

HLA-B27 Subtypes Distribution among Moroccan Patients with Ankylosing Spondylitis

Amribet Meryem^{1,2,*}, Bourhim Nouredine¹, Mkinsi Slaoui Ouafaa³, Belhouari Abderrahmane⁴, Naya Abdellah¹, Fadli Mohamed⁵, Bennani Siham²

¹Biochemistry and Molecular Biology laboratory, Ain Chock Faculty of Science Hassan II University, Casablanca, Morocco

²Immunohistocompatibility and Molecular Biology laboratory, Pasteur Institute, Casablanca, Morocco

³Rheumatology Department, Ibn Rochd Hospital, Casablanca, Morocco

⁴Ben M'Sik Faculty of Science, Departement of Statistics, Casablanca, Morocco

⁵Lagitre Laboratory, One Lambda, Milano, Italy

*Corresponding author: amribet.meryem@gmail.com

Received January 14, 2013; Revised January 31, 2013; Accepted February 15, 2013

Abstract The association of HLA-B27 with ankylosing spondylitis accounts for 20 to 50% of the total disease risk. It varies markedly among racial and ethnic populations. The main purpose of the present study is to perform an investigation regarding the distribution of the human leukocyte antigen HLA-B27 and its subtypes in Moroccan healthy controls and in patients with AS and to compare this with other reports from other populations. One hundred twenty-five controls and 116 patients with AS were evaluated in this study. Among patients, three cases were associated to acute anterior uveitis. Typing of the HLA-B27 alleles was performed by microlymphocytotoxicity and polymerase chain reaction amplification with sequence-specific primers. A significant association between ankylosing spondylitis and B27 was identified; HLA-B*27 allele was carried by 46.5% of patients and only one subject from healthy controls was found to be positive ($p < 0.001$, RR= 2.95). Among all positive patients, 64.8% were males and 53.7% belonged to 20–29 and 30–39 age groups. Being male aged 30–39 was significantly associated with B27 positivity. Four HLA- B*27 alleles were observed in this study: B*2705 (44.4%), B*2702 (29.6%), B*2703 (9.2%) and B*2708 (16.7%). The only positive subject from healthy controls carried B*2705 allele. B*2708 subtype was identified in all cases with uveitis. This allele showed a significant association with patients being female. Concluding, HLA-B27 is strongly associated with ankylosing spondylitis in Moroccan population. Our results showed a restricted number of HLA-B27 subtypes with predominance of HLA-B*2705, 02 alleles. The B*2708 allele detected for the first time in North Africa, seems to be associated with ankylosing spondylitis in the studied population.

Keywords: Ankylosing spondylitis, PCR-SSP, HLA-B27 subtypes, Moroccan population

1. Introduction

Ankylosing spondylitis (AS) is a prototype of a group of inflammatory diseases formerly known as spondyloarthropathies, and currently called spondyloarthritis and its incidence range from 0.2 to 1.1% [1]. It is a form of seronegative inflammatory arthritis that affects primarily the sacroiliac joints and the axial skeleton (spine) and, less frequently, peripheral joints and other extra-articular organs such as the eyes, skin, and cardiovascular system [2]. This rheumatic disease can lead to structural and functional impairments and a decrease in quality of life. It usually develops in the second or third decade of life affecting young men more frequently than women with a ratio Male/Female of roughly 2 to 1 [3]. The exact cause of AS is unknown, but substantial evidence indicates that it arise as a consequence of abnormal immune response, which may be triggered by a complex of genetic and environmental factors [4]. In 1973, two groups working independently reported a striking association between what we now

know as human leukocyte antigen-B27 (HLA-B27) and ankylosing spondylitis [5,6], however the exact role of B27 molecule in the etiology of the disease still remains elusive. To explain its pathogenic role, a number of theories confirmed by transgenic rats, have emerged relating to various aspects of the HLA-B27 molecule, from its classical role in antigen presentation to its propensity to misfold during assembly in the endoplasmic reticulum and to undergo dimerization [7,8].

There is considerable heterogeneity within the HLA-B27 group of alleles referred to as subtypes. Most of them differ from each other in a few amino acid residues occupying defined locations in the peptide-binding groove [9]. It has been well established that HLA-B27 subtypes differ their ethnic distribution, which may be the result of different genetic and geographic origins [10]. Although the ethiopathogenesis of AS is not completely understood, it is generally accepted that the strength of various B27 subtypes in association with AS is different. While the two B*2706 and B*2709 subtypes appear to be unassociated or perhaps weakly associated with the AS. The relatively common subtypes B*2705, B*2702, B*2704, B*2701, B*2707, B*2708, B*2710, B*2714,

B*2715 and B*2719 were reported to be positively associated with the disease. Other subtypes have not been yet fully studied for disease association. So, it becomes essential to study the definitive conclusion of the disease-associated subtypes in different ethnic group, which is greatly advantageous in clarifying the role of B27 in the AS [11].

In Morocco, HLA-B27 allele frequencies has never been studied before, hence the main objective of the present study was to unravel the B*27 subtypes prevalence in positive AS patients and healthy controls from the Moroccan population and to compare this with other populations previously reported.

2. Material and Method

2.1. Study Population

One hundred and sixteen consecutive adult patients with AS and 125 unrelated healthy individuals were included in the study. The control group consisted of healthy bone marrow and kidneys volunteer donors of both genders (sex-ratio (M/F) = 0.86) with no family history of AS or other kind of rheumatic symptoms suggesting presence of a serious disease. Ankylosing spondylitis diagnosis was in accordance with the modified New York criteria [12]. Patients were recruited in the study between February 2009 and April 2010, from those followed at the rheumatology department of the Ibn Rochd Hospital, Casablanca, Morocco. A complete clinical evaluation was done for all patients; three of them had acute anterior uveitis. Only one subject from each family was included. All subjects provided written informed consent. This study was approved by the ethnics review committee of the Pasteur Institute of Morocco, in accordance with the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects.

2.2 Laboratory Analyses

HLA typing and allele frequency was carried out at the Immune histocompatibility laboratory of Immunology and Molecular Biology Department at the Pasteur Institute in Casablanca. Using the commercial QIAamp DNA Blood Midi Kit, (Quiagen, QIAamp DNA Midi and Blood Midi handbook), genomic DNA was extracted from 5ml peripheral blood of each one of the studied populations. HLA-class I (-A,-B) typing was undertaken by microlymphocytotoxicity test using HLA typing Terasaki plate (One Lambda, USA). Molecular typing were performed by polymerase chain reaction amplification method (PCR-SSO) with specific primers for -A and -B loci followed by a probe hybridization reaction (Line Probe Assay, Innogenetics INNO-LIPA HLA B Update plus).

HLA- B*27 alleles were typed at high resolution using the PCR-sequence-specific primers (PCR SSP) (One Lambda's Micro SSP™ Generic and High Resolution HLA Typing Trays). PCR products were visualized in 2.5% agarose gel under UV illumination following ethidium bromide staining and documented by photography.

2.3 Statistical Analysis

Statistical analysis was carried out using SPSS 17.0 software. Chi-squared test was used to compare the genotypes distribution or allele frequencies between AS patients and controls. The allelic frequencies of each one of the B27 subtypes were determined by direct count. The confident interval (CI) of the calculated odds ratio (OR) was estimated by approximate 95%. Results were considered significant when the *p*-value was less than 0.05.

3. Result

A total of 116 AS patients and 125 controls were recruited in this study. Demographic variables and baseline characteristics of patients were given in Table 1. The mean age \pm standard deviation (SD) was 37.94 ± 13.44 (range: 10-74 years) in patients and 42 ± 13.66 (range: 16-68 years) in control group, respectively. There were 62 (53.45%) female and 54 (46.55%) male in patient group, in the control group it was 67 (53.6%) and 58 (46.4%), respectively. All the subjects were native residents of Morocco.

Table 1. Distribution by Age and Sex of Patients with Ankylosing Spondylitis

Age	Patients N=116	%	Sex M n=54 (46.55%)	Sex F n=62 (53.45%)
10-19	13	11.20	9	4
20-29	23	19.82	12	11
30-39	31	26.72	12	19
40-49	28	24.13	12	16
50-59	14	12.06	6	8
60-69	6	5.17	2	4
70-79	1	0.86	1	0

In all patients with ankylosing spondylitis, Fifty-four (46.55%) was HLA- B27 positive. Among these subjects, 64.8% (35 cases) were males and 35.2% (19 cases) were female (Table 2). The majority of patients (29/54) was within the 20-29 and 30-39 age groups, which corresponds to 53.7% of the total positive patients. In the same groups, 58.3% and 64.7% were male, respectively. No significant difference in HLA-B27 positivity was encountered with different age groups and sex except for 30-39 age group ($p < 0.05$).

Table 2. Distribution of HLA-B27+ Ankylosing Spondylitis Patients by age

Age	n= 54	%	Sex M n=35 (64.8%)	Sex F n=19 (35.2%)	<i>p</i> - value
10 - 19	7	12.96	6	1	0.068
20 - 29	12	22.22	7	5	0.211
30 - 39	17	31.48	11	6	0.041
40 - 49	9	16.66	5	4	0.358
50 - 59	5	9.25	3	2	0.433
60 - 69	3	5.55	2	1	0.460
>70	1	1.85	1	0	0.433

Among healthy controls, only one out of 125 participants was B27 positive. This patient was male subject who belonged to age group 20-29. The difference

between patients with AS and controls is found to be highly significant ($p < 0.001$, $RR = 2.95$).

While up to the subtype level, out of the ninety-eight subtypes reported worldwide (<http://hla.alleles.org/class1.html>), only four alleles, B*2702, B*2703, B*2705 and B*2708 were found in the present study. Table 3 shows the distribution of HLA-B*27 alleles in the studied groups and the statistical analysis for disease association. B*2705 and B*2702 were clearly the predominant subtypes; they were detected in 24 (44.4%) and 16 (29.6%) of all 54 patients, respectively, whereas B*2703 and B*2708 were present in 9.2% and 16.6% of the positive subjects.

The B*2708 subtype was associated with 26.3% of female patients (5/19), compared with the male positive subjects, this difference was statically significant ($p = 0.03$, Table 4). The B*2705, B*2702 and B*2703 subtypes were relatively equally distributed between both sexes. No significant difference in the subtypes distribution between males and females was found.

Table 3. Distribution of HLA-B27+ Ankylosing Spondylitis: Patients by Subtype and Sex

Subtype	Patients n	%	Sex M (%)	Sex F (%)	p-value
B*2705	24	44.4	16 (45.71)	8 (42.10)	0.319
B*2702	16	29.6	12 (34.28)	4 (21.05)	0.915
B*2708	9	16.7	4 (11.42)	5 (26.31)	0.031
B*2703	5	9.2	3 (8.57)	2 (10.52)	0.460
Total	54	100	35 (64.8)	19 (35.2)	

Concerning the healthy group, the only HLA-B*27 positive case found was represented by HLA-B*2705 variant.

4. Discussion

The association of HLA-B27 with ankylosing spondylitis has been known for over 39 years and has been well established in many ethnic groups. It remains one of the best examples of a disease association with a hereditary marker. [6,13]. In Morocco little is known regarding HLA-B27 prevalence. To the best of our knowledge this is the first report about B27 subtype frequencies in Moroccan patients with AS.

The prevalence of HLA-B27 varies greatly in the different ethnicities ranging from 0% in African Bantu and Australian Aborigines to 50% in Native Americans [14]. In our healthy population, the frequency of B27 is found to be very low; only one subject was positive for HLA-B27 out of 125 tested participants. This prevalence was lower than that noted in Caucasians and some prevalence reports from other Arabic countries such as Tunisia, Syria, and Emirates [15,16,17].

Regarding patients with ankylosing spondylitis, the B27 incidence (46.55%) was lower than that documented in Middle Eastern and Western European populations [18], [19]. However, our estimate was much higher compared to what has been reported in the prior year by Atouf et al. in which the estimated HLA-B27 prevalence rate was 29.3% [20]. The difference between frequencies reported in that study and present report is likely due to the lower size of their studied population. In the other hand, analysis by gender and age reveals that HLA prevalence among males is significantly higher than that found in females (64.8% vs. 35.2%, $p < 0.001$) and showed two peaks among males aged 20-29 and 30-39 years. It is worth noting that our

studied population included 62 females and 54 males, thus this is the lowest male/female ratio (0.75) reported in the literature [3,21].

Genetic heterogeneity of HLA-B27 alleles is increasing due to different population studies and high-resolution techniques [22]. Not all of these alleles are equally associated with AS and are not equally distributed over the world [23,24]. In the present report we identified 4 HLA-B*27 variants in our studied groups. In fact, we could not accurately define the contribution of each one of the identified HLA-B*27 subtype to AS risk, because of the very low B*27 frequency in the healthy group; among 125 studied subjects, only one (0.81%) was found to be positive HLA-B*27, this case was represented by HLA-B*2705 variant.

The distribution of subtypes in the patients included in this study follows the world tendency: B*2705 was the most frequent subtype (44.44%). It seems to be the allele from which all subtypes derived [25]. It is well established that it's clearly associated with ankylosing spondylitis and related spondyloarthropathies around the world. It is the predominant subtype in almost all populations, particularly among Euro-Caucasians. The B*2702 was present in 29.62% of patients. It is prevalent in Mediterranean populations, showing a high frequency in the Middle Eastern Arabs and Berbers [26,27]. This allele was found over-represented in the Jewish population (54%) [28], having a similar distribution to that found in North African Caucasians from Algeria (52%) and Tunisia (43%). In Turks, overall frequency in AS patients is 2.6%, represented by B*2702 and B*2705 as the susceptibility disease markers [25,29]. B*2703 was seen in 9.2% of our patients. It has previously been detected in American blacks [30]. This allele was a predominant subtype of HLA-B27 in West Gambia, West Africa [31]. It was also found that B*2703 has a subtly higher frequency amongst the low incidence of rare subtypes in Wuhan populations [11]. Similar findings have been described in Taiwanese Chinese population [32]. B*2708 is a rare European subtype that was first observed in Britain and has been reported in association with AS in a large family from the Azores Islands in the Atlantic Ocean, a territory of Portugal [33,34]. It is an extremely rare subtype, and its relationship to disease has not been defined. In the present study, among all positive subjects 16.7% have B*2708 and its prevalence was significantly higher in female than male patients ($p = 0.03$). Although the presence of this allele should be interpreted carefully, according to Gonzalez et al., each B27 allele was considered to be susceptible or predisposing subtype if at least one patient could be detected in one population [26]. Consequently, this prompt suggestion that, B*2708 may be a disease-associated subtype in Moroccan population, which is intriguing and perhaps worthy of further investigation. Our study also suggests that this subtype could have been introduced as a result of an admixture with European genes too.

Acute anterior uveitis (AAU) occurs in approximately 20%–30% of the patients with AS, being considered the most common extra-articular manifestation [35]. It relates to neither joint disease exacerbation nor severity [36]. In the present study, three of our patients suffer from AAU. HLA-B*2708 allele was represented in all subjects with AAU. This may suggest a possible role of this allele in the occurrence of this clinical feature.

In conclusion, the conundrum of this remarkable association of HLA-B27 with AS, still remains as puzzling as ever and it continues to challenge our minds. Meanwhile, the present report provides interesting information on the HLA-B*27 association with AS in Moroccan patients. The low B*27 prevalence in the healthy group, coupled with the remarked strong AS risk demonstrated by our results. This study showed that HLA*B2705, HLA*B2702, HLA*B2703 and HLA*B2708 are the subtypes found in our population. As all over the world B*2705 was the predominant subtype. Furthermore, we suggest a possible association with AS for HLA*B2708 found for the first time in North Africa. Nevertheless, the definitive conclusion of the disease-associated subtype needs to be confirmed in further studies and in other populations.

Acknowledgement

We thank Baha Warda (PhD student, Molecular Biology laboratory of Pasteur Institute) for critical revision of the manuscript.

Statement of Competing Interests

There is no conflict of interests between the different authors of this work.

List of Abbreviations

AS: Ankylosing spondylitis
 PCR: Polymerase chain reaction
 CI: Confident Interval
 OR: Odds Ratio
 SD: Standard Deviation
 AAU: Acute anterior uveitis

Reference

- Moschos, M.M., Gatzoufas, Z. and Margetis, I., "Intravitreal Triamcinolone Acetonide for Macular Edema in HLA-B27 Negative Ankylosing Spondylitis," *Case Rep Ophthalmol*, 1 (2). 105-109. Dec.2010.
- Bezerra Gouveia, E., Elmann, D. and Ávila Morales. M.S., "Ankylosing spondylitis and uveitis: overview," *Rev Bras Reumatol*, 52 (5). 742-756. Jun.2012.
- Feldtkeller, E., Khan, M.A., Van der Heijde, D., Van der Linden, S. and Braun, J., "Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis," *Rheumatol Int*, 23 (2). 61-66. Mar.2003.
- Pham, T., "Pathophysiology of ankylosing spondylitis: what's new?," *Joint Bone Spine*, 75 (6). 656-660. Dec.2008.
- Caffrey, M.F. and James, D. C. O., "Human lymphocyte antigen association in ankylosing spondylitis," *Nature*, 242. 121. Mar.1973.
- Schlosstein, L., Terasaki, P.I., Bluestone, R. and Pearson, C.M., "High association of an HLA antigen, W27, with ankylosing spondylitis," *N Engl J Med*, 288 (14). 704-706. Apr.1973.
- Colbert, R.A., DeLay, M.L., Layh-Schmitt, G. and Sowders, D.P., "HLA-B27 misfolding and spondyloarthropathies," *Prion*, 3 (1). 15-26. Jan-Mar.2009.
- Antoniou, A.N., Lenart, I., and Guiliano, D.B., "Pathogenicity of Misfolded and Dimeric HLA-B27 Molecules," *International Journal of Rheumatology*, Article ID 486856, 9 pages doi:10.1155/2011/486856. 2011.
- Gonzalez, S., Garcia-Fernandez, S., Martinez-Borra, J., Blanco-Gelaz, M.A., Rodrigo, L., Sanchez del Rio, J., López-Vazquez, A., Torre-Alonso, J.C., and López-Larrea, C., "High variability of HLA-B27 alleles in ankylosing spondylitis and related spondyloarthropathies in the population of northern Spain," *Hum Immunol*, 63 (8). 673-676. Aug.2002.
- Shankarkumar, U., Ghosh, K. and Mohanty, D., "HLA B27 polymorphism in Western India," *Tissue Antigens*, 60 (1). 98-101. Jul.2002.
- Liu, X., Hu, L.H., Li, Y.R., Chen, F.H., Ning, Y. and Yao, Q.F., "The association of HLA-B*27 subtypes with ankylosing spondylitis in Wuhan population of China," *Rheumatol Int*, 30 (5). 587-590. Mar.2010.
- Van der Linden, S., Valkenburg, H.A. and Cats, A., "Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for modification of the New York criteria," *Arthritis Rheum*, 27 (4). 361. Apr.1984.
- Brewerton, D.A., Hart, F.D., Nicholls, A., Caffrey, M., James, D.C.O. and Sturrock, R.D., "Ankylosing spondylitis and HLA-27," *The Lancet*, 301 (7809). 904-907. Apr.1973.
- Khan, M.A., "HLA-B27 and its pathogenic role," *J Clin Rheumatol*, 14 (1). 50-52. Feb.2008.
- Ayed, K., Ayed-Jendoubi, S., Sfar, I., Labonne, M.P. and Gebuhrer, L., "HLA class I and HLA class II phenotypic, gene and haplotypic frequencies in Tunisians by using molecular typing data," *Tissue Antigens*, 64 (4). 520-532. Oct.2004.
- Harfouch, E.I. and Al-Cheikh, S.A., "HLA-B27 and its subtypes in Syrian patients with ankylosing spondylitis," *Saudi Med J*, 32 (4). 364-368. Apr.2011.
- Al-Attia, H.M. and al-Amiri, N., "HLA-B27 in healthy adults in UAE. An extremely low prevalence in Emirian Arabs," *Scand J Rheumatol*, 24 (4). 225-227. Jan.1995.
- Mustafa, K.N., Hammoudeh, M. and Khan, M.A., "HLA-B27 Prevalence in Arab Populations and Among Patients with Ankylosing Spondylitis," *J Rheumatol*, 39 (8). 1675-1677. Aug.2012.
- Salvarani, C. and Fries, W., "Clinical features and epidemiology of spondyloarthritides associated with inflammatory bowel disease," *World J Gastroenterol*, 15 (20). 2449-2455. May.2009.
- Atouf, O., Benbouazza, K., Brick, C., Saoud, B., Benseffaj, N., Amine, B., Hajjaj-Hassouni, N. and Essakalli, M., "Distribution of HLA class I and II genes in ankylosing spondylitis patients from Morocco," *Pathol Biol*, 60 (6). 80-83. Dec.2012.
- Rivera, S., Hassanhi, M., Márquez, G., Fuenmayor, A., Monzón, J. and Avila, J., "Relation of spondyloarthropathy and HLA-B27 antigen in patients from the state of Zulia, Venezuela," *Sangre (Barc)*, 41 (6). 473-476. Dec.1996.
- Ma, H.J. and Hu, F.P., "Diversity of human leukocyte antigen-B27 alleles in Han population of Hunan province, southern China," *Tissue Antigens*, 68 (2). 163-166. Aug.2006.
- Khan, M.A., "Update: the twenty subtypes of HLA-B27," *Curr Opin Rheumatol*, 12 (4). 235-238. Jul.2000.
- Nicknam, M.H., Mahmoudi, M., Amirzargar, A.A., Ganjalikhan Hakemi, M., Khosravi, F., Jamshidi, A.R., Amirkhani, A., Ansari-pour, B., Pourpak, Z., Moin, M. And Nikbin, B., "Determination of HLA- B27 subtypes in Iranian patients with ankylosing spondylitis," *Iran J Allergy Asthma Immunol*, 7 (1). 19-24. Mar.2008.
- Alaez, C., Gazit, E., Ibarrola, B., Yaron, M., Livneh, A., Avishai, O. and Gorodezky, C., "Distribution of HLA-B27 Subtypes in Ankylosing Spondylitis in an Israeli Population," *Archives of Medical Research*, 38. 452-455. May.2007.
- Gonzalez-Roces, S., Alvarez, M.V., Gonzalez, S., Dieye, A., Makni, H., Woodfield, D.G., Housan, L., Kononkov, V., Abbadi, M.C., Grunnet, N., Coto, E. and López-Larrea, C., "HLA-B27 polymorphism and worldwide susceptibility to ankylosing spondylitis," *Tissue Antigens*, 49 (2).116-123. Feb.1997.
- Gorodezky, C., Alaez, C., Vazquez-García, M.N., de la Rosa, G., Infante, E., Balladares, S., Toribio, R., Pérez-Luque, E., Muñoz, L., "The genetic structure of Mexican Mestizos of different locations: tracking back their origins through MHC genes, blood group systems, and microsatellites," *Hum Immunol*, 62 (9). 979-991. Sep.2001.
- Gonzalez-Roces, S., Brautbar, C., Pena, M., Dominguez, O., Coto, E., Alvarez, V., Segal, R., López-Larrea, C., "Molecular analysis of HLA-B27 haplotypes in Caucasoids. Frequencies of B27-Cw in Jewish and Spanish populations," *Hum Immunol*, 41 (2). 127-134. Oct.1994.

- [29] Brinici, A., Bilgici, A., Kuru, O., Durupinar, B, "HLA-B27 polymorphism in Turkish patients with ankylosing spondylitis," *Rheumatol Int*, 26 (4). 285-287. Feb.2006.
- [30] Gul, A., Uyar, F.A., Inanc, M., Ocal, L., Barrett, J.H., Aral, O., Koniç, M. and Saruhan-Direskeneli, G, "A weak association of HLA- B*2702 with Behcet's disease," *Genes Immun*, 3 (6). 368-372. Sep.2002.
- [31] Choo, S.Y., St John, T., Orr, H.T. and Hansen, J.A, "Molecular analysis of the variant alloantigen HLA-B27d (HLA-B*2703) identifies a unique single amino acid substitution," *Hum Immunol*, 21 (3). 209-219. Mar.1988.
- [32] Yang, K.L., Chen, I.H., Hsiao, C.K., Cherng, J.M., Yang, K.Z., Chang, C.C., Yeh, C.C. and Lin, P.Y, "Polymorphism of HLA-B27 in Taiwanese Chinese," *Tissue Antigens*, 63 (5). 476-479. May.2004.
- [33] Cipriani, A., Rivera, S., Hassanhi, M., Marquez, G., Hernandez, R., Villalobos, C. and Montiel, M, "HLA-B27 subtypes determination in patients with ankylosing spondylitis from Zulia, Venezuela", *Hum Immunol*, 64 (7). 745-749. Jul.2003.
- [34] Shankarkumar, U., Ghosh, K. and Mohanty, D, "HLA B27 polymorphism in Western India," *Tissue Antigens*, 60 (1). 98-101. Jul.2002.
- [35] Rosebaum, J.T, "Acute anterior uveitis and spondyloarthropathies," *Rheum Dis Clin North Am*, 18 (1). 143-152. Feb.1992.
- [36] Brophy, S., Pavy, S., Lewis, P., Taylor, G., Bradbury, L., Robertson, D., Lovell, C., Calin, A, "Inflammatory eye, skin, and bowel disease in spondyloarthritis: genetic, phenotypic, and environmental factors," *J Rheumatol*, 28 (12). 2667-2673. Dec.2001