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# DETECTION OF HLA-B27 BY FLOW CYTOMETRY: RETROSPECTIVE STUDY IN A TERTIARY CARE HOSPITAL

**Pathology**

**Dr. Suhani Kunria** Junior Resident, Department of Pathology, Government Medical College & Hospital, Sector 32, Chandigarh

**Dr. Anita Tahlan** Associate Professor, Department of Pathology, Government Medical College & Hospital, Sector 32, Chandigarh

**Dr. Nayan Koul*** Demonstrator, Department of Pathology, Government Medical College & Hospital, Sector 32, Chandigarh *Corresponding Author

**Dr. S. Lehl** Professor of Medicine, Department of Pathology, Government Medical College & Hospital, Sector 32, Chandigarh

## ABSTRACT

**Introduction:** HLA-B27 is a class I major histocompatibility complex (MHC) antigen which is encoded on MHC region of chromosome 6 and a non-MHC encoded beta chain, β2 microglobulin. It is associated with ankylosing spondylitis (AS) in 90-95% cases.

**Material and Methods:** All peripheral blood samples of spondyloarthropathy patients received for HLA-B27 typing were included in the study. Cells were stained with monoclonal antibodies against HLA-B27 and CD3 using lyse wash procedure. Cell acquisition and analysis was done on BD FACS Calibur flow cytometer.

**Results:** A total of 133 cases of spondyloarthropathy were studied over a period from 2008-2018. A total of 133 cases were studied under this period. These included 98 males (73.1%) and 36 females (26.8%) with Out of 133 cases, only 34 (25.5%) were reported as positive, 21 cases were indeterminate (15.7%) and 78 cases were negative(58.6%).The flow cytometric analysis was repeated after 4 weeks in 21 indeterminate cases and only 1 case(4.7%) came out to be positive.

## KEYWORDS

HLA-B27, Cytometry, Spondyloarthropathy, Major Histocompatibility Complex (MHC).

**INTRODUCTION**:
Spondyloarthropathies are distinct disorders that encompass psoriatic arthritis, reactive arthritis, juvenile idiopathic arthritis, arthritis related to inflammatory bowel disease (IBD) and AS[1]. AS has a very strong association with HLA-B27 antigen.[2] The routine HLA typing tests including the classical lymphotoxicity testing, polymerase chain reaction, or DNA sequence analysis, are time consuming and expensive. Screening by flow cytometry is quite inexpensive and easy to perform as compared to the above mentioned tests.[3]

**AIMS AND OBJECTIVES**:
The study was performed in order to determine the utility of flow cytometry in detecting HLAB27 antigen in north Indian population.

**MATERIAL AND METHODS:**
The present study was conducted in the department of pathology in collaboration with department of medicine, Govt. Medical College and Hospital, Chandigarh. All the suspected cases of spondyloarthropathy were analyzed retrospectively from April 2008-December 2018 and HLA-B27 testing was carried out using BD FACS Calibur flow cytometer. The Blood was collected by venepuncture into a sterile EDTA blood collection tube and was processed within 4 hours. Anti HLA-B27 fluorescein isothiocyante (FITC) and anti CD 3 phycoerythrin (PE) monoclonal antibodies for staining of cells were procured from Becton-Dickinson Biosciences, San Jose, CA, USA. Isotype control used was mouse anti IgG1FITC/IgG2PE. Staining of cells was done with standard lyse wash procedure according to manufacturer's instructions and the samples were analyzed on a BD FACS Calibur (2 Laser, 4 colours) flow cytometer. At least 10,000 lymphocytes were selected for analysis through forward scatter side scatter (FSc / SSc) gating technique. The expression /absence of HLA-B27 were determined by quadrant application using isotype control as quality control. Intensity was evaluated as median channel flouroscence of the histogram peak.

**RESULTS**:
In this study, all the test peripheral blood samples were subjected to screening for HLA-B27 by flow cytometry method. A total of 133 cases were studied under this period. These included 98 males (73.1%) and 36 females (26.8%) with male: female ratio of 2.7:1.The age of patients ranged from 18 to 78 years (mean 28 years). Out of 133 cases, only 34 (25.5%) were reported as positive, 21 cases were indeterminate (15.7%) and 78 cases were negative(58.6%).The flow cytometric analysis was repeated after 4 weeks in 21 indeterminate cases and only 1 case(4.7%) came out to be positive. (Fig.1, 2).

**DISCUSSION:**
In presence of clinical features, diagnostic probability of AS increases from 50% to 90% if HLA-B27 is positive.[4] Therefore, HLA-B27 inclusion in presence of symptoms has been advocated since radiological findings appear late. In our study out of 133 cases of spondyloarthropathy, only 34 (25.5%) were reported as positive. There are very few studies related to HLA-B27 association with AS in Indian population with a broad range of frequency from 18% to 94%.[5]

The limitation of our study was that we could not discriminate HLA-B27 allelic variants that could have been done by DNA sequence analysis, which may be crucial for proper diagnosis.

**CONCLUSION:**
Flow cytometry for testing HLA-B27 is a rapid and relatively inexpensive methods but have been reported to lack specificity. Molecular biology is a more reliable but costly technique. DNA typing can be used as a complementary technique and can be applied to samples whose HLA-B27 phenotype cannot be determined by flow cytometry

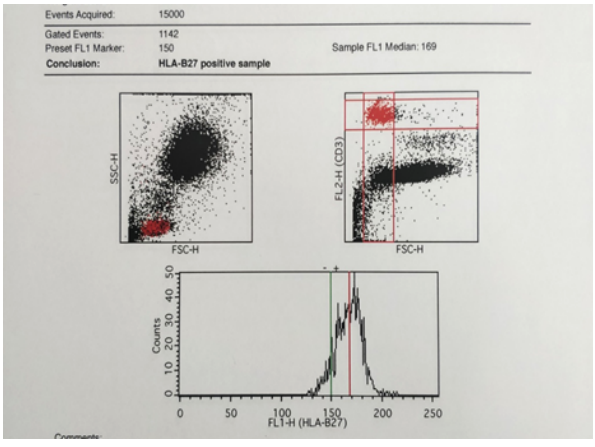


**Fig:1. HLA b27 positive sample**

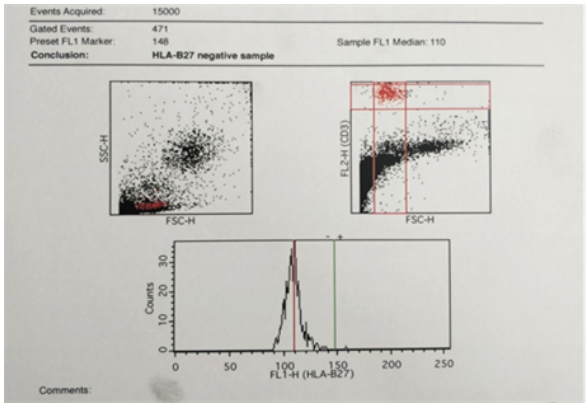


**Fig:2: HLA b27 negative sample**

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