# A Treatise about Anomalous Laboratory Investigation results accompanying HLA-B27 positive Higher age group population

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## ABSTRACT

HLA-B27 test is generally positive in spondyloarthritis (SpA) and ankylosing spondylitis (AS). Several anomalous laboratory test results are frequently found in HLA-B27-positive patients. In this study, we intended to evaluate two groups of HLA-B27 positive patients- one group belonging to the 13-40 years age group and another group belonging to the 41-71 years age group. The rationale of this partition was based on the age when AS first emerged and the age when the disease was set up for quite a few years respectively. We anticipate alterations of several familiar laboratory test outcomes between these two groups. After our analysis, we found that in the upper age group, ESR and uric acid are significantly decreased, while SGPT, creatinine, and HbA1C are significantly increased. Other values like ct value in PCR, RA seropositivity, and TC of WBC are decreased a bit in the higher age group; haemoglobin and CRP also marginally increased in this set which are not statistically significant. The plausible explanations behind these changes are discussed.

**Keywords:** HLA-B27 test, spondyloarthritis, ankylosing spondylitis, PCR, ESR, uric acid, SGPT, creatinine, HbA1C.

## INTRODUCTION

The surface of white blood cells has a particular protein antigen known as HLA-B27 (human leukocyte antigen B27). It is a component of the class I major histocompatibility complex (MHC) molecules, which are essential to the immune system because they expose immune cells to antigens. HLA-B27 has a strong correlation with several inflammatory and autoimmune disorders, especially spondyloarthritis (SpA) and ankylosing spondylitis (AS).

Although it raises the risk, having the HLA-B27 gene marker does not guarantee that a person will get these illnesses. Although the precise connection between HLA-B27 and these disorders is not entirely understood, it is thought that HLA-B27 may cause aberrant immune reactions that result in tissue damage and inflammation, particularly in the joints and spine. In people who have the HLA-B27 marker, a genetic predisposition along with environmental variables probably plays a role in the development of various illnesses [1].

The spondyloarthritides (SpA), of which ankylosing spondylitis (AS) is the prototype, are a category of prevalent inflammatory rheumatic disorders that are strongly associated with HLA-B27. The SpA collectively has an impact on 0.6–1% of adult US citizens. It is important to know how HLA-B27 naturally functions, the diseases it has been linked to, and the current hypotheses on its pathogenic role, which despite much research is still unknown.

Epitopes or antigens displayed by histocompatibility antigens (HLAs) on antigen-presenting cells (APCs) control adaptive immunity. Particular immunological receptors on lymphocytes (T cell — TCR; B cell — BCR or immunoglobulins) specifically recognize this peptide-HLA (pHLA) combination. One of these illnesses where immunological repertoire research could have a significant effect is ankylosing spondylitis (AS). The 'seronegative spondyloarthropathies', which also include psoriatic arthritis, reactive arthritis (caused by bacterial gastrointestinal or urinary infections), and arthritis complicating inflammatory bowel illness, include AS as its model disease. These diseases affect between 2-3% of people of European heritage and at least 1% of people in Asia overall [2]. Significant back pain, stiffness, decreased function, and eventually the fusion of the spine and pelvis are symptoms of AS. Early-onset axial spondyloarthritis, also known as non-radiographic spondyloarthritis (nr-axSpA), is how AS first manifests in early adulthood. Subsequently, typical sacroiliac radiographic abnormalities appear, which help to define the progression of AS. Psoriasis, inflamed bowel illness, and acute anterior uveitis are common extra-skeletal connections with AS. Depression and anxiety are negatively correlated with disease activity indicators, and AS has a lifelong negative effect on patients [3]. The function of the MHC gene HLA-B27, a member of the HLA Class I family, is to provide CD8 T lymphocytes with peptide antigens.

There is strong evidence of gene-gene interaction between the HLA-I risk allele and ERAP1 in AS, psoriasis, and Behcet's disease, indicating that they must work closely together to influence disease risk[4,5]. The HLA-I antigenic processing and presentation (APP) pathway involves the M1-aminopeptidase genes ERAP1 and/or ERAP2.

The most typical extra-articular AS manifestation is uveitis. Additionally, many AS patients also suffer from inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. Axial/peripheral arthritis, on the other hand, is the most prevalent extra-intestinal consequence of IBD, particularly in individuals with CD[6,7]. Nearly 50% of AS patients have latent gut inflammation, and 15% of IBD patients have peripheral SpA; both disorders can manifest simultaneously or sequentially [8,9]. There is significant overlap among the genetic risk factors for AS, CD, and PsA, including IL23R, IL12B, STAT3, ORMDL3, and CARD9, in addition to similar and overlapping illness manifestations [10].

This study aimed to find out abnormal laboratory test results associated with HLA-B27 test positive results in a population of Eastern India.

## MATERIALS AND METHOD

**Sample Types:**

EDTA Whole Blood samples were collected from suspected patients advised by the doctors for the HLA-B27 test as a routine laboratory procedure. Patients' demographic and clinical records were noted. In this study, all samples and records were maintained anonymous as per Institutional Ethical Committee guidelines.

**Nucleic Acid Extraction:**

This was done with the QIAamp® DNA Mini kit following all the instructions provided in the Manufacturer's kit.

**Real-Time PCR test:**

This was done with TRUPCR® HLA-B27 Real-Time PCR Kit (Kilpest India Ltd. Govindpura, Bhopal, India, Marketed by - 3B BlackBio Biotech India Ltd. Govindpura, Bhopal, India). Both Qiagen Rotor-Gene Q Real-time PCR & BIORAD CFX96 Thermal cycler were used for this study. A Real-Time Amplification test for the qualitative detection of the HLA-B27 allele in human blood is the HLA-B27 Real-Time PCR Kit. The sample's DNA is extracted, amplified in real-time, and detected with fluorescent reporter dye probes designed to look for the HLA-B27 allele. An oligonucleotide probe that is specific to the target DNA sequence is used in real-time PCR to produce the fluorescence signal. On its 5' end, the probe has a fluorescent dye molecule, and on its 3' end, a quencher molecule. One of the chains of the amplified fragment and the probe hybridize.

The Taq DNA polymerase, which has 5' - 3' exonuclease activity, cleaves the probe during the synthesis of a complementary chain. Because of the separation of the fluorescent dye and quencher dye, the total fluorescence of the reaction volume rises in direct proportion to the number of amplicon copies produced by the PCR. Every cycle of the reaction is monitored for the fluorescent signal, and the resultant curve is used to calculate the threshold cycle value. The threshold cycle enables qualitative comparisons between the examined and control samples because it is proportionate to the starting number of DNA copies in a sample. The study results are given in Table 1 and Table 2.

**Table 1: Some common laboratory test results in HLA-B27 positive cases.**

|  | Age group (13-40 years) Mean±SD | Age group (41-71 years) Mean±SD |
|---|---|---|
| ct value | 26.53 +/- 3.21 | 27.15 +/- 2.92 |
| RA (Seronegative/Seropositive) | 11.02 +/- 6.16 | 8.71 +/- 0.29 |
| Haemoglobin (gm/dl) | 12.94 +/- 2.02 | 13.11 +/- 1.55 |
| TC of WBC (/Cu mm) | 9267 +/- 2463 | 8204 +/- 2094 |
| Neutrophil (%) | 62 +/- 11 | 66 +/- 7 |
| Lymphocyte (%) | 27 +/- 7 | 27 +/- 7 |
| ESR (mm) | 32.6 +/- 28.63 | 18.91 +/- 12.35 |
| SGPT (U/L) | 29.25 +/- 12.94 | 38 +/- 18.48 |
| Creatinine (mg/dl) | 0.71 +/- 0.11 | 0.82 +/- 0.16 |
| Uric acid (mg/dl) | 5.16 +/- 1.26 | 4.35 +/- 1.23 |
| HbA1C (%) | 5.47 +/- 0.52 | 5.85 +/- 0.15 |
| CRP (mg/dl) | 19.27 +/- 15.1 | 22.4 +/- 25.54 |

**Table 2: Statistical analysis of the common test results in the two defined groups of HLA-B27 positive cases.**

|  | t value | p value |
|---|---|---|
| ct value | 0.801 | 0.43 |
| RA (Seronegative/Seropositive) | 1.905 | 0.06 |
| Haemoglobin | 0.367 | 0.71 |
| TC of WBC | 1.828 | 0.07 |
| Neutrophil | 1.656 | 0.1 |
| Lymphocyte | 0 | 1 |
| ESR | 2.304 | 0.02 |
| SGPT | 2.295 | 0.02 |
| Creatinine | 3.36 | 0.001 |
| Uric acid | 2.599 | 0.01 |
| HbA1C | 3.625 | 0.0006 |
| CRP | 0.636 | 0.53 |

## RESULTS AND DISCUSSION

It is well known that males are more HLA-B27 positive than females [11, 12]**.** In this study, 80.9% of patients were males in the 13-40 years age group and 76.9% were males in the 41-71 age group. This difference is not statistically significant and thus in all subjects, 79.4% were males. Although the average ct value in PCR was slightly higher in higher age groups, this difference was not significant. This may indicate a mild decrease in genetic expression in the higher age group, which is quite natural. Similarly, differences between haemoglobin levels, TC of WBC, percentages of neutrophils and lymphocytes, and CRP levels were not significant (Table 1, 2). A mild increase in haemoglobin may be due to a good balanced diet in the higher age group in this population. However, haemoglobin is usually decreased in the higher age group and HbA1C is increased in the higher age group [13]. The decrease of TC of WBC may be due to senility and increased CRP and neutrophils may be due to relatively increased inflammation in higher age groups. In this study, ESR was significantly decreased in the higher age group. It is important to note that AS usually begins in the age group 15-30 years with more activity of the disease at the beginning when ESR is significantly high [12], in the higher age group the activity decreases and thus ESR also decreases. In normal subjects in some studies, SGPT is mildly decreased in higher age groups [14]. In this study, SGPT is significantly increased in the higher age group, which may be associated with altered liver function in higher age groups in HLA-B27 positive cases. Creatinine level is mildly increased in higher age groups in normal subjects, but the difference is not so pronounced [15]. The increase in creatinine in the higher age group in this study is attributed to impaired renal function, which is common in this group. There is an increased tendency of uric acid with age [16]. In this study, however, uric acid is significantly decreased in higher age groups, which may be due to changing dietary habits.

## CONCLUSION

HLA-B27 test is usually positive AS and SpA. In this study, among the two groups of HLA-B27 positive patients- one group belonging to the 13-40 years age group and another group belonging to the 41-71 years age group, in the upper age group, we found ESR and uric acid are significantly decreased, while SGPT, creatinine, and HbA1C are significantly increased.

## CONFLICT OF INTEREST

The author declares no conflict of interest.

## AUTHOR'S CONTRIBUTION

Bipasha Dey Sutradhar and Joydeb Mallick carried out the experiment. Satadal Das designed the study procedure and analyse the data. Bhaskar Narayan Chaudhuri and Partha Guchhait reviewed and edited the manuscript.

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