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Original article

Association of Vitiligo with ABO/Rh System and its Influence on Thyroid Stimulating Hormone and Vitamin D

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ABSTRACT

This study was conducted to determine if there is a relationship between vitiligo and ABO blood groups, the Rhesus (Rh) factor, thyroid stimulating hormone (TSH) and vitamin D. For vitiligo analysis, two hundred subjects participated in this study, 100 vitiligo patients and 100 control cases (without vitiligo). ABO blood grouping and Rh typing were tested by a slide method. TSH testing involved 80 vitiligo patients and 80 controls (without vitiligo) and the hormone was analyzed by separating the serum in a centrifuge for two minutes and the results were obtained by Beckman fully automatic analyzer. For vitamin D, 50 vitiligo patients and 50 healthy people (without vitiligo) were included. The data on vitamin D were obtained from private laboratory services. Statistical analysis was performed using IBM SPSS version 26. P<0.05 was considered significant. Most patients with vitiligo had a significantly lower level of serum vitamin D compared with controls (p-value < 0.05), while no statistically significant difference in TSH serum levels between vitiligo cases and controls, was found (p-value > 0.05). Furthermore, despite showing that subjects with blood group O are more susceptible to vitiligo as compared to other groups, there was no significant association of vitiligo with ABO blood groups (p-value > 0.05). Similarly, the incidence of Rh positive and Rh negative was not statistically different between the two groups (p-value > 0.05). This study showed that vitiligo patients are often vitamin D deficient. This study highlights the need to evaluate vitamin D status in vitiligo patients to improve the level of skin pigment loss. It remains unknown whether vitamin D deficiency causes vitiligo. However, a collection of larger sample sizes of different ethnicities should be required to achieve a precise conclusion.

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INTRODUCTION

Vitiligo is an autoimmune disease caused by the immune system when attacking the melanocytes [1]. Melanocytes are cells that produce melanin located at the stratum basale of the epidermal layer of the skin, the uveal layer of the eye, the inner ear, the meninges, the heart and the bones [2,3]. Melanin is the natural pigment in the human body and is responsible for the color of the skin, hair, and eyes [4]. It is synthesized via multiple processes called melanogenesis and stored within a special organelle called the melanosome [2].

According to a recent review conducted by Lili Guo *et al.*, 2023 reported that melanin has five basic types namely; eumelanin, pheomelanin, neuromelanin, allomelanin and pyomelanin [5]. The most common type is eumelanin, which is divided into forms, brown eumelanin and black eumelanin. These two forms are chemically different and are

responsible for yellow and grey hair respectively. Pheomelanin exists in the lips, glans of the penis, nipples and vagina and is responsible for the color of red hair and when mixed with a small amount of brown eumelanin gives the hair an orange color [6]. Neuromelanin on the other hand is a black pigment produced in specific neurons in the brain and its function is still not yet known. However, some studies are suggested to play an important role in the process of apoptosis [7]. Allomelanin is nitrogen-free melanin often found in fungi [8]. Lastly, pyomelanin is an extracellular red brown pigment lacking nitrogen and produced by many types of bacteria and fungi [9].

Melanin levels in our skin are naturally controlled by genetics and determine the color of the skin, eyes and hair. Interestingly, in dark-skinned people, melanocytes produce more melanin than they do in those with light skin tones [3]. Obviously, there is a direct correlation between the geographic distribution of solar radiation and the distribution of skin pigmentation around the world in which some people have dark skin while others have light colored skin [4]. In addition to providing pigmentation in human skin, melanin also plays an important role by giving protection against damage from direct sunlight and harmful UV radiation [10]. European people for instance predominantly have white and fair skin due to the lack of melanin pigment which makes them more vulnerable to skin cancer and sunburns.

Vitiligo is not an infectious disease. It can affect anyone regardless of their age, ethnicity, or gender and around 1% of the global population has vitiligo [11]. Vitiligo is identified through white patches on the skin that are easily visible. This is generally a clear indication of the disease [12]. It is more noticeable on darker skin and can affect any part of the body, adopting with it different shapes and can develop as well as spread in a short period of time whilst covering multiple areas of the body simultaneously [12].

Despite the possibility of lacking any association of the ABO blood group with vitiligo [13-16]. A study conducted by Ghaderi R. and Alipour A., 2007 pointed out that the etiology of this disorder might be due to the genetic factors and ABO blood groups [17]. In fact, the association between ABO blood type and vitiligo seems to be controversial, as previous reports indicated that the frequency of AB blood group is higher in vitiligo cases while other observations declared that blood group B is more common in vitiligo [17, 18]. Furthermore, some studies confirmed that either no relationship between ABO blood groups and vitiligo [13,15,16,18]. People's blood types are different. The ABO blood group system includes; A, AB, B, and O with +, – or null denoting RhD status, and each blood group is different due to its possession of specific antigens (surface specific carbohydrate sugar markers) found on the red blood cells and are also expressed on the surface of most epithelial, endothelial cells including skin cells [18]. Therefore, scientists have predicted that these antigens of the ABO blood group might play an important role in the pathophysiology of vitiligo disease.

It has been demonstrated that the etiology of vitiligo is complex and multifactorial including; autoimmune (self-destructive mechanism) as well as genetic, inflammatory and environmental origin [19]. Numerous studies have reported the association between vitiligo and autoimmune disorders such as thyroid disease [1, 20-23]. Both Graves' disease and Hashimoto's disease have been shown to be correlated with vitiligo incidence, as individuals were more prone to thyroid disturbance [22, 24]. In contrast, the risk of vitiligo due to genetic factors is estimated at up to 83%, but it is still a multifactorial disease with a polygenic pattern of inheritance [19,25], whereas various studies have illustrated the importance of vitamin D3 in treating vitiligo patients [26-30].

The number of Libyan patients with vitiligo seems to have steadily increased in recent years. According to Eltrabulsi Aisha *et al.*, 2019, vitiligo is a major health problem in Libya [31]. This study set out to shed light on the possible linkage between ABO/Rh systems and Libyan vitiligo patients and further evaluate as well as compare the levels of TSH and vitamin D between vitiligo and normal healthy subjects.

METHODS

Study setting

This study was conducted by enrolling patients with vitiligo who made random visits to the Bir Usta Milad Hospital in Tripoli district (Libya) and were confirmed to have vitiligo by a dermatologist between March 2021 and August 2021. After informed consent, the subjects underwent the following tests: ABO/Rh blood group test (ABO typing), TSH level test and vitamin D test. Participants with any chronic diseases such as diabetes mellitus, thyroid disease, heart disease and pregnant women were excluded. A questionnaire was used in this study to seek the etiology of vitiligo.

Study population and sample collection

In general, the study population was divided into two groups; the first group represents vitiligo patients and the second group hosts the healthy control samples (without vitiligo). For ABO/Rh blood group test, the number of collected samples was 100 samples of vitiligo patients (n=100) and another 100 samples were collected from the control healthy blood donors (n=100) from both genders. ABO/Rh blood group test was performed in all patients and controls using

the slide technique. Briefly, blood samples were collected and mixed with anti-A and anti-B serums in slides. If the agglutination reactions were observed in the "Anti-A" mark on a slide, this indicates blood type A. Blood group type B refers to agglutination in the "Anti-B" mark, and agglutination in both marks represents blood type AB. No agglutination in either mark indicates blood type O, and lastly agglutination in the “Anti-D” tube indicates an Rh +ve blood type. Quality control measures were employed, and ethical guidelines were followed.

In the case of TSH analysis, 80 blood samples for each group (vitiligo n=80 and control n=80) were separately collected to measure the level of TSH hormone. TSH analysis was conducted by Beckman fully automated autoanalyzer. Similarly, 50 blood samples for each group (vitiligo n=50 and control n=50) were also separately collected to check the plasma level of vitamin D which was recorded by private laboratory services. Vitamin D levels were classified into three levels based on this study: low (<30 ng/ml), normal (30-60 ng/ml), and high (>60 ng/ml). Conversely, the normal range for TSH was 0.5–5.5 mIU/L.

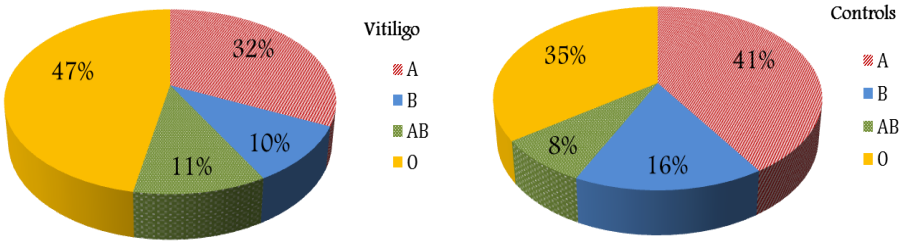
Statistical analysis

All data were analyzed using SPSS IBM version 26.0. Continuous variables are reported as mean ± standard deviation and categorical variables are displayed as percentages. An independent (unpaired) t-test was used to compare the means of vitamin D variants between the two groups, while a chi-square analysis was used to assess the distribution of ABO blood groups among the study groups. In cases where the data were abnormally distributed, the Mann-Whitney test was employed to compare the two groups. For all tests, the significance level was set at $p<0.05$.

RESULTS

Among 200 subjects included in this study 100 cases (100%) were patients with vitiligo and 100 subjects (100%) were normal healthy individuals. The incidence of ABO blood groups among cases of vitiligo and controls is shown in Figure 1. Blood groups A, B, AB and O in vitiligo patients were found to be 32%, 10%, 11% and 47%; while they were 41%, 16%, 8% and 35% in the controls, respectively (Figure 1A). In this regard, vitiligo patients showed the highest prevalence of the O blood group compared to controls. More precisely, the frequency of ABO/Rh blood group systems was calculated in 200 participants and presented in figure 1B. The prevalence of Rh positive and Rh-negative vitiligo patients was found to be 96% and 4% compared to 90% and 10% in the controls respectively (Table 1A). The Blood group O+ was found to be most prevalent followed by the blood group A+ (Figure 1C). Nonetheless, no statistically significant difference was found between vitiligo and ABO and Rh blood group system P>0.05 (Table 1B & 1A).

A



B

Vitiligo

Blood Group	Frequency	Percent
A–	3	3.0%
A+	29	29.0%
B+	10	10.0%
AB+	10	10.0%
AB–	1	1.0%
O+	41	41.0%
O–	6	6.0%
Total	100	100%

Controls

Blood Group	Frequency	Percent
A+	41	41.0%
B+	15	15.0%
AB+	8	8.0%
O+	32	32.0%
O–	3	3.0%
B–	1	1.0%
Total	100	100%

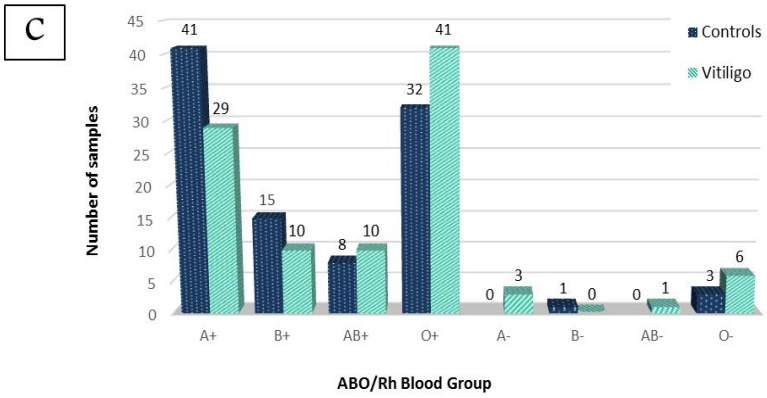


Figure 1. Distribution of ABO/Rh blood groups among participants enrolled in the study. *[A] Percentage distribution of ABO blood groups in both vitiligo and control groups. [B] Detailed percentage distribution of ABO/ Rh blood group systems ("+" Rh positive & "–" Rh negative) in the study groups. [C] Number of ABO blood groups with their Rh factor samples distribution between the study groups showing in a bar chart.*

Table 1. Compared Rhesus factor (Rh) between 100 vitiligo patients and 100 healthy people (without vitiligo) [A]. Compared blood groups between 100 vitiligo patients and 100 healthy people (without vitiligo) [B].

A

	Rh System			
Study groups	Rh +ve	Rh –ve	Total	P-value*
Vitiligo	96	4	100	0.16
Control	90	10	100	
Total	186	14	200	
*Chi-square test (X^2) = 1.9, P = 0.16				

B

	Blood Groups					
Study groups	A	B	AB	O	Total	P-value*
Vitiligo	32	10	11	47	100	0.19
Control	41	16	8	35	100	
Total	73	26	19	82	200	
*Chi-square test (X^2) = 4.72, df = 3, P = 0.19						

In regards to TSH, the hormone levels in vitiligo patients were found 12.5% above the normal range (>5.5ulU/ml), 6.25% below the normal range (<0.5ulU/ml) and about 81.25% at normal levels, whereas in control cases the results were 15%, 8.75% and 76.25% respectively (Table 2). According to SPSS analysis, there was also no specific association established (not statistically significant) between vitiligo and TSH P>0.05.

Table 2. TSH comparison between 80 vitiligo patients and 80 healthy people (without vitiligo)

	Vitiligo patients (n=80)				Healthy people (n=80)					
TSH	High	Low	Normal	Total	High	Low	Normal	Total	Mann-Whitney Test	P-value
Frequency	10	5	65	80	12	7	61	80	3183.5	0.955
%	12.5%	6.25%	81.25%	100%	15%	8.75%	76.25%	100%		

Considering the important role of vitamin D in melanogenesis and its responsibility for skin pigmentation, the levels of vitamin D were determined in vitiligo cases and compared with controls (Table 3). The levels of vitamin D in vitiligo patients were found to be 0% high, 84% low and 16% at normal ranges compared to control cases which were 0%, 26%, and 74% respectively. Interestingly, we observe that patients with vitiligo had significantly lower vitamin D (p=0.004) compared to controls which is significantly different between the study groups P>0.05 (Table 3).

Table 3. Compared vitamin D levels between 50 vitiligo patients and 50 healthy people (without vitiligo)

	Vitiligo patients (n=50)				Healthy people (n=50)					
Vitamin D	High	Low	Normal	Total	High	Low	Normal	Total	T-test	P-value
Frequency	0	42	8	50	0	36	14	50	1.449	0.004*
%	0%	84%	16%	100%	0%	72%	28%	100%		

DISCUSSION

The data obtained in this study indicate that blood group O was the most common blood group among vitiligo cases. However statistically, there was no significant association of vitiligo with ABO blood groups (p>0.05) (Table 1B). The data presented in Figure 1 and Table 1 showed the frequency of ABO/Rh systems among 100 vitiligo patients, blood group O was more susceptible (47%) while groups AB (11%) and B (10%) had lower susceptibility. Furthermore, the incidence of Rh positive and Rh negative was not statistically different between the two groups (Table 1A). In fact, this data was contradicted by an older study conducted by Ghaderi R. *et al.*, 2007 but agreed with them in the Rh group which did not show any differences. Ghaderi R. and co-workers showed that blood group B is more susceptible in vitiligo patients [17]. Furthermore, a case-control study in Kashmir, Pakistan conducted by Rather *et al.*, 2014 to examine the relationship between ABO blood groups and different skin diseases including vitiligo also contradicted our data by finding that in vitiligo patients (n=76), the B blood group was the most common blood group among vitiligo patients 47.4% [32]. Nevertheless, our findings were consistent with several previous publications which indicate that statistically there was no significant association between ABO blood groups and vitiligo [13, 15, 16]. Actually, many dermatological diseases were shown to be linked to blood types and hence it was envisaged that producing antimelanocyte antibodies in vitiligo would be likely triggered by blood type alloantigens [20, 33]. Instead, recent studies shed new light on the possibility of contributing to the stress, psychological and genetic factors in such diseases [25,34,35].

Indeed, stressful events and psychological disorders have shown to have a role in the onset and aggravation of vitiligo [35,36]. This was evident during this study when the vitiligo patients were asked if they were been exposed to psychological disorders or suffered from any hereditary problems during their livelihood. 77% of patients showed symptoms after some weeks from exposure to shock or psychological disorder and 23% of patients had vitiligo in the family. It is well known that the consequence of exposure to shock or psychological disorders such as depression would affect the human body's immune system due to an alteration in the levels of cortisol and dehydroepiandrosterone (DHEAS) leads to a reduction of defense mechanisms and redox potential, heightening local and systemic levels of inflammation with worsening and multiplication of vitiligo patches [34]. Therefore, it is possible that stress and psychological disorders may play a golden role in vitiligo [35].

Numerous studies have provided evidence of the association of vitiligo with autoimmune thyroid diseases [19, 21-24, 37- 39]. For example, an interesting review conducted by Vrijman C. *et al.*, 2012 includes forty-eight studies published between 1968 and 2012 looking at evidence of the prevalence of thyroid diseases in vitiligo patients. This review indicated a higher risk and greater susceptibility to thyroid disease (autoimmune) in individuals with vitiligo compared to those without the condition [22].

Recently, a study conducted by Bashrahil *et al.*, 2022 has also demonstrated that an association between age and thyroid biomarkers (T3 (triiodothyronine), T4 (thyroxine), TSH, thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TGAb) was detected in vitiligo patients when compared to controls [23]. Inconsistent with these findings, our findings indicated no significant differences between patients with vitiligo and healthy subjects in TSH p>0.05 (Table 2). In line with this, Sara Saniee and colleagues reported that TSH levels did not significantly differ between vitiligo patients and healthy individuals [40]. Additionally, in 2014 Gopal and co-workers revealed no link between the age, sex, or length of the condition and the prevalence of hypothyroidism among vitiligo cases [39]. It seems that in the literature there are conflicting data regarding TSH levels in patients with vitiligo.

Although several comprehensive studies have described a possible relationship between TSH and vitiligo, very limited studies have been established so far in Libya. Indeed, to the best of our understanding, there has been only one study conducted in our country by El-Dibany *et al.*, 2017 that investigated the potential connection between serum T3, T4, TSH and vitiligo concluding that both T3 and T4 serum levels were normal and the frequency of high TSH was statistically significant (P = 0.016) in vitiligo cases compared to controls [41]. We predicted that the relationship could

be traceable in our study and unfortunately, this was not achieved. On the other hand, we found that most of the vitiligo patients (84%) had very low levels of vitamin D. Although the variances were almost consistent between vitiligo patients and healthy people, these differences were statistically significant p=0.004 (Table 3). Similar to our finding, a recent study by Mahmmod *et al.*, 2021 reported a high incidence of vitamin D deficiency among vitiligo patients [42]. Furthermore, a systematic review and meta-analysis by Upala S. and Sanguankeo A. also demonstrated lower vitamin D levels in patients with vitiligo compared to healthy controls [43]. Nevertheless, some studies contradicted our results by demonstrating that no statistical difference in vitamin D levels between individuals with vitiligo and healthy controls [40 and 44]. A possible explanation for these conflicting results can be related to several factors including vitiligo history, age, direct sun exposure, seasonal variations and the nature of the job (indoor-outdoor type of work) [45].

Substantial evidence exists supporting the potential for vitamin D in immunosuppressive activity and its low levels are linked with autoimmune conditions such as vitiligo [46 and 47]. Vitamin D increases melanogenesis of cultured human melanocytes due to its antiapoptotic property and thus showed promising results when applied topically in combination with phototherapy to treat vitiligo patients [43 and 48].

CONCLUSION

This study showed that in comparison to healthy controls, vitamin D levels were significantly low in vitiligo patients. TSH levels were however insignificant. Blood group O is more susceptible to vitiligo but there was no significant association between vitiligo and ABO/Rh blood groups. In order to minimize the extent of the disease, it would be beneficial to use vitamin D supplements during vitiligo treatment. Further investigations are required to explore additional insight into the association between vitiligo and the topics. For instance, a collection of much larger samples from different geographical areas may be necessary to achieve a precise conclusion.

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Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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إرتباط البهاق بنظام فصائل الدم و تأثيرة على هرمون الغدة الدرقية و فيتامين د

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المستخلص

البهاق هو مرض جلدي مناعي ذاتي، يتميز بفقدان لون الجلد (الصباغ) ويؤثر على حوالي 1-2٪ من سكان العالم. البهاق ليس مرضًا معديًا ويمكن أن يؤثر على جميع الأعمار من كلا الجنسين. على الرغم من الأبحاث العديدة التي تم نشرها حول البهاق، إلا أن أسباب المرض نفسه تظل غامضة. من الواضح أن حالات البهاق في منطقة طرابلس - ليبيا تزايدت في السنوات الأخيرة ولذلك قمنا بهذه الدراسة لفهم سبب هذا المرض الجلدي. الهدف من هذه الدراسة هو تحديد ما إذا كانت هناك علاقة بين البهاق وفصائل الدم ABO وعامل الريسوس (Rh) والهرمون المحفز للغدة الدرقية (TSH) وفيتامين د. لتحليل البهاق، شارك في هذه الدراسة مائتي شخص، 100 مريض بالبهاق و 100 حالة سيطرة (بدون البهاق). تم اختبار فصائل الدم ABO والعامل Rh بطريقة الشريحة. شمل اختبار هرمون الغدة الدرقية 80 مريضاً بالبهاق و 80 حالة سيطرة (بدون البهاق) وتم تحليل الهرمون عن طريق فصل المصل في جهاز الطرد المركزي لمدة دقيقتين وتم الحصول على النتائج بواسطة محلل بيكمن الآلي بالكامل. بالنسبة لفيتامين د، تم تضمين 50 عينة لمرضى بالبهاق و50 شخصاً سليماً (ليس لديهم البهاق). تم الحصول على البيانات المتعلقة بفيتامين د من خدمات المختبرات الخاصة. تم إجراء التحليل الإحصائي باستخدام SPSS الإصدار 26. واعتبر $P>0.05$ مهمًا ذو دلالة إحصائية. كان لدى معظم المرضى الذين يعانون من البهاق مستوى أقل بكثير من فيتامين د في الدم مقارنة مع مجموعة التحكم (قيمة الدالة الإحصائية $p>0.05$)، في حين لم يتم العثور على فروق ذات دلالة إحصائية في مستويات هرمون الغدة الدرقية بين حالات البهاق والمجموعة الضابطة (قيمة $p<0.05$). علاوة على ذلك، على الرغم من إظهار أن الأشخاص ذوي فصيلة الدم O هم أكثر عرضة للإصابة بالبهاق مقارنة بالمجموعات الأخرى، لم يكن هناك ارتباط كبير بين البهاق وفصائل الدم ABO (القيمة $p<0.05$)، لم يكن معدل Rh الإجابي و السلبي ذو إختلاف كبير ما بين المجموعتين 96%، 4% في مرضى البهاق مقارنة بـ 90%، 10% في السيطرة على التوالي و هذا الإختلاف لم يكن أيضا ذو دلالة إحصائية (قيمة $p<0.05$). أظهرت هذه الدراسة أن مرضى البهاق غالبا ما يعانون من نقص فيتامين د. تسلط هذه الدراسة الضوء على الحاجة إلى تقييم حالة فيتامين د لدى مرضى البهاق لتحسين مستوى فقدان صبغة الجلد. لا يزال غير معروف ما إذا كان نقص فيتامين د يسبب البهاق. لذلك في المستقبل، ينبغي أن تكون هناك حاجة إلى تجميع أعداد أكبر من العينات من مختلف الأعراق للوصول إلى نتيجة دقيقة.

الكلمات الدالة. البهاق، هرمون الغدة الدرقية، فيتامين د، وفصائل الدم ABO وعامل الريسوس (Rh).