, 432 (86.4%) were negative and 21(4.2%) were inconclusive [Ref. Table 1]. Out of these 21 inconclusive cases, 7 patients were found positive for HLA B27 (33%) by molecular PCR Tech. [Ref. Table 2]. Remaining 14 (66%) were border line positive on the basis of decision Marker- Suffix value of Beads. These patients have complaint of joint pain, neck pain, and shoulder pain etc.

The total number of HLA B27 positive cases were 54 with distribution shown in Table 3.

Out of 54 cases which were positive (+) ve for HLA-B27, 51 cases were males (94·4%) and 03 were females (5.6%), Ratio and M: F of 94:6 (Table 1).

Age distribution: Positive male patients were of age group - (10-60) yrs. (93.6%), however, majority were 21-40 yrs. In female - age group, 31-40 yrs. (6.4%), (Table No 2).

Further, 21 (4.2%) patients analyzed on FCM (Flow Cytometer) which were inconclusive, analyses using multiplex DNA –PCR molecular SSO technology.

Out of these 21 inconclusive patients, 7 were found Positive (33%) by PCR method and remaining 14- (66%) were border line positive on the basis of Decision Marker - suffix value of Beads (The Target value). These patients have complaints of joint pain, neck pain, shoulder pain etc.

Uveitis

Total 18 cases of Uveitis were referred for detection association of HLA-B27 antigen, 3 patients were positive (16%), one was inconclusive on FCM and was later confirmed positive (+ve) by PCR tech. (22%). Remaining 14-patients (77%) were negative (- ve) for HLA-B27.

All 18 cases of suspected Uveitis have H/O lower back pain, neck pain, stiffness, pain- in axial skeleton polyarthritis, knee swelling, R/E glaucoma with total atrophy, gastro-intestinal problem, morning inflammation, fever. One of these patients had H/o Tubectomy of R/E, Redness for 8-10 days, nodular Episcleritis | RE, conjunctivitis of L/E.

Psoriasis

2 patients of psoriasis were referred for detection and association of HLA B-27 were found negative.

Table 1: The Results of HLA-B27 on FCM.

Results	No of cases	Percentage
Positive	47	9.4%
Inconclusive	21	4.2%
Negative	432	86.4%
Total	500	100%

Table 2: Age and Sex distribution of HLA - B27 Positive Cases.

Age Group	Male	Female	Total
10-20	07	-	07
21-30	18	-	18
31-40	15	03	18
41-50	09	-	09
51-60	02	-	02
Total	51	03	54
Percentage	93.6%	6.4%	

Table 3: Inconclusive Results of FCM Confirmed on DNA PCR Mol. Tech.

Diagnosis	Technique		Total
	FCM	PCR	
AS	45	6	51
Uveitis	2	1	3
Total	47	7	54

Table 4: LA Typing by using PCR tech. of Inconclusive Cases.

	Alleles of B Locus	
Case (1)	HLA B* 13:01:01:03	B* 27 = 86 (FN)
Case (2)	HLA B* 15:39:01:01	B* 27:05:02:01
Case (3)	HLA B* 27:03	B* 40:06:01:05
Case (4)	HLA B* 27:07:01	B* 40:03:02:01
Case (5)	HLA B* 18:01:01:01	B* 27:04:03
Case (6)	HLA B* 27:05:02:01	B* 40:06:01:01
Case (7)	HLA B* 07:01:01:01	B* 37:01:01:01

Discussion

The Spondyloarthritides (SpA) is a group of auto-immune diseases which includes/shares certain clinical features and association with Ankylosing Spondylitis (AS), Reiter's syndrome (RS), Reactive Arthritis (ReA), Enteropathic spondylitis (Cohn's disease and ulcerative colitis), Uveitis, Psoriatic arthropathy (PsA) and undifferentiated Spondylitis (USpA). These are chronic inflammatory diseases that begin primarily in the sacroiliac joints [25] and goes on to involve the spine and large joints. Reactive arthritis (ReA) is an acute non purulent arthritis complicating infection elsewhere in the body, usually genitourinary infection with chlamydia trichromatic, enteritis due to gram negative Entrobacteria such as *Shigella, Salmonella* or *campylobacter* species reported by Huges and Wright (1973). Some patients usually young adults of 20-40-years age present with features of Spondyloarthritis (SpA) but lack criteria for these diagnosis. For example, a patient may present with inflammatory synovitis of knee, Achilles Tendinitis and dactilitis of one digit or sacroilites in the absence of other criteria for AS. Such patients are said to be having undifferentiated Spondyloarthritis (USpA).

Ankylosing Spondylitis is a variant of Spondyloarthritis (SpA) that affects young adults. It causes inflammation in the spine and sacroiliac joints causing chronic pain and stiffness in the back. This disease is predominantly found in joints and inflammation at the attachment sites of tendons and ligaments to the bones. It develops through environmental factors [15,19,24] and interaction of genetic factors. Patients with inflammatory bowel disease may develop Spondyloarthritis suggesting that certain diseases mechanisms are shared [11,26] Ankylosing Spondylitis (AS) is characterized by axial arthritis, involving sacroiliac joints and spine, peripheral arthritis, Entheses with extra articular features, such as uveitis, inflammatory bowel disease and psoriasis Brown., *et al*. 2016 [11].

AS is a typical Arthritis in which there is a long standing inflammation of the joints of the spine [4,21]. Typically, the spine joints and pelvis also affected. Occasionally other joints such as the shoulders/or hips are also involved. Eye and bowel problems may occur. It is believed to involve a combination of genes and environmental factors reported by Harda SI., *et al*. (2003) [22].

Common symptoms of AS include long standing back pain, stiffness (worsen at night), fatigue, painful swellings, joint heel pain, episodes of eye inflammation.

The human leukocyte antigen (HLA) class I molecules are essential in immune regulation, especially in defence against intra cellular infections (viz. viruses) [2,21]. HLA alleles have evolved as the most polymorphic loci in the genome with 6919 Cl I and 1875 Cl II alleles having been reported as of January 2013 which confirmed susceptibility to specific immune mediated diseases.

MHC has six polymorphic genes HLA-A, HLA-B, HLA-C, HLA-DP, HLA-DQ and HLA- DR, Schlosstein T; and Terasaki I; (1973) [3,4], HLA-AB&C being HLA Cl I molecules, Tiwari., *et al*. (1985) [3] that binds and presents a range of intracellular peptides to cytotoxic CD +8 – T and CD4+ T Cells presented by ClI [27]. It also controls the innate immune response of human body and is significant component of the immune system. They are expressed on the surface of almost all nucleated cells. Class II molecules HLA DP DQ DR are expressed on B- lymphocytes, Antigen presenting cells and dendritic cells. The Class II molecule initiate a general immune response and need to be present on immunological active cells. Hildebrand., *et al*. (2013), [28] and Abbas., *et al*. [10] described B- lymphocytes and macrophages encoded by 4-MP region of human chromosome 6p21 and it is the most variable region in the human genome.

A haplotype is the set of HLA antigens inherited from each parents (Mother/Father) [13]. The studies carried out over last decade have identified long human diseases that are significantly more common among individuals that carry particular HLA genes/specificities. For example, HLA-B antigens (HLA B*07:02 and B*27:05) with AS, HLA DQB1* 06:02 associated with seropositive Rheumatoid arthritis (RA), HLA-DRB*alleles; DR B*:01,04:04:05, and few others that code for sequence motif in the HLA- DR Beta chain called shared Epitopes (SE) are associated with seropositive Rheumatoid arthritis, reported by Holoshitz J; 2013, [29] Han C; [20] Robbinson DW; 2006 [7].

The association between HLA antigens (specificities) and increased susceptibility to various disease has been almost established, though the specific mechanism involved has not been elucidated.

Currently, it is believed that HLA specificities are genetic markers of linkage disequilibrium with disease related genes most likely regulate immune functions. The associations of some of the diseases with HLA Haplotypes, support this hypothesis, Abbas., *et al*. (2014) [10]. This would mean that combination of specificities types results from genes.

Association between HLA – B27 and AS (Ankylosing Spondylitis) was first reported by Brewerton., *et al*. (1973) [9] with other member of the SpA group and was later confirmed by C-lopez-Larea., *et al*. 1995 [30] Kankonkar., *et al*. (1998) [12] and Shankar Kumar., *et al*. (2002 and 2003) [31].

Various studies have shown viz. Han C., *et al*. 2006) [20] that 90-94% of AS patients have HLA B27 alleles positivity, while 5-9% of general population, AS may have other contributory factors for positivity of HLA- B27 [16,20,24,32,33].

For the first time Khan., *et al*. (1997) [13] reported that association of HLA B27- and its CREG antigens HLA-B7, B22, B27, B40,13, and 42 [11-13] with AS patients among American Blacks. Subsequently, these findings were confirmed among AS and RS (Reiter's syndrome) by Arnet., *et al*. (1977) [34]. Similarly, HLA-B27 CREG antigen were found associated with Brazilian USpA patients by Cedoz., *et al*. (1995) [35] from Israel and association with inflammatory arthritis patients from France were reported by Samao-Barros., *et al*. 2003 and Shankar Kumar [31] as an immune mediated chronic disease characterized by inflammation of Axial skeleton as well as extra spinal involvement, and Etio-pathogenesis which was not fully understood. Genetic factors play a key role in [33]. AS due to susceptibility.

As mentioned earlier, the prevalence of AS in Caucasians has been estimated at 5-100 per 1000 adults. It is important to note that form and degree of disease expressions are extremely variable reported by Tiwari., *et al*. 1985 [3]. The frequency of HLA B27 ranges from 88-90% of patients of AS, compared to 4-8% in controls, races show different rates of association [3].

HLA-B 27 is a well-documented genetic risk factor allele (genes/proteins) for AS, examined in the world population. covers over 1000 HLA B*27 alleles reported in 2017 [18]. These include

- B* 27: 02 – European and middle East Jewish Mediterranean population.
- B*27:04 – Chinese [27] Taiwanese and Japanese
- B*27: 03 – In American population, West Africans [35,36,37]
- B*27:05 – In almost all populations (Indian) major in Caucasians, in addition, B*27:01, B*27:02, B*27:08 and B*9 [27,38,39]
- B*27:06 – south Indian and Asian [40-42]
- B*27:07:07- in Sardinia and south Italy
- B*27:07 – May be protective, individuals [30]
- B*27:22 – Rare subtype in south east population [36]
- B*27:06 – Indian and Thai (Indian Normal public patients) [30,42]
- B*27:08 – Relatively rare subtype
- B*27:09 – In Sardinia and south Italy [43]

In addition to HLA B*27:05, most alleles like HLA B*:01, B*:02, B*:03, B*:04, B*:10, B*:13, B*:14, B*15 are known to be linked with AS, (Herbert., *et al*. 2016 [41], Taurog., *et al.* [40]). As far as Association of HLA B-27 alleles in different population in India is concerned, there are very few studies connecting HLA-B antigen with the population and clinical manifestations of AS but there are no reports of HLA-B genotype association in south Indian AS patients (Shankar Kurnar, 2003 [31,40] and Radhia KB;., *et al.* 2008) [25]. All different population studies were described by various investigators [35-37,39,45].

Earlier it was estimated that the population prevalence of AS was 0.25 to 2.2 per 1000 [13]. But recent study by Colin and Fries [46] reported that AS positive healthy males and females have striking prevalence. However, it must be noted that, form and degree of disease expression are extremely variable [1,3]. The frequency of HLA B27 ranges from 88-90% of patients of AS compared to 4-8 in controls. However, different races show different rates of association Tiwari JI; 1998., *et al* [3] and Kankonkar., *et al*. 1998 [12].

In our study of 500 clinical suspected cases, we found 50 cases were B27 positives, showing positivity rate of 10%. Age at presentation play an important role in the positivity and association with HLA B 27 and AS cases [47]. We also observed that the disease was active in the age group of 14-47 years (except one case of 60 years of age). Similarly, Achuthan., *et al*. 1990 [48] and Kankonkar., *et al*. 1998 [12] reported that the disease was found active in patients of age group 20-40 years, Van der linden., *et al*. 1977 [47] and Woodrow and Esmond 1978 [49] have reported that the overall age of onset of clinical symptoms was definitely higher in the HLA-B27 negative patients as compared to the HLA- B 27 positive patients.

In normal subjects, we found frequency of HLA-B*27 in 13 patients (18.5%) and B*40 in 16 patients (22.8%) and B*7 and B*40 both antigens together in a same patient was found in 2 patients (2.8%).

In all autoimmune diseases, the tendency for the disease condition, to run in the family is well documented (Thomson., *et al*. 1983) [14] and the association of HLA- B27 with AS is consistent with a dominant or additive mode of inheritance. It has also been reported that HLA -B 27 positive relatives of AS patients are much more likely to be affected than unrelated HLA-B27 positive individuals [14,23].

We did not test related members hence; this phenomenon could not be confirmed in the present study.

Our study shows, a preponderance of males over females in HLA B*27 positive patients: 47 males patients (age group 14 - 47) and 3 females (33-37 age group) (Table 2). Reasons for these findings could be that the woman in our country (Indians) do not complain of their symptoms and neglect their health and seek no medical advice. This finding is contrary to the findings of Vander Linder., *et al*. (1978) [47], Kankonkar., *et al*. (1998) [12].

It has also been reported that association of Vitamin D and HLA - B27 is an important factor for collaborative clinical manifestations. Deficiency of Vitamin D can cause problem in bones viz. reduced density and weakening [21,26] and shows symptoms relating to spondylitis. Vitamin B12 deficiency causes anaemia which is also one of the symptoms of AS [19,21,26]. In our study, we could not study deficiencies of vitamin D and B12 in the positive patients.

Iridiocyclities has been found to be frequently associated with systemic diseases, Tuberculosis, Syphilis, Brucellosis. Brewerton.,

et al. [9] were to first to report the association between uveitis and HLA –B27 and since than many other workers have independently confirmed such an association and, have even reported that patient developing uveitis after Yersinia infection had 100% association with HLA B27.

In the present Study, we received 4 cases with redness of eyes, low vision, rash on the body.

These were found negative for HLA- B27 antigen.

Uveitis is the most common form of intra ocular inflammation of irisciliary body or choroid, which presents predominantly as anterior uveitis (Chang., *et al*. 2005) [18], Han C [20]; Robinson., *et al*. (2006) [7]. Acute uveitis accounting for approximately 90-95% of all cases. The disease is typically acute in onset, unilateral, non-granulomatous inflammation with recurrent attacks. Approximately 50% acute anterior uveitis cases are associated with the alleles of HLA-B*27. Environmental factors play a critical role in the pathogenesis [19], Chang., *et al*. 2005 [18]; Marceilla MC., *et al.* 2016 [32], Dashti., *et al*. 2018 [16], Sharman N., *et al*. 2019 [24]. PCR Molecular Typing confirms allelic association with HLA-B*27. (Tekeuchi, N., *et al*. 2015). In the present study, 17 suspected cases of uveitis were referred for detection and association for HLA-B*27 and alleles. Out of 17cases, 3 patients were found positive (17%), 14 patients (82.5%) were negative.

Various studies have shown (Han C., *et al*. 2006) [20] that 90-94% of AS patients have HLA-B27 alleles positivity, while 5-90% of general population, AS may have other contributory factors for positivity (Rober [20,24,28,29,33,37]).

Identification of HLA-B27 by PCR technique supports the diagnosis of AS in symptomatic individuals and negative/inconclusive results exclude the diagnosis. This disease, we observed, in our patient's acute onset unilateral granulomatous involving iris and tendency of recurrence, looks environmental factors could be playing a critical role in pathogenesis (Chang., *et al*. 2005) [18].

Role of HLA -B alleles and clinical presentation of B27 Negative Spondyloarthritis patients from Mumbai showed, frequency of HLA B*07 was significantly found increased, whereas frequently of B*40 was decreased. It was concluded that B*07 was associated with B*27 negative Spondylosis-Arthropathy from western India.

This is our initial study. It is in agreement with the findings of many other authors (workers) who have shown a considerably high association of HLA B27 with Ankylosing Spondylitis (*AS*).

Hence, study testing for detection of HLA-B27 antigen/ alleles and other autoimmune diseases could be to useful.

We could not study in our referred cases, community wise and family wise association of HLA-B27 and other auto immune diseases; we analysed as and when cases were referred by clinicians from various departments to confirm clinically suspected cases of auto immune diseases for HLA B27.

Conclusion

Hence, for the diagnosis of AS, HLA-B27 and alleles/Subtypes association are important and conventional PCR technology is a promising diagnostic method could be considered.

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