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Relationship Between Catechol-O-Methyltransferase Polymorphism and Vitiligo in A Sample of Iraqi Peoples

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Abstract

Background Vitiligo is a common condition, highly distributed in Iraq and in the world. It is an acquired disorder of the skin and mucous membranes. It may appear at any age; cases have been reported as early as 6 weeks after birth. Segmental vitiligo often occurs in a dermatomal pattern, This observation led to a neural hypothesis that proposes certain chemical mediators released from nerve endings may cause decreased melanin production, elevated neuropeptide levels have been demonstrated in skin affected by vitiligo.

Objective To shed light on some demographic parameter and detect the association between vitiligo and Catechol-O-Methyltransferase (COMT) gene polymorphisms in a sample of Iraqi population.

Methods This case-control study was performed to detect the polymorphism of COMT 158 G/A by polymerase chain reaction restriction length polymorphism trial in 110 individuals of two groups of peoples, the first group represent the patients and the other group was apparently healthy as control, with age range from 3-62 years. Ninety-six Iraqi vitiligo patient were selected from vitiligo patients attended the Consulting Clinic of Al-Mussyab Hospital and private clinic of dermatology in Al-Mussyab City during the period from the beginning of June 2014 till ending of September 2014. Approximately 2 ml of blood samples were collected from all individuals in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes with aseptic technique and kept in -20°C.

Results The demographic study revealed that the disease was appeared in females 59 (61.46%) more than that in males 37 (38.54%) with a highly significant difference (P ≤0.01) and the ratio of females to males was 1.59: 1. The mean age of the patient group was 20.67±13.36 years with age range 3-62, the highest number of patients affected by vitiligo was found in the age range 10-19 years with a highly significant difference (P <0.01). The most frequent vitiligo type was segmental followed by generalized, mucosal, acrofacial, then focal type, lately, the universal type with a highly significant difference (P <0.01). In this study, psychological factors were seen in 85% of cases, 10% nutrition may act as triggering factor in these cases, and 5% unknown cause, the result of this study count no differences in COMT polymorphism between vitiligo patients and the control persons.

Conclusion There was no significant association between vitiligo and COMT 158 G/A polymorphisms in the Iraqi people.

Keywords Vitiligo, Catechol O-methyl transferase gene, polymerase chain reaction

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List of abbreviations: COMT = Catechol O-methyl transferase, PCR-RFLP = Polymerase chain reaction-restriction fragment length polymorphism), SNPs = Single nucleotide polymorphism

Introduction

Vitiligo is an idiopathic, acquired, and worldwide common problem, affecting persons of all ages and both sex. It

presented by well circumscribed, depigmented macules and patches, which occur after selective destruction of melanocytes. Patients lose their skin color, mostly in progressive ways. The cause of the disease is still unknown [1].

Vitiligo can be psychologically problem, the precise pathogenesis remains evasive; however, several studies have been submitted to explain the loss of epidermal melanocytes in this disorder. The mechanisms fall within the item of autoimmune, oxidant-antioxidant, biochemical, viral and neural. Studies had also pointed to an important role of genetic susceptibility to vitiligo [2]. It appears genetically in a polygenic, multifactorial method, and the complex genetics of vitiligo problem includes multiple susceptibility loci, genetic heterogeneity and incomplete penetrance with gene-environment and also gene-gene interaction [3].

In the melanocytes, catechol O-methyl transferase (COMT) can prevent the creating of toxic O-quinones through melanin synthesis (4). Human COMT has two length, the soluble and membrane-bound (MB) (5). A single base-pair change (G/A) in exon 4 of the COMT gene, resulting in the changing of amino acid (Val/Met) at codon 108 of S-COMT or codon 158 of MB-COMT, which reduces the activity and thermo stability of the enzyme (6). Since COMT participate in the metabolic and autocytotoxic failure of melanocytes and the other epidermal cells in vitiligo disorder, so the COMT 158 G/A polymorphism might be playing a role in the etiology of vitiligo [7]. Identification of the genes, which involved in the etiology of vitiligo can aid in the diagnosis, treatment and preventions of disease development.

This study was conducted to shed light on some demographic parameters and detect the association between vitiligo and COMT gene polymorphisms in a sample of Iraqi population.

Methods

This case-control study carried out to detect the COMT gene by molecular analysis. The work was carried out on 110 individuals of two groups of peoples, the first group represented the patients and the other group was apparently healthy as control, with age range from 3-62 years. Whole blood were taken from 96 patients who were selected from vitiligo patients attended the Consulting Clinic of Al-Mussyab Hospital and special clinic of dermatology. The study extends from the beginning of June 2014 till ending of September 2014.

Inclusion criteria

Patients must be previously diagnosed with vitiligo (they were have acquired patchy depigmentation with typical distribution; face, hands, legs and genital area) and reserved certain type of drug regimen), and 14 apparently healthy individuals were selected with no vitiligo, they had no family history of this disease and corresponding to patients group in their age and sex.

Exclusion criteria

Those individuals with atypical lesion distribution as congenital like birthmarks, or due to inflammatory skin disease (psoriasis, lichen planus) or due to use of melanocytotoxic chemicals, post traumatic causes and postinfection.

Blood sample collection and DNA extraction

Approximately 2 ml of blood samples, which collected from all individuals in EDTA anticoagulant tubes with aseptic technique then kept in -20°C. Genomic DNA was extracted from blood sample by using Genomic DNA Kit (Genaid, Taiwan), which provides an efficient way for purifying total DNA from human whole blood. The outcome demonstrated that the DNA in all samples, which sufficiently for polymerase chain reaction (PCR) analysis and appeared as compact separated band by electrophoresis on 1% agarose gel with ethidium bromide at 60 V for half hour (Figure 1).

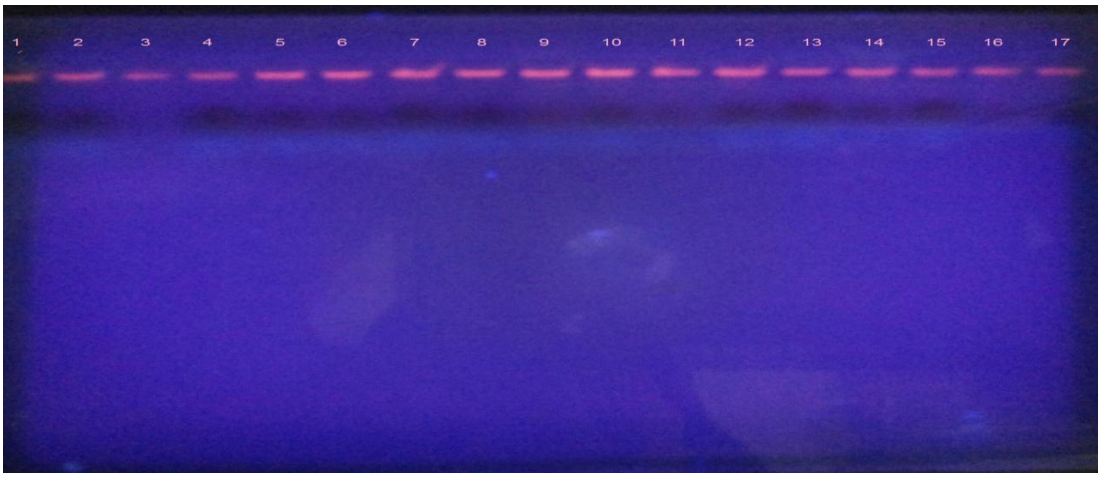

Figure 1. The chromosomal DNA purified from vitiligo patients on 1% agarose gel with ethidium bromide at 60 V for half hour

Genotyping and polymorphisms

The COMT 158 G/A single nucleotide polymorphism (SNP) was detected by using PCR/RFLP method according to [8]. For amplification the DNA fragment (158 pb), the type of primers was used:

'5-GGAGCTGGGGGCCTACTGTG'-3, forward

'5-GGCCCTTTTTCCAGG TCTGACA-'3, reverse

In PCR protocol, DNA samples (50 ng) submit initial denaturation at 94°C for 3 min (1 cycle) then followed by 35 amplification cycles in the thermal cycler PCR express, each cycle including, denaturation at 94°C for 1 min, then annealing at 58°C for 1 min, and extension at 72°C for 1 min, with a final extension step at 72°C for 7 min. So finally, the product of 185 bp was digested with the NlaIII enzyme (Promega, USA) for 3 h at 37°C. To identify the COMT polymorphisms, the digested fragments were separated by 3% agarose gel and then stained with (ethidium bromide) stain. A 100-bp marker (100 bp DNA ladder) was considered as a size standard for each gel pathway [9].

Ethical issue

This research has been approved by Scientific Council of Dermatology; verbal consent has been obtained from the patients.

Statistical analysis

Analysis of data was carried out using statistical package for social sciences (SPSS) software; version 13 and P value obtained by Chi-square test. P value less than 0.05 was considered as significant.

Results

Demographical parameters of vitiligo patients

Distribution of vitiligo patients according to sex

In this study, the disease was present more in females 59 (61.46%) than that in males 37 (38.54%) with a highly significant difference (P ≤0.01) and the ratio of females to males was 1.59:1 as shown in table (1) and figure (2).

Distribution of vitiligo patients according to age

The mean age of the patients' group was 20.67±13.36 years with age range 3-62. The mean age of control group was 26.3 years with age range 3-40 years. Distribution of patients among age group, showed that 19 (19.79%) patients were in ≤9 years age group, 33 (34.38%) patients were in 10-19 years age group, 15 (15.63%) patients were in 20-29 years age group, 16 (16.67%) patients were in 30-39 years age group, 11 (11.46%) patients

were in 40-49 years age group, while only 2 (2.08%) patients were in ≥50 years age group. In present study, the highest number of patients affected by vitiligo was found in the age range 10-19 years as shown in table (2) and figure (3).

Table 1. Distribution of vitiligo patients according to sex

Sex	No.	Percentage (%)
Male	37	38.54
Female	59	61.46
Total	96	100
P value	<0.01	

P value by Chi square test

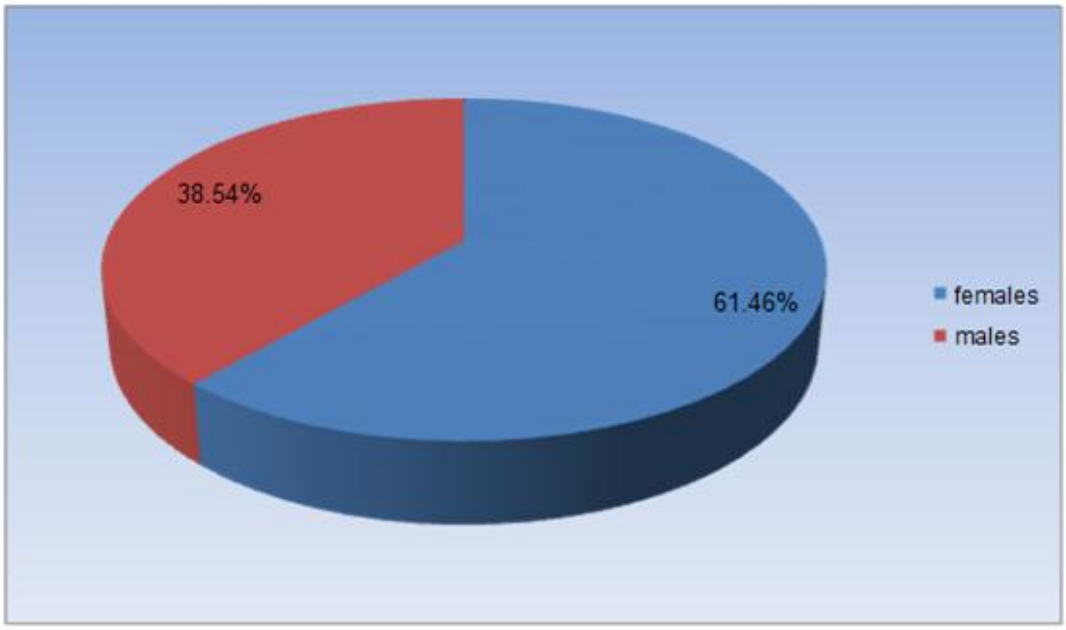

Figure 2. Distribution of vitiligo patients according to sex

Table 2. Distribution of vitiligo patients according to age and sex

Age (year)	Male		Female		P value	Total	
	No.	%	No.	%		No.	%
≤ 9	5	13.51	14	23.73	≤0.05	19	19.79
10-19	11	29.73	22	37.29	≤0.05	33	34.38
20-29	5	13.51	10	16.95	>0.05	15	15.63
30-39	8	21.62	8	13.56	≤0.05	16	16.67
40-49	6	16.21	5	8.47	≤0.05	11	11.46
≥ 50	2	5.41	0	0	>0.05	2	2.08
Total	37	100	59	100	…	96	100
P value	≤0.01		≤0.01			≤0.01	

P value by Chi square

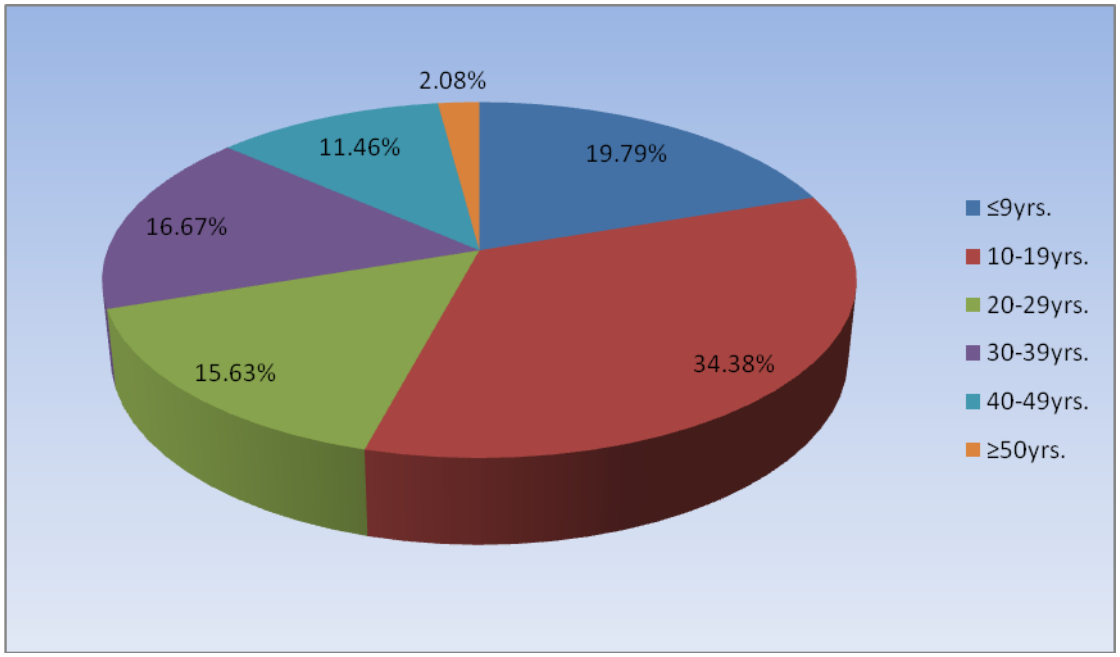

Figure 3. Distribution of vitiligo patients according to the age

Distribution of vitiligo patients according to type of vitiligo

Vitiligo types were determined for all patients, the most frequent vitiligo type was segmental with a percentage 57.29%, followed by generalized with a percentage 33.33%, mucosal with a percentage 3.13%, acrofacial with a percentage 3.13%, then focal type with a percentage 2.08%, lately, the universal type with a percentage 1.04% with a highly significant difference (P ≤0.01).as shown in table (3) and figure (4).

Table 3. Distribution of vitiligo patients according to non-segmental and segmental

Non-segmental					Segmental
Generalize	**Universal**	**Focal**	**Mucosal**	**Acrofacial**	
32	1	2	3	3	55
33.33 %	1.04%	2.08%	3.13%	3.13%	57.29%
P value			≤0.01		

P value by Chi square

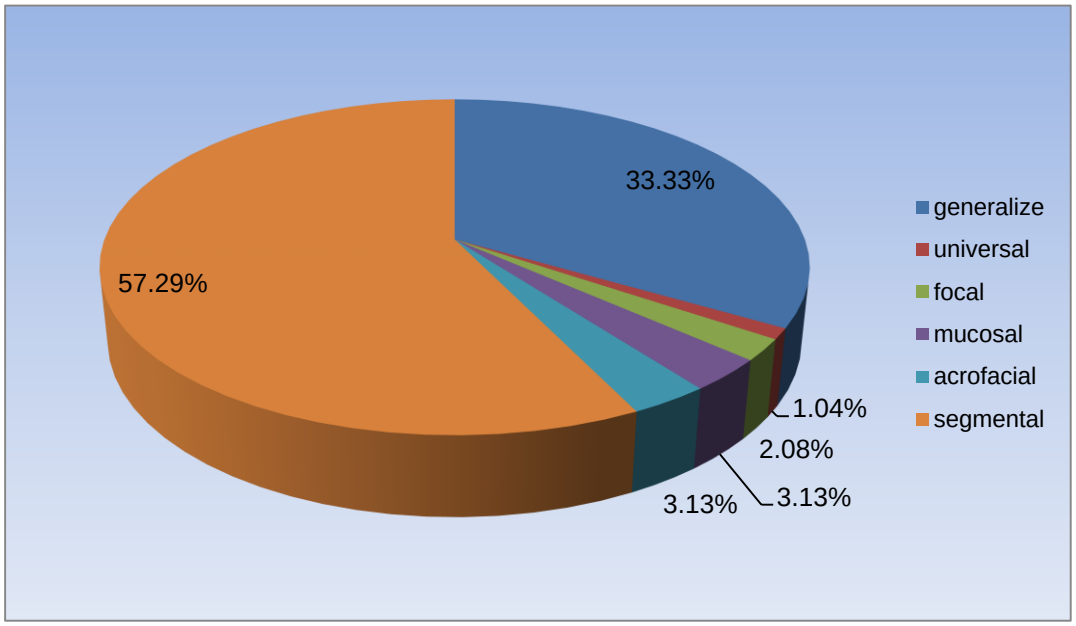

Figure 4. Distribution of vitiligo patients according to the age

Distribution of vitiligo patients according to risk factor

In this study, psychological factors were seen in 85% of cases, 10% nutrition may act as triggering factor in these cases, and 5% unknown cause. It has been become evident that vitiligo is quite prevalent and continues a major psychological health problem in Iraq.

In current study 28.94% patients were associated with positive family history of vitiligo, while 71.05 had no family history of vitiligo, statistically no significant correlation was found between vitiligo and positive family history as shown in figure (5).

Molecular study

The result of using primer ('5-GGAGCTGGGGGCCTACTGTG'-3, forward and '5-GGCCCTTTTTCCAGGTCTGACA-'3, reverse) showed amplified fragment (185 bp), as a clear band by electrophoresis on a 2% agarose gel at 60 V for 30 min as shown in figure (6).

PCR products underwent restriction digestion with NIa III (Neisseria Lactamica) as shown in figure (7) and restriction fragment length polymorphism (RFLP) was used in this study for identification of various alleles in COMT gene (COMT-158 G/A polymorphism). The genetic result of present study revealed that H/H(bp), H/L(bp), L/L(bp) genotypes were in 62.5%, 21.87% and 15.63%, respectively, in vitiligo patients and there is no changes in COMT-HH, COMT-LL and COMT-HL polymorphism found between vitiligo patients and the control persons, and that indicated there was no significant association between 158 G/A COMT polymorphisms and the occurrence of vitiligo.

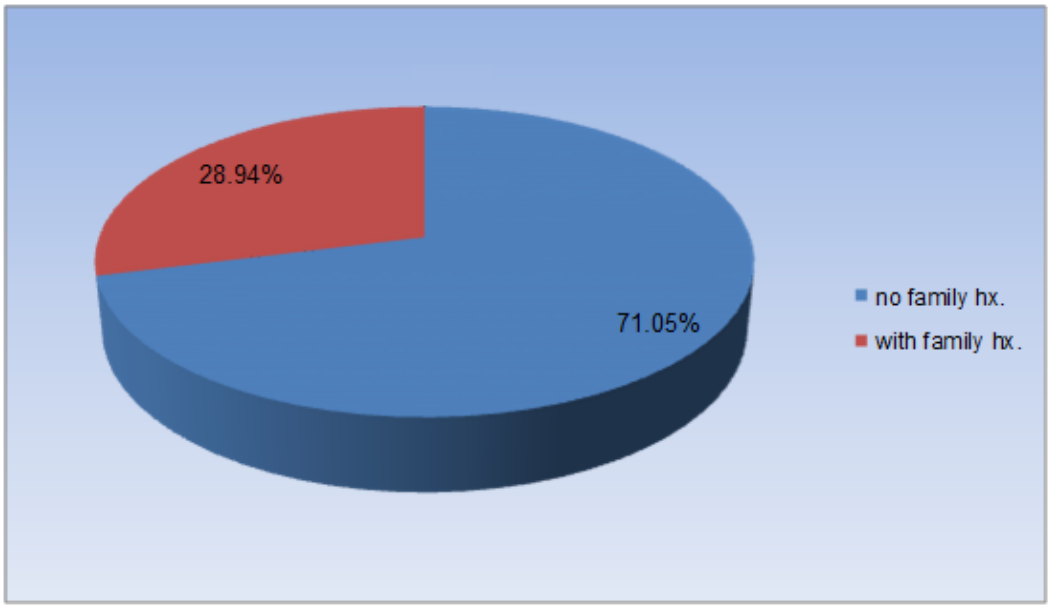

Figure 5. Distribution of vitiligo patients according to family history

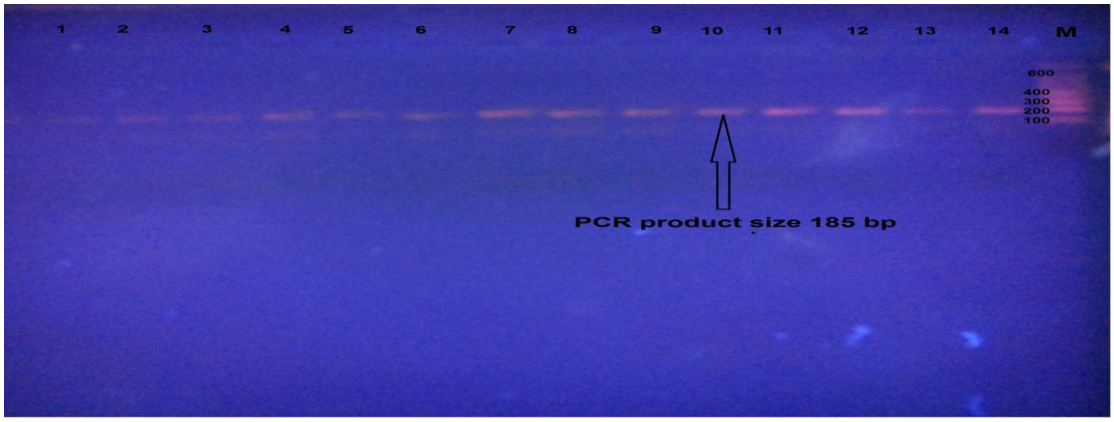

Figure 6. Detection of PCR product DNA bands of COMT gene by using primer set (185 bp). The amplified fragment was separated by electrophoresis on 2% agarose gel, colored with ethidium bromide at 60 V for 30 min. The photographed under UV light. 1. M: Marker DNA ladder Size (100bp). 2. Lanes (1-14) patients gave band for amplified fragment

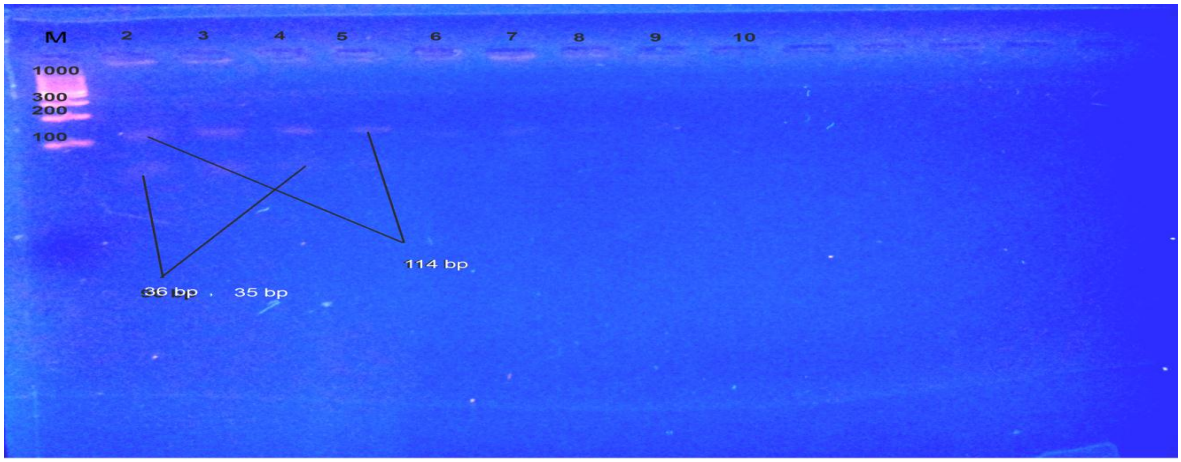

Figure 7. PCR product digested with NIa III, fragment was separated by electrophoresis on a 3% agarose gel, and colored with ethidium bromide at 100 V for 30 min, under UV light. 1. M: Marker DNA ladder Size (100bp). 2. Lanes (2-8) RFLP analysis of vitiligo patients, COMT-HH, represented by 114, 36, 35 bp

Discussion

Vitiligo is multifactorial and polygenic. The precise pathogenesis remains elusive; however, several theories have been proposed to explain the loss of epidermal melanocytes in this disorder. Proposed mechanisms fall under the rubrics of autoimmune, biochemical, oxidant-antioxidant, neural, and viral. Study has also pointed to a significant role of genetic susceptibility to vitiligo [2].

Oxidative stress may also play an important pathogenic role in vitiligo. There was study suggest that accumulation of free radicals that are toxic to melanocytes leads to their destruction. Cultured melanocytes and the serum of patients with vitiligo often have increased nitric oxide levels, suggesting that nitric oxide could lead to auto destruction of melanocytes [10]. The mean age of onset is earlier in those with a positive family history, which ranges from 7.7% to more than 50% [11].

In the current study, vitiligo was more females than in males and mainly at age group (10-19 years) in both sexes. This result agrees in part with Kyriakis et al. [12] who also found that vitiligo was significantly more prevalent in young women (30 years of age) than young men. However, current result disagrees with their finding regarding age group in males as they mention that the male peak prevalence was in the fifth decade of life.

Halder [13] mention that segmental vitiligo is the most common clinical type observed in children, the frequency of is significantly increased in children as compared to adults, this is close to current finding regarding age and that the most common type was segmental.

The melanocytes COMT gene was chosen in this study because, the COMT can prevent the formation of toxic O-quinones during melanin synthesis as mentioned by Pavel et al. [4] who stated that the cause of vitiligo is unknown, but may related to genetics and environmental factors, studies indicates that certain types of genes involved in melanin synthesis and oxidative stress initiate development of vitiligo.

The vitiligo is a social problem so early diagnosis of vitiligo is very important to treat the patient as early as possible. It is important to avoid stress which play a causative role in vitiligo, especially when there is mutation in COMT that responsible for catalysis the O-methylation of biologically active or toxic catechol during stress condition, there are several environmental factors such as stress; extreme exposure to sunlight or pesticides plays a role in the etiology of vitiligo [14]. Also Barisić-Drusko and Rucević [15] found that the

most frequent triggers in children patients with vitiligo were psychological factors (56.9%), inflammatory focus (30.8%), less often physical trauma (9.2%) and other triggers (3.1%).

Mehaney et al. [16] noticed that genotype and the allele distributions of COMT 158 G/A polymorphism in vitiligo patients were not importance different from those of control peoples, which agree with results of this study. There was similar study by Türsen et al. [9] who reported that the distribution of the H/H, H/L, L/L genotypes were 24%, 54%, 22%, respectively, in vitiligo patients, and no differences in COMT-HH, COMT-HL and COMT-LL polymorphism were detected between vitiligo patients and the control subjects.

Also in current study, majority of vitiligo patients had no family history of vitiligo, which agree with Handa and Kaur (17), Al Mutairi and Sharma [18] Rahman et al. [19], who also found the same results that may be attributed to the role of genetic factors in the pathogenesis of vitiligo.

In conclusion, vitiligo is more common in females than males. The highest number of patients affected by vitiligo were found with age range 10-19 yrs. The most frequent vitiligo type was segmental, followed by generalized, mucosal, acrofacial, then focal type, lately, the universal type. The genetic study revealed that H/H, H/L, L/L genotypes were 62.5%, 21.87% and 15.63%, respectively of vitiligo patients. No differences in COMT-HH, COMT-HL and COMT-LL polymorphism were found between vitiligo patients and the control subjects. There was no significant association between 158 G/A COMT polymorphisms and susceptibility to vitiligo.

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Author contribution

Al-Hamadani: Study design, collection of samples, writing the manuscript. Al-Thwani: Supervised all the steps of the study.

Conflict of interest

The authors declare there is no conflict of interest.

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