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ORIGINAL: Evaluation of Serum Levels of Vitamin D in Patients with Vitiligo: A Case-Control Study

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ABSTRACT

Introduction: Vitiligo is an autoimmune disease that is caused by the destruction of melanocytes and is appeared as depigmented patches on skin and mucosa. There have been various reports of serum Vitamin D levels in patients with vitiligo. The present study aimed to Evaluation of serum Vitamin D levels in patients with vitiligo.

Material and Methods: A case-control study was performed on 138 samples among people referring to Sabzevar Vasei clinic in the period of 2018-2019. The research units meeting the inclusion criteria were selected using convenience sampling and then divided into two group case and control (69 patients and 69 healthy people). After obtaining informed consent and completing the checklist of demographic characteristics, blood samples were taken from both groups and serum levels of Vitamin D in both groups were evaluated and compared. Data were analyzed using SPSS 20 via the independent t-test, chi-square test, Mann-Whitney test, Spearman correlation at a significance level of less than 0.01.

Results: In total, 131 participants completed the study; the two groups did not differ significantly in terms of age nor gender. At the end of the study, the mean of serum levels of Vitamin D in was significantly lower in the patients with vitiligo than in the people without vitiligo ($P < 0.001$).

Conclusion: The results showed that serum Vitamin D levels in patients with vitiligo are lower than in those who do not have vitiligo. Therefore, it is recommended to measure the serum level of Vitamin D in these patients and if it is low, a treatment regimen should begin for these patients.

Introduction

Vitiligo is an acquired skin disease, with unknown etiology, characterized by multiple or single depigmented macules and spots with distinct margins on the skin or mucosa (1, 2). The disease tends to develop over time, in which melanocytes are destroyed in parts of the skin, mucous membranes and retina. 1-2% of the world's population (10-20 million people) are known to have vitiligo (2, 3). There are

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many causes including autoimmune disorders, genetics, spontaneous destruction of melanocytes and neurological causes for this disease, but the exact cause of the disease is still (4).

Many autoimmune diseases, such as rheumatoid arthritis, diabetes mellitus, and multiple sclerosis, are commonly associated with decreased Vitamin D levels. However, there is limited research looking at the association between vitiligo and decreased Vitamin D levels (5). Vitamin D, due to its receptor on B and T lymphocytes, macrophages and dendritic cells, has many effects on the innate and acquired immune system. Some studies indicate the potential role of this vitamin in the prevention of autoimmune diseases, but current information is insufficient to prove a significant relationship between hormone levels and the incidence of autoimmune diseases (5). This vitamin differentiates dendritic cells and inhibits T helper 7 and T helper 1 cells, and thus is an important regulatory factor in autoimmune diseases and inhibits the inflammatory response through its receptors (6). Vitamin D is used topically to treat autoimmune skin diseases, especially in children with psoriasis and vitiligo, and the combination of Calcipotriene and steroid drugs topically re-pigments pigments in psoriasis and vitiligo (7). Therefore, it is hypothesized that a decrease in Vitamin D levels in the body is associated with the development of vitiligo.

Previous studies have examined serum levels of Vitamin D in patients with vitiligo. For example, in Egypt in 2013, Saleh et al. conducted a study comparing the serum levels of Vitamin D in patients with vitiligo with and without autoimmune disease. The results of this study showed that the serum level of Vitamin D in patients with vitiligo was lower than in those without vitiligo (8). Other studies have been performed for this purpose; however, it is apparent that there is still a significant lack of research in this area. Research by Saleh et al., identifies that re-pigmentation, as a result of topical use of Vitamin D, leads to a decrease in Vitamin D levels. It is suggested that this may play a role in the development of vitiligo, but that it is also an area that requires further research.

Due to the fact that research in this area is limited and that a study for this purpose has not been done in Sabzevar University of Medical Sciences, it is hoped that a study would be beneficial. This study endeavors to evaluate the relationship between Vitamin D and vitiligo and in doing so, can help to guide future treatment and prevention of the disease. Therefore, the aim of this study is to evaluate the relationship between serum Vitamin D levels in patients with vitiligo.

Methods

This study is a case-control study with a statistical population of 138 persons (69 persons affected with vitiligo and 69 persons without vitiligo). Patients affected with vitiligo participated in the research, attended Vasei clinic in Sabzevar on Mar. 2018 till Mar. 2019 and filled an application of agreement to take part in the study. Participants with a record of Vitamin D use, auto-immune disease, a family history of vitiligo, orthopedic surgery, affection to rheumatic diseases, and any disagreement of continuing cooperation with the researcher during the research were marked out of study. As there was a limited number of research samples, the sampling was competed quickly and easily. All persons affected with vitiligo were placed in the case group under an expert’s view and according to clinical criteria. Whereas the healthy persons were chosen based on anamnesis and an approval for lack of skin diseases (witness group), who were similar with each other concerning age, gender and social-economic status, and had gone to the research location (Vasei clinic in Sabzevar) at the time of researcher's presence and had the conditions for participating in the study. The age range was ±3.

After receiving approval from qualified persons, the information needed for completing the check list of demographic specifications were recorded by the researcher through interviewing persons

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under research and also using the information in their medical records. The level of Vitamin D in both groups were examined; a full blood sample was taken from both groups and Vitamin D serum level was analyzed and compared for same.

After filling the check list of study by the researcher, the collected information was moved to SPSS 20 software. Then it was presented to an expert in statistics for the mathematical analysis. Data description was indicated in the form of tables and charts using standards in descriptive statistics such as mode and standard deviation of mean ±. For data analysis variance analysis, statistical independent t-test, Mann-Whitney test, average age of patients in case group was 25.97±18.6 years and in witness group was 25.23±18.7 years. Most of the patients were under the twenty years of age average. For comparison of age average in both group of case and witness, Mann-Whitney test was used and no considerable difference was witnessed between both groups' averages (P=0.74).

Therefore, both groups were similar regarding age. The average Vitamin D serum level in case group was 17.08±14.81 and in control group was 25.62±20.87. Results of Mann-Whitney test showed that there was a meaningful difference in average of Vitamin D serum level between two groups of case and control (P<0.001) (***Table 1***).

Table 1. Comparison of the average serum level of vitamin D in case and control groups

Group	Standard deviation ± mean	Mann Whitney	Probability value
Case	19.01 ± 12.11	0.001	>0.001
Control	21.92 ± 20.19		

Vitamin D serum level was also analyzed relating to gender, which revealed that that the average in women was 17.11±17.22 and in men was 17.04±10.29. The results of Mann-Whitney test indicated that there was no considerable difference between the averages of Vitamin D serum level between spearman correlation and chi-square were used. A considerable level of statistics P<0.05 is thought through.

Results

In this study, 138 persons including 42 women (60.9%) and 27 men (39.1%) in the case group and 39 women (60.9%) and 30 men (56.5%) in the control group participated in the study. For analyzing the equalization of both groups concerning gender, chi-square test was used and no considerable difference was noticed (p=0.6). As a result, both groups were similar regarding gender. The

the identified genders.

For analyzing the relation between age and serum level of Vitamin D, spearman correlation test was used that its results showed as the age of patients rises, serum level of Vitamin D increases (P=0.04, P=0.24). Paradoxically, in analysis of the relation between inception of disease duration and serum level of Vitamin D, results of spearman correlation coefficient test indicated that there was no relation between these two variables (P=0.51, P=0.11).

Finally, we also analyzed the relation between skin rash areas (face, neck, body, extensor level, periorificial, flexor level interference and generalized interference) and serum level of vitamin D in patients affected with vitiligo. The results showed that there was not a considerable difference between averages of Vitamin D serum level in both groups (based on face interference and non-interference (P=0.93), neck (P=0.34), body (P=0.7), extensor level (P=0.44), periorificial (P=0.059), flexor level (P=0.64), generalized interference (P=0.66)); therefore, according to the results mentioned above, there was no relation between serum level of Vitamin D and any of skin rash areas.

Discussion

This research has discovered that the average

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Vitamin D serum level in the group affected with vitiligo is less than the Serum Vitamin D levels in the group of people without vitiligo. It was also found that there is a correlation between an increase in patient's age and the increase of serum level of Vitamin D. However, there was no considerable difference in the average Vitamin D serum levels amongst vitiligo patients concerning gender, inception of disease duration and skin rash areas (face, neck, body, extensor level, periorificial, flexor level interference and also generalized interference).

In most studies related to vitiligo patients, the ratio of women and men affected has been almost equal. In the study of Yaghoobi et al. 45.3% were female patients and 54.7% were men (9) However, in the study of Shamir et al. (10) on 90 patients with vitiligo, the male to female ratio was 1/2 to 1) more common in men (but in our study, in contrast to the study of Shamir, the disease was more common in women 90% female and 36.1% were male. This discrepancy may be due to the fact that in the society where our study was performed, women are more likely to seek help regarding skin issues and therefore, are more likely to be referred. Literature has also shown that peak age of disease diagnosis is between 10 and 30 years old (9). The mean age of patients in our study is 21.69. 11.9 and based on this, the present study is consistent with the existing literature on the age of patients with vitiligo.

The results of study of Beheshti et al. in 2014 demonstrated consistencies with our study in relation to prevalence in the female gender and the average age range of patients (11).

The average of Vitamin D serum level in the case group was 17.08±14.81 and in the control group was 25.62±20.87. These results indicate that the average of Vitamin D serum level in the group affected with vitiligo was less than the group of healthy persons. The results of statistical tests showed a meaningful difference between the average of Vitamin D serum level in both groups affected with vitiligo and healthy persons ($P<0.001$). Danilo et al. in 2013 completed research with the subject of analysis in long-term use of Vitamin D medicine's effect on patients affected with psoriasis and vitiligo. In this study 16 patients affected with vitiligo received 35000 Vitamin D units per day for 6 months accompanied by a diet high in calcium and good hydration of at least 2.5 liters per day for 6 months. The level of Vitamin D in all patients at the beginning of study was less than 30 nano-grams per milliliter. After the medical course was finished, Vitamin D serum level had considerably increased (18.4 ± 8.9 to 132.5 ± 37 nano-grams per milliliter) and PTH serum level had decreased (55.3 ± 25 to 25.4 ± 10.7 pico-grams per milliliter). Results of this study indicates that high use of Vitamin D affects treatment of patients affected with vitiligo (12). Doss et al. conducted a study on 2015 in Egypt with the subject of Vitamin D receptor expression. Examinations showed that only in 10 patients (33.3 %) serum level of Vitamin D was normal and sufficient, in 12 patients (40 %) Vitamin D serum level was insufficient and in 8 patients (26.7 %) it was low resulting in a diagnosis of Vitamin D deficiency. Most persons of control group (96.7 %) had a normal and sufficient serum level of Vitamin D. The average serum level of Vitamin D in patients affected with vitiligo was meaningfully low compared to the control group ($p<0.001$). The VDR-mRNA expression in the affected group was considerably lower than control group ($p<0.001$). Results of this study showed that lack of Vitamin D influences vitiligo disease's expansion and by affecting the immune system it will cause melanosis (13). The results of both aforementioned studies had correlation with results of our study regarding low Vitamin D serum levels in patients affected with vitiligo compared to healthy persons and highlighted the importance and effective role of Vitamin D in such patients' treatment.

In confirming results of our study, we could point out to Jonathan and his colleagues' study in 2010 in USA with the subject of analysis in serum level of Vitamin D in patients affected with vitiligo. In their study, Vitamin D serum level of patients was

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measured, and based on that the patients were divided into three: 31.1 % normal (more than 30 nano-grams per milliliter), 55.6% insufficient (less than 30 nano-grams per milliliter) and 13.3 % very low (less than 15 nano-grams per milliliter). Insufficient serum level of Vitamin D was related with high fitzpatrick (C195% = 1.12 –2.77, OR = 1.76). Very low serum level of Vitamin D was related with autoimmune disorders (C195% = 1.06 –94.7, OR = 10). Results of this study implied that very low levels of Vitamin D are a suitable screening tool for patients affected with autoimmune disorders. In addition, examinations showed a relation between Vitamin D serum levels and phototype fitzpatrick in replacement of race which needs to be further analyzed (14).

Karagun et al. conducted a study on 2016 in Turkey with the subject of analysis in role of Vitamin D serum level. In this study, Vitamin D serum level was analyzed among 50 patients affected with vitiligo and 47 healthy persons. The analysis indicated the Vitamin D serum level was lower in patients affected with Vitiligo compared to the control group but that this difference was not meaningful (p=0.570). Discoveries of this study implied that Vitamin D serum level was related to vitiligo disease but that it is unknow if Vitamin D deficiency causes vitiligo (15).

Ustun et al. conducted research in 2014 in Turkey with the subject of analyzing Vitamin D serum levels among patients affected with vitiligo. In this study, the average of vitamin D serum level among patients affected by vitiligo and the control group was orderly 15.2±5.2 and 14.4±6.2 nano-grams per milliliter (p>0.05). 48% was insufficient Vitamin D serum level patients (less than 30 nano-grams per milliliter) and in 52% was very low Vitamin D serum level patients (less than 15 nano-grams per milliliter). Very low and insufficient Vitamin D serum level is seen more commonly among patients affected with vitiligo, while this was not consistent within the control group. Nevertheless, more examinations are needed and necessary for analyzing effect of vitamin D serum level on the pathogenesis disease (16). The results of their study demonstrated correlations with ours, especially in regard to low Vitamin D serum level in patients affected with vitiligo compared to healthy persons. There were also dissimilarities which ensured that our study was meaningful.

Beheshti et al. conduct a study on 2014 in Qazvin (Iran) with the subject of analysis in Vitamin D serum level among patients affected with vitiligo. Among the participants 42 persons were men and 58 were women. The average age of participants in the study was 28.7±1.17. Average of Vitamin D serum level among the persons was equal to 42±24.14 nano-grams per milliliter that this amount had a considerable difference with normal persons (p<0.04). Results of this study implied that average Vitamin D Serum Level in patients affected with vitiligo had a great difference compared with that of healthy persons (11).

Nanvazadeh et al. conducted research in 2016 in Iran with the intent of analyzing Vitamin D 25 Hydroxy Serum Levels among patients affected with vitiligo comparing to the healthy control group. The examinations showed that average levels of Vitamin D in patients' group was 13.3±9.75 ng/mL and in the control group was 12.73±8.29 ng/mL. No meaningful difference was seen between the two groups. Results of this study implied that there wasn’t any difference between the two groups and that maybe the reason of low Vitamin D 25 Hydroxy Levels could be seen within the whole society. This study also indicated that family history was the only effective factor in serum level of Vitamin D among patients affected with vitiligo (17). Their study's results were different to ours concerning low average of vitamin D serum level in the group affected with vitiligo in comparison to the group of healthy persons. This subject indicates this issue that there are still paradoxical discoveries regarding vitiligo disease and that further and more generalized research is needed in this field.

Other results showed that average Vitamin D serum level did not have a considerable difference among vitiligo patients regarding gender (P=0.3), inception of disease duration

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(P=0.51, p=0.11) and skin rash areas (face interference (P=0.93), neck interference (P=0.34), body interference (P=0.7), extensor level interference (P=0.44), periorificial interference (P=0.059), flexor level interference (P=0.64), and generalized interference (P=0.66)). Saleh et al. noticed in their study on 2013 that there's no meaningful relation between serum level of Vitamin D and disease duration and skin rash areas in patients affected with vitiligo (8) which was in agreeance with our study. In this field, Jonathan et al. noticed in their study in 2010 that serum level of Vitamin D was not related to age, gender, and race, time of disease inception, body's interfered area and autoimmune disease infection in family history (14). Ustun et al. (2014) also explored the relationship between serum level of Vitamin D and disease duration and skin rash areas in patients affected with vitiligo. The conclusion that was reached was that there is no meaningful relation (16) which is consistent with our findings.

Overall, the results of this study are consistent with other studies concerning serum level of vitamin D among vitiligo patients. Research shows that in such patients' vitamin D serum level differs with healthy persons and is usually seen to be lower than normal. Yet there are still paradoxical studies regarding this matter that indicates lack of relation or considerable difference in vitamin D serum level among vitiligo patients. Since vitiligo disease usually has a crucial effect on patients' lives, active treatment preferring it to be in the first steps of the disease, is advised. Present treatment methods are concentrated on preventing the disease's development and attaining pigmentation. Treatments including corticosteroids, localized immune moderators, photo (chemo) therapy, surgery, combined treatment and depigmentation of pigmented skin (18). Vitiligo's treatments in general, are not well targeted. It is for this reason that we do not fully understand vitiligo's etiology or reasons of its cause and the interactions that might affect its prevalence. Therefore, more studies are needed to confirm vitiligo's cause (19).

In general, low levels of Vitamin D appear to be related with different autoimmune diseases including multiple sclerosis, systemic lupus erythematosus, indistinct connective tissue disease and rheumatoid arthritis. According to this study's results, it seems that vitiligo as an autoimmune disease, has been formed or accentuated by low levels of Vitamin D and also according to other similar studies, receiving sufficient levels of Vitamin D in vitiligo patients' diet can help such patients' recovery and treatment.

Consistent with our study, Silverberg and his colleagues also examined 25 hydroxy levels of Vitamin D among 45 vitiligo patients. More than 50% (55.6%) of patients had 25 hydroxy insufficient levels of Vitamin D. In addition, the aforementioned writers discovered a group of vitiligo patients (13.3%) with 25 hydroxy very low levels (deficiency) of vitamin D who had autoimmune disorders at the same time. The study suggested that vitamin D deficiency might be the danger cause for vitiligo development and monitoring levels of vitamin D among vitiligo patients might identify those who are at a greater risk of developing a secondary autoimmune disorder.

Nevertheless, studies completed in this field are extremely limited and all of them have not factored in other demographic profiles of their patients. In Iran, the studies are even more limited regarding this matter compared to other areas of the world. Consequently, there is no complete consensus concerning the relation between serum levels of Vitamin D and vitiligo, therefore requiring further examination.

Conclusion

The nature of Vitamin D and the various reasons affecting its levels, including diseases and symptoms such as delayed of child growth, bone transformation, along with autoimmune disorders, heart disease and sepsis must be reckoned as one of the more important priorities for health. Therefore, enhancing public health awareness through

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designing and performing plans such as national prevention and control of Vitamin D deficiency can aid in the improvement in the general public's health literacy regarding this issue. This can be achieved through performing educational programs, nutrients enrichment plan, creating a culture for healthy lifestyle, sports and walking promotion and also using food supplements in personal lives as a cheap, easy and useful strategy for defeating Vitamin D deficiency.
According to the results of this study and the relation between serum level of Vitamin D and vitiligo disease, it is suggested that in such patients, serum level of Vitamin D be measured and in the case that it is low, treatment must be commenced early.

Ethical standards statement

Special thanks to Sabzevar Vasei clinic's cooperation and Clinical research and development center of Sabzevar Vasei Hospital, affiliated to research office of Sabzevar University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

Authors' contributions

All authors have participated in the design, implementation, and writing of all sections of the present study.

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