Rapid HLA-B27 Test with Real-Time PCR in Suspected Ankylosing Spondylitis Iraqi Patients

Introduction:
HLA-B27 is a MHC class 1 molecule that is strongly associated with the Ankylosing Spondylitis and Seronegative Spondyloarthropathies. Various methods have been developed for the identification of the HLA-B27 allele. The PCR-based HLA-typing methods, including the standard PCR with sequence-specific primers (SSP), sequence specific oligonucleotide (SSO) have become widely used alternatives to serology. This study is to assess a real-time TaqMan PCR assay for genotyping HLA-B*27 in the Iraqi patients with suspected Ankylosing Spondylitis. The study consisted of 50 Iraqi Arab Muslims patients suspected to have ankylosing spondylitis consulted Orthopedic and Rheumatology department in Al-Kindi Teaching Hospital in Baghdad from September 2012 to May 2013. The second control group consisted of twenty apparently healthy volunteers' age matched with first group from staff employees served as controls. Blood samples have been obtained from them and genomic DNA was extracted using using reila prep spin column kit (Promega, USA). Real-time PCR reactions were performed using an Exicycler TM 96 Real-time Quantitative Thermal Block (Bioneer, Daejeon, Korea) and the AccuPower, HLA-B27 real-time PCR kit (Bioneer, Daejeon, Korea). The age of patients in this study were range from 16-35 years with a mean of 28.2 ± 3.5 years. Control ages range from 17-37 years with a mean of 30.28 ± 2.87. All positive samples had Ct FAM values <28. Ten out of sixty (20%) of patients showed positive HLA-B27 results and did not detected in control group. This results gave concordant with PCR-SSO results. Real-time PCR methods appear to be reliable and very fast in the detection of HLA-B27 and could be potential alternatives to conventional PCR and serology methods.

Patients and methods:
The study consisted of 50 Iraqi Arab Muslims patients suspected to have ankylosing spondylitis consulted Orthopedic and Rheumatology department in Al-Kindi Teaching Hospital in Baghdad from September 2012 to May 2013. The study took place at the HLA typing research Unit, Al-Kindi College of Medicine, Baghdad University. The age of patients in this study were range from 16-35 years with a mean of 28.2 ± 3.5 years. Control ages range from 17-37 years with a mean of 30.28 ± 2.87.

The exclusion criteria were patients previously had prior or recent history of rheumatoid arthritis, autoimmune diseases and other metabolic diseases. Those patients had been previously typed with conventional PCR-SSO method using SSO HLA type B update plus Kit and Master mix for HLA-B multiplex plus Kit -Innogenetics-Belgium using automated method by Autolipa-48–Innogenetics-Belgium.. The second control group consisted of twenty apparently healthy volunteers' age matched with first group from staff employees served as controls.

In this study we try to assess “B27” associated with ankylosing spondylitis using real time PCR in Iraqi patients.

KEYWORDS: Real time, PCR, Ankylosing Spondylitis
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Statistical analysis:
Was done using MiniTab version 3. P-value less than 0.05 was considered positive.

Results:
All positive samples had Ct FAM values <28. All samples had Ct TAMRA values <26 (Fig. 1).

A Total of 50 clinical samples who were suspected to have AS and twenty controls were used to compare the real time Taq Man PCR with traditional PCR-SSP method. Ten out of fifty (20%) of patients showed positive HLA-B27 results and did not detected in control group with “B27” FAM Ct value = 26 and thirty samples had FAM Ct value=33 and ten samples their “B27” FAM Ct did not determined and give negative results Figure-2. These results gave concordant with PCR-SSO results. The conventional PCR-SSO method takes approximately two days. In comparison, the real-time PCR systems can analyze 96 samples at same time with in one and half hour.

Fig. 1. Fluorescence signal versus cycle number plot during the amplification of HLA-B27 positive cases of human DNA samples of patients suspected to have AS with primers specific for HLA-B27 (green signal) and GAPDH (black signal)(NTC).

Abbreviation: GAPDH, glyceraldehyde-3-phosphate dehydrogenase. NTC, Non Template Control.

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Fig. 2. Histogram representation showing the distribution of Ct FAM (HLA-B27) and Ct TAMRA (GAPDH) (NTC) from 50 samples on genotyping by Taqman allelic-specific amplification. All samples had Ct TAMRA values <26, and 10 positive samples had Ct FAM values <28. Abbreviation: GAPDH, glyceraldehyde-3-phosphate dehydrogenase. NTC, Non Template Control.

Discussion:
Serological typing had been the main technique used for HLA Class I analysis. However, due to the beginning of molecular biology, the last twenty years has seen an exponential growth in the application of DNA technology to the field of Histocompatibility and Immunogenetics. DNA based typing focuses on defining differences in genes and may identify differences of little biologic relevance (8). There are many limitation for serological tests include lack of availability of specific antisera for all alleles of HLA-B27 because the number of known HLA-B27 alleles has increased, the test must be performed within 6 hours after drawing the blood, the amount of blood used must not be less than 5 ml, difficult in confirming homozygosity and it is difficult to be done in immune suppressed patients (9). False-negative serological HLA-B27 typing results may be due to altered antigenic epitopes but it can be detected by polymerase chain reaction (10). Thus, molecular typing techniques, which give more accurate results, have replaced the Microlymphocytotoxicity. Most genotyping methods are based on the group-specific amplification of HLA-B alleles by the PCR. The PCR-SSP and PCR-SSO is more widely used to detect HLA-B27 alleles. However, conventional PCR-based genotyping requires post-PCR manipulations that increase the risk of cross-contamination between samples; further, these post-PCR steps are laborious, especially when genotyping large numbers of samples. Therefore, real-time PCR, which allows the simultaneous amplification and detection of a specific DNA target, is an attractive alternative to conventional PCR-SSP (11, 12). In our study we use real time PCR method for detection HLA-B27 in suspected AS Iraqi patients and detected 20% of them were “B27” positive and this results was in accordance with results done by SSO method. There is no other studies in Iraq about “B27” subtypes. The 20 percent is so low because other studies found 90% is association between “B27” and AS because the study patients group are suspected to have AS and not proved by their physisans by other tests like x-ray and other investigations. Thus, Real time-PCR accurately determined HLA-B27 genotypes and substantially reduced the labor-intensive steps and the total processing time (2 hr, including interpretation) when compared to the usual time required for PCR-SSP (5 hr). Our results in agreement with other study done in Dutch and other countries (6, 13). Thus real time PCR technique is superior to serology, SSP and SSO as fresh and aged samples can be tested and only a small amount of blood is used. The results can be obtained even with a low concentration of DNA (0.1 ng/ml). Moreover, this technique is reliable, simple, convenient, and more cost effective for routine laboratories. The technique is fast and easy to perform and to handle specimens, because the viable cells necessary for serological typing are not needed. Thus this techniques can be employed as a part of routine clinical practice for exact diagnosis of Seronegative Spondyloarthrisitis and it can be envisaged that in the near future HLA-typing by serology will be replaced by real time PCR in routine clinical practice.In addition to that HLA-B27 gene can be detected by using a spectral plasmon resonance imaging system(14).

Conclusions:
Real-time PCR methods appear to be reliable and very fast in the detection of HLA-B27 and could be potential alternatives to conventional PCR and serology methods.
Conflict of interest:
There is no conflict of interest.

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