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Clinical Efficacy of HLA B27for Diagnosis of Ankylosing Spondylitis

KEYWORDS: HLA-B27; Ankylosing spondylitis

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ABSTRACT ***Aim:*** *The aim of our study was to find an involvement of HLA-B27 specific allele in relation to age & sex in the symptomatic & suspected cases of ankylosingspondylitis(AS) using Sequence Specific Allele PCR (SSA-PCR) method.*

Study design: *A hospital based cross sectional study was conducted on pt's having chronic low back ache attending the Outpatient department of Orthopaedics, Government Meical College Jammu. 67cases (47male & 20 female) in the age group (15-40 yrs) suspected of AS were selected randomly for this study. They were then divided into two groups according to age & sex 15-35 yrs & 36-60 yrs and were subjected to SSA-PCR for detection of HLA-B27.*

Methodology: *All the 67 cases both male & females suspected of AS were selected and sequence specific allele-PCR technique was used to detect the HLA-B27 specific allele.*

Specimens were collected from patients attending the Out Patient Department of Orthopaedics. Whole blood samples were collected from suspected pt's with symptoms of low backache and the samples were transported at 4°C to the laboratory for further processing. Silica column based nucleopore DNA extraction kit was utilized for high yield nucleic acid extraction. Eluted DNA was used for amplification of sequence specific allele of HLA-B27 by PCR method. Then Agarose gel electrophoresis was performed, the gel was stained with Ethediumbromide which gets intercalated into DNA, and fluoresce under U.V light. After electrophoresis gel is illuminated with an U.V lamp. The ethidium bromide flueseces reddish orange in the presence of DNA, since it has intercalated with the DNA. The gel is then photographed with a digital or Polaroid camera fitted inside the gel imager.

Result: *The study showed that, out of 47 males included in the study 17 males (36.2%) were detected +ve with HLA-B27 and 4 females (20%) out of 20 females were HLA-B27 +ve. Out of the 47 males, 24 males lying in the age group (15-35 yrs) 10 were HLA-B27 +ve & 7 out of 23 males in the age group (36-60 yrs) were +ve for HLA-B27.Of the 12 females in (15-35 yrs), 3 were +ve for HLA-B27 and 1 out of 8 females in (36-60yrs) was +ve for HLA-B27.*

Introduction:

Human leukocyte antigen- B27 is a class I surface antigen encoded by B locus in the major histocompatibitity complex (MHC) on the short (p) arm of chromosome no.6 at position 21.3 from base pair 31,429,845 to base pair 31,432, 923 and presents microbial antigens to T cells(1).

Ankylosing spondylitis is a long term disease that involves inflammation of joints between the spinal bones and joints between the spine and pelvis(2). These joints become swollen and inflamed as ankylosing spondylitis is a form of chronic inflammation of the spine and sacroiliac joints. Chronic inflammation in these areas causes pain and stiffness in and around the spine. Over a period of time, chronic inflammation of spine (Spondylitis) can lead to a complete cementing together (fusion) of the vertebra, a process referred to as ankylosis(3). Ankylosis leads to loss of mobility of the spine.The disease starts in the sacroiliac joints and spreads to the spine in the majority of patients.2 The axial inflammation comprises sacroiliitis, spondylitis, spondylodiscitis, and spondylarthritis.3 Another major characteristic and pathognomonic sign of AS is new bone formation. Osteoproliferative processes occur often at previously inflamed areas and are detected by imaging techniques as syndesmophytes, calcification, and ankylosis of spinal joints, entheses, and ligaments. These structures are subject to different imaging procedures for assessments of the diagnosis and the course of the disease.4 Conventional radiography which has been the "gold standard" in the imaging of AS for the past decades has been included in the internationally well accepted ASAS core set of assessments in AS.5

Pelvic x ray measurements are critical for a diagnosis of AS6 and spinal x ray measurements have been used to quantify spinal lesions. Scoring systems such as the Stokes AS spinal score (SASSS7), the modified SASSS,8 and the Bath Ankylosing Spondylitis Radiological Index (BASRI9) have been evaluated. In a recent study the SASSS and the BASRI were found to be reproducible, but both had a rather low sensitivity to change.10 Furthermore, the modified SASSS was found to be the most reliable in comparison with the original SASSS and the BASRI for scoring chronic spinal lesions in AS.11 All three x ray scoring systems assess only parts of the spine—the SASSS only the lumbar spine, the modified SASSS the cervical and the lumbar spine, and the BASRI the lumbar and the cervical spine and the sacroiliac joints.

Magnetic resonance imaging (MRI) can visualise both acute and chronic sinflammation in the sacroiliac joints,12–14 in peripheral joints,15 entheses and the spine,15,16 of patients with AS and other spondyloarthritides. Regression of active spinal lesions after treatment with anti-tumour necrosis factor (TNF) agents has been described by several groups.17–19 Recently proposed a new scoring system to evaluate MR images in the acute and chronic stages of AS.20 The activity score proposed in that system was shown to be reliable and sensitive to change already after 3 months of anti-TNF therapy.20

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The erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP) are the two most common laboratory measurements of systemic inflammation in clinical practice. Both of them have been shown to be useful indicators of disease activity in other rheumatic diseases such as rheumatoid arthritis21. The ESR measurement is a simple measurement of the velocity (in mm/ hr) of sedimentation of erythrocytes in anticoagulated freshly drawn blood in a standardized vertical tube. Inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and IL- 1 stimulate the liver to produce acute phase reactant proteins (fibrinogen, immunoglobulins, hapto-globin, CRP and others). These proteins, in particular fibrinogen and immunoglobulins, increase the dielectric constant in the blood, allowing erythrocytes to form rouleaux and increasing the velocity of their descent in the tube (22). The function of CRP is unknown. It is produced in the liver and found only in low levels in normal human serum. High levels are found during acute infection, tissue injury, or inflammatory disease. The molecule is capable of activating the complement cascade and initiate both the opsonic and lyticpotentials of this system23-25. These characteristics suggest that CRP may play an important role in the mediation of inflammatory responses.

Ankylosing spondylitis is also a systemic disease, it can cause inflammation of other joints as well as other organs such as eyes heart, lungs and kidneys(26).

Ankylosing spondylitis is 2 to 3 times more common in men than in women. It affects all age groups, including children when it affects children, it is referred to as juvenile Ankylosing Spondylitis. The most common age of onset of symptoms is in the 2nd & 3rd decades of life & affects usually male in the ratio of 2:1. The incidence of HLA B27 is approximately 7% in female population(27).

The tendency to develop A.S is believed to be genetically inherited, & a majority (nearly 90%) of people with AS are born with a gene known as HLA-B27 gene.

Although exact cause of A.S is unknown but genetics plays an important role. Most individuals who have A.S also have a gene that produces a genetic marker, which is a protein called HLA-B27, which is a major histocompatibility complex class I molecule, that is strongly associated with the disease. A.S.HLA-B27 gene is a perfectly normal gene found in 8% of the general population. Only 2% of people born with this gene will eventually develop spondylitis.

Material & methods:-
67 samples of blood collected from routine pt's attending the OPD of Orthopaedics of Government Medical College with Lower Back Ache from Jan 15th 2 till Aug 15th(8 months). These samples were selected randomly and in house sequence specific Allele PCR technique was used to detect HLA-B27 Sepcific allele.

Sample Preparation:-
Whole Blood:- Samples were mixed on a rotating mixer for 1 hr and an aliquot of 0.5µl was removed & mixed with 6µl of formamide and 3.5µl of water and incubated at 95°C for 5 min's.

HLA-B27 Specific Amplification:-The test primers are (5' primer, B 27ex 294 F: 5'CTACGTGGACGACAC GCT-3'; 3'primer, B27 ex 2199RC: 5'-AGTCTGTGCCTTGGCCTTGC-3') and amplify a region of 141 bp in exon 2 of HLA-B. The primers are specific for all subtypes of HLA-B27 (HLA-B 2701-HLA B2713) except HLA-B2712. The control primers HGH I (5'-CAGTGCCTTCCCAACCATTCCCTTA-3') and HGH2 (5,-ATCCACTCACGGATTTCTGTTGTGTTTC-3') have been described previously and amplify a conserved region in the human growth hormone gene, yielding a product of 439 bp(28)

PCR was performed in a final volume of 50µl and contained 1µM of each test primer, 0.4 µM of each control primer, 240 µM of each dNTP, 10mM MgCl2, 0.01% gelatin, 0.01% Triton X-100, 2.5 U of Amplitaq polymerase (PE Biosystems, Foster city, USA), and 10µl of the sample mix.

PCR was performed on Bench top model 9600 thermocycler with an initial denaturation fo 94°C for 5min, 35 repetitive cycles of amplification (30s at 94°C, 30 s at 61°C and 72°C for 30 s) & a final elongation at 72°C for 5 min.

The products were resolved by electrophoresis in a 2 % agarose gel containing ethidium bromide at 150 V for 15-20 minutes.

Results:
A total of 67specimens (47 males& 20 females) were included in the study. Out of 47 males included in the study 17 males (36.2%) were detected +ve with HLA-B27 and 4 females (20%) out of 20 females were HLA-B27 +ve.

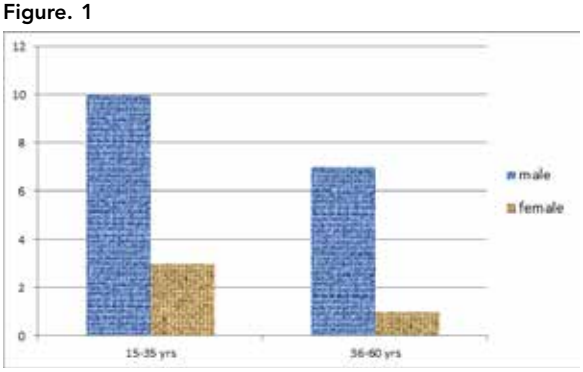
Of the 47 males, 24 males lying in the age group (15-35 yrs) 10 were HLA-B27 +ve & 7 out of 23 males were +ve in the age group (36-60 yrs).

Of the 12 females in (15-35 yrs), 3 were +ve for HLA-B27 and 1 out of 8 females in (36-60yrs) was +ve for HLA-B27. These results are depicted in Table. 1 and shown graphically in Figure. 1.

Table. 1

S.No	Age group	Males (47)		Age group	Females (20)	
		+ve (17) (36.2%)	-ve (30)		+ve (4) (20%)	-ve (16)
1	15-35yrs (24)	10	14	15-35yrs (12)	3	9
2	36-60 yrs(23)	7	16	36-60yrs (8)	1	7

Figure. 1



Discussion:
The HLA-B27 gene appears only to increase the tendency of developing AS, while some additional factors perhaps environmental are necessary for the disease to appear or become expressed. While 7% of the United States popula-

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tion has the HLA-B27 gene only 1% of the population actually has the disease A.S. In Northern Scandinavia 1.8% of the population has A.S, while 24% of the general population has the HLA B-27 gene(29).

Testing for HLA-B27 is of clinical importance for the diagnosis of Ankylosing Spondylistis . Excluding HLA-B27 virtually excludues A.S(30).

It has been well established that association of the HLA-B27 antigen in 90-95% of pt's with A.S are HLA-B27 specific allele +ve(31). Identification of HLA-B27 by PCR supports the diagnosis of A.S in symptomatic individual and –ve results exclude the diagnosis(32). Studies show that 90-94% of AS sufferers have HLA-B27 allele +ve, While 5-9% of the general population with AS may have other contributory factors for positivity of HLA-B27. SSAP is a novel, rapid, cost effective and standard method for the detection of HLA-B27 alleles(33).

Serological techniques such as microcytotoxicity and flow cytometry for testing HLA-B27 require viable cells that adequately express HLA-B27 and may give false –ve results if HLA-B27 is down regulated or masked. Flow cytometry is rapid and relatively inexpensive, but lacks specificity, especially in presence of Ag's that cross-react with HLA-B27, such as HLA-B7(31).

Present observations confirm the significance of HLA-B27 allele as a new, rapid molecular markers for diagnosis of A.S.

Conclusion:

HLA-B27 gene, is strongly associated with A.S. It is one of the most frequent gene investigated by clinicians for diagnosis & prognosis of A.S, besides an increased ESR, Anaemia, Xray and bone scan showing characteristic changes. The PCR SSAP technique used in this study is reliable, simple, convenient & more cost effective for routine laboratories. It is easy to perform & handle the specimens.

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