Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 305 – 311
Received 25 April 2021; Accepted 08 May 2021.

The Role of Some Blood Parameters Andmethylenetetrahy Drofolate Reductase Polymorphisms in Vitiligo

KarrarAbd-Ali JahilandLiqaa H. Saqban[1*]

[1]Departmentof Biology, College of Sciences, University of Al-Qadisiyah, Iraq

***Corresponding author: rasha.albukhlate@qu.edu.iq**

Abstract

The aim of study was to determine the relationship between Methylenetetrahydrofolate Reductase Polymorphisms in vitiligo with some clinical and blood parameters which include amount of hemoglobin in whole blood , RBCs and WBCs , the study included 60 patienthave been diagnosed with Vitiligo and 40 healthy people as a control group . The samples were collected from, Al-Kafeel Specialist Hospital, Imam Al-Hussein Teaching Hospital, blood were examined for all patients and control, and genetic polymorphism of gene rs1801133 C / T were studied using PCR-ARMS technology, the results were as follows:

According to the of age of the subject were classified into five groups (5-15 years, 16-25 years, 26-35 years, 35-45 years, 46-55 years) the number and percentage of each category in vitiligo patients was 5 (8.33%), 6 (10%), 12 (20%), 17 (28.33%), 20 (33.34%) respectively, and while the healthgropuswere as follows: 4 (10%), 5 (21.5%), 8 (20%), 10 (25), 13 (32.5%), respectively. As the results showed by dividing the healthy and patient according to gender, the percentage of males was found in 34 (56.66%) and females 26 (43.34%), compared with the control group 50% for both sexes, allele frequency and genotype frequency. On the other hand the Methylenetetrahydrofolate reductase polymorphism was determined as the following : AA genotype, was recorded as 13 patients, AT genotype 27 and TT genotype was 20, while in healthy subjects 10, 11, 19 for each of the TT, AT and AA genotypes, respectively.

Keywords:vitiligo, rs1801133 C / T, ARMS-PCR.

Introduction :

Vitiligo, s a condition in which the skin loses its pigment cells (melanocytes), it can be hereditary or acquired, it was an aggressive disease characterized by depigmentation of skin color as a result of the gradual loss of function Melanocytes. It is also common throughout the world,It can be seen in all age groups [1]. Generally it is defined as limits Symmetrical, sometimes one-sided, and often dematomal, milky white spots of varying size and localization are the clinical features of the disease. These specifications was encountered equally in both sexes. The prevalence rate of these diseaae was varying depending on ethnicity and geographical distributions [2]. Although the etiology of Vitiligo has not yet been established Fully understood, autoimmune, cytotoxic, neurotoxic, Hypotheses based on biochemistry are taken into

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consideration [3]. The Genetic predisposition and trigger factors have roles in the onset of the disease [4,13]. Some of studies have shown that oxygen roots are reactive ROS plays a role in the pathogenesis of Vitiligo [5] and some of them demonstrated that MTHFR gene polymorphisms may play a vital role in genetic susceptibility to vitiligo [6]. The goal of study was to clinical and blood parameters inblood of patient have vitiligo, as well as estimated MTHFR rsgene polymorphisms and compare the results with apparently healthy.

Materials and methods:

Subjects:

The study group consisted of 60 non-related patients. Vitiligo (men, women; mean age: 38.61 ± 14.767 Standard deviation SD years) and 40 (20 men and 20 women; Median age: 27.48 ± 10.154 years (SD) apparentlyhealthy controls. Vitiligo patients were taken consecutively of those who are fully and prospectively treated continued in the department of dermatology in Al-Hussein teaching hospital, Karbala, Iraq. The diagnosis of Vitiligo has been confirmed clinically. The control group included apparentlyhealthy individuals who have normal in routine physical examination, and did nothave chronic diseases. Including exclusion criteria for both groups inflammatory diseases that accompany infection diseases, autoimmune disorders and oncological diseases, Patients whose immune system is suppressed, diseases of the liver and kidneys, Diabetes and familial hypercholesterolemia and the presence of a chronic disease. Moreover, patients have had photo chemotherapy with Vitiligo and or using systemic medications for the past three months as well as the topical medications were also excluded

Clinical and demographic characteristics including age, Sex, disease initiation and type of disease registered. Karbalalocal Ethics Committee was obtained from , Faculty of Medicine, University of kerbala and written informed consent Obtained from the study participants

Molecular assay for detection rs1801133 T/C polymorphism:

DNA extraction from peripheral blood leukocytes EDTA Anti-Coagulant Blood uses AccuPrep® Genomic DNA Extraction Kit - Bioneer (korea) according to manufacture instructions. Methylenetetrahydrofolate Reductase Polymorphisms Single nucleotide polymorphism (SNP)rs1801131, Thetetra-primer amplification refractory mutation system (Tetra-ARMS) method was used to genotype MTHFR polymorphisms rs1801133 T/C. The specific primers were synthesis according to [7] table 1, 0.5 µl per Primer PCR (10 ng / ml) (Peshgaman Iran Company), 12 µl Master mixture (amplicon, Denmark), 2 µl (~ 1ng / mL) genomic DNA, 10 µl DNase-Free Distilled Water (Parstous Biotech , Iran) mixed to final size 25 micro liter, tetra ARMS-PCR product was visualized in 2% PCR agarose gel electrophoresis and placed on 254 nm (ultra-high-altitude optoelectronics UV light, USA) after staining with ethidium bromide dye

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Table 1: Oligonuclotides primers for MTHFR polymorphisms rs1801133 T/C

Type of primer	5-3 direction	product
FO	CATATCAGTCATGAGCCCAGCCACTCAC	**456 bp**
RO	AGGAGATCTGGGAAGAACCAGCGAACTC	
FI	TTGAAGGAGAAGGTGTCTGCGGGCGT	**282 bp**
RI	CAAAGAAAAGCTGCGTGATGATGAAATAGG	**231 bp**

The method was followed according to the researcher's instructions [8] in a technique of amplification refractory mutation system , the optimization of PCR conditions for tetra-ARMS PCR genotyping of rs1801133 T/C polymorphisms. As the following; 5:00 at 95C Initial denaturation followed by 35 cycles as 30 secondat 95Cdenaturation , 30 second at 66C as annealing temperature and 45 second at 72 as Extension temperature, and finally five minutes at 72C as Final extension.

Statistical analysis

Analyze the results with SPSS V.16Windows (SPSS Corporation, Chicago, Illinois, USA).Use an independent t-test for comparisonData between the patient and the control group.Chi-square and logistic regression are usedSelect T2D andSNP. A P value less than 0.05 are important.

Results and Discussion

Clinical and demographic characteristics of vitiligo Patient (age, smoking, married statues, and family history of vitiligo) Stratification based on MTHFR polymorphisms As shown in Table 1. Clinical and demographic characteristics of vitiligo Patients and MTHFR polymorphisms ($p< 0.05$) (Table 2).

Table 2: Clinical and demographic characteristics between vitiligo Patients and health.

variables	control	Patient	odd ratio	%95 CI	X^2	P value
age						
5- 15 years	4 (10)	5 (8.33)	-	-		
16-25 Years	5 (21.5)	6 (10)	-	-	0.314	0.986
26-35 Years	8 (20)	12 (20)	-	-		
36-45 Years	10 (25)	17 (28.33)	-	-		
46-55 Years	13 (32.5)	20 (33.34)	-	-		
Smoking						
Smoking	4 (10)	11(18.3)	0.49	0.14-	1.3	0.25

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				1.68			
Non smoking	36 (90)	49(81.7)					
Family history							
Family history	-	57(95)	-	-	-	-	
No family history	-	3(5)	-	-	-	-	
Married statues							
married	15(37.5)	51 (85)	0.1	0.04-0.275	24	0.001	
No married	25(62.5)	9 (15)					

Our result found the most cases of vitiligo in the age 46-55 years as 20 cases (33.34) it was no significant differences (X2 0.314, P> 0.05)with other groups, It has not been proven in previous studies that there is a relationship between Vitiligo exclusively with age, Anyone of any age can develop vitiligo, but it is very rarely reported to be present at birth. In a Dutch study, 50% of people reported that the disease appeared before the age of 20 years[9].

The study also indicated that there was no effect of smokers with vitiligo patients, so the patients were divided into a group of smokers with number and percentage was 11 (18.3%) and a group of non-smokers 49 (81.7%). The 95% confidence interval ranges from (0.14 to 1.68). it was found chi-square value was (1.3) at a probability level of 0.25, the number and percentage of the family history of the patients was 57 (95%) and the patients without a family history was 3 (5%), respectively.

The results of the differences between the blood parameters shown in the figure (1) revealed a variation in the levels of these blood parameters in vitiligo patients compared with the control groups under the probability level (0.05). The mean hemoglobin was 12.85 ± 1.6 grams per deciliter for patients have Vitiligo, it was lesser than control group, which had an average hemoglobin (14.21 ± 1.54) grams per deciliter, on the one hand, the mean of RBCs in patients was (4.7 ± 0.56) million red blood cells per microliter (MCL). It is lower than the control group as they had an average of RBCs (5.18 ± 0.56) million RBCs per one microliter (MCL) below the likelihood level (P <0.05).

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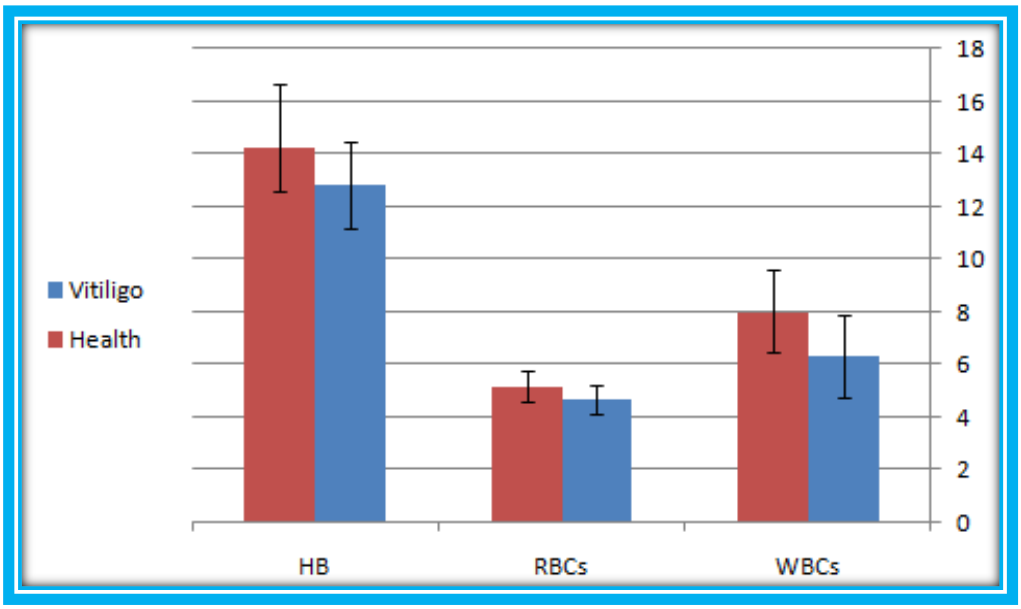

Figure 1: Mean and standard deviation of blood parameters between patient and control.

It differs from what the researcher stated [10]. Nearly a quarter of vitiligo patients suffer from anemia, but they did not differ statistically from control in Saudi Arabia, where he showed that vitiligo patients were 2.4 times more likely to have anemia with low Significantly average MCV value compared to healthy controls. Vitiligo patients may be more likely to develop celiac disease, an autoimmune disorder known to be associated with hypovolemia. In spite of what [11] showed about a lower prevalence (9.9%) of anemia in vitiligo patients, as this prevalence was similarly not statistically different from the control group. On the other hand, [10] found A statistically higher prevalence of anemia among vitiligo patients (20%) compared to the control group (3%).

The results of the current study showed about the analysis of the genetic variance in groups and its estimation through alleles, which include both types: allele frequency and genotype frequency. The AA genotype, was recorded as 13 patients, AT genotype 27 and TT genotype was 20, while in healthy subjects 10, 11, 19 for each of the TT, AT and AA genotypes, respectively, as shown in the table 3 figure 2.

Table 3: Odd ratio and confidence interval between genotype frequency and allele frequency among patient and control

Genotype frequency	health	patient	odd ratio	Confidence interval	P value
AA	91	13	-	-	-
AC	11	27	3.58	1.327-9.69	0.001
CC	10	20	2.92	1.03-8.24	0.04
Allele frequency					

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A	24	34	-	-	-
C	16	26	1.14	0.501-2.58	0.74

The genotype of the wild type AA and the allele A was a reference, and the allele frequencies and genotype were significantly different in patients and healthy subjects. The MTHFR gene polymorphism, our results found there is a significant difference in the genotype and the allele between the genotype that carries the type A allele, with a probability of more than three times (3.58) and confidence intervals as 1.327-9.69 compared to the genotype carrying AC and also exceeding the two-fold (2.92) and the confidence interval range between 1.03-8.24 for the genotype carrying the CC allele.

The study disagreement with [11] who showed that there is no correlation in the three genotypes with each other for vitiligo patients with healthy subjects, wild type allele A and genotype A, as it was shown that the CC genotype increased significantly in patients compared to controls (13). % Versus 19%, respectively, p = 0.015) and determined as a risk genotype (OR = 1.56; CI = 1.09--2.25).

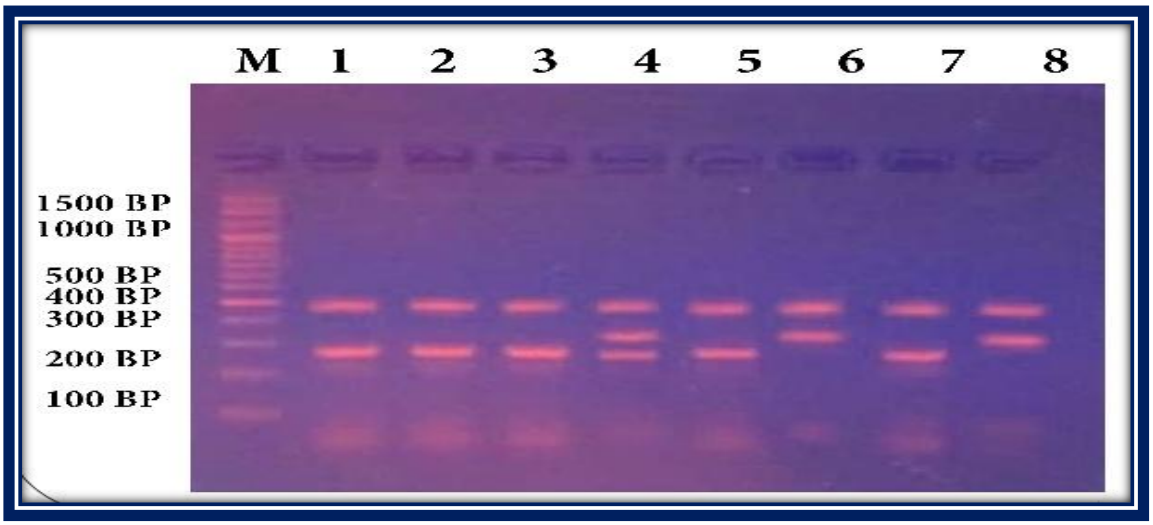

Figure 2: polymorphisms rs1801133 T/C in agrose gel electrophoresis, M : DNA marker, lane 1, 2, 3, 5 and 7 represented TT genotype, Lane 4 represented AT genotype while the lane 6 and 8 represented AA genotype.

References

[1] Kemp, E. H., Gavalas, N. G., Gawkrodger, D. J., &Weetman, A. P. (2007). Autoantibody responses to melanocytes in the depigmenting skin disease vitiligo. Autoimmunity Reviews, 6(3), 138-142.

[2]-Zhang, Y., Cai, Y., Shi, M., Jiang, S., Cui, S., Wu, Y., ... & Chen, H. D. (2016). The prevalence of vitiligo: a meta-analysis. PloS one, 11(9), e0163806.

[3]-Singh, S., Singh, U., & Pandey, S. S. (2011). Increased level of serum homocysteine in vitiligo. Journal of clinical laboratory analysis, 25(2), 110-112.

[4]-Mohammed, G. F., Gomaa, A. H., & Al-Dhubaibi, M. S. (2015). Highlights in pathogenesis of vitiligo. World Journal of Clinical Cases: WJCC, 3(3), 221.

[5]- Yeh, C .K., S. E. Harris, et al. (2012). "Hyperglycemia and xerostomia are key determinants of tooth decay in type 1 diabetic mice." Lab Invest 92(6): 868-882.

[6]-El Tahlawi, S., Halim, D. M. A., El Hadidi, H., Fawzy, M. M., Hegazy, R. A., Ezzat, M., ... & Sayed, K. S. (2020). Estimation of homocysteine level and methylenetetrahydrofolate reductase (MTHFR) gene and cystathionine B synthase (CBS) gene polymorphisms in vitiligo patients. Skin pharmacology and physiology, 33(1), 38-43.

[7]Poodineh, M., Saravani, R., Mirhosseini, M., &Sargazi, S. (2019). Association of two methylenetetrahydrofolate reductase polymorphisms (rs1801133, rs1801131) with the risk of type 2 diabetes in South-East of Iran. Reports of biochemistry & molecular biology, 8(2), 178.

[8]Zabeel, A. K., Al-Bazi, W. G. M., & Muhammed, H. A. (2018). Study the association of PIT1 gene polymorphism with milk yield and body weight traits of local breed Iraqi cattle in Kerbala province. Biochemical and Cellular Archives, 18(2), 1867-71.

[9] Jaisankar, T. J., Baruah, M. C., & Garg, B. R. (1992). Vitiligo in children. International journal of dermatology, 31(9), 621-623.

[10] Mubki, T., Alissa, A., Mulekar, S., Albargawi, S., Youssef, M., &AlJasser, M. (2017). Association of vitiligo with anemia, vitamin B12 deficiency, diabetes mellitus, and thyroid dysfunction in Saudi Arab patients: A case control study. *Journal of Dermatology & Dermatologic Surgery*, *21*(2), 72-76.

[11]Ataş, H., &Gönül, M. (2017). Increased risk of metabolic syndrome in patients with vitiligo. Balkan medical journal, 34(3), 219.

[12] Jadeja, S. D., Mansuri, M. S., Singh, M., Patel, H., Marfatia, Y. S., & Begum, R. (2018). Association of elevated homocysteine levels and Methylenetetrahydrofolate reductase (MTHFR) 1298 A> C polymorphism with Vitiligo susceptibility in Gujarat. Journal of dermatological science, 90(2), 112-122.

[13] JALIL, A. T., DILFY, S. H., KAREVSKIY, A., & NAJAH, N. (2020). Viral Hepatitis in Dhi-Qar Province: Demographics and Hematological Characteristics of Patients. *International Journal of Pharmaceutical Research*, *12*(1).