OPEN ACCESS

Saudi Journal of Biomedical Research
Abbreviated Key Title: Saudi J Biomed Res
ISSN 2518-3214 (Print) | ISSN 2518-3222 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

A Treatise about Some Anomalous Laboratory Investigation Results Accompanying HLA-B27 Positive Higher Age Group Population

Jeegisha Verma[1], Bhaskar Narayan Chaudhuri[2], Partha Guchhait[2], Bipasha Dey Sutradhar[3], Joydeb Mallick[3], Ahana Talukdar[1], Isha Majumdar[1], Arup Kumar Dawn[2], Satadal Das[2*]

[1]Dept. of Biotechnology, Kalinga Institute of Industrial Technology, Bhubaneswar, Odisha - 751024, India
[2]Dept. of Microbiology and Molecular Biology, Peerless Hospitex Hospital and Research Center Limited, Kolkata-700094, India
[3]Dept. of Microbiology, Swami Vivekananda University, Barrackpore, West Bengal- 700121, India

DOI: 10.36348/sjbr.2024.v09i04.003 | **Received:** 11.05.2024 | **Accepted:** 26.06.2024 | **Published:** 29.06.2024

***Corresponding author:** Satadal Das
Dept. of Microbiology and Molecular Biology, Peerless Hospitex Hospital and Research Center Limited, Kolkata-700094, India

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, neutrophil percentage and CRP levels were significantly increased, while lymphocyte percentage was significantly decreased. ESR levels also decreased but were not statistically significant. There was practically no change in average PCR ct values, haemoglobin levels, total count of leucocytes, uric acid, creatinine, SGPT, or HbA1C levels between the two groups. 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 [Bowness P, 2015].

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 [Dean L. E *et al.*, 2014]**.** 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 [Healey E. L *et al.*, 2011]**.** 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 [Cortes A *et al.*, 2015] [Kirino Y *et al.*, 2013]. 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 [D'Incà R *et al*., 2009] [Kumar A *et al.*, 2020]. Nearly 50% of AS patients have latent gut inflammation, and 15% of IBD patients have peripheral SpA; both disorders can manifest simultaneously or sequentially [Gracey E *et al*., 2019] [Karreman M *et al*, 2017]. 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 [Richard-Miceli C *et al.*, 2012]. 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 METHODS

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**
PCR ct value	26.35 ± 2.93	26.82 ± 2.92
RA (Seronegative/Seropositive)	11.02 ± 6.16	8.71 ± 0.29
Haemoglobin (gm/dl)	13.26 ± 1.94	12.43 ± 2.25
TC of WBC (/Cu mm)	8994 ± 2287	8323 ± 2033
Neutrophil (%)	62 ± 11	69 ± 11
Lymphocyte (%)	29 ± 7	24 ± 10
ESR (mm)	29.2 ± 26.90	25.71 ± 18.28
SGPT (U/L)	36 ± 24.03	35.8 ± 18.64
Creatinine (mg/dl)	0.72 ± 0.12	0.78 ± 0.19
Uric acid (mg/dl)	5.29 ± 1.25	5.02 ± 1.72
HbA1C (%)	5.48 ± 0.55	5.60 ± 0.46
CRP (mg/dl)	22.08 ± 13.95	36.25 ± 25.91

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

	t value	p value
PCR ct value	0.722	0.47
RA (Seronegative/Seropositive)	1.905	0.06
Haemoglobin	1.818	0.07
TC of WBC	1.369	0.17
Neutrophil	2.863	0.005
Lymphocyte	2.754	0.007
ESR	0.646	0.519
SGPT	0.040	0.97
Creatinine	1.823	0.07
Uric acid	0.849	0.40
HbA1C	1.036	0.30
CRP	3.361	0.0012

RESULTS AND DISCUSSION

A total of 89 patients' test results were analysed in this study. It is well known that males are more HLA-B27 positive than females [Xiong J *et al*., 2014; Ji X *et al*., 2018]. A total of 89 patients tested positive for HLAB-27 from a sample of 250 patients that got tested in the hospital, where the study was conducted. In this study, for the 13-40 age group, a total of 58 HLA-B27 positive samples were tested, 47 patients were male, and 11 patients were female. Thus 81% of patients were males in the 13-40 years age group. For the 41-71 age group, 31 HLA-B27 positive samples were tested, 24 patients were male and 7 patients were female. 77.4% were males in the 41-71 age group. This difference is not statistically significant and thus in all subjects, 79.7% 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. The reason might be that the gene expression may be affected by changes in epigenetic information influenced by internal factors such as alteration of chromatin structure and external factors like how an individual faces more environmental changes with age [Pal S *et al.*, 2016]. Similarly, differences between haemoglobin levels, and TC of WBC were not significant (Table 1,2). The percentage of neutrophils was significantly higher and percentages of lymphocytes were significantly lower in the high-age group (Table 1,2). Haemoglobin levels for the higher age group in this study were found to be decreased, whereas HbA1c levels were increased. Haemoglobin is usually decreased in the higher age group and HbA1C is increased in the higher age group [Wu L *et al*., 2017]. The mild decrease of TC of WBC may be due to senility and significantly increased CRP and neutrophils may be due to relatively increased inflammation in higher age groups. In this study, ESR was decreased in the higher age group, but the difference was not significant. 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 [Ji X *et al*., 2018], 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 [Saxena T *et al*., 2014]. We found similar results in this study. There is an increased tendency of uric acid with age [Das M *et al*., 2014]. In this study, however, uric acid is decreased in higher age groups, which may be due to changing dietary habits. Creatinine level is mildly increased in higher age groups in normal subjects, but the difference is not so pronounced [Verma M *et al.*, 2006]. The increase in creatinine in the higher age group in this study is attributed to impaired renal function, which is common in this group. The patients in both age groups had a few that got an anti-CCP test and were found to be negative for anti-CCP, which is an auto-antibody and a prominent marker to support the diagnosis of rheumatoid arthritis (RA). Rheumatoid arthritis coexisting with ankylosing spondylitis is very rare [Barczyńska T. A *et al*., 2015]. For testing the patient samples, we used the RT-PCR technique which is proven to be a better and more sensitive method to detect HLA-B27, as compared to the traditional serological procedure of flow cytometry, which might give ambiguous results, and thus the samples might need genotyping [Jang H. S *et al*., 2020].

However, as we used TRUPCR® HLA-B27 Real-Time PCR Kit, a limitation to the observation was that this method is only used for qualitative detection of HLA-B27; we cannot detect the subtype of HLA-B27 antigen by using this process. HLA-B27 sub-types are differently related to the incidence of ankylosing spondylitis, and thus it is necessary to take it into account. 105 known subtypes of HLA-B27 exist and HLA-B*27:06 and HLA-B*27:09 subtypes are known to not be associated with ankylosing spondylitis [Chen B *et al*., 2017].

FRET PCR of exon 2 and exon 3 and further FRET PCR of another exon 2 fragments can help in confirming the HLA-B27 positive results and add to the diagnosis by further helping in differentiating the HLA-B27 subtypes [Jang H. S *et al*., 2020], however, such procedures were not conducted in this study.

Another limitation of the study is not conducting associated bacterial infection tests as these

infections can cause elevated levels of CRP and aid in the pathogenesis of AS [Zhu L *et al.*, 2023]. Furthermore, we did not check for elevated levels of IgA, IgG, and IgM as these immunoglobulins in the patients' samples as these markers can be correlated to rheumatic inflammation if elevated [Zhu L *et al.*, 2023]. Specifically, IgA levels if elevated provide more value to the diagnosis of AS [Zhu L *et al.*, 2023]. If these tests were conducted, we could have more improved judgement of whether a HLA-B27 positive patient would manifest AS.

CONCLUSION

HLA-B27 test is usually positive for 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 that neutrophil percentage and CRP levels were significantly increased, while lymphocyte percentage was significantly decreased.

Conflict of Interest: The author declares no conflict of interest.

Author's Contribution

Jeegisha Verma, Bipasha Dey Sutradhar and Joydeb Mallick under supervision of Arup Kumar Dawn 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.

Funding Source: Any funding did not support this study.

Acknowledgement

We hereby acknowledge the Managing Director, Peerless Hospitex Hospital & Research Centre Limited, Kolkata for providing with the opportunity to pursue this research work in this institute.

ABBREVIATIONS:

HLA - Human Leukocyte Antigen
SpA - **S**pondyloarthritis
AS - Ankylosing Spondylitis.
ESR - Erythrocyte Sedimentation Rate
SGPT - Serum Glutamate Pyruvate Transaminase
HbA1C - Glycated Haemglobin
RA - Rheumatoid arthritis
Ct - Threshold Cycle
CRP - C-Reactive Protein
TC of WBC - Total Count of White Blood Cells
MHC - Major Histocompatiblity Complex
ERAP1 - Endoplasmic reticulum aminopeptidase 1
IBD - Inflammatory Bowel Disease
CD - Celiac Disease
IL23R - Interleukin 23 Receptor
IL12B - Interleukin 12B
STAT3 - Signal transducer and activator of transcription 3
ORMDL3 - ORMDL sphingolipid biosynthesis regulator 3
CARD9 - Caspase Recruitment Domain Containing Protein 9
Anti- CCP - Anti- Cyclic Citrullinated Peptide

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