The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90 (2), Page 2563-2566

Evaluation of Vitamin D Receptor Gene Polymorphism in Vitiligo in Sharkia Governorate

Mahmoud Y Abdelmawla[1], Somia H Abdullah[2], Mohamed M Nasr[1], Yasmine E Abd-Elghany[1]
Dermatology, Venereology and Andrology Department[1],
Medical Biochemistry Department[2], Faculty of Medicine, Zagazig University, Egypt.
Corresponding Author Name: Yasmine Elsayed Abd-Elghany
Phone Number: +2 0103555489**, Email:** Yasminsayed829@gmail.com, **ORCID:** 0000-0002-9630-2147

ABSTRACT

Background: Vitiligo is a depigmentation disorder with macular depigmentation and white patches as the main clinical feature. Etiology of vitiligo remains unclear, but multiple factors have been implicated in the development of the disease, including impaired melanocyte migration, genetic susceptibility and autoimmunity. **Objective:** The aim of the study was to identify if there is any relation between vitamin D gene affection and having vitiligo.

Patients and Methods: This was an analytical case control study; in which patients were selected from the outpatient clinic of Dermatology, Venereology and Andrology Department, Faculty of Medicine, Zagazig University Hospitals. The study was carried out on 60 subjects, divided into 2 groups (30 vitiligo patients, and 30 healthy controls), using socio-medical questionnaire, Detailed dermatological examination, CBC, liver and kidney function tests and DNA extract from blood and detection of gene polymorphism.

Results: There was no significant difference between the vitiligo and control groups regarding age, sex, residence, and family history of vitiligo. The most found genotype in vitiligo patients was GA (80% of cases) with high statistical significant difference found between the two groups. while the most found genotype in controls was GG (70% of them), with high statistical significant difference found between the two groups. Vitiligo score was higher in GA genotype patients compared to GG and AA genotype patients, but with no significant difference.

Conclusion: This study suggests an association between vitamin D receptor (VDR) gene polymorphisms in vitiligo in Egyptian population.

Keywords: Vitamin D receptor gene polymorphism, Vitiligo, Association.

INTRODUCTION

Vitiligo is a depigmentation disorder with macular depigmentation and white patches as the most common clinical feature, cause of vitiligo remains unclear, but multiple factors have been implicated in the development of the disease, including impaired melanocyte migration, genetic susceptibility and autoimmunity **(1)**. Vitamin D receptor (VDR) is a member of the steroid/thyroid hormone receptor superfamily. It has been detected that the expression and function of the VDR may be influenced by polymorphisms of the VDR gene. Vitamin D analogues have been used in vitiligo intervention alone or in combination with other treatment **(2)**.

Vitamin D receptor gene polymorphism is considered main cause in several autoimmune diseases including vitiligo **(3)**. It has effective role in the regulation of T cell function and the biological function of melanocytes **(4)**. Also it has master regulator of epidermal barrier function, inflammation, keratinocytes proliferation, and microbial defense **(5)**.

The aim of the study: to identify if there is any relation between vitamin D gene affection and having a vitiligo.

PATIENTS AND METHODS

An analytical case control study was carried out on 60 subjects divided into 2 groups (30 vitiligo patients, and 30 healthy controls), were selected from the outpatient clinic of Dermatology, Venereology and Andrology Department, Faculty of Medicine, Zagazig University Hospitals, from the first of February 2019 to the end of February 2020.

Inclusion criteria:

All patients enrolled in the study had:

- Adult vitiligo of both sexes.

Exclusion criteria:

- Other dermatological diseases (such as systemic lupus erythematosus, atopic dermatitis).
- Pregnant and lactating females.
- History of tuberculosis or any active infectious disease.
- Systemic illness (e.g., diabetes, hypertension, liver diseases, renal disorders, rheumatoid disease or other arthropathy disorder).

Study tools:

All patients were subjected to:

1- Complete history taking:
   a- Personal history including age, residence, socioeconomic status, marital status and any marital consanguinity plus nutritional conditions.
   b- Family history of similar conditions and other diseases.
   c- Past history of previous medication, psychological stress and surgery.
2- Complete general examination with blood pressure measurement.

Received: 27/06/2022
Accepted: 03/09/2022

3- Detailed dermatological examination: vitiligo patients were assessed by vitiligo area severity index **(VASI)** [6].
4- Complete blood picture (CBC), liver and renal functions tests were carried out.

Procedure of DNA extraction from Blood

A-Components belong to SYNC™ Total DNA extraction kit

DNA extraction:

The reagents were highly pure analytical PCR material. All of the pipettes, tips, and tubes used for DNA extraction were DNase and RNase-free tubes were supplied by Gentra (Minneapolis. USA) to prevent contamination. By the gSYNC™ DNA Extraction Kit and EDTA anticoagulated peripheral blood leukocytes, genome DNA was extracted as directed by the manufacturer (Geneaid Biotech Ltd .USA).

Quantification and purity of DNA:

This was performed to determine the concentration of DNA and to assess the purity of DNA. This was achieved by calculating the A260/A280 ratio. This ratio was determined to be between 1.7 and 1.9 for pure double-stranded DNA. The process involved adding 20 µL of each isolated DNA sample to 1 ml of deionized water, then using the Milton Roy Spectronic 3000 Array to detect absorbance at 260 and 280 nm wavelengths.

B-Detection of gene polymorphism:

VDR Apa-I polymorphisms were analyzed by means of PCR restriction fragment length polymorphism (RFLP) method. VDR Apa-I amplification was done using the primer 50 -CAGAGCATGGACAGGGAGCAAG-30 and antisense primer 50 -GCAACTCCTCATGGCTGAGGTCTCA-30. PCR was performed in a final volume of 25 µl containing 5.5 l µl of H_2O, 5 µl of genomic DNA, 1 µl of each primer (1 lM), and a 29 Super Hot PCR Master Mix (12.5 µl) (Bioron, Ludwigshafen am Rhein, Germany). PCR protocol was performed at 94°C for 5 min, followed by 35 cycles at 93°C for 45 s, at 66°C for 30 s, and at 72°C for 45 s. A final extension step was carried out at 72°C for 7 min. The PCR products were digested overnight at 65 C with Taq-I restriction enzyme.

Detection and quantitation of extracted DNA

Agarose gel electrophoresis

The quality of the isolated DNA was determined by running 5 µl of each sample on ethidium bromide stained 1.0% agarose gel. The DNA sample was after that visualized on a Gel documentation system[7].

Ethical approval

The study was approved by Sharkia Health Directorate and approval letter was sent to the selected health center. Written consent was taken from the participants after explaining the objectives and methodology of the study. Each participant expressed as a code to ensure confidentiality. The Institutional Review Board (IRB) gave its formal consent (ZU-IRB # 5395). This work has been carried out in accordance with the code of ethics of the world medical association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis

Data were imported into Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. According to the type of data; qualitative were represented as number and percentage and quantitative continuous group represented by mean ± standard deviation (SD). Difference and association of qualitative variable were calculated by Chi square test (X^2). T test was used to compare two groups of normally distributed quantitative data. Differences between more than 2 groups of quantitative abnormally distributed independent data was done by Krusskal-Wallis test. P value was set at <0.05 for significant results and <0.001 for high significant result.

RESULTS

This table shows no significant difference between vitiligo patients and their controls regarding age, sex, and residence ***Table (1) and figure (1)***.

Table (1): Demographic characteristics of vitiligo cases and controls

Variables		Vitiligo cases *(n=30)*	Controls *(n=30)*	Test	P
Age (years) Mean± SD (Range)		37.7 ± 11.6 20 - 40	36.5 ± 11.4 20 - 38	0.713	0.81 NS
Sex, n (%)	***Male***	10 (33.3)	12 (40%)	0.29#	0.59 NS
	Female	20 (66.7)	18 (60%)		
Residence, n (%)	***Rural***	15 (50%)	16 (53.3%)	0.067#	0.796 NS
	Urban	15 (50%)	14 (46.7%)		

SD: Standard deviation. * Mw test # Chi square test. NS: non-significant

This table shows statistically significant difference between the two groups regarding family history of vitiligo ***Table (2)***.

Table (2): Family history of vitiligo group and controls

Variables	Vitiligo cases *(n=30)*		Controls *(n=30)*		x^2	P
	N	*%*	*N*	*%*		
Positive history of vitiligo	11	36.7	2	6.6	7.9	0.005 S
Negative family history of vitiligo	19	63.3	28	93.3		

x: Chi square test [2]. S: significant

This table shows that the most found genotype in vitiligo patients was GA (80% of cases) while the most found genotype in controls was GG (70% of them), with high statistical significant difference found between the two groups ***Table (3)***.

Table (3): Vitamin D receptor gene genotype distributions between vitiligo group and controls

Genotype	Vitiligo cases *(n=30)*		Controls *(n=30)*		x^2	P
	N	*%*	*N*	*%*		
GG	4	13.3	21	70	27.1	<0.001 HS
AA	2	6.7	5	16.7		
GA	24	80	4	13.3		
Allele distribution						
G	32	53.3	46	76.7	7.18	0.007 S
A	28	46.7	14	23.3		

x: Chi square test [2]. HS: highly significant. S: significant

This table shows that vitiligo score was higher in GA genotype patients compared to GG and AA genotype patients, but with no significant difference ***Table (4)***.

Table (4): Vitiligo area scoring index in different genotypes among vitiligo patients

Variables		GG *(n=4)*	AA *(n=2)*	GA *(n=24)*	KW	P
Score	***Mean± SD***	3.1 ± 1.94	2.1 ± 1.96	3.6 ± 2.43	0.78	0.68 NS
	Median	2.38	2.13	3.5		
	(Range)	1.75 – 6	0.75 - 3.5	0.5 – 9		

Kruskal-Wallis test. NS: non-significant

DISCUSSION

Analysis of our findings revealed that the mean± SD of age of vitiligo group was 37.7 ± 11.6 years and in control group was 36.5 ± 11.4 years. No significant difference was found between vitiligo patients and their controls regarding age, sex, and residence. The present study also revealed that there was no statistical significant difference between the groups regarding age, sex, and residence, as they are matched groups.

In comparison with the study of **Hassan *et al.*** [(8)], age and gender ratio were not substantially different for each variable among patients with vitiligo (61 females, and 39 males) and healthy controls (60 females, and 40 males). The age group of patients ranged from 4 to 58 years with a mean age of 28.66 ± SD 11.98 years. In the present study; there was 36.7% had positive family history of vitiligo among vitiligo group, and there was no statistically significant difference between the two vitiligo and healthy groups regarding family history of vitiligo. In **Butt *et al.*** [(9)], study, 22% of patients showed positive family history of vitiligo. This is in comparison with **Shajil *et al.*** [(10)], who found positive family history of vitiligo in 21.93% of patients. **Tanioka *et al.*** [(11)], noted positive family history in 26% of patients, which also support our results. Another Indian study of **Gopal *et al.*** [(12)], reported 36% of patients with family history of vitiligo. All these studies establish the genetic basis of vitiligo.

The current study revealed that the most found genotype in vitiligo patients was GA (80% of cases) while the most found genotype in controls was GG (70% of them), with high statistical significant difference found between the two groups.

In addition to above findings, the present study revealed that vitiligo score was higher in GA genotype patients compared to GG and AA genotype patients, but with no significant difference.

Unfortunately only few association studies regarding VDR polymorphisms studies for vitiligo patients are available. One study of **Birlea *et al.*** [(13)], from Romania showed that mutant CC homozygous for APa1 was significantly associated with susceptibility to vitiligo, which contradicts our results; may be the reason of this difference because this study is working in a different allele. In comparison with the study of **Hassan *et al.*** [(8)], we illustrated that the most found genotype in in controls was GG (70% of them) and the commonest genotype among vitiligo cases was GA (80%), with high statistical significant difference found between the 2 groups, which is similar to our results.

Noronha *et al.* [14], aimed to evaluate the potential association between VDR polymorphisms and vitiligo susceptibility and (ii) To estimate the serum levels of 25–hydroxyvitamin D in case and control groups. Their study included 34 participants (17 with vitiligo and 17 age-and gender-matched healthy controls). They found that, there was no significant difference in the distribution of VDR polymorphisms between the case and control groups. In their study, no significant difference was observed in the distribution of Fok1, Bsm1, Apa1, and Taq1 VDR polymorphisms between the case and control groups. However, they observed a significant association between the Fok1 polymorphism and serum 25(OH) vitamin D levels in vitiligo patients studied.

It seems that a generalized wide genomic study warranted to study VDR gene polymorphism on vitiligo and other inflammatory skin disorders.

Limitations of our study were small sample size. We also did not consider seasonal variation in serum vitamin D levels. Further Egyptian studies with larger sample size are needed to evaluate the association of VDR gene polymorphisms and serum 25-hydroxyvitamin D levels in patients with vitiligo.

CONCLUSION

This study suggests an association between VDR gene polymorphisms in vitiligo Egyptian population. Further association and functional studies of additional VDR single nucleotide polymorphisms (SNPs) and other genes are required in a diverse ethnic and large-sample population to identify the genetic factors associated with vitiligo. The single nucleotide gene polymorphisms of various VDR genes as found in the cases might lead to vitamin D deficiency, due to alteration of VDR. In turn, this could lead to an increase in the susceptibility of developing vitiligo, due to the stimulatory role of vitamin D in melanogenesis, and we disclosed that polymorphisms of VDR gene were associated with Egyptian patients with vitiligo. Allelic variance in the VDR gene or other genes in linkage with disequilibrium of this gene might predispose to the development of vitiligo.

RECOMMENDATIONS

We recommend conducting more studies to assess the level of vitamin D in our community in order to detect the appropriate cut points (levels of deficiency and inadequacy) and to detect them as reference values and to study the prevalence of vitamin D deficiency in our community as regard these cut points.

We conclude that patient with vitiligo are high risk group for vitamin D deficiency, therefore, it is reasonable to screen level of vitamin D routinely in all patients with vitiligo and replenish its deficiency, which might benefit psoriasis treatment. So, we also recommend performing interventional studies with vitamin-D supplementation to detect their effect on vitiligo and its possible future use as part of treatment plans for vitiligo.

More studies to analyze changes of vitamin D in patients during the seasons and their relationship to clinical changes in the disease, as well as more studies of vitamin D levels according to the clinical forms with a larger sample numbers are warranted. Future studies looking at the exact mechanism that explains the association between low levels of vitamin D and vitiligo are important to reveal the exact relations.

Acknowledgement: the authors should thank all the participants who contributed to the study and all the managerial system that facilitated this work.

No Fund

No conflict of interest

REFERENCES

1. **Alikhan A, Lesley M, Meagban D *et al.* (2011):** Vitiligo: A comprehensive overview. J Am Acad Dermatol., 65:473-49.
2. **Reichrath J, Zouboulis C, Vogt T *et al.* (2016):** Targeting the vitamin D endocrine system (VDES) for the management of inflammatory and malignant skin diseases: a historical view and outlook. Rev Endocr Metab Disord., 17:405–417.
3. **Silverberg J, Silverberg A, Malka E *et al.* (2010):** A pilot study assessing the role of 25 hydroxy vitamin D levels in patients with vitiligo vulgaris. J Am Acad Dermatol., 62(6):937–941.
4. **Watabe H, Soma Y, Kawa Y *et al.* (2002):** Differentiation of murine melanocyte precursors induced by 1, 25-dihydroxyvitamin D3 is associated with the stimulation of endothelin B receptor expression. J Invest Dermatol., 119:583–9.
5. **Hawker N, Pennypacker S, Chang S *et al.* (2007):** Regulation of human epidermal keratinocyte differentiation by the vitamin D receptor gene. J Invest Dermatol., 127:874.
6. **Hamzavi I, Jain H, McLean D *et al.* (2004):** Parametric modeling of narrowband UV-B phototherapy for vitiligo using a novel quantitative tool: the Vitiligo Area Scoring Index. Archives of Dermatology, 140(6): 677-683..
7. **Green M, Sambrook J (2019**): Agarose gel electrophoresis. https://pubmed.ncbi.nlm.nih.gov/30602561
8. **Hassan I, Bhat Y, Haq S *et al.* (2019):** Association of vitamin D receptor gene polymorphisms and serum 25-hydroxy vitamin D levels in vitiligo–A case-control study. Indian Dermatology Online Journal, 10(2): 131.
9. **Butt G, Faria A, Uzair W *et al.* (2015):** Familial frequency of vitiligo and its association with autoimmune disorders; Journal of Pakistan Association of Dermatologists, 25 (2):101-104.
10. **Shajil E, Agarwal D, Vagadia K *et al.* (2006):** Vitiligo: Clinical profiles in Vadodara, Gujarat. Indian J Dermatol., 51:100- 4.
11. **Tanioka M, Yamamoto Y, Katoh M *et al.* (2009):** Vitiligo vulgaris and autoimmune diseases in Japan. A report from vitiligo clinic in Kyoto university Hospital Dermatoendocrinology, 1:43-5.
12. **Gopal K, Ramarao G, Kumar Y *et al.* (2007):** Vitiligo: a part of a systemic autoimmune process. Indian J Dermatol Venereol Leprol., 73:162-5.
13. **Birlea S, Birlea M, Cimponeriu D *et al.* (2006):** Autoimmune diseases and vitamin D receptor Apa-I polymorphism are associated with vitiligo in a small inbred Romanian Community. Acta Derm Venereol., 86:209-14.
14. **Noronha T, Girisha B, Kumari S *et al.* (2022):** A study of vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D levels in vitiligo patients and controls. Clin Dermatol Rev.,6:47-52.