

Association of Human Leukocyte Antigen-(HLA-B 27) Gene Polymorphism with Vitiligo Disease

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ABSTRACT

Vitiligo have been recorded in different ages in population it is appeared by interaction between different factors causes de-pigmentation in skin, the present study conducted to estimate HLA 27 gene polymorphism in vitiligo patients using single specific primer-polymerase chain reaction (PCR-SSP), DNA was extracted from whole blood for patients and control, then amplification was implemented, the results of demographic show that the means of age were 33.093 and 32.809 for patients and control, disease was recorded in female than male 79.54%, 18.18%, about 36.36% of patient had family history and 56.81% of them, their parents had family related. High percentage of patients had dispersive sites of de-pigment sites 54.54% while 40.90% of them in hands and legs. The genotyping results show that there was no association between HLA B27 and vitiligo disease, HLA – was 95.83% in patients while it was 85.71% in control in non-significant differences. This study concluded that there was no association between HLA B 27 and vitiligo disease in Iraqi population.

Keywords: vitiligo disease, of Human Leukocyte Antigen- (HLA- B 27), PCR-SSP.

Introduction

Vitiligo is an autoimmune disease resulted from melanocytes destruction which caused depigmentation in some part of skin and hair. The real cases of vitiligo have been studied, all reasons of vitiligo were based on the Loss of melanocytes function, However, some factors like autoimmune, genetic polymorphism and mutation, viral infections and oxidative stress may be contributed in vitiligo incidence and development¹.

There were three theories for explaining the mechanisms of vitiligo pathology, the convergence theory is that stress, toxic compounds accumulation, autoimmunity, genetic mutations, changes in cellular environment and melanocyte migration and proliferation impaired have major role in varying features of vitiligo etiopathology².

Studies reported association between MHC class I region SNPs and vitiligo in the vicinity of the *HLA-A*

gene^{3,4} found high-risk allele when they analysis DNA sequence encoding to canonical HLA-A2 specificity, which also present in variety of auto-antigens derived from melanocyte proteins like tyrosinase⁵, TRP2⁶, OCA2⁷ and MART-1/melan-A⁸.

Materials and Method

Study Design: Case-control study was carried out at the DNA lab/Babylon unv./in Babylon province/Iraq.

Study Population: The study subjects enrolled 44 patients suffer from vitiligo that diagnostic by specialist physician and these patients under biological therapy randomly selected from Karbala teaching hospital. All subjects in this study were taken written consent before participation in this study according to ethical approval of Iraqi ministry of health. Questionnaire taken from the patient included age, sex smoking habit, alcohol intake, and family history, past medical history.

Blood samples were collected in EDTA tube for DNA extraction, DNA was extracted from whole blood using (Genaid extraction kit) according to⁹, After DNA extraction; consternation and purity of DNA

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were estimated using nanodrp. PCR conditions were performed as a following.

PCR Amplification single specific primer-polymerase chain reaction (PCR-SSP) using, a set of primers including (forward primer: 5F: 5'- GCTACGTGGACGACACGCT-3', R:5'-CTCGGTCAGTCTGTGCCTT -3', R:5'-TCTCGGTAAGTCTGTGCCTT A-3' (149 bp) and HgH as positive control F5'-TGCCCTTCCCAACCATTCCTTA-3' M R5'CCACTCACGGATTCTGTTGTTGTGTTTC (434bp) ¹⁰.

PCR conditions it performed as a following; perdenaturation for 5 min at 94°C, then 35 cycles (60s at 94°C, 2 min at 65°C, 60 s at 72°C, and finally 10 min at 72°C). PCR products were determined by electrophoresis pattern in agarose gel (1.5% agarose, 70 V, 20 mA for 45 min) with ethidium bromide staining, the results were statically analysis using χ^2 and odd ratio at CI 95% And p value <0.05).

Results and Discussion

Vitiligo is depigmentation disorder disease caused by interaction of different factors, one of these is genetic polymorphisms and mutations, present study was conducted to detection HLA -27 gene polymorphism in vitiligo patients using PCR-ARMS technique. according to data that collected from patients and control the demographic the distribution of study groups show in table 1, there is no significant differences between patients and control in age mean (33.093 and 32.809) years at p value 0.928.

The prevalence of diseases in present study was in high in males than females, about 79.54% of samples were males several studies show that females were more frequent than males as in ¹¹ for three years respectively in koria. In Indian ¹², recorded that the ratio of females to males was 1.5:1, other study deal with present results that males more frequent than females ^{13,14}, on the other hand there was equal incidence between male and female ¹⁵. This distribution depending on different factor like life style, genetic predisposition, genetic of gender, nutrition, and environmental factors.

Table 1: distribution of study subjects according age and sex

Categories	Patients	Control	Statics
Age	33.093 ± 15.00	32.809 ± 15.066	t = 0.0894 p= 0.9289
Sex			
Male	79.54%	80.95%	Qi-square 0.006, P = 0.938
Female	18.18%	19.04%	

Table 2, show chractisite of patients, 36.36 % of patients had family history while 63.63 didn't have family history, ¹⁶ mention in their study that vitiligo in the family history is caused by autosomal dominant inheritance. ¹⁷ clarified the relation between family history and children vitiligo, the earlier onset of pediatric vitiligo is linked to a family history. The information's of family history of disease in Iraq was poor, especially genetic mutation and polymorphism in addition of other factor which contribution in disease incidence. In addition of family history about 56.81 % (table 2) of patinas their parents were related, this because culture of relative marriage in population.

Table 2: some features of vitiligo patients

Categories	Percentages %
Family history	
Yes	36.36
No	63.63

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Sites	
Dispersive	54.54
Hands and legs	40.90
Abdomen	2.27
Face	2.27
Father and mother relation	
Yes	56.81
No	43.18

About 54.54% of patient had dispersive de-pigments area while 40.90 % of them the de-pigment area in terminal and 2.27 % in the abdomen and face ¹² explained this phenomena that de-pigmentation occurs simultaneously or subsequently at various unrelated distant sites.

The results of DNA extraction was in figure (1, A) all of patients and control had high concentrated and pure DNA.

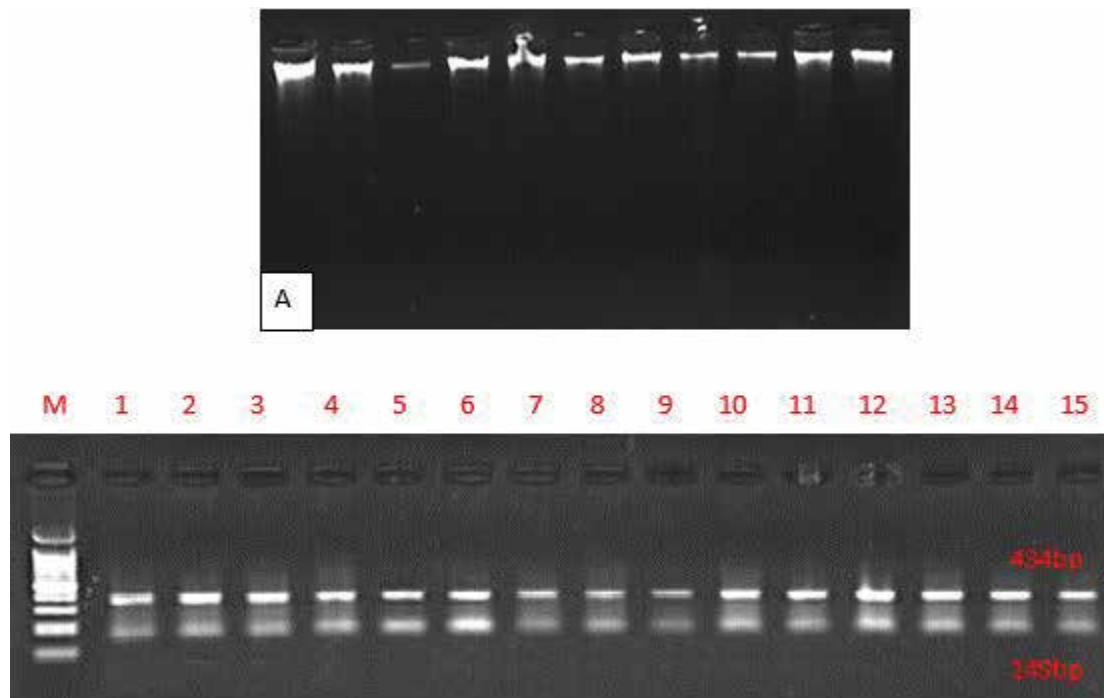


Figure 1: Electrophoresis pattern of A, DNA extracted from patient and control (70 V, 20 mA, 30 min, 0.5 X TBE buffer) . B PCR- SSP products for of hela - 27 genotyping in patient and control . lane 1, 7 line and 8,15 HLA +.

Table (3) and figure (2,B) show results of HLA genotyping, HLA– was more frequent in patients 95.83% than control 85.71% while HLA+ was high percentage in control 14.28% than patients 4.166%. these differences were non-significant at p value 0.2921, odd ration 0.265 (CI 0.0214 to 3.1778). A study implemented by ¹⁸ didn't deal with present study, they improved association several HLA alleles like B 27 with vitiligo disease especially in familial history. the association of HLA alleles with vitiligo were diverse among population, B27 was associated with this disease in Italian population ¹⁹.

As a results of HLA alleles role in immune response against viral infected cells in serious pathways, it may be contributed in response to vitiligo caused by viral infection which improved by detected CMV in DNA of de-pigments cells ^{20,21}.

Investigators consider HLA gene polymorphism as genetic marker of vitiligo disease and predisposition because of closed association between HLA alleles and disease incidence in different population ^{22,23} also the strongly linkage between HLA loci and other sites in the region of major histocompatibility complex (MHC) in chromosome 6p. ^{24,25}.

Table 3: Genotype frequency in patents and control

Genotype	Patient	Control	Odd ratio
HLA+	4.166%	14.28%	0.2609
HLA -	95.83%	85.71%	CI% 0.0214 to 3.1778 P = 0.2921

Conclusion

This study concluded that there was no association between HLAB 27 and vitiligi disease in Iraqi population.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: Self-funding

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