A Cross-sectional Study of the Catalase Genetic Polymorphism (-262 cytosine/ thymine) and Blood Catalase Activity among Jordanian Vitiligo Patients

Ebtesam H Alhawmdeh[1], Nailya R Bulatova[1], Al Motassem F Yousef [1], Mohammed A AlAbbadi[1], Ethar A Omer[1]*

[1] Department of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy, The University of Jordan, Jordan.

ABSTRACT

Vitiligo is brought on by functional melanocyte loss and manifests as white maculae that may cover the whole body's skin. There is a genetic background in the pathogenesis of vitiligo. Polymorphisms in different parts of catalase gene may affect the disease activity and result in less functional catalase, thus, accumulation of hydrogen peroxide, one of the oxidative factors that damage melanocytes. We evaluated the CAT 262 genetic polymorphism of vitiligo patients using the polymerase chain reaction (PCR) technique with at least one C and at least one T model. The study included 48 vitiligo patient and 51 control individuals. Family history of vitiligo was present in 27.1% of patients and autoimmune disease were diagnosed in 16.7% of patients. Three quarters of vitiligo patients (75.0%) reported that emotional stress was the major triggering factor for their disease. The CC genotype was predominant (56.2% in vitiligo patients and 62.7% in control) with no significant difference between the study groups (p=0.7). Catalase activity in blood was comparable between the study arms (159.1±21.6 MU/L in vitiligo patients and 151.3±25.4 MU/L in controls (p=0.15). We conclude that neither genetic polymorphism in CAT 262 C/T nor blood catalase activity is associated with vitiligo.

Keywords: Genetic Polymorphism, Blood Catalase, Vitiligo, CAT Gene (-262 cytosine/thymine), Jordanians.

INTRODUCTION

The most prevalent pigmentary condition, vitiligo, is brought on by functional melanocyte loss and manifests as white maculae that may cover the whole body's skin[1].

The etiology of vitiligo appears to be multifactorial, including mainly environmental, genetic, and immunologic factors which may synergistically cause melanocyte destruction[2]. The two main types of vitiligo are non-segmental vitiligo, which is the most prevalent form and often manifests as bilateral white patches, and segmental vitiligo, which has a unilateral distribution[3] and contributes to 5–16% of cases[4]. In 87% of cases the disease started before the age of 30 years and in 41.3% it started before the age of 10[3].

In Jordan, the prevalence of vitiligo in children was found to rise with age (0.45% 1 years, 1% 1-5 years, 2.1% 5-12 years). The prevalence of vitiligo was found to range from 0.06% to 2.28% in the general population and from 0% to 2.16% in juvenile populations. In 92.9% of vitiligo patients, non-segmental form was present[5].

Studies using histology and immunohistochemistry demonstrated that the afflicted skin region is devoid of melanocytes, despite their occasional presence[6]. High level of both organ- and non-organ-specific autoantibodies have been reported in the serum of vitiligo patients [7]. The antimelanocyte and antinuclear antibodies were shown to be higher in vitiligo patients as opposed to controls, furthermore, these antibodies levels were shown to

correspond with disease activity[8]. These autoantibodies, which belong to the class G immunoglobulins, were also discovered in the lesional vitiligo epidermis' basal layer, together with deposits of complement component 3 (C3)[9]. Tyrosinase, tyrosinase-related protein-1 (TRP-1), TRP-2, Pmel17 (also known as gp100), the transcriptional factors SOX 9 and SOX 10, and the type 1 membrane receptor for melanin-concentrating hormone are the major melanocytic antigens (MCH-R1)[10,53]. Additionally, people with vitiligo have high frequencies of cytotoxic T lymphocytes that are reactive to melanocytes in their peripheral blood[11], capable of releasing type B granzyme, perforin, and IFN, while vitiligo epidermis exhibits perilesional T-cell infiltration[12]. It was shown that CD8+ T lymphocytes that have the characteristics of skin-homing, are oriented toward type-1 effector function, and are significantly cytotoxic being clustered around disappearing melanocytes[13].

Recent studies of the pathophysiology of vitiligo highlighted the significance of reactive oxygen species (ROS) and their function in melanocyte-intrinsic abnormalities as potential major triggers of the whole inflammatory cascade[14].In oxidative stress, superoxide is a primary oxygen radical produced when an oxygen molecule receives one electron. Superoxide dismutase (SOD) converts the superoxide to hydrogen peroxide (H_2O_2) that, in the presence of free ferrous iron, may produce hydroxyl radicals and exacerbate diseases[15]. Normally, the antioxidant system defends the cell from ROS, however, during oxidative stress, this system becomes unbalanced[16,54], in addition to H_2O_2 buildup brought on by environmental trauma like UVB exposure (290-320 mm)[17]. Together, autoimmunity and oxidative stress have a synergistic impact on melanocytes, causing cell death and depigmentation[18]. The immune system creates a long-lasting inflammatory environment where ROS build and damage nearby cells[19].

One of the antioxidant enzymes is catalase which is responsible of the conversion of hydrogen peroxide, one of

ROS, to water and oxygen. A number of studies investigated the catalase activity in both plasma and epidermis of vitiligo patients with contradictory results[7,17, 20].

It has been suggested that a relation exists between genetic factors and susceptibility of vitiligo.

Genetic polymorphisms in different regions in CAT gene (a gene that encodes for catalase) have been studied in relation to vitiligo[21]. The gene for human catalase has been mapped to chromosome 11, band p13, and is split into 13 exons by 12 introns and spans[22]. Numerous polymorphisms have been described in the promoter, 5´ and 3´- untranslated regions (UTRs), exons and introns[23,24].

This study focuses mainly on the most common CAT gene polymorphism -262 cytosine/thymine (-262 C/T) in the promoter region in which C to T substitution occur at position -262 (db SNP ID: rs1001179) and that has been found to be associated with alteration in the CAT activities. The 262 C/T polymorphism may affect CAT transcription by modulation of the transcriptional factor binding position and increased basal CAT expression in various cell including erythrocytes[24]. The latter study investigated the relation between 262 C/T genetic polymorphism and the level of catalase in general, not in relation to any specific disease, and found that individuals carrying T allele have significant higher level of catalase in comparison with individuals carrying homozygote C allele[24]. On the contrary, another group of investigators showed that CC homozygotes had higher activity of CAT compared to those with CT or TT genotypes[25]. A later study found no association between the CAT262C/T polymorphism and CAT activity[26].

With regards to the association of CAT262C/T polymorphism and vitiligo in particular we found two studies, and both demonstrated lack of such association[17,27].

The aim of the study was dual: to assess the relation between the CAT 262 C/T polymorphism of and the susceptibility to vitiligo in Jordanian patients; in addition to the study of blood catalase activity in Jordanian vitiligo patients.

METHODOLOGY

Study design

This is a cross-sectional study that involved patients diagnosed with vitiligo and apparently healthy individual matched by age and gender who served as control.

Sample size

a) Using the online software https://clincalc.com/stats/samplesize.aspx, we have calculated the required sample size for detecting differences in blood catalase activity between vitiligo patients and found the size to be 24 per group based on the data from the previous publication[28]. However, we included 48 patients and 51 controls.

Clinical Setting and Patients

The vitiligo patients (cases) were recruited from the Dermatology Clinics at Al-Basheer Hospital related to Jordanian Ministry of Health and Kind Hussein Medical City related to the Royal Medical Services, Amman, Jordan over the period of May 1, 2015 to June 17, 2015. Vitiligo diagnosis was established by dermatologists using standard diagnostic criteria[3]. The active vitiligo was defined as the progression or appearance of new lesions in the last 3 months and the stable vitiligo was defined as the absence of new lesions or progression in the last 6 months, respectively[29].Healthy volunteers (control) were recruited from the outpatient clinics.

Inclusion criteria for vitiligo cases

1. Jordanian nationality.
2. Age between 18 and 60 years.

Exclusion criteria for cases

1. Any acute or chronic disease
2. Patient with other skin diseases

Inclusion criteria for controls:

1. Jordanian nationality
2. Age-matched (by decades) and gender-matched to control apparently healthy individuals.

Exclusion criteria for control

1. Any acute or chronic disease
2. History, either personal, or family, of any autoimmune diseases
3. No personal or family history of vitiligo.

Ethical consideration

The privacy and rights of the human participants were upheld throughout the investigation. The Institutional Review Boards' research ethical permissions were obtained from Jordanian Ministry of Health (approval number 4822 on March 10, 2015) and Royal Medical Services (approval number 3542 on February 25, 2015). Written informed consent form was provided to each patient, who was then requested to read and sign the form. The use of a code number for each participant rather than his name or file number were used to protect the anonymity and confidentiality of the information, and the potential study recruits were notified that participation in the study is voluntary and that they have the choice to decline without consequence.

Assessment of the CAT 262 polymorphism

The CAT 262 genetic polymorphism was evaluated using the polymerase chain reaction (PCR) with at least one C and at least one T model.

DNA extraction

Venous blood samples were collected from the patients and healthy subjects in K3 EDTA-coated tubes. The tubes were kept in icebox and DNA extraction was performed in the same day. Using the "Promega Wizard genomic DNA purification kit, Promega Corporation, USA" in accordance with the manufacturer's instructions, genomic DNA was isolated from the whole blood.

Assessment of DNA yield and purity

DNA was kept at -20° C until further investigation and the quality of the DNA was assessed using 0.5% agarose gel electrophoresis.

Genomic amplification

Using the forward and reverse primers, a 320 bp amplicon containing the targeted site, CAT 262 (rs1001179), was sequenced using PCR.

Measurement of blood catalase activity

Blood samples were collected by using EDTA tubes;

all samples were prepared after 4 hours of being collected to have consistency in our results. Erythrocyte hemolysis was induced by using saponin due to the high concentration of catalase in erythrocytes. Spectrophotometric assay was used to measure catalase activity in the blood based on the formation of stable complex between hydrogen peroxide and ammonium molybdate based on the method described by Goth (1992)[30]. In more details, one hundred μL of blood was dissolved it in 2 ml of 10% saponin solution. After finishing sample preparation 180 μL of lysate was taken and incubated it at 37° C for 60 second with 1.5 ml of 65 μmol/ml hydrogen peroxide, then 1.5 ml of 32.4 mmol/l ammonium molybdate was added to stop the enzymatic reaction resulting in a yellow solution that was measured against blank 3 at 405 nm.

The following equation was used to measure catalase activity:

Blood catalase activity (A) (kunits/l) = A (sample) - A (blank 1)/ A (blank 2) - A (blank 3) x 4 26 x 105

In which:

Blank 1: 1.5ml hydrogen peroxide + 1.5 ml ammonium molybdate + 1.5 PBS + 180 μL sample

Blank 2: 1.5ml hydrogen peroxide + 1.5 ml ammonium molybdate + 1.5 PBS

Blank 3: 1.5 ml ammonium molybdate + 1.5 PBS

Data management and statistical analysis

Continuous variables were reported as mean ± standard deviation (SD) and the comparison between the groups was conducted using independent sample t-test or one-way ANOVA test, as appropriate. Levene's test was utilized to examine the homogeneity of variance, and Kolmogrov-Smirnov test was employed to evaluate the normality of distribution. Chi-square or Fisher exact test was used to compare frequency of alleles and genotypes between the vitiligo and the control groups, where allele frequency = number of copies of an allele in a population /total number of all alleles for that gene in a population, and genotype frequency = number of individuals with a particular genotype in a population/total number of all individuals in a population. Genotype and allele frequencies were matched to expectation by Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium was assessed utilizing the formula:

$(a+b)^2$ with degree of freedom of 1,

where a is the frequency of one allele, and b is the frequency of the other allele. As a measure of the relationship between genotypes, odds ratios (OR) and their 95% confidence intervals (CI) were determined. The Mantel-Haenszel statistics were used to estimate the common odds ratios, their CI, and their p-values. For all comparisons, a p-value of 0.05 or less was regarded as statistically significant.

RESULTS

Study subject's description:

The study flow chart is shown in Figure1. Among 90 vitiligo patients approached initially, 42 were excluded, while among 121 healthy individuals approached initially, 70 were excluded from the study. The ultimate number of study participants was 48 vitiligo patients 51 control subjects.
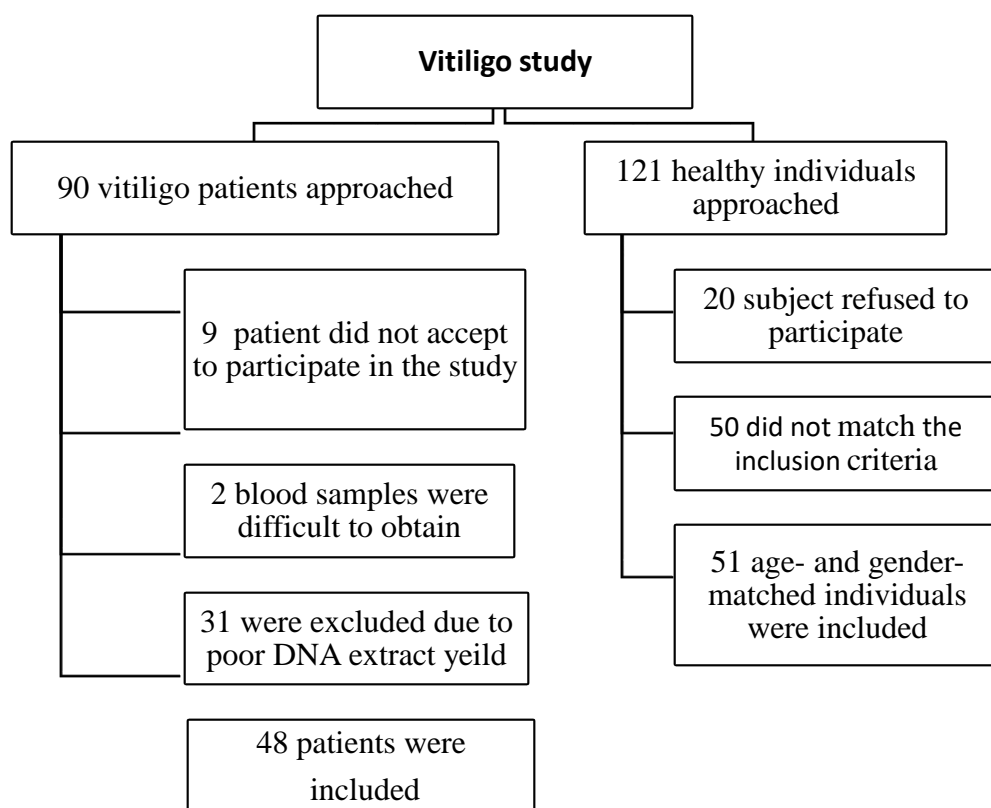
```
                    ┌─────────────────────────┐
                    │      Vitiligo study     │
                    └────────────┬────────────┘
           ┌─────────────────────┴─────────────────────┐
┌──────────────────────────────┐      ┌──────────────────────────────┐
│ 90 vitiligo patients approached│      │ 121 healthy individuals       │
│                                │      │ approached                    │
└──────────────────────────────┘      └──────────────────────────────┘
```

| 9 patient did not accept to participate in the study |

| 20 subject refused to participate |

| 2 blood samples were difficult to obtain |

| 50 did not match the inclusion criteria |

| 31 were excluded due to poor DNA extract yeild |

| 51 age- and gender-matched individuals were included |

| 48 patients were included |

Figure 1. Study flow chart

Demographic and clinical characteristics of vitiligo patients:

Demographic and clinical characteristics of vitiligo patients are shown in Table 1. The mean age of vitiligo patients was 33.7 (±13.2) years.

The major proportion of vitiligo patients were females (72.9%)). Non-segmental vitiligo was diagnosed in majority of patients (93.7%), and the rest of patients had segmental vitiligo. Active disease was documented in more than half (53.9%) of cases. Three-quarters of patients (75.0%) reported that the emotional stress served as a triggering factor for their disease. Family history of vitiligo was present in 27.1% of vitiligo patients, while total of 16.7% of patients reported presence of autoimmune disease.

Blood catalase activity

The mean catalase activity in the blood (MU/L) did not differ significantly between the vitiligo patients (159.1±21.6) and the controls (151.3±25.4) (p=0.15). The catalase activity was also similar among the vitiligo patients with and without active disease (161.4±22.3 and 156.3±21.0, respectively, p=0.9). Furthermore, the catalase activity did not differ between vitiligo patients who received and who did not receive Psoralen plus ultraviolet A (PUVA) therapy in Table 2.

Table 1. Clinico-demographic characteristics of vitiligo patients (N=48)

Characteristic	
Age, mean (SD)	33.7 (13.2)
Gender, N (%)	
Males	13 (27.1)
Females	35(72.9)
Education level, N (%)	
Elementary	9 (18.87)
High school	27 (56.3)
Diploma	8 (16.7)
University	5 (10.4)
Localization of vitiligo, N (%)	
Segmental	3 (6.3)
Non-segmental	45 (93.7)
Disease activity, N (%) (assessed in 39 patients)	
Active	21 (53.9)
Stable	18 (46.1)
Presence of risk factors for vitiligo, N (%)	
Emotional stress	36 (75.0)
Chemicals exposure	3 (6.3)
Sun exposure	2 (4.2)
Others	7 (14.6)
Positive family history of vitiligo, N (%)	
Yes	13 (27.1)
No	35 (72.9)
Presence of autoimmune diseases, N (%)	
Thyroid disorder	7 (14.6)
Rheumatoid arthritis	1 (2.1)

Table 2. Comparison of blood catalase activity in vitiligo patients according to treatment with PUVA therapy

PUVA therapy	N*	Mean (±SD)	p**
Treatment-naive	12	156.2 (21.9)	
<6 months treatment	13	157.9 (17.5)	0.9
>6 months treatment	11	162.3 (27.4)	

*The total number of patients was less than 48 due to missing data

** by one-way ANOVA test

Genotype & Sequencing results

Figures 2-4 show sequencing results for the complementary strand of CAT gene. Data were analyzed by using Chromas program; one black band means CC genotype, one green band means TT genotype and 2 bands mean CT genotype.

As shown in Table 3, the most prevalent genotype in both vitiligo cases and controls was CC genotype (56.2% and 62.7%, respectively). There was no difference in all three genotypes distribution between the vitiligo patients and the controls (p=0.7). Similarly, the allele distribution did not differ between the vitiligo patients and the controls (p=0.6).

Table 3. Genotype and allele frequencies among vitiligo patients and controls

	Cases (N=48), N (%)	Controls (N=51), N (%)	Total	p
Genotype				
CC	27 (56.2)	32 (62.7)	59 (59.6)	
CT	18 (37.5)	15 (29.4)	33 (33.3)	
TT	3 (6.2)	4 (7.8)	7 (7.1)	0.7*
Allele				
C	72 (75.0)	79 (77.5)	151 (76.3)	
T	24 (25.0)	23 (24.0)	47 (23.7)	0.6**

* By Fisher exact test

** By independent-sample t-test
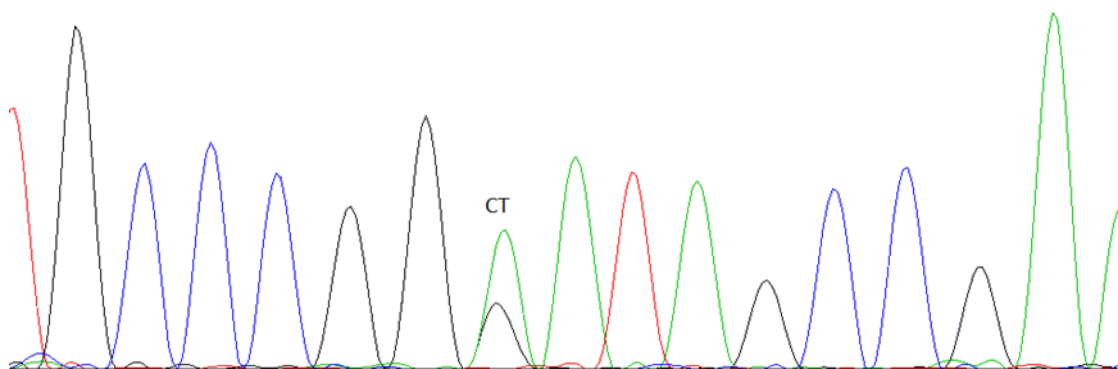
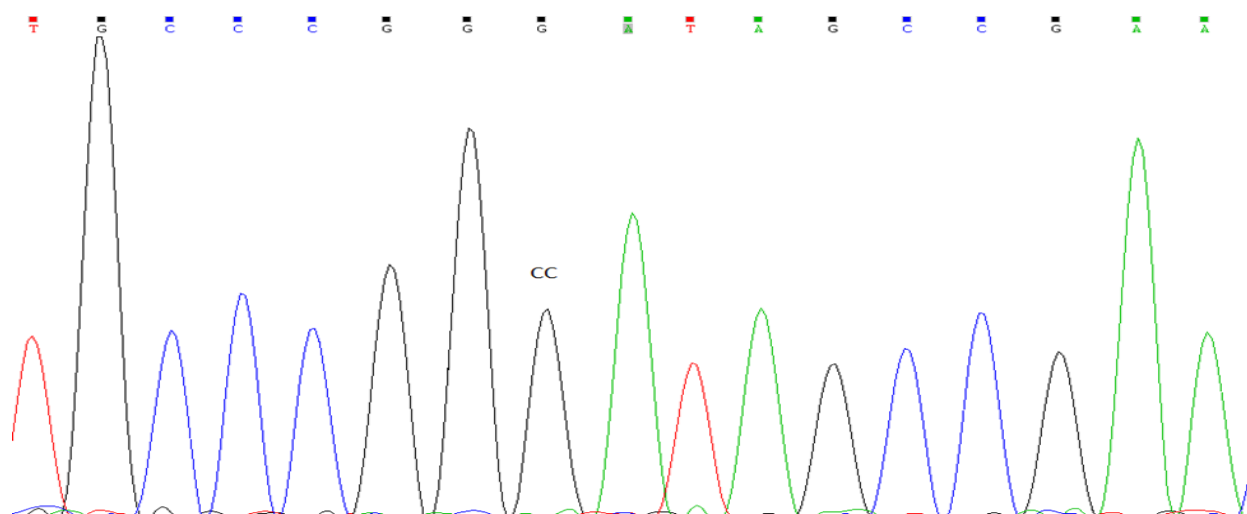**Figure 2. Genotype sequencing (CT)**
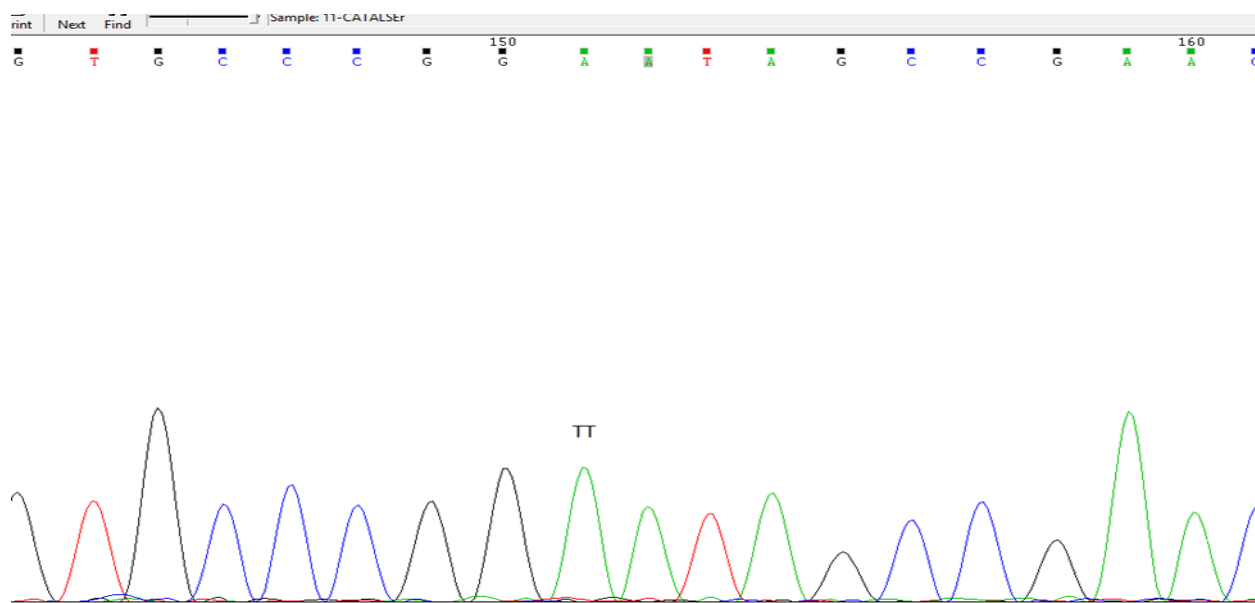
Figure 3. Genotype sequencing (CC)

Figure 4. Genotype sequencing (TT)

As shown in Table 4, the occurrence of at least one T was not different between the vitiligo patients and controls ($p=0.5$), likewise, the occurrence of at least one C did not differ between the two study arms ($p=0.8$).

Table 4. Recessive and dominant genotype distribution among vitiligo patients and controls

		At least one T model		
Study group	**CC**	**CT+TT**	**Total**	**p**
Cases, N (%)	27 (56.3)	21 (43.7)	48 (100)	
Controls, N (%)	32 (62.7)	19 (37.3)	51 (100)	0.5
	At least one C model			
Study group	**CC+CT**	**TT**	**Total**	**p**
Cases, N (%)	45 (93.8)	3 (6.2)	48 (100)	
Controls, N (%)	47 (92.2)	4 (7.8)	51 (100)	0.8

DISCUSSION:

Importance of the study

Among the hypotheses for vitiligo, oxidative stress is considered as the initial pathogenic event in melanocyte destruction[7]. It was recently demonstrated that oxidative stress, in addition to producing multiple forms of melanocyte death, can induce the formation of the inflammatory cytokines, such as IL-6, IL-8, CXCL12, CCL5, CXCL10, and CXCL16 from stressed melanocytes or keratinocytes, resulting in initiation or promotion of the autoimmune reaction toward melanocytes[31].

It is widely accepted that there is a genetic background in the pathogenesis of vitiligo. A few studies were conducted in Jordan to investigate associations of SMOC2[32], NALP1[33], PTPN22 1858C/T[34], PTPN22 and SMOC2[35] and NLRP1[36]genes, the latter two in relation to autoimmune thyroid disease. However, the association of catalase gene with vitiligo has not been investigated in Jordanian population, therefore, we decided to focus on the CAT gene polymorphism 262 C/T as it is less frequently reported than other CAT gene polymorphisms.it was previously shown that polymorphisms in catalase gene may affect the enzyme activity and result in less functional catalase and accumulation of hydrogen peroxide and, on the other hand, these polymorphisms were associated with vitiligo in some populations. However, it was suggested that the controversy concerning the CAT activity in vitiligo patients may be at least partially related to the polymorphisms in the catalase gene[31].A meta-analysis demonstrated that the 389 C/T polymorphisms in CAT were not associated with the risk of vitiligo in Asians and Turks, on the contrary, the CT genotype was proposed to be a genetic risk factor for susceptibility to vitiligo, while in Western Europeans the CC genotype might decrease the risk of vitiligo[37]. A number of studies demonstrated that CAT -89A/T, -262G/A, and -262T/C polymorphisms in the promoter region and -20T/C in 5′-untranslated region have detrimental effects on the catalase expression or function[21,24,38,39]. In both Chinese and Indian populations, CAT -89A/T variants were associated with a significant decrease in CAT activity and a genetic predisposition for vitiligo, especially in active and generalized vitiligo patients[40,41]. On the other hand, the CAT –262G/A variant showed no change in CAT activity or risk of vitiligo in Indian population[41]. Furthermore, in Northwestern Mexicans, CAT 419 C/T gene polymorphism was not informative, -89 A/T was associated with risk, while 389 C/T conferred protection against vitiligo along with AT haplotype. Additionally, although the serum CAT activity was lower in vitiligo patients, there was no association with any of the polymorphisms[42].

Previous studies demonstrated that the CAT 262 C>T polymorphism may affect the transcription of reporter genes and the binding of transcription factors [4,40,43]. Thus, the purpose of this study was to determine whether there is association between the CAT 262 polymorphism, as well as the blood catalase activity, with vitiligo in Jordanians.

Study findings

Our study showed that 27.1% of patient had family history of vitiligo, lower than reported in a retrospective analysis conducted in Saudi Arabia (42.8%)[43] but higher than in the previous study from Jordan (19.2%)[32] and the US and UK populations (6.1%)[44].

In our study, 16.7% of patients had autoimmune disease in line with the Saudi study where 90.5 % of patient had no history of autoimmune disease[43]. Our data regarding prevalence of thyroid autoimmune disease (14.6%) are comparable with another report where thyroid issues were present in 21.1% of vitiligo patients[45].

Genotype

Our results reveal that CC genotype was predominant in both study groups (58.3% in vitiligo patients vs. 62.7% in control). This frequency was somewhat in between the Hungarian and the English populations (Hungarian 43% in vitiligo patients and 36% in controls[17], English 72.3% in vitiligo patients and 75.1% in controls[27]), but the lack of difference in genotype frequency between the vitiligo patients and the controls was consistent among the three studies. Notably, a recent comprehensive meta-analysis and prioritization study to identify vitiligo associated coding and non-coding single-nucleotide variants (SNV) using web-based bioinformatics tools prioritized CAT gene, among 13 SNVs, from a set of 291 SNVs, as a candidate contributing to vitiligo pathogenesis[46].

Catalase activity

There is controversy regarding changes in serum catalase activity in vitiligo patients. Patients with active localized vitiligo showed significant decrease in the catalase levels when compared with healthy controls in studies conducted in Turkey [47,48], Nepal[20] and India[7] Notably, lower catalase activity was found in segmental vitiligo patients, whereas in non-segmental vitiligo patients the enzyme activity was normal[49]. More recently, significantly reduced catalase activity, along with other oxidant-antioxidant systems changes were found in serum of Chinese patients with nonsegmental vitiligo compared with healthy controls (HC)[31]. The current study found no difference in blood catalase activity between the vitiligo patients and the control individuals, in line with the studies from Hungary**[17]** Tunisia[50] and Turkey[51]. Furthermore, in a study from India, there was a significant decrease in serum catalase activity in the active phase group (p value=0.044) but not in the static group (p=0.095) in comparison with healthy group[7], however, our data did not support this observation. Notably, blood catalase activity in our patients was not affected by PUVA therapy.

Study limitations

Selection bias might occur as this research was a hospital-based cross-sectional study. Female participants were represented more than males and the gender interference with genotype prevalence cannot be excluded. Furthermore, since the data on triggering factors for vitiligo were retrospectively collected, this may increase the risk of recall bias, especially for events that date back to years ago like excessive sun exposure or emotional stress. Additionally, catalase activity was detected only in the blood, and not in the epidermis, which is more invasive but could be more accurately detect the oxidative processes at the local disease site. Genomewide association study, the best type of investigation to detect genetic loci involved in vitiligo, was not conducted.

CONCLUSION

Genetic polymorphism CAT 262C>T is not related to the incidence of vitiligo. There is no difference in blood catalase activity between vitiligo patients and healthy individuals, between patients with active and stable disease, or between patients receiving PUVA therapy and those without such treatment.

REFERENCES

1. Ahmed jan N, Masood S. Vitiligo. [Updated 2022 Aug 8]. *In: StatPearls[Internet]*. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559149/..

2. Guerra L, Dellambra E, Brescia S, Raskovic D. Vitiligo: pathogenetic hypotheses and targets for current therapies. *Curr Drug Metab*. 2010 Jun 1;11(5):451-67. doi:10.2174/138920010791526105.

3. Ezzedine K, Lim HW, Suzuki T, Katayama I, Hamzavi I, Lan CC, Goh BK, Anbar T, Silva de Castro C, Lee AY, Parsad D, van Geel N, Le Poole IC, Oiso N, Benzekri L, Spritz R, Gauthier Y, Hann SK, Picardo M, Taieb A; Vitiligo Global Issue Consensus Conference Panelists. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. *Pigment Cell Melanoma Res*. 2012 May;25(3):E1-13.
https://doi:10.1111/j.1755-148X.2012.00997.x.

4. Babusikova E, Jesenak M, Evinova A, Banovcin P, Dobrota D. Frequency of polymorphism -262 c/t in catalase gene and oxidative damage in Slovak children with bronchial asthma. *Arch Bronconeumol*. 2013 Dec;49(12):507-12. doi:10.1016/j.arbres.2013.04.002.

5. Al-Refu K. Vitiligo in children: a clinical-epidemiologic study in Jordan. *Pediatr Dermatol*. 2012 Jan-Feb;29(1):114-5. https://doi:10.1111/j.1525-1470.2011.01478.x.

6. [6] Kaur G, Punia RS, Kundu R, Thami GP. Evaluation of active and stable stages of vitiligo using S-100 and human melanoma black-45 immunostains. *Indian J Dermatopathol Diagn Dermatol* 2020;7:2-6. https://doi:10.4103/ijdpdd.ijdpdd_44_19.

7. Deo SS, Bhagat AR, Shah RN. Study of oxidative stress in peripheral blood of Indian vitiligo patients. *Indian Dermatol Online J*. 2013 Oct;4(4):279-82. https://doi:10.4103/2229-5178.120637.

8. [8] El-Gayyar MA, Helmy ME, Amer ER, Elsaied MA, Gaballah MA. Antimelanocyte Antibodies: A Possible Role in Patients with Vitiligo. *Indian J Dermatol*. 2020 Jan-Feb;65(1):33-37. doi:10.4103/ijd.IJD_344_18.

9. [9] Said-Fernandez SL, Sanchez-Domínguez CN, Salinas-Santander MA, Martinez-Rodriguez HG, Kubelis-Lopez DE, Zapata-Salazar NA, Vazquez-Martinez OT, Wollina U, Lotti T, Ocampo-Candiani J. Novel immunological and genetic factors associated with vitiligo: A review. *Exp Ther Med*. 2021 Apr;21(4):312. doi:10.3892/etm.2021.9743.

10. [10] Prasad, D. Pathogenesis of vitiligo. *In: Handbook of Vitiligo: Basic Science and Clinical Management*. Hamzavi I.H., Mahmoud B.H., Isedeh P.N. (Eds.); Jaypee Brothers Medical Publishers (P) Ltd., 2016; 1st edition, Chapter 03, pp. 37–37. https://doi.org/10.5005/jp/books/12775_4.

11. Katz EL, Harris JE. Translational Research in Vitiligo. *Front Immunol*. 2021 Mar 2;12:624517. doi:10.3389/fimmu.2021.624517.

12. Frączek A, Owczarczyk-Saczonek A, Placek W. The Role of TRM Cells in the Pathogenesis of Vitiligo-A Review of the Current State-Of-The-Art. *Int J Mol Sci*. 2020 May 18;21(10):3552. doi:10.3390/ijms21103552.

13. Yang L, Wei Y, Sun Y, Shi W, Yang J, Zhu L, Li M. Interferon-gamma Inhibits Melanogenesis and Induces Apoptosis in Melanocytes: A Pivotal Role of CD8+ Cytotoxic T Lymphocytes in Vitiligo. *Acta Derm Venereol*. 2015 Jul;95(6):664-70. https://doi:10.2340/00015555-2080.

14. Marchioro HZ, Silva de Castro CC, Fava VM, Sakiyama PH, Dellatorre G, Miot HA. Update on the pathogenesis of vitiligo. *An Bras Dermatol*. 2022 Jul-Aug;97(4):478-490. doi:10.1016/j.abd.2021.09.008.

15. Fujii J, Homma T, Osaki T. Superoxide Radicals in the Execution of Cell Death. *Antioxidants (Basel)*. 2022 Mar 4;11(3):501. https://doi:10.3390/antiox11030501. .

16. Zheleva A, Nikolova G, Karamalakova Y, Hristakieva E, Lavcheva R, Gadjeva V. Comparative study on some oxidative stress parameters in blood of vitiligo patients before and after combined therapy. *Regul Toxicol Pharmacol*. 2018 Apr; 94: 234-239. https://doi:10.1016/j.yrtph.2018.02.008.

17. Kósa Z, Fejes Z, Nagy T, Csordás M, Simics E, Remenyik E, Góth L. Catalase -262C>T polymorphisms in Hungarian vitiligo patients and in controls: further acatalasemia mutations in Hungary. *Mol Biol Rep*. 2012 Apr;39(4):4787-95. https://doi:10.1007/s11033-011-1272-6.

18. Xuan Y, Yang Y, Xiang L, Zhang C. The Role of Oxidative Stress in the Pathogenesis of Vitiligo: A Culprit for Melanocyte Death. *Oxid Med Cell Longev*. 2022 Jan 22;2022:8498472. doi:10.1155/2022/8498472.

19. Solleiro-Villavicencio H, Rivas-Arancibia S. Effect of Chronic Oxidative Stress on Neuroinflammatory Response Mediated by CD4+T Cells in Neurodegenerative Diseases. *Front Cell Neurosci*. 2018 Apr 27;12:114. doi:10.3389/fncel.2018.00114.

20. [20] Agrawal S, Kumar A, Dhali TK, Majhi SK. Comparison of oxidant-antioxidant status in patients with vitiligo and healthy population. *Kathmandu Univ Med J (KUMJ)*. 2014 Apr-Jun;12(46):132-6. https://doi:10.3126/kumj.v12i2.13660.

21. Kodydková J, Vávrová L, Kocík M, Žák A. Human catalase, its polymorphisms, regulation and changes of its activity in different diseases. *Folia Biol (Praha)*. 2014;60(4):153-67.

22. Quan F, Korneluk RG, Tropak MB, Gravel RA. Isolation and characterization of the human catalase gene. *Nucleic Acids Res*. 1986 Jul 11;14(13):5321-35. doi:10.1093/nar/14.13.5321

23. Hebert-Schuster M, Fabre EE, Nivet-Antoine V. Catalase polymorphisms and metabolic diseases. *Curr Opin Clin Nutr Metab Care*. 2012 Jul;15(4):397-402. https://doi:10.1097/MCO.0b013e328354a326.

24. Forsberg L, Lyrenäs L, de Faire U, Morgenstern R. A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. *Free Radic Biol Med*. 2001 Mar 1;30(5):500-5. doi:10.1016/s0891-5849(00)00487-1.

25. Ahn J, Nowell S, McCann SE, Yu J, Carter L, Lang NP, Kadlubar FF, Ratnasinghe LD, Ambrosone CB. Associations between catalase phenotype and genotype: modification by epidemiologic factors. *Cancer Epidemiol Biomarkers Prev*. 2006 Jun;15(6):1217-22. doi:10.1158/1055-9965.EPI-06-0104.

26. Mak JC, Ho SP, Yu WC, Choo KL, Chu CM, Yew WW, Lam WK, Chan-Yeung M. Polymorphisms and functional activity in superoxide dismutase and catalase genes in smokers with COPD. *Eur Respir J*. 2007 Oct;30(4):684-90. doi:10.1183/09031936.00015207.

27. Gavalas NG, Akhtar S, Gawkrodger DJ, Watson PF, Weetman AP, Kemp EH. Analysis of allelic variants in the catalase gene in patients with the skin depigmenting disorder vitiligo. *Biochem Biophys Res Commun*. 2006 Jul 14;345(4):1586-91. doi:10.1016/j.bbrc.2006.05.063

28. Sample size calculator. Available at: https://clincalc.com/stats/samplesize.aspx. Last accessed on December 6, 2022.

29. Kemp EH, Emhemad S, Akhtar S, Watson PF, Gawkrodger DJ, Weetman AP. Autoantibodies against tyrosine hydroxylase in patients with non-segmental (generalised) vitiligo. *Exp Dermatol*. 2011 Jan;20(1):35-40. doi:10.1111/j.1600-0625.2010.01181.x.

30. Góth L. Two cases of acatalasemia in Hungary. *Clin Chim Acta*. 1992 Apr 30;207(1-2):155-8. https://doi:10.1016/0009-8981(92)90160-r.

31. Li S, Dai W, Wang S, Kang P, Ye Z, Han P, Zeng K, Li C. Clinical Significance of Serum Oxidative Stress Markers to Assess Disease Activity and Severity in Patients With Non-Segmental Vitiligo. *Front Cell Dev Biol*. 2021 Dec 16; 9:739413. https://doi:10.3389/fcell.2021.739413.

32. Alkhateeb A, Al-Dain Marzouka N, Qarqaz F. SMOC2 gene variant and the risk of vitiligo in Jordanian Arabs. *Eur J Dermatol*. 2010 Nov-Dec;20(6):701-4. doi:10.1684/ejd.2010.1095.

33. Alkhateeb A, Qarqaz F. Genetic association of NALP1 with generalized vitiligo in Jordanian Arabs. *Arch Dermatol Res*. 2010 Oct;302(8):631-4. doi:10.1007/s00403-010-1064-1. Epub 2010 Jun 24.

34. Alkhateeb A, Qarqaz F, Al-Sabah J, Al Rashaideh T. Clinical characteristics and PTPN22 1858C/T variant analysis in Jordanian Arab vitiligo patients. *Mol Diagn Ther*. 2010 Jun 1;14(3):179-84. https://doi:10.1007/BF03256371.

35. [35] Alkhateeb A, Marzouka NA, Tashtoush R. Variants in PTPN22 and SMOC2 genes and the risk of thyroid disease in the Jordanian Arab population. *Endocrine*. 2013 Dec;44(3):702-9. doi:10.1007/s12020-013-9908-z.

36. [36] Alkhateeb A, Jarun Y, Tashtoush R. Polymorphisms in NLRP1 gene and susceptibility to autoimmune thyroid disease. *Autoimmunity*. 2013 May;46(3):215-21. doi:10.3109/08916934.2013.768617.

37. Lu L, Liu L, Ji Y, Jin H, He L. Association of the 389 C/T polymorphism of the catalase gene with susceptibility to vitiligo: a meta-analysis. *Clin Exp Dermatol*. 2014 Jun;39(4):454-60. doi:10.1111/ced.12340.

38. Komina AV, Korostileva KA, Gyrylova SN, Belonogov RN, Ruksha TG. Interaction between single nucleotide polymorphism in catalase gene and catalase activity under the conditions of oxidative stress. *Physiol Res*. 2012;61(6):655-8. doi:10.33549/physiolres.932333.

39. [39] Bastaki M, Huen K, Manzanillo P, Chande N, Chen C, Balmes JR, Tager IB, Holland N. Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. *Pharmacogenet Genomics*. 2006 Apr;16(4):279-86. https://doi:10.1097/01.fpc.0000199498.08725.9c.

40. Liu L, Li C, Gao J, Li K, Zhang R, Wang G, Li C, Gao T. Promoter variant in the catalase gene is associated with vitiligo in Chinese people. *J Invest Dermatol*. 2010 Nov;130(11):2647-53. https:// doi:10.1038/jid.2010.192.

41. Mansuri MS, Jadeja SD, Singh M, Laddha NC, Dwivedi M, Begum R. The catalase gene promoter and 5'-untranslated region variants lead to altered gene expression and enzyme activity in vitiligo. *Br J Dermatol*. 2017 Dec;177(6):1590-1600. doi:10.1111/bjd.15681.

42. Ochoa-Ramírez LA, Díaz-Camacho SP, Becerra-Loaiza DS, Verdugo-Nieto L, Muñoz-Estrada VF, Servín-Vázquez LA, Osuna-Ramírez I, Rodríguez-Millán J, Velarde-Félix JS. Catalase but not vitamin D receptor gene polymorphisms are associated with nonsegmental vitiligo in Northwestern Mexicans. *Int J Dermatol*. 2019 Nov;58(11):1264-1269. doi:10.1111/ijd.14508.

43. Alissa A, Al Eisa A, Huma R, Mulekar S. Vitiligo-epidemiological study of 4134 patients at the National Center for Vitiligo and Psoriasis in Central Saudi Arabia. *Saudi Med J*. 2011 Dec;32(12):1291-6.

44. Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. *Pigment Cell Res*. 2003 Jun;16(3):208-14. https://doi:10.1034/j.1600-0749.2003.00032.x.

45. Nejad SB, Qadim HH, Nazeman L, Fadaii R, Goldust M. Frequency of autoimmune diseases in those suffering from vitiligo in comparison with normal population. *Pak J Biol Sci*. 2013 Jun 15;16(12):570-4. https://doi:10.3923/pjbs.2013.570.574.

46. Dutta T, Mitra S, Saha A, Ganguly K, Pyne T, Sengupta M. A comprehensive meta-analysis and prioritization study to identify vitiligo associated coding and non-coding SNV candidates using web-based bioinformatics tools. *Sci Rep*. 2022 Aug 25;12(1):14543. https://doi:10.1038/s41598-022-18766-9.

47. Arican O, Kurutas EB. Oxidative stress in the blood of patients with active localized vitiligo. *Acta Dermatovenerol Alp Pannonica Adriat*. 2008 Mar;17(1):12-6.

48. Ozel Turkcu U, Solak Tekin N, Gokdogan Edgunlu T, Karakas Celik S, Oner S. The association of FOXO3A gene polymorphisms with serum FOXO3A levels and oxidative stress markers in vitiligo patients. *Gene*. 2014 Feb 15;536(1):129-34.
https://doi:10.1016/j.gene.2013.11.055

49. Shajil EM, Begum R. Antioxidant status of segmental and non-segmental vitiligo. *Pigment Cell Res*. 2006 Apr;19(2):179-80.
https://doi:10.1111/j.1600-0749.2006.00299.x.

50. Ines D, Sonia B, Riadh BM, Amel el G, Slaheddine M, Hamida T, Hamadi A, Basma H. A comparative study of oxidant-antioxidant status in stable and active vitiligo patients. *Arch Dermatol Res*. 2006 Sep;298(4):147-52.
https://doi:10.1007/s00403-006-0680-2

51. Hazneci E, Karabulut AB, Oztürk C, Batçioğlu K, Doğan G, Karaca S, Eşrefoğlu M. A comparative study of superoxide dismutase, catalase, and glutathione peroxidase activities and nitrate levels in vitiligo patients. *Int J Dermatol*. 2005 Aug;44(8):636-40.
https://doi:10.1111/j.1365-4632.2004.02027.x.

52. Saja Hamed, Fatma Afifi, Iman Mansi, Yasser Bustanji, Hatim S Alkhatib. Screening of commonly used plant extracts in Jordanian skin lightening folkloric recipes for their tyrosinase inhibitory activity: An in vitro study. Jordan Journal of Pharmaceutical Sciences. 2021;14(2).

53. Sandipta Ghosh, Tribeni Chatterjee, Anirban Sardar, Ishita Chatterjee, Arup Bose, Angana Dasgupta, Akash Malitha, Krishnendu Acharya. Antioxidant Properties and Phytochemical Screening of Infusion and Decoction Obtained from Three Cultivated Pleurotus Species: A Comparative Study. Jordan Journal of Pharmaceutical Sciences. 2020 ; 13(2).

عدم وجود ارتباط بين تعدد الأشكال الجيني الكاتليز (262السيتوزين>الثايمين) مع التعرض للبهاق بين الأردنيين: دراسة حالة مراقبة

ابتسام الحوامدة* [1]، نايليا بولاتوفا [1]، المعتصم يوسف [1]، محمد العبادي [1] وإيثار عمر [1]

[1] قسم الصيدلة والتكنولوجيا الصيدلانية، كلية الصيدلة الجامعة الأردنية، عمان، الأردن.

ملخص

يتم جلب البهاق عن طريق فقدان الخلايا الصباغية الوظيفية ويظهر على شكل بقع بيضاء قد تغطي جلد الجسم كله. هناك خلفية وراثية في التسبب في البهاق. تعدد الأشكال في أجزاء مختلفة من الجينات الكاتلاز قد تؤثر على نشاط المرض وتؤدي إلى تقليل وظيفة الكاتليز، وبالتالي، تراكم بيروكسيد الهيدروجين، واحدة من العوامل المؤكسدة التي تضر الخلايا الصباغية، واحدة من العوامل المؤكسدة التي تضر الخلايا الصباغية. قمنا بتقييم تعدد الأشكال الجيني CAT 262 من مرضى تعدد الاشكال الوراثي البهاق باستخدام تقنية تفاعل البلمرة المتسلسل (PCR) مع واحد على الأقل سي ونموذج تي واحد على الأقل. وشملت الدراسة 48 مريض البهاق و 51 عينة ضابطه. كان التاريخ العائلي للبهاق موجودا في 27.1 ٪ من المرضى وتم تشخيص أمراض المناعة الذاتية في 16.0 ٪ من المرضى. أفاد حوالي ثلاثة أرباع مرضى البهاق (75.0٪) أن التوتر النفسي كان العامل الرئيسي المسبب لمرضهم. كان النمط الجيني CC السائد (56.2% لمرضى البهاق و 62.7% للظابطه) مع عدم وجود فرق كبير بين مجموعة الدراسة (P=0.7). كان نشاط الكاتليز في الدم متقارب بين أطراف الدراسة (159.1± MU 21.6 وحدة/لتر في مرضى البهاق و151.3 ± 25.4 وحدة/لتر في العينه الضابطه (P=0.15). نستنتج أنه لا يرتبط تعدد الأشكال الجيني في CAT262 ولا نشاط الكاتليز في الدم بالبهاق.

الكلمات الدالة: تعدد الأشكال الجيني، الكاتليز في الدم، البهاق، الجين Cat 262- سيتوسين، ثيامين، الاردنيون.