



A Case Control Study: Evaluation of Serum Vitamin D Levels and Vitamin B12 Levels in Vitiligo

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Abstract

Background: *Vitiligo is a common, acquired, often heritable, multifactorial, polygenic pigmentary disorder. Low levels of Vitamin D, an essential hormone synthesized in the skin with diverse effects on immune system have been associated with several autoimmune diseases including vitiligo.*

OBJECTIVES: *To study the association of the serum levels of Vitamin B12 and D3 in patients with vitiligo vulgaris in a tertiary care centre.*

Materials and Methods: *This is a case-control study conducted over a period of four months that included 30 clinically diagnosed cases of vitiligo and 30 age and sex matched controls. The serum levels of Vitamin D3 and B12 levels were measured in all subjects by Chemiluminiscent immunoassay. Statistical analysis was conducted by using Student's t test and Mann Whitney test of the data collected by questionnaire.*

Results: *The mean value of serum Vitamin D3 levels was 22.10, values in males being significantly lower among the cases compared to the controls. Mean value of serum vitamin B12 (348.6) was significantly decreased in vitiligo group as compared to controls.*

Conclusions: *The present study demonstrated that there is an association between low serum levels of Vitamin D3 and Vitamin B12 with Vitiligo respectively suggesting the screening of these parameters to be made routine in Vitiligo patients.*

Keywords: *Vitiligo, Vitamin B12, Vitamin D3*

Introduction

Vitiligo is a common, acquired, often heritable, multifactorial, polygenic pigmentary disorder with a complex pathogenesis and prevalence of 0.5-1% of population¹. It is characterized by depigmented macules on skin due to loss of melanocytes.²⁻⁴. The resulting depigmentation whenever involves

the cosmetically obvious areas like face and limbs may result in psychosocial morbidity^{3, 4}. Extensive studies in China, India and Denmark have estimated its prevalence to be 0.093%, 0.005% and 0.28% respectively, the highest prevalence of 8.8% being reported from Gujarat province of India^{2,3}. The cause of disease is

unknown, but there are different theories including autoimmune, genetic, toxic metabolites or oxidative stress, neural causes and the lack of melanocyte growth factors³.

Vitamin D is a fat soluble essential hormone synthesized in the skin on exposure to ultraviolet B (UVB) present in the sunlight responsible for bone and Calcium homeostasis exerting its effects via Vitamin D receptors (VDR). It is also known to have immunoregulatory role on Innate and Adaptive systems contributing to the immunotolerance of the cell structures. It has been shown that vitamin D may reduce the generation of certain cytokines like interleukins 2, 6, 12 and TNF α . A lack of vitamin D may cause defective macrophage function^{5, 6}. It may be logical to consider the role of Vitamin D in vitiligo, as Vitiligo is considered to be primarily an autoimmune disease, an aberration of immune surveillance leading to dysfunction and destruction of melanocytes causing depigmentation.

Vitamin B 12 is a water soluble vitamin primarily synthesized by gut bacteria used by every cell of the body for metabolism. Vitamin B12 helps in conversion of homocysteine to methionine, its deficiency leading to accumulation of homocysteine. Breakdown of homocysteine leads to the production of reactive oxygen species, harmful free radicals and thus increased oxidative stress in the skin, all leading to destruction of melanocytes^{7, 8}. It is believed that patients with vitiligo are more likely to have pernicious anemia and vitamin B12 deficiency^{3, 5}. Vitamin B12 facilitates the conversion of folate to tetrahydrofolate, which is essential in various enzyme reactions^{6, 9}.

With this background, we conducted a case-control study to compare serum vitamin D3 and vitamin B12 levels between patients with vitiligo and controls.

Materials and methods

Total of 60 subjects with age ranging from 10 to 70 years were enrolled for the study comprising of 30 new clinically diagnosed patients of vitiligo

and 30 controls from the outpatient department of Dermatology and Venereology of Narayana Medical college and hospital, Nellore, Andhra Pradesh, India.

Exclusion criteria were as follows: age <10 years and > 70 years, patients suffering from major cardiovascular diseases, renal, hepatic and major gastrointestinal disorders, those on supplements of folic acid, vitamin B12 and vitamin D, pregnancy. All the individuals studied were subjected to a complete assessment of history and clinical examination including the extent of vitiligo, estimated by the rule of nine, distribution of the lesion, clinical type of vitiligo, and bilaterality of lesions. The activity of the disease was assessed as follows: stable disease- no change in the vitiligo lesions during the 3 months before the study as observed by the patient and progressive disease-enlargement of already present lesions and/or the appearance of new lesions within the 3 months before the study as observed by the patient.

Serum vitamin D levels were measured via the electrochemiluminescence assay using commercial kits. Regarding serum vitamin D levels, the subjects were categorized into three groups: normal who had vitamin D levels >30 ng/ml; insufficient who had vitamin D levels between 20 to 30 ng/ml; and deficient who had vitamin D levels <20 ng/ml. Serum Vitamin B12 levels were measured in both patients and controls by chemiluminescent assay. Vitamin B12 levels <211pg/ml were considered deficient, levels \geq 211pg/ml being considered normal.

Statistical analysis of the data was done using student's t-test for difference of mean of vitamin B12 and Vitamin D on SPSS for windows (version 16.0) statistical package (SPSS Inc., Chicago, IL) computer statistics program. Comparisons between vitamin D levels on one hand and sex, age, and time of onset of disease on the other hand were made using Mann Whitney test. P values \leq 0.05 was taken to be statistically significant with a confidence level of 95%.

Results

The range of age of the sample included under the study was 10-70 years, the mean age of vitiligo patients and controls being 43.9 ± 14.60 and 49.8 ± 16.00350 years respectively. Among the thirty vitiligo cases, 11 were males and 19 were females. [Table 1]

The mean value of serum vitamin B12 among the cases was 348.6 ± 134.90 which was sufficiently lower when compared to the controls included under the study (479.88 ± 299.4). There was a statistically significant difference between the cases and controls regarding the same as evident by the p value of 0.000. [Table 1]

Mean values of serum vitamin D3 were 22.10 ± 13.05 and 24.23 ± 13.19 among the cases and controls respectively. This finding of increased number of cases with significantly lower values of Vitamin D3 as compared to the

controls was statistically significant (p value 0.000). [Table 1]

Regarding the sex of the patients, the mean value of vitamin B12 level was higher in male compared to the female patients without statistical significance (349.5 ± 132.62 Vs 344.9 ± 138.52). Similarly the mean value of Vitamin D serum level was higher among the males in comparison with the females, but without statistical significance (24.51 ± 14.25 Vs 21.15 ± 12.98). [Table 2]

Statistically significant association between low levels of Vitamin D3 with Vitiligo has been more readily observed among the males compared to the females (p values 0.04 Vs 0.12). [Table 3]

Statistically significant association has been observed between duration of disease and deficiency of vitamin B12 (p value 0.039), but not with deficiency of Vitamin D3 (p value 0.532). [Table 4]

Table 1: Serum levels of vitamin B12 and Vitamin D3 in vitiligo and controls

Groups	Vitamin B12 Mean \pm SD (pg/ml)	Vitamin D Mean \pm SD (ng/ml)
Cases	348.6 ± 134.90	22.10 ± 13.05
Controls	479.88 ± 299.4	24.23 ± 13.19
P value	0.000	0.000

Table 2: Serum levels of vitamin B12 and Vitamin D3 in male and female cases

Groups	Vitamin B12 Mean \pm SD (pg/ml)	Vitamin D Mean \pm SD (ng/ml)
Males (11)	349.5 ± 132.62	24.51 ± 14.25
Females (19)	344.9 ± 138.52	21.15 ± 12.98
P value	0.852	0.758

Table 3: Sexual distribution of serum levels of Vitamin D3 in cases and controls

Groups	Vitiligo cases	Controls	P value
Males (11)	21.24 ± 12.48	24.59 ± 12.86	0.04
Females (19)	23.56 ± 13.12	24.12 ± 13.17	0.12

Table 4: Correlation of serum vitamin B12 and Vitamin D3 with duration of disease

Duration of disease (years)	Total (30) No (%)	Vitamin B12 Mean \pm SD (pg/ml)	Vitamin D3 Mean \pm SD (ng/ml)
≤ 1	13	421.96 ± 125.70	24.28 ± 14.22
2-5	14	370.54 ± 125.07	23.59 ± 13.27
> 5	3	342.56 ± 59.61	22.26 ± 12.41
P value		0.039*	0.532

Discussion

Vitiligo is a common, multifactorial, polygenic pigmentary disorder with a complex pathogenesis and psychological morbidity. It may be logical to consider the role of Vitamin D, an immunoregulatory hormone in vitiligo, as Vitiligo is considered to be primarily an autoimmune disease, an aberration of immune surveillance leading to dysfunction and destruction of melanocytes causing depigmentation. Vitamin B 12 is a water soluble vitamin involved in oxidation and antioxidation system through homocysteine- methionine pathway. Breakdown of homocysteine leads to the production of reactive oxygen species, harmful free radicals and thus increased oxidative stress in the skin, all leading to destruction of melanocytes⁸. It is believed that patients with vitiligo are more likely to have pernicious anemia and vitamin B12 deficiency^{8,10}. The finding of range of age of the sample included in the current study (10 to 70 years) is in accordance with the existing reports of the age distribution of vitiligo¹¹. The mean age of vitiligo patients and controls was 43.9 ± 14.60 and 49.8 ± 16.00350 years respectively. [Table 1] Mean values of serum vitamin D3 were 22.10 ± 13.05 and 24.23 ± 13.19 among the cases and controls respectively. This showed the increased association of Vitiligo cases with deficiency of Vitamin D 3 in a statistically significant manner (p value 0.000). [Table 1] This finding concorded with the Silverberg JI et al, and Xu X, et al. study, which showed a deficient level of Vitamin D3 in Vitiligo patients, without statistical significance^{12, 13}. This finding coincided with a case report Nunes JP et al, which showed low levels of vitamin D (equal to 12 ng/mL) to be associated with vitiligo patient¹⁴. Despite lower serum levels of vitamin D in women, there was no statistically significant relationship between sex and serum vitamin D levels in our study. However, when compared to healthy men, serum vitamin D levels were significantly lower in male patients with vitiligo. [Table 2, Table 3] This finding correlated with

findings of Khurram et al study and Xu X et al.^{13, 15}. On the other hand, there was no significant difference between serum vitamin D levels between the women in case and control groups, that can be explained by their reduced duration of sun exposure compared to males, on account of their remaining indoors.

In our study we found that there was a significant decrease in serum vitamin B12 levels in vitiligo cases when compared to controls. This finding coincides with Singh S et al study and Karadag As et al study but not with Yasar Ali et al¹⁶⁻¹⁸. Sex did not affect the levels of vitamin B12 in the current study. This is in accordance with the studies of Montes et al^{7, 8}, Batawi et al⁸ Singh S et al¹⁶ and Gonul et al¹⁹.

Statistically significant association has been observed between duration of disease and deficiency of vitamin B12 (p value 0.039). This finding coincided with the findings of Singh S et al.²⁰ There was no statistically significant association between the duration of disease and Vitamin D3 deficiency (p value 0.532). [Table 4] Measurement of serum levels of vitamin D3 and B12 at the time of diagnosis of vitiligo is recommended and their supplementation is suggested to those suffering from the deficiencies of the same. Further studies with larger sample size and cohort studies are recommended to investigate the effects of vitamin D 3 and B12 deficiency and their supplementation on the incidence of vitiligo.

Conclusion

Nutritional deficiencies of Vitamin D3 and B12 can be considered as precipitating factors in the pathogenesis of vitiligo in predisposed individuals due to their role in free radical mediated damage of melanocytes. Hence we recommend routine screening for their deficiencies to prevent the occurrence and progression of Vitiligo.

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