

# Rheumatoid Factor, Anti-cyclic Citrullinated Peptide and HLA-B Locus Antigens in Psoriatic Arthritis Patients in Bangladesh

Bhowmik D<sup>1</sup>, Sarker MC<sup>2</sup>, Tarafder S<sup>3</sup>, Jahan H<sup>4</sup>, Tarana MN<sup>5</sup>, Sultana SS<sup>6</sup>, Hussain MR<sup>7</sup>

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### Abstract

**Introduction:** Psoriatic arthritis (PsA) is established as a multifactorial disease resulting from a complex interplay between genetic, environmental and immunological factors. It is a seronegative arthritis but rheumatoid factor may be present in up to 15% of PsA patients. Antibodies recognizing a cyclic citrullinated peptide are highly specific for rheumatoid arthritis (RA) but their role in PsA remains unclear. An increased prevalence of anti-CCP antibody in PsA is also reported. Study shows that HLA-DRB1 shared epitope is significantly associated with the presence of anti-CCP antibody in PsA patients but this type of association is not found with other human leukocyte antigens.

**Objectives:** The aim of this study was to investigate the frequency of anti-CCP and RF in PsA patients and their associations with HLA-B locus antigens.

**Methods:** In this cross sectional study, we selected 50 unrelated consecutive patients with PsA according to CASPAR criteria for PsA. 6 ml of blood was collected from each patient for HLA-B locus typing, RA test and test for anti-CCP. Patient's serum samples were tested for RF by Nephelometric system and tests for anti-CCP were done by ELISA. HLA-B locus typing was done by PCR with sequence specific primer.

**Results:** Among 50 PsA patients, 27 (54%) are female and 23 (46%) are male. RA test is positive in 10 (20%) patients and anti-CCP is positive in 7 (14%) patients. Significant association was found between HLA-B\*37 and RF ( $p$  value = < 0.001).

**Conclusion:** RF is present in 10 (20%) and anti-CCP is present in 7 (14%) PsA patients. HLA-B\*37 was significantly found in RF positive patients.

### Key Words:

Psoriatic Arthritis (PsA), Anti-Cyclic Citrullinated Peptide (anti-CCP), Rheumatoid Factor (RF), Human Leukocyte Antigen (HLA), Polymerase Chain Reaction (PCR).

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1. Dr. Devolina Bhowmik, Assistant Professor, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka, Bangladesh.
2. Dr. Manash Chandra Sarker, Junior Consultant, NITOR, Dhaka, Bangladesh.
3. Prof. Dr. Shirin Tarafder, Professor, Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.
4. Dr. Hosne Jahan, Assistant Professor, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka, Bangladesh.
5. Dr. Mst. Naznin Tarana, Assistant Professor, Department of Microbiology, National Institute of Laboratory Medicine and Referral Centre, Dhaka, Bangladesh.
6. Dr. Sabia Shahin Sultana, Lecturer, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka, Bangladesh.
7. Dr. Muhammad Raquib Hussain, Resident Doctor, NITOR, Dhaka

**Correspondence to:** Dr. Devolina Bhowmik, Assistant Professor, Department of Microbiology, Shaheed Suhrawardy Medical College, Dhaka, Bangladesh. Mobile: 01710258846, Email: drdbhowmik@yahoo.com.

### Introduction

Psoriatic arthritis (PsA) is an inflammatory arthritis associated with psoriasis. The prevalence of PsA among patients with psoriasis ranges from 3% to 30%.<sup>1,2</sup> Genetic, environmental and immunological factors are responsible for developing of PsA. Genetic factors are highly associated with PsA and may account for approximately 30%-40% of the genetic susceptibility.<sup>3</sup> Genetic factors are also important in both susceptibility to and the expression of PsA. PsA is a seronegative arthritis but rheumatoid factor may be present in up to 15% of PsA patients, which could be expected that this is a chronic inflammatory state.<sup>4</sup> The prevalence of RF is 19.2% in patients with PsA in Bangladesh and 11% in UK.<sup>5,6</sup> Sometimes, it is difficult to differentiate PsA from rheumatoid arthritis (RA) when the disease is manifested by a polyarticular pattern and the rheumatoid factor (RF) is positive. Acute-phase reactants in PsA patients are frequently normal or

minimally elevated. There is no proven blood biomarker for predicting the diagnosis of PsA.<sup>7</sup> Antibodies recognizing a cyclic citrullinated peptide are highly specific for rheumatoid arthritis (RA) but their role in PsA remains unclear. Recently, a study has reported, an increased prevalence of anti-CCP in PsA as 15.7%.<sup>8</sup> In Romania, the prevalence of anti-CCP is 12.5% and 15.6% in UK among PsA patients.<sup>9,6</sup> Anti-CCP is not always associated with the presence of RF and the presence of anti-CCP in PsA patients is significantly associated with the HLA-DRB1 shared epitope.<sup>6</sup> There are few studies found to see the association of HLA-B locus antigens with RF factors and anti-CCP and regarding this aspect there is no data found in our country.

So the aim of this study is to observe the distribution of anti-CCP and RF in PsA patients and to assess their associations with HLA-B locus antigens.

### Materials and methods

This study was conducted from March, 2015 to February, 2016 in the department of Microbiology and Immunology, BSMMU, Shahbagh, Dhaka.

Before starting work it was approved by BSMMU institutional review board (BSMMU/2015/9693, date-29/07/2015). This study was funded by the department of Microbiology and Immunology, BSMMU. In this cross sectional study, we selected 50 unrelated consecutive patients with PsA according to CASPAR criteria for PsA from Department of Rheumatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. All patients were carefully phenotyped by rheumatologists. History of other associated autoimmune disease and past medical history were taken from previous records. Patients having other diagnosed autoimmune diseases in association with PsA were excluded for the present study. 6 ml of blood was collected from each patient for HLA-B locus typing, RA test and anti-CCP. Laboratory works were performed in the Department of Microbiology and Immunology, BSMMU, Shahbag, Dhaka.

### Detection of RF by Nephelometry

Patient's serum samples were tested for RF by Nephelometric system. The lot no. and expiry date was checked. All components were allowed to reach room temperature prior to use. The test was done according to manufacturer instructions. RF was detected quantitatively and recorded as IU/ml. All steps were performed automatically by the system. RF > 15 IU/ml was interpreted as positive. In order to ensure a valid test result controls was included in each test run. If the control values outside the range, the results of the assay were considered to be invalid.

### Detection of Anti-CCP by ELISA

Tests were done by ELISA using EURO-Diagnostica, Sweden ELISA kit. Result of anti-CCP was considered positive if it was  $\geq 25$  IU/ml. In order to ensure a valid test result controls was included in each test run.

### HLA-B locus typing

Purified genomic DNA isolation was performed by DNA extraction kit according to manufacturer's instruction supplied with the PCR kit (QIAamp DNA Mini and Blood Mini Handbook 04/2010). After DNA extraction it was stored at  $-20^{\circ}\text{C}$  until PCR was done. Low-resolution single specific primer-polymerase chain reaction (SSP-PCR) was performed with SSP kit (DNA amplification was done by using Morgan™ HLA SSP B following manufacturer's instruction.). As low-resolution PCR reaction cannot always distinguish between closely related alleles. In this study, only serological level data were analyzed to avoid ambiguity. The amplified DNA was examined by agar gel electrophoresis that separates the DNA fragments by size. Specific HLA-B locus antigen was determined using the worksheet (supplied along with the kit).

### Statistical Analysis

All data, after collection by data sheet were checked, entered in computed based SPSS 22.0 for windows software. Categorical parameters are expressed as frequency and percentage. Comparisons between groups (continuous parameters) were done by unpaired t test. Statistical analysis of HLA-B locus antigens with anti-CCP and RF were determined by chi-square test. A *p*-value of <0.05 was considered significant.

### Results

Table I shows among 50 PsA patients, 27 (54%) are female and 23 (46%) are male. Mean Age of patients is  $44.60 \pm 12.16$  years. RA test is positive in 10 (20%) patients and anti-CCP is positive in 7 (14%) patients. Most common clinical pattern of RA test positive PsA patients is symmetrical polyarthritis pattern 8(80%) and most common clinical pattern of Anti-CCP positive PsA patients is symmetrical polyarthritis pattern 9(90%)

Table II shows distribution of HLA-B locus antigens in anti-CCP positive and anti-CCP negative PsA patients. Among anti-CCP positive patients, most common identified HLA-B locus antigen was HLA-B\*7(42.85%) and among anti-CCP negative patients, most common identified HLA-B locus antigen was HLA-B\*15(39.53%). In this study, HLA-B\*15 was significantly found in anti-CCP negative patients (*p* value= 0.041) because HLA-B\*15 was found as 00% in anti-CCP positive and 39.53% in anti-CCP negative patients.

Table III shows distribution of HLA-B locus antigens in RF positive and RF negative PsA patients. Among RF positive patients, most common identified antigen was HLA-B\*7(30%), HLA-B\*15(30%), HLA-B\*37(30%) and among RF negative patients, most common identified antigen was HLA-B\*15(35%). When RF positive patients were compared with RF negative patients, association was found between HLA-B\*37 and RF (*p* value= < 0.001) because HLA-B\*37 was found as 30% in RF positive and 00% in RF negative patients.

## Results

**Table I**

*Demographic and clinical features of the patients with psoriatic arthritis (n = 50).*

Features	No. of patients
Number of female patients	27(54%)
Number of male patients	23(46%)
Female: male ratio	1.2:1
Mean Age $\pm$ SD (Year)	44.60 $\pm$ 12.16
RA test positive PsA patients	10(20%)
Most common clinical pattern of RA test positive PsA patients	Symmetrical polyarthritis pattern 8(80%)
Anti-CCP antibody positive PsA patients	7(14%)
Most common clinical pattern of Anti-CCP positive PsA patients	Symmetrical polyarthritis pattern 7(100%)

Note: Figure within the parenthesis indicates percentage; The percentage does not matches the total no.

**Table II**

*Distribution of HLA-B locus antigens among anti-CCP positive and anti-CCP negative psoriatic arthritis patients (n= 50).*

HLA-B locus Antigens	Anti-CCP-positive patients (n=7)	Anti-CCP negative patients (n=43)	<i>P value</i>
HLA-B*07 (n=13)	3(42.85)	10(23.25)	0.273
HLA-B*13 (n=5)	2(28.57)	3(6.97)	0.077
HLA-B*15 (n=17)	00(00)	17(39.53)	0.041
HLA-B*27(n=12)	00(00)	12(27.90)	0.109
HLA-B*35 (n=6)	1(14.28)	5(11.62)	0.841
HLA-B*37 (n=3)	1(14.28)	2(4.65)	0.319
HLA-B*38 (n=10)	1(14.28)	9(20.93)	0.683
HLA-B*39 (n=3)	00(00)	3(6.97)	0.471
HLA-B*40 (n=5)	1(14.28)	4(9.30)	0.683
HLA-B*44 (n=6)	00(00)	6(13.95)	0.292
HLA-B*51 (n=5)	1(14.28)	4(9.30)	0.683
HLA-B*52 (n=7)	2(28.57)	5(11.62)	0.231
HLA-B*55 (n=1)	00(00)	1(2.32)	0.683
HLA-B*56 (n=2)	1(14.28)	1(2.32)	0.134
HLA-B*57 (n=3)	1(14.28)	2(4.65)	0.319
HLA-B*58 (n=2)	00(00)	2(4.65)	0.560

HLA= Human leukocyte antigen ; Figure within the parenthesis indicates percentage.

**Table III***Distribution of HLA-B locus antigens among RF positive and RF negative psoriatic arthritis patients (n= 50).*

HLA-B locus Antigens	RF-positive Patients (n= 10)	RF-negative patients (n=40)	<i>P value</i>
HLA-B*07 (n=13)	3(30)	10(25)	0.747
HLA-B*13 (n=5)	2(20)	3(7.5)	0.238
HLA-B*15 (n=17)	3(30)	14(35)	0.765
HLA-B*27 (n=12)	00(00)	12(30)	0.092
HLA-B*35 (n=6)	00(00)	6(15)	0.192
HLA-B*37 (n=3)	3(30)	00(00)	<0.001
HLA-B*38 (n=10)	2(20)	8(20)	1.00
HLA-B*39 (n=3)	00(00)	3(7.5)	0.371
HLA-B*40 (n=5)	1(10)	4(10)	1.00
HLA-B*44 (n=6)	00	6(15)	0.191
HLA-B*51 (n=5)	1(10)	4(10)	1.00
HLA-B*52 (n=7)	2(20)	5(12.5)	0.541
HLA-B*55 (n=1)	00(00)	1(2.5)	0.613
HLA-B*56 (n=2)	2(20)	00(00)	0.06
HLA-B*57 (n=3)	1(10)	2(5)	0.552
HLA-B*58 (n=2)	00(00)	2(5)	0.470

Note: HLA= Human leukocyte antigen; Figure within the parenthesis indicates percentage.

### Discussion

Psoriatic arthritis (PsA) is a genetically complex autoimmune disease and is a member of the spondyloarthopathy group. The CASPAR criteria, can facilitate the diagnosis of PsA and it is an internationally accepted criteria.<sup>10</sup> PsA is a seronegative arthritis. Recently some studies have reported that RF and anti-CCP are also present in psoriatic arthritis patients.

There were 54% female and 46% male patients in this study. Female: male ratio was 1.2:1 which is almost similar with the findings of study on Caucasian patients. In which female: male ratio was 1:1.<sup>11</sup> Study on patients of Iran showed that the prevalence of PsA among men was not significantly different from that of women.<sup>12</sup> But study in Bangladesh and in South India, there was a different feature, female: male ratio was 1:1.74 and 1:1.7 respectively.<sup>5,13</sup> So, in this aspect, different pictures are seen in different study. However, it is not clear whether this gender-related difference is secondary to differences in occupational physical activity, hormonal changes or other factors.

In this study, Mean age of PsA patients was 44.60±12.16 (mean±SD) years and patients age range is in between 19-

70 years which is almost similar to the findings of other studies. Study on Bangladeshi patients, Mean age of patients was 38.85±13.28 years.<sup>5</sup> Another study on patients of Iran, the Mean age of patients was 44.8±13.2 years.<sup>14</sup> Above findings indicate that PsA develops predominantly at 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> decade of life.

Anti-CCP is highly specific for rheumatoid arthritis (RA) but their role in PsA remains unclear. In our study, 14% patients were positive for anti-CCP. Study in Romania showed that the prevalence of anti-CCP was 15.7% in PsA patients.<sup>8</sup> Another study in Romania, showed the prevalence of anti-CCP was 12.5%.<sup>9</sup> Study in UK, showed prevalence of anti-CCP was 15.6% in PsA patients.<sup>6</sup> These results are more or less similar with this study. This result confirmed that anti-CCP is present in PsA patients but the prevalence is lower than rheumatoid arthritis (RA) patients because in rheumatoid arthritis, 95.7% patients were positive for anti-CCP.<sup>6</sup>

PsA is a seronegative arthritis but rheumatoid factor may be present in up to 15% of PsA patients.<sup>4</sup> In this study 20% patients were positive for RF. Similar type of finding was found on Bangladeshi patients and the prevalence of RF was 19.2%; study on Canadian patients the prevalence

of RF was 15% in PsA patients.<sup>5,4</sup> This result confirmed that RF is present in PsA patients but prevalence is low than rheumatoid arthritis patients because in rheumatoid arthritis, 100% patients were positive for RF.<sup>6</sup> So, genetic characteristics of PsA are different from RA.

All patients positive for anti-CCP had symmetric polyarthritis pattern and 80% RF positive patients had symmetrical polyarthritis pattern in our study. Study on patients of Bangladesh shows that symmetrical polyarthritis pattern is the predominant pattern found in all RF and anti-CCP positive PsA patients.<sup>15</sup> Study on patients of Cairo, Egypt also found the same feature that all anti-CCP antibody positive PsA patients had symmetrical polyarthritis pattern and one had axial joint involvement.<sup>16</sup> In UK, symmetrical polyarthritis was the predominant clinical pattern in anti-CCP and RF positive PsA patients. There is evidence that anti-CCP antibody positive PsA patients are highly associated with RA-like polyarticular involvement and may be associated with the HLA-DRB1 shared epitope (SE).<sup>6</sup> It may be possible that anti-CCP or RF positive typical PsA patients may have concomitant rheumatoid arthritis. So, HLA-DRB1 typing is needed in anti-CCP and RF positive PsA patients to justify this type of association.

Genes in the major histocompatibility complex (MHC) or human leukocyte antigen (HLA) have been mapped to chromosome 6p21.3. They encode a series of glycoproteins that play an important part in immunological self and non-self discrimination by presenting antigens to T cells.<sup>10</sup> A lot of work has been done to find out the degree and nature of association of human leukocyte antigens and PsA. Due to high degree of polymorphism within the genes of HLA, the precise genetic cause of the association has been difficult to define.

In this study, HLA-B\*15 was found statistically more significant in anti-CCP negative patients ( $p$  value= 0.041) because the frequency of HLA-B\*15 was 17(39.53%) in anti-CCP negative patients and 00(00%) in anti-CCP positive patients. In my knowledge, this type of association was not found in any study. This finding suggests that the genetic cause of PsA is different but proper explanation regarding this type of finding was not found. So, HLA-B\*15 may have protective role against anti-CCP antibody in Bangladeshi PsA patients.

HLA-B\*37 was statistically significant in RF positive patients ( $p$  value <0.001) in this study. I did not find any study in which this type of association was found. This finding indicates that HLA-B\*37 allele confer an association with RF positive patients in Bangladesh. The role of RF in the pathogenesis of PsA is not well established.

It may be possible that these RF positive patients had concomitant rheumatoid arthritis. Study on patients in UK, 60% RF positive PsA patients carry HLA-DRB1 shared epitope.<sup>6</sup> So, HLA-DRB1 typing is needed in RF positive PsA patients to justify this type of association. There is evidence that false positive RF was found in 13% of patients with PsA.<sup>16</sup> In our study, only one patient had high titer for RA test (402 IU/ml) and the titer of RA test of other patients was low (32.8- 48.5 IU/ml). We exclude PsA patients suffering from hepatitis, SLE, other joint diseases such as typical rheumatoid arthritis, ankylosing spondylitis, gout and autoimmune disease. By using a cut off at a higher specificity, it may be possible to reduce the number of false positive results. So, further study with a standard cut off value for RA test is however needed to justify this type of associations. In my knowledge, two studies were found regarding the distribution of HLA-B locus antigens in relation to anti-CCP and RF in PsA patients. Study in Italy, showed that there was no association found between HLA-B locus antigens and anti-CCP in PsA patients.<sup>17</sup> Study in UK, showed that HLA-B\*27 was not associated with anti-CCP and RF positive PsA patients.<sup>6</sup> In that study, all anti-CCP positive PsA patients carry HLA-DRB1 shared epitope.<sup>6</sup> So, along with HLA-B locus typing, HLA-DRB1 typing should be needed in PsA patients.

### Conclusions

20% and 14% PsA patients are positive for RF and anti-CCP antibody respectively in this study. In relation to anti-CCP, HLA-B\*15 was associated with anti-CCP negative patients. In relation to RF, HLA-B\*37 was significantly found in RF positive patients. Because of small sample size, study population in this study may not represent the entire PsA patients in Bangladesh. So, before drawing firm conclusion further study is needed on large group of PsA patients to justify this type of association.

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