Molecular and Serologic Detection of HLA-B27 among Ankylosing Spondylitis Patients with Some Clinical Correlations

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ABSTRACT:

BACKGROUND:

HLA-B27 can effect clinical presentation and course of ankylosing spondylitis. Different detection techniques of HLA-B27 are available with variable sensitivities and specificities.

OBJECTIVE:

To compare serologic and molecular diagnostic techniques of detecting HLA-B27 status and to correlate it with some clinical variables among ankylosing spondylitis patients.

PATIENTS AND METHODS:

A cross-sectional study was conducted on 83 Iraqi patients with ankylosing spondylitis. Clinical and laboratory evaluations were reported. HLA-B27 status was determined in all patients by real-time PCR using HLA-B27 RealFast™ kit; ELISA method was used as well to detect soluble serum HLA-B27 antigens using Human Leukocyte Antigen® kit.

RESULTS:

The mean age of patients ± SD was (38.4±9.8) years. Male to female ratio was 9:1. Disease onset occurred <30 years in 78% of cases. All (100%) cases had lower back pain, 44 (54%) patients had enthesitis, 14 (16.9%) had peripheral arthritis, 12 (14.5%) had eye involvement, while cardiovascular disease and psoriasis were present in one patient (1.2%) each. HLA-B27 was detected in 55 (66.3%) patients by real-time PCR. The disease onset was earlier and disease duration was longer in HLA-B27-positive patients (p-value<0.05 for both). Uveitis was significantly associated with HLA-B27 positivity (p=0.032). HLA-B27 positive cases by ELISA test were 32 (38.6%) patients. Comparing HLA-B27 gene detection by real-time PCR with sHLA-B27 detection by ELISA revealed that the sensitivity of ELISA was 38.18%, specificity was 60.71% and positive predictive value (PPV) was 61.8%.

HLA-B27 typing by microlymphocytotoxicity (MLCT) test from initial old serologic typing was available for a subgroup of 28 (33.7%) cases. Comparison of ELISA and MLCT results with real-time PCR in this subgroup of 28 cases revealed that ELISA method showed a sensitivity of 60.6%, specificity of 72.72% and PPV of 86.95%, while MLCT showed a sensitivity of 68.96%, specificity of 80%, and PPV of 90.9%

CONCLUSION:

Real-time PCR detection of HLA-B27 status is superior to serological techniques (ELISA and MLCT) as the latter two yielded high false results, with MLCT being relatively better than ELISA.

KEYWORDS: Ankylosing spondylitis, HLA-B27, real-time PCR, ELISA, MLCT

INTRODUCTION:

Ankylosing spondylitis (AS) is a systemic inflammatory disease affecting the sacroiliac joints, spine and not infrequently peripheral joints. [1] It starts in the second or third decade of life with a male to female ratio between 2:1 and 3:1. [2] A strong genetic predisposition for AS and a strong association with HLA-B27 positivity was established. [3,4,5] HLA-B27 is present in >90% of white AS patients and 50-80% of non-white AS patients. [1] Several theories have been created to illustrate this association, such as arthritogenic peptide, molecular mimicry, free heavy chain and unfolded protein response theories. [6]

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HLA-B27 positivity is shown to be linked to earlier and more severe clinical course and increased disease activity. [7] Serologic methods were used to be the standard tests for detection of HLA-B27 such as MLCT test which is based on detecting HLA-B27 antigen on WBC surface or by enzyme immune assays or ELISA by detecting the soluble antigen in serum but serologic techniques were replaced by DNA-based typing because serologic methods are sensitive to down regulation or conformational changes of the antigens, cannot detect the protein structure differences caused by single or limited nucleotide polymorphism, [8,9] have false-negative and false-positive results. [9] Several reasons can explain this high false results e.g. cross reactivity to other antibodies with different HLA class I antigens due to the extensive similarity within the class, [10] altered antigenic epitopes, platelet or erythrocyte contamination. [10,12]

PCR-based techniques can detect the presence / absence of HLA-B27 gene and also can detect allelic differences at the nucleotide level [12]; It is rapid and selective for detection of HLA-B*2701 to HLA-B*2728 subtypes, is capable of detecting a single base difference in DNA sequence between two alleles, [14] and can be applied on any source of good quality DNA regardless of cell viability. [15]

MATERIALS AND METHODS:

This is a cross sectional study that enrolled a total of 83 AS cases from the Rheumatology and Rehabilitation Consultation Clinic at the Medical City – Baghdad / Iraq between December 18th, 2016 and February 22nd, 2017. The diagnosis of AS was based on modified New York Criteria for AS or based on Assessment of Spondyloarthritis International Society (ASAS) classification criteria of axial spondyloarthropathies. All patients were personally interviewed to obtain their demographic and clinical data, investigations performed and on-going treatment regimes.

Consents were taken from all patients and the study was approved by the Ethical Committee at the College of Medicine / University of Baghdad and that of the Training and Development Centre / Ministry of Health, Baghdad / Iraq.

A 3-5 ml peripheral blood sample was aspirated from all enrolled cases for detection of HLA-B27 by ELISA and real-time PCR. ELISA method was performed at the Teaching Laboratories / Medical City – Baghdad while DNA extraction and real-time PCR were performed at Genetics Unit of the Central Public Health Laboratory / Baghdad.

DNA was extracted using Wizard® Genomic DNA Purification Kit, Promega Corporation, USA. Amplification was performed by real-time PCR using RealFast™ Assay kit. Master Mix was prepared for all reactions (samples, positive and negative controls). The components of master mix were (10µl of realfast™ 2x genotyping mix + 5 µl of HLA-B27 assay mix) for each reaction; 15 µl Master Mix was pipetted into each tube; a 5 µl purified patients' DNA or control template (positive control) was added to reach a final reaction volume of 20 µl into each tube. The real-time PCR device was programmed according to the manufacturer's instructions for quantitation with two targets / reporter dyes (HEX and FAM) from AB 7500 Fast software. The fluorescent levels and corresponding amplification curves were displayed in amplification plots in real time PCR software. The DNA samples positive for HLA-B27 as well as the HLA-B27 positive control showed amplification in both HEX and FAM channels, while HLA-B27 negative samples showed amplification in the HEX channel only.

Serologic detection of soluble HLA-B27 status from serum samples was performed by sandwich-ELISA method using (human leukocyte antigen) ELISA kit, MyBioSource™, USA. Standards or samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for HLA-B27 and Avidin-Horseradish Peroxidase (HRP) conjugate was added to each micro plate well and incubated at 37°. Free components were washed away. The substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of sHLA-B27.

As for MLCT technique, the white blood cells (WBCs) are isolated from the blood and are placed in wells in a microtiter plate and then antibodies are added to different wells specific for various class I (including HLA-B27) and class II antigens. The microtiter plate is incubated and then a complement is added. If the WBCs

express MHC allele for which the particular monoclonal antibody (MAB) is specific, then cells lysis occurs and these dead cells will uptake a dye such as trypan blue which can be visualised under the microscope. [16]

Statistical analysis included the descriptive measures of frequency, percentage, mean and standard deviation (SD) and was carried out using IBM-SPSS ver. 20. The data were analysed using Chi square (X^2) test to compare categorical variables and t-test to compare continuous variables in different groups. In all tests, a p-value of <0.05 was considered as statistically significant. The sensitivity, specificity and positive predictive value were measured according to the following equations:

Sensitivity = a / a+c Specificity = d / b+d PPV: = a / a+b

where a = true positive; b = false positive; c = false negative and d = true negative

RESULTS:

The mean age of patients ± SD was 38.4±9.8 years. The average age of disease onset ± SD was 25.55±8.056 years; average age at diagnosis ±SD was 32.133±9.08 years with a male to female ratio of 9:1. The mean of disease duration ±SD was 6.5±5.8 years. All (100%) patients had lower back pain; enthesitis was observed in 44 (53%) cases, peripheral joints involvement in 14 (16.9%) cases, uveitis in 12 (14.5%) cases, cardiac problem and psoriasis each was found in 1 (1.2%) case only. A positive family history was seen in 19 (22.9%) cases. Radiological findings in the sacroiliac area (unilateral sacroiliitis (US) or Bilateral asymmetrical sarcoiliitis (BAS) or BAS with fusion) was seen in 52 (62.7%) cases; 31 (37.3%) cases had radiological findings in sacroiliac area plus the spine (S) [sacroiliitis+ Bamboo spine (BS) and dorsal kyphosis ± cervical fusion (CF)]. Disease activity markers (ESR & C-reactive protein 'CRP') were measured; elevated ESR was present in in 74 (89.2%) patients while the remaining 9 (10.8%) patients had normal ESR. As for CRP, 15 (71.4%) of cases had positive result and the remaining 6 (28.6%) cases were negative.

The frequency of HLA-B27 in the studied patients by real-time PCR was (66.3%) as it was detected in 55 / 83 cases. In this study, HLA-B27 was prevalent in male patients. The disease onset was earlier and disease duration was longer in in HLA-B27-positive patients (p-value=<0.005). There was a statistically significant association of HLA-B27 with male gender, uveitis, age at disease onset and disease duration, (p-values= 0.002, <0.005, 0.014 and 0.032, respectively); while no significant statistical association of HLA-B27 was found with age at diagnosis, enthesitis, peripheral arthritis, family history, disease activity markers (CRP and ESR) radiological findings groups and biologic and methotrexate treatment (p-values=0.051, 0.647, 0.125, 0.096, 0.337, 0.843 and 0.805, respectively); table (1).

The frequency of sHLA-B27 positivity by ELISA test was 38.6% as it was detected in 30 /83 cases. Comparison of ELISA results against real-time PCR results with all studied variables (table 1) revealed that 34 (66.7%) cases had false-negative results (real-time PCR-positive but ELISA-negative). Therefore, the sensitivity of ELISA technique was only 38.18%, while there were 11 (34.4%) false-positive results (negative real-time PCR but positive ELISA). So, the specificity of this method was 60.71%.

A subgroup of 28 cases, already on treatment, had older initial HLA-B27 typing by MLCT test. In those 28 cases, HLA-B27 typing was available by all 3 methods (MLCT, ELISA and real-time PCR). Comparing serological (ELISA and MLCT) results with molecular (real-time PCR) results that is used as a control, revealed that false negative results by ELISA were 13/28 (46.4%) and the false positives were 3/28 (10.7%) [Sensitivity= 60.6%; specificity =72.72%, PPV=86.95%] while MLCT showed that 9/28 (32.1%) results were false negatives and 2/28 (7.1%) were false positives [sensitivity=68.96%, specificity=80%, PPV=90.9%]; as shown in Table (2).

Table 1: Comparison of HLA-B27 status by ELISA and real-time PCR methods with patients' demographic, clinical and radiological variables.

Studied variables		ELISA HLA B27 status					real-time PCR HLA-B27 status				
		Positive		Negative		P-value	Positive		Negative		P-value
		Count	%	Count	%		count	%	count	%	
Sex	Male	29	90.6	47	92.2	0.807	54	98.2	22	78.6	0.002*
	Female	3	9.4	4	7.8		1	1.8	6	21.4	
Age groups (Years)	≤30	5	15.6	13	25.5	0.325	13	23.6	5	17.9	0.523
	31-40	10	31.3	21	41.2		20	36.4	11	39.3	
	41-50	14	43.8	13	25.5		19	34.5	8	28.6	
	51+	3	9.4	4	7.8		3	5.5	4	14.3	
Family History	P	7	21.9	12	23.5	0.861	16	29.1	3	10.7	0.096
	N	25	78.1	39	76.5		39	70.9	25	89.3	
Lower Back Pain	P	32	100	51	100	1	55	100	28	100	0
	N	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
Peripheral Joint involvement	P	4	12.5	10	19.6	0.55	12	21.8	2	7.1	0.125
	N	28	87.5	41	80.4		43	78.2	26	92.9	
Enthesitis	P	16	50.0	28	54.9	0.821	28	50.9	16	57.1	0.647
	N	16	50.0	23	45.1		27	49.1	12	42.9	
Uveitis	P	6	18.7	6	11.8	0.523	11	20.0	1	3.6	0.032*
	N	26	81.3	45	88.2		44	80.0	27	96.4	
Age at disease onset		-	-	-	-	-	23.255	6.86	30.18	8.68	<0.005*
Disease duration		5.78	5.08	7.02	6.25	0.349	7.49	6.49	4.68	3.65	0.014*
Age at diagnosis		32.28	9.73	32.04	8.75	0.907	30.75	9.095	34.86	8.575	0.051
Bilateral asymmetrical sarcoiliitis	P	14	43.8	21	41.2	0.817	23	41.8	12	42.9	0.928
	N	18	56.2	30	58.8		32	58.2	16	57.1	
Bilateral asymmetrical sacroiliitis With fusion	P	17	53.1	27	52.9	0.987	30	54.5	14	50.0	0.817
	N	15	46.9	24	47.1		25	45.5	14	50.0	
Bilateral sacroiliitis	P	13	40.6	15	29.4	0.293	20	36.4	8	28.6	0.624
	N	19	59.4	36	70.6		35	63.6	20	71.4	
Cervical fusion	P	12	37.5	16	31.4	0.636	19	34.5	9	32.1	0.827
	N	20	62.5	35	68.6		36	65.5	19	67.9	
Unilateral sacroiliitis	P	1	3.1	2	3.9	0.850	1	1.8	2	7.1	0.262
	N	31	96.9	49	96.1		54	98.2	26	92.9	
Wedge shape Lumber vertebrae	P	0	0.0	3	100	0.281	3	5.5	0	0.0	0.547
	N	32	100	48	0.0		52	94.5	28	100	
Biological treatment	P	29	90.6	43	84.3	0.517	48	87.3	24	100	0.843
	N	3	9.4	8	15.7		7	12.7	4	0.0	

Methotrexate P N	2 30	6.3 93.8	11 4	21.6 78.4	0.071	9 46	16.4 83.6	4 24	16.7 83.3	0.805
Total No. (%)	32 (38.6)		51 (61.4)		Total	55 (66.7)		28 (33.73)		83 (100)

P=Positive; N=Negative; PCR=Polymerase Chain Reaction; HLA=Human Leukocyte Antigen;
* = presence of a significant association ($p<0.05$)

Table 2: Comparison of HLA-B27 typing obtained from microlymphocytotoxicity (MLCT), ELISA and real-time PCR techniques among 28 AS cases.

Case No.	MLCT	ELISA	real-time PCR	Case No.	MLCT	ELISA	real-time PCR
1	N	N	P	15	N	P	N
2	N	N	p	16	P	N	P
3	N	N	P	17	P	N	P
4	N	N	P	18	P	N	P
5	N	N	P	19	P	N	N
6	N	N	N	20	P	N	P
7	N	N	N	21	P	N	P
8	N	N	P	22	P	N	N
9	N	N	N	23	P	N	P
10	N	N	P	24	P	P	P
11	N	P	N	25	P	P	P
12	N	P	p	26	P	P	P
13	N	P	N	27	P	P	P
14	N	P	P	28	P	P	P

ELISA: False Positives=3, False Negatives=13; MLCT: False Positives=2, False Negatives=9.
ELISA sensitivity= 60.60%; specificity=72.72%; PPV =86.95%.
MLCT sensitivity = 68.96%; specificity= 80%. PPV = 90.90%.

PPV=Positive Predictive Value, N=Negative, P=Positive

Table 3: Comparison of some clinical variables in different Iraqi studies on AS patients.

Variables	Current study	Gorial et al [18]	Al-Qadi et al [19]	Al-Bedri [20]	Al-Rawi et al. [21]
Year	2018	2016	2015	2014	1978
No. of patients	83	200	41	318	53
Mean Age ± SD (year)	38.4±9.8	35.2±8.6	32.8±5.8	-	-
Disease duration Mean ± SD (year)	6.5±5.8	10.9±6.7	5.4±3.5	-	-
M:F ratio	9:1	13.3:1	4.1:1	9:1	8:1
Enthesitis No. (%)	44 (53)	96 (48)	-	256 (80.5)	29 (54.7)
Peripheral arthritis No.(%)	14 (16.9)	127 (63.5)	17 (41.5)	44 (13.8)	3 (5.66)
Uveitis No.(%)	12 (14.5)	43 (21.5)	5 (12.2)	75 (23.6)	5 (9.4)
Positive Family history No.(%)	19 (22.9)	52 (26)	15 (36.6)	10 (3.1)	4 (7.55)

SD=Standard Deviation

Table 4: Comparison of the frequency of HLA-B27 in different studies on AS patients.

Study	Year	Country	HLA-B27 Positivity (%)	No. of cases	Method of detection	Reference No.
Al-Rawi	1978	Iraq	21 (84)	25	MLCT	23
Al-Arfaj	1996	Saudi Arabia	8 (67)	11	Not mentioned	34
Nickman et al	2008	Iran	82 (68.9)	119	Conventional PCR	33
Harfouch & Suadi	2011	Syria	30 (60)	50	SSP-PCR	35
Otom & Al-Ahmar	2013	Jordan	72 (72)	100	Not mentioned	36
Inanır et al.	2013	Turkey	76 (73.89)	103	Not mentioned	24
Al-Badri	2014	Iraq	175 (55)	318	MLCT	22
Gorial	2015	Iraq	61 (69.5)	139	Not mentioned	20
Al-Qadi	2016	Iraq	27 (65.9)	41	SSP-PCR	21
Current	2018	Iraq	55 (66.3)	83	Real time PCR	-

PCR= Polymerase Chain Reaction; SSP= Sequence-Specific Primer

DISCUSSION:

This study is one of few studies that determined the frequency and association of HLA-B27 with ankylosing spondylitis among Iraqi patients, probably the second that used molecular detection of HLA-B27 typing by real-time PCR and investigating disease associations. To the best of the present knowledge, the current study is the first that compared serological (ELISA and MLCT) methods with molecular method (real-time PCR) with measurement of sensitivity and specificity of the two serological tests against real-time PCR.

Literature showed that AS patients with HLA-B27 had longer disease duration, higher markers of disease activity, poorer functional status, poorer quality of life and more extra-articular manifestations, which is reflected in the percentage of patients in need of biologic therapies. [17, 18, 19] For this reason, different variables were studied, including patients' demographic features, clinical, laboratory and radiological variables; all were correlated with ELISA and real-time PCR results for HLA-B27 status among Iraqi AS patients.

The findings of the current study were compared with other local studies (Table 3), and other neighbouring studies.

In this study, the mean age of patients ±SD with Ankylosing Spondylitis (AS) was 38.4±9.8 years with an age range between (16-62) years.

A previous Iraqi study [20] reported that the mean age of was 35±8.6 years (range 18-66) years, which is comparable with the current study.

In this study, there was a male predominance (M/F ratio was 9:1). This ratio was higher than Al-Qadi study of 4.1:1 [21] but similar to Al-Bedri and Al-Rawi studies. [22, 23]

M:F ratio in Turkish [24] and Iranian [25] studies in 2013 conducted on 103 and 320 AS patients was (1.6:1 and 3.8:1) respectively. This male predominance in Iraqi AS patients can be attributed to genetic and environmental factors that play a role in etiology and disease manifestation. [26, 27] It can also be caused by delayed diagnosis in women due to incomplete and slow progression of disease [28,29] which makes females more likely to have milder pain at first, thus making the diagnosis of AS more difficult in women. [30]

Enthesitis was observed in 44 (53%) of cases, peripheral arthritis in 14 (16.9%), and eye involvement in 12 (14.5%) of cases; these results disagree with other local studies [summarized in table (3)] and these differences might be due to variability in sample size, disease duration of the enrolled cases and treatment modality used.

Disease activity markers were not conclusive in our study as most patients were already on treatment (NSAID with or without disease modifying drugs).

Family history of a similar condition was found in 19 (22.9%) cases, which was higher than Al-Bedri, [22] and Turkish [24] studies where positive family history was 10 (3.1%) and 21 (20.38%) respectively but lower than Al-Qadi [21] and an Iranian [25] studies as they showed 15 (36.6%) and 96 (30%) positivity respectively. Being a multifactorial disease with a variation in genetic susceptibility in different ethnic groups might explain this difference. [31]

Sacroiliitis was present in all patients but axial involvement was found in 31 (37.3%) cases only. These findings do not agree with Al-Bedri study [22] which showed axial involvement in 217 (68.2%) cases and only 17 (5%) cases had a disease limited to sacroiliac and lumbar region. This is due to difference in number of enrolled patients, disease duration and the fact that most

patients [n=72 (86.7%)] in the present study were taking biologic treatment from the start as TNF-inhibitors slows the rate of radiographic progression in AS. [32]

The frequency of HLA-B27 in this study was 66.27% which was nearly similar to Gorial, [20] Al-Qadi, [21] an Iranian, [33] and a Saudi [34] studies while it was higher than AlBedri, [22] and a Syrian [35] studies but lower than Al-Rawi, [23] Jordanian, [36] and a Turkish [24] studies; Table (4).

The variations in the frequency of HLA-B27, although small, might be due to difference in genetic susceptibility, number of patients enrolled, and differences in detection method of HLA-B27. Each test can show different frequency of positivity on the same sample of patients. [37, 38]

In this study, there were statistically significant associations between HLA-B27 positivity and sex, age of disease onset, disease duration and occurrence of uveitis.

HLA-B27 positive patients tend to have increased disease progression than negative ones and subsequently earlier onset and diagnosis; [39] HLA-B27 positive patients have high concentration of TNF-α in the aqueous humour than negative ones; the pro-inflammatory cytokine TNF-α may play role in the pathogenesis of clinical uveitis. [40]

A previous Iraqi study [21] suggested that there was a tendency of the disease to affect more HLA-B27 positive men than HLA-B27 positive women and the appearance of the symptoms at younger age in the presence of HLA-B27 gene was associated with early onset of the disease. These findings were in concordance with findings of the present study.

The current study showed that there were no significant associations of HLA-B27 with age at diagnosis, family history, enthesitis, peripheral arthritis, disease activity markers, radiological findings and treatment types.

A previous Iraqi study [21] showed that there was a significant relation between HLA-B27 and family history, which disagrees with findings of the present study. Sample size might have a role in this difference.

Atagunduz study [17] in 2010 showed that HLA-B27 positive male patients were 3-4 times more likely than HLA-B27 negative male patients to have radiological progression which does not correspond to the present results as most patients were already taking biologic disease-modifying agents, causing delayed radiological progression.

The frequency of HLA-B27 by ELISA method was 38.5%, which was much lower that the frequency by real-time PCR, with a relatively high false negative and false positive results, although ELISA is known to be reliable, accurate and with a high sensitivity. [41]

ELISA method used in this study was based on detection of soluble HLA-B27 antigens in serum samples. [42, 43, 44] This method showed good correlation with traditional serologic technique. [37]

The low concentration of the soluble HLA-B27 detected in this study could be explained by type of treatment they were put on as all enrolled patients were on NSIAD and most of them were also on disease-modifying agents; a possible loss of some sHLA-B27 during blood clotting might also be a factor. [37]

A higher positivity rate can be obtained by using an enhancing solution with anti-HLA-B7 MAB (clone MB40.2, subclass IgG1) as used in Dunky study; [37] its content (monoclonal antibody) does not cross react with HLA-B27, binds to soluble HLA-B7 leading to accumulation of HLA-B7 molecules thus preventing cross reaction that causes false positive results and acts as a bridge antibody that allows the distinction between HLA-B27 positivity and negativity. [37]

False-negative results of MLCT may be due to altered antigenic epitopes, platelet or erythrocyte contamination, Salmonella enteritidis or Yersenia infection. [10]

Lack of availability of specific antisera for all alleles of HLA-B27 [45] makes it impossible to detect all currently known HLA molecular variants due to families of alleles whose HLA B products share some serologic markers while these alleles encode distinct molecular variants. [46]

Cross reaction with HLA-B7 can also cause false positive result. HLA-B27 and B7 antigens are members of large cross-reactive group (CREG) which contains; the HLA-B7, B13, B22, B27, B40, B41, B42, B47, and B48 antigens and these members are known to share common epitopes. Thus the monoclonal anti-B27 may cross-react with any members of this group. [11]

Comparing result of HLA-B27 from a subgroup of 28 cases in the current study by MLCT, ELISA and real-time PCR using the latter as a control method (as it detects the gene not the antigen) revealed that the sensitivity of ELISA was 60.6% in contrast to 68.96% for MLCT, specificity of ELISA was 72.72% versus 80% for MLCT, while PPV was 86.95% for ELISA and 90.90% for MLCT.

A previous study [37] conducted on 81 patients with suspected inflammatory disorder reported six wrong results in enzyme immune assay (EIA) method when compared with PCR results with four false negatives, one false positive results plus one undeterminable sample. Not adding an enhancing solution, patients' use of biologic treatment, and possible loss of sHLA-B27 during blood clotting of samples might explain the differences from the present results. The same study reported only two wrong results in MLCT, both were false negatives. This also differs from the current findings possibly due to cross-reaction with large cross-reactive group, not using large panel of antisera against HLA-B27, not excluding altered antigenic epitopes, or less likely due to platelet or erythrocyte contamination as in acute phase of Salmonella or Yersenia infection. [10]

A Korean study [47] conducted on 328 patients compared MLCT HLA-B27 results of these patients with those of sequence-specific primer PCR and revealed that 14% (14/100) were false positive and 3.9% (3/228) were false negative ones. The false positive results in that study was due to cross reaction with HLA-B7.

CONCLUSION:

Real-time PCR detection of HLA-B27 typing is superior to that of ELISA and MLCT; the latter two yielded high false negative or positive results. If molecular diagnostic test was not available, enzyme immune assay (EIA) to detect cellular not serum antigens or flow cytometry (FC) are recommended alternatives as MLCT and ELISA on serum samples showed low sensitivity, specificity and positive predictive values.

Conflict of interest:

All authors declare no conflict of interest.

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