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Original Research Article

Here's what really matters in vitiligo; Vitamin D3 and lifestyle

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ABSTRACT

Epidermal melanocyte deficit is the basis of Vitiligo. It is a prolonged condition that may be inherited or acquired. Vitiligo affects 1-2 percent of the global population of all races. Several processes have been hypothesized for the breakdown of melanocytes in Vitiligo. These include genetic, autoimmune, oxidative stress, inflammatory mediator production, and melanocyte detachment processes.

Vitamin D suppresses UVB-induced apoptosis in keratinocytes and melanocytes by reducing IL-6, IL-8, TNF-a, and TNF-c production. It reduces the autoimmune linked to Vitiligo.

We conducted a case-control study in which we compared the level of Vitamin D in patients with Vitiligo and healthy cases. We confirmed our diagnosis with biopsy and utilized the Elisa method to assess the level of Vitamin D.

The concentrations of Vitamin D in individuals with Vitiligo were much lower than in controls; however, we did not find a significant effect of vitamin D deficiency on the progression of Vitiligo lesions. Therefore we conclude that Vitamin D is involved in the genesis of Vitiligo, and replenishing the levels may help the patient recover faster.

1. Introduction

Vitiligo is caused by the paucity of melanocytes in the epidermis. It is a persistent ailment and could be hereditary or acquired. Globally, 1-2 percent of the population of all races is affected by Vitiligo.[1] Numerous factors play a role in the etiopathogenesis of Vitiligo, including genetic, immunological, autoimmune, and neurogenic. Deficiency of melanocyte growth factors, environmental factors, biochemical defects, inadequate free-radical protection, and inherent flaw of melanocyte attachment also contribute to Vitiligo.[2]

1.1. Protective role of Vitamin D

Vitamin D3 promotes the survival of melanocytes by regulating T cell activation and coordinating melanogenic cytokines [endothelin 3 (ET-3)] with the SCF/c-Kit pathway. Hence it acts as an antioxidant to protect from Vitiligo.[3] Vitamin D also reduces UVB-induced apoptosis in keratinocytes and melanocytes by inhibiting the expression of IL-6, IL-8, TNF-a, and TNF-c. It diminishes the autoimmunity associated with Vitiligo.[3] Vitamin D regulates calcium levels in the body. The epidermal keratinocytes of Vitiligo sufferers are deficient in calcium transportation. Thus, Vitamin D may play a function in Vitiligo treatment by increasing the availability of calcium.[4] In addition, the enzyme tyrosine kinase requires calcium during the synthesis of melanin. Therefore, a calcium deficit could hinder melanin synthesis.

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In conjunction with the Department of Pharmacology, we sought to determine the relationship between Vitiligo and vitamin D3 in patients who visited the outpatient department of Dermatology, Venereology, and Leprosy at Govt. Medical College, Amritsar.

2. Materials and Methods

2.1. Study design

We conducted a case-control study in collaboration with the Department of Pharmacology at Govt. Medical College, Amritsar.

2.2. Sample selection

One hundred fifty patients of either gender between the ages of 12 and 60 who visited the Outpatient Department of Dermatology, Venereology, and Leprosy were prospectively enrolled. After receiving clearance from the institution's ethics and thesis committee, participants were separated into Groups A and B. Group A consists of 75 Vitiligo patients clinically and histopathologically diagnosed. Group B consisted of 75 healthy volunteers of the same age and gender Department of Dermatology, Venereology, and Leprosy.

2.3. Inclusion criteria

1. Patients suffering from all types of Vitiligo
2. Age group between 12-60 years of either sex

2.4. Exclusion criteria

1. Any chronic medical disease like diabetes mellitus, autoimmune disease, hypertension, and tuberculosis
2. Current consumption of vitamin D3 (within two months
3. Patients receiving concomitant treatments with the ability to influence vitamin D3
4. Patients suffering from bowel disease with malabsorption of vitamin D3
5. Patient reporting to Dermatology, Venereology, and Leprosy Department with Acute Drug Reaction

We conducted the following routine and special tests in both cases and controls, excluding skin biopsy performed only in patients.

2.5. Classification of Vitiligo

In 2011, a worldwide agreement distinguished Segmental Vitiligo (SV) from all other kinds of Vitiligo and defined Vitiligo as referring to all forms of Non-Segmental Vitiligo (NSV). "Mixed Vitiligo," in which SV and NSV coexist in one patient, is classified as a subset of NSV. Distinguishing SV from various varieties of Vitiligo was one of the most crucial conclusions made by the committee, mainly because of prognostic consequences. NSV encompasses the acrofacial, mucosal, generalized, universal, mixed and unusual versions. [1]

Type of Vitiligo [1,5]	**Subtypes**
Non Segmental	Focal, Mucosal Acrofacial
Segmental	Focal Uni-segmental Bi-segmental
Mixed	Combination of the above types
Unclassified	Focal at onset but evolves to other types later on

2.6. Assessment of clinical cases

Parameters to access the severity/activity of vitiligo: Vitiligo area severity index (VASI).

The percentage of Vitiligo involvement is calculated in terms of hand units (which compasses the palm plus volar surface of all digits) is approximately equivalent to 1% of the total body surface area. The degree of pigmentation is estimated to be the nearest of the following percentage:

100%	Complete depigmentation, no pigmentation present
90%	Specks of pigmentation present
75%	Depigmented area exceeds the pigmented area
50%	Pigmented and depigmented areas are equal
25%	Pigmented area exceeds the depigmented area
10%	Only specks of depigmentation present

VASI= $\sum$ all body sites (Hand Units) x (extent of depigmentation)

2.7. Routine investigations

a) Hb b)TLC,DLC c)SGOT,SGPT d)Platelet count e)PBF f)ESR g) Urine C/E h) FBS i) B. Urea j) S. Creat

2.8. Specialized investigations

1. Serum Vitamin D3 estimation using Sandwich-ELISA technique.
2. Skin Biopsy for histopathological examination.

The Department of Clinical Biochemistry of Government Medical College, Amritsar, conducted investigations (a-j).

3. Histopathology

A fully grown lesion was selected for histological evaluation. Aesthetically significant locations (such as the face), injury-prone, movable sites (such as joints and bony prominences), lower legs (prone to stasis changes), and areas susceptible to bleeding (scalp) or infection (perianal region) were omitted. We preferred a 4 mm punch for biopsy. A 2 percent lignocaine with 1:1000 adrenaline

was administered to anesthetize the vascular sites locally, whereas 2 percent lignocaine alone was utilized for fingers, toes, and the penis. After a biopsy, we applied firm pressure with saline-soaked sterile gauze. The specimen was put in an acceptable amount of 10% neutral buffered formalin and brought to the laboratory before it could dry.

3.1. Data collection

The orthopedics interpreted the vitamin D value and categorized the participants into three groups: deficient (20 IU), inadequate (20-30 IU), and standard (>30 IU).

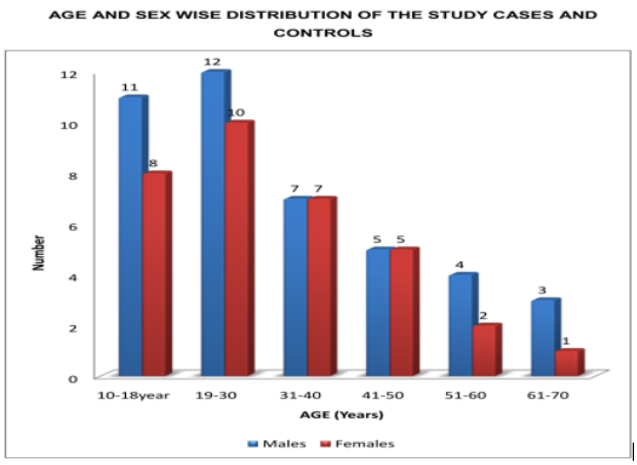
4. Statistical Analysis

Version 22 of the SPSS Statistics software was utilized for statistical analysis (IBM, Armonk, NY). Quantitative factors are represented by the median and interquartile range (IQR). A p-value less than 0.05 were regarded as statistically significant.

5. Results

The following tables and graphs represent our results.

5.1. Demographics



Graph 1: Shows a greater foot all of male patients in our outpatient department.

Demonstrates increased prevalence of Vitiligo in males and younger population.

42 (56 percent) of the 75 cases and 75 controls were male, whereas 33 (44 percent) were female. Nineteen Vitiligo patients aged between 10 - 18 years, 22 Vitiligo patients were in the age group 19 - 30, 14 Vitiligo patients between the ages of 31 - 40, and 10 Vitiligo patients between the ages of 41-50, and 10 patients over the age of 51.

Of the 75 case subjects, 39 (52 percent) were from urban areas and 36 (48 percent) from rural regions. 32

Table 1: Age and sex wise distribution of the study cases and controls

Age	Cases (n=75		Total	Controls (n=75)		Total (Control)
	Males number (%age)	Females Number(% age)		Males Number (% age)	Females Number (%age)	
10-18	11(14.6%)	8(10.66%)	19(25.33%)	11(14.6%)	8(10.66%)	19(25.33%)
19-30	12(16%)	10(16%)	22(29.33%)	12(16%)	10(16%)	22(29.33%)
31-40	7(9.33%)	7(9.33%)	14(18.66%)	7(9.33%)	7(9.33%)	14(18.66%)
41-50	5(6.66%)	5(6.66%)	10(13.33%)	5(6.66%)	5(6.66%)	10(13.33%)
51-60	4(5.33%)	2(2.66%)	6(8%)	4(5.33%)	2(2.66%)	6(8%)
61-70	3(4%)	1(1.33%)	4(5.33%)	3(4%)	1(1.33%)	4(5.33%)
71-80	0	0	0	0	0	0
	42(56%)	33(44%)	75(100%)	42(56%)	33(44%)	75(100%)

Table 2: Urban Vs. Rural Disease prevalence

Group	Urban	Rural	Total
A	39 (52%)	36(48%)	75(50%)
B	32(42%)	43 (58%)	75(50%)

(42 percent) of the 75 control participants belonged to urban areas, whereas 43 (58 percent) to rural areas. We could not find a significant association between cases and controls regarding their residential locations. The increased prevalence suggests that urban living conditions may contribute to the development of Vitiligo. The urban style of life and Vitiligo must be the subject of additional research to establish a conclusive link.

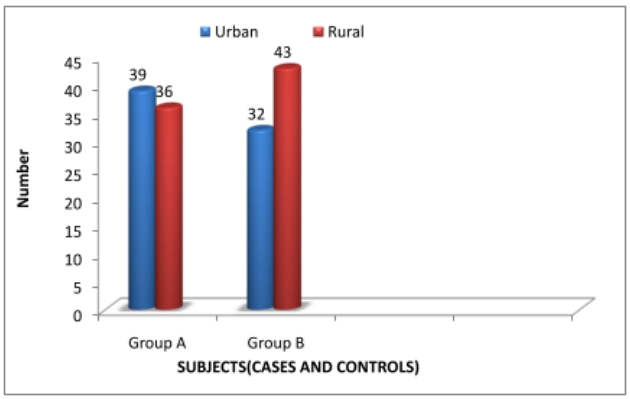


Fig. 1: Subjects (Cases and controls)

Occupation in cases vs. controls

Table 3: Occupation of the study cases and controls

Code	Occupation	Cases	Control	Total
1.	Student	25(33.3%)	29(38.7%)	54(36%)
2.	Housewife	18(24.1%)	19(25.3%)	37(24.7%)
3.	Service	20(26.6%)	16(21.4%)	36(24%)
4.	Farmer	4(5.3%)	1(1.3%)	5(3.3%)
5	Businessman	1(1.3%)	10(13.3%)	18(12%)
	Total	75(100%)	10(13.3%)	18(12%)

X^2-17.901 df-11 p value-084 not significant

In both the case and control groups, most patients who visited our outpatient clinic were students, reflecting that the younger generation is more conscious of skin ailments. Even though we were unable to achieve a statistically significant difference between cases and controls, our study demonstrates that the prevalence of Vitiligo among younger populations has escalated.

Table showing vegetarian and non-vegetarian patients vs. controls 45 (60%) of the 75 Vitiligo patients were vegetarian, while only 30 (40%) of the controls were vegetarian. The data was statistically significant (p-value. 014), indicating that dietary factors are essential in developing Vitiligo. A non-Vegetarian diet abounds with

Table 4: Dietary factors and role in Vitiligo

Variable	Cases	Controls
Vegetarian	45(60%)	30(40%)
Non vegetarian	30(40%)	45(60%)
Total	75(100%)	75(100%)

essential amino acids, proteins Vitamin B12 and Vitamin D3. These nutritional elements may have a significant role in developing Vitiligo, or retrospectively, improving diet may lead to a decrease in Vitiligo patches.

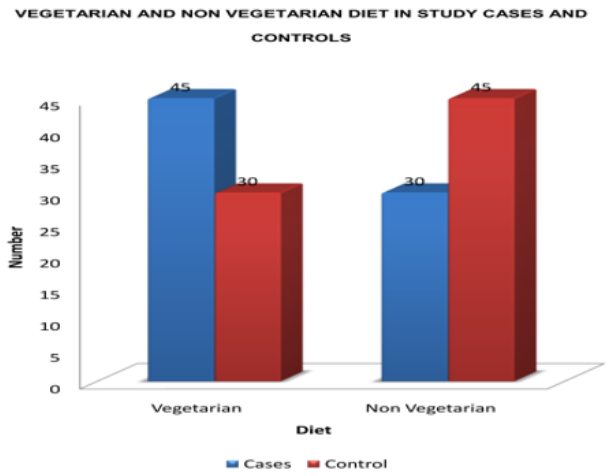


Fig. 2: Bar graph representing the relation betweenvitiligo and dietary pattern.

Table 5: Tablebody surface area involvement in vitiligo and its comparison

BSA	Number of cases	Percentage
1-10%	61	81.3
11-20%	7	9.3
21-30%	0	0
31-40%	3	4
41-50%	1	1.3
51-60%	1	1.3
61-70%	1	1.3
>70%	1	1.3
Total	75	100

Table showing relationship between Body Surface Area and the number of cases.

The majority of our patients had body surface involvement between 1-10 percent.

Table representing the most prominent type of Vitiligo

Non-segmental Vitiligo is the predominant type of Vitiligo. The percentage in our study was eighty-one percent which was close to the prevalence in the rest of the world (ninety percent).

Table 6: Types of vitiligo and their demographics

Type	Number	Percentage
Acrofacial	4	5.33
Focal	4	5.33
Mixed	2	2.66
Mucosal	1	1.33
Nsv	61	81.33
Segmental	3	4
Total	75	100

Table 7: Vitamin D levels in cases and controls

Variable	Cases Mean± sd	Controls Mean± sd	't value'	P-value
Vitamin D_3	24.0748±3.795	38.170±9.541	-10.421	<.001 highly significant

Vitamin D3 levels were insufficient in 62 (82.7%) of 75 patients but only 12 (16%) of the controls. In contrast, levels were adequate in 13 (17.3%) cases and 63 (84%) of controls. The data was statistically highly significant (p value.001). The mean vitamin D level in cases was 24.0748±3.795, while controls had 38.170±9.541IU.

Table showing Age-wise Vitamin D3 levels in cases and controls 17 (89.5%) of 10-19-year-olds had low vitamin D3 levels, 19 (86.4%) of 19-30-year-olds, 12 (85.7%) of 31-40-year-olds, 9 (90%) of 41-50-year-olds, 4 (66.7%) of 51-60-year-olds, and 1 (25%) of 61-70-year-olds. Vitamin D3 levels did not vary with age (p =.567), but the percentage of people lacking Vitamin D3 is higher at a younger age.

Insufficient vitamin D3 levels were found in 32 (97 percent) of the 33 females with Vitiligo and 30 (71.4 percent) of the 42 males. The data was statistically significant (p value.004), indicating that females had more significant vitamin D3 insufficiency than males. The outcome is shown in the above table.

Thirty-six cases of Vitiligo from rural areas had insufficient vitamin D3 levels (72.2%), while 39 cases of Vitiligo from urban areas had insufficient vitamin D3 levels (89.7%). The result was statistically significant (p-value =.048), indicating that urban patients with Vitiligo had higher vitamin D3 insufficiency than rural instances. The overall incidence of Vitamin D insufficiency is more in urban than the rural population both in cases and controls. This could be attributed to the more sedentary lifestyle of the urban population.

6. Discussion

In this analysis, the majority of Vitiligo patients had low vitamin D values relative to the comparison group, with a significant percentage having inferior values. The result was statistically meaningful (p0.05).

Fig. 3: Non-segmentalvitiligo, acrofacial subtype

Fig. 4: Segmental Vitiligo: Uni-segmental subtype

Although in our study, males outnumbered females in cases of Vitiligo, females made up the majority of those with inadequate vitamin D. Women in our location (North India) don clothing covering nearly all of their bodies, with face and hands visible. The females dress so due to religious and ethnic concerns and the long summers in our region, where most people are dark-skinned and highly anxious about being sun-tanned. The increased prevalence of Vitiligo may be attributed to more male participants having reported the condition due to occupational stigma.

In our work, the preponderance of participants was between 10 and 40. The mean age in study cases and controls was 32.07±14.863. According to a study conducted by Shajil EM et al., the peak incidence of Vitiligo occurs between 10 and 30.[6] Most of the patients with low vitamin D were young in our study though it had no significant relation to the age group (p-value .567). This result could be attributable to the notion that most patients in this sample were teenagers and young adults because of a current

Table 8: Age-wise level of Vitamin D in cases and controls

	Cases		Control	
Age (Years)	Insufficient (Vitamin D_3)	Sufficient (Vitamin D_3)	Insufficient (Vitamin D_3)	Sufficient (Vitamin D_3)
10-18	17(89.5%)	2 (10.5%)	4(21.1%)	15(78.9%)
19-30	19(86.4%)	3 (13.6%)	5(22.7%)	17(77.3%)
31-40	12(85.7%)	2 (14.3%)	2(14.3%)	12(85.7%)
41-50	9(90%)	1 (10%)	0	10(100%)
51-60	4(66.7%)	2 (33.3%)	1 (16.7%)	5(83.3%)
61-70	1(25%)	3 (75%)	0	4(100%)
71-80	0	0	0	0
Total	62(82.7%)	13 (17.3%)	12 (16%)	63 (84%)
	c^2=11.645df=5p value= .567; not significant		c^2=3.801df= 5p value=.578; not significant	

Table 9: Vitamin D3 levels and sex distribution in Vitiligo

	Cases		Control	
Sex	Insufficient (Vitamin D_3)	Sufficient (Vitamin D_3)	Insufficient (Vitamin D_3)	Sufficient (Vitamin D_3)
Females	32 (97%)	1(3%)	1(3%)	32(97%)
Males	30 (71.4%)	12(28.6%)	11(26.2%)	31(73.8%)
	c^2=8.413df=1p value= .004;significant		c^2=7.375df=1p value= .007;significant	

Table 10: Vitamin D3 levels in rural and urban populations in both cases and controls

	Cases	Cases	Controls	Controls
Area	Insufficient Vitamin D3	Normal Vitamin D3	Insufficient VitaminD3	Normal Vitamin D3
Rural	26(72.2%)	10(27.7%)	20(46%)	23(54%)
Urban	35(89.7%)	4(10.2%)	6(19%)	26(81%)
	p-value significant	p-value significant		

lifestyle pattern among the youngsters of our culture, which entails inverting the sleep-wake cycle and rising late in the morning.

39 (52 percent) of the 75 cases were urban dwellers, whereas 36 (48 percent) were rural dwellers. Mehta NR's study also revealed an urban majority of Vitiligo instances, resulting from more significant environmental degradation in urban settings and acting trigger for Vitiligo.[7]

60 percent of the 75 instances of Vitiligo were vegetarian, whereas 40 percent were non-vegetarian, indicating a modest preference for vegetarians. Therefore, vegans may be more susceptible to developing Vitiligo due to their reduced protein intake. This outcome in our study was consistent with that of Behl PN's study.[8]

6.1. Occupation

25 (33.3%) of the 75 cases were pupils, 18 (24.1%) were housewives, 20 (26.6%) were office staff, 4 (5.3%) were farmers, and 1 (1.3%) was an entrepreneur.

6.2. Relation of the level of vitamin D3 and rural /urban distribution of cases

Our investigation showed a strong correlation between serum vitamin D3 levels and rural/urban case location (p-value 0.048). 89.7% of cases in urban regions and 72.2% in rural areas had inadequate vitamin D3 levels. The findings were compared with those of Nurbazlin M. et al. who discovered that the vitamin D3 concentration of rural women was considerably greater than that of urban women (p0.001) because countryside women spent more extended hours in the sunlight than urban women.[9]

Serum vitamin D3 levels did not correlate with Vitiligo-associated BSA (p-value 0.942). Our findings were similar to those of Jonathan I. Silverberg, Ustun I et al. and Esmat et al., who also found no correlation between BSA and serum vitamin D3 levels in Vitiligo patients.[10–12]

Moreover, Karagüzel et al. observed that providing vitamin D supplements to Vitiligo people with decreased levels reduced lesion sizes from 66.1 58.3 cm2 to 48.0 52.6 cm2 after six months of therapy (p0.001), compared to a rise in lesion size from 34.8 48.1 cm2 to 53.5 64.9 cm2 (p0.01) in patients who only got topical therapy.[13] Based on our understanding, vitamin D substantially affects melanocytes and keratinocytes. According to research, vitamin D3 boosts

tyrosinase activity and melanogenesis in vitro, resulting in the repigmentation of Vitiligo cutaneous patches.

As vitamin D analogs, calcipotriol and tacalcitol are also documented to stimulate repigmentation in Vitiligo patients. Vitamin D has immunomodulatory properties via reducing the expression of interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-alfa, and TNF-gamma, according to another research. Furthermore, it has been observed that the active form of vitamin D inhibits ultraviolet B-induced apoptosis in melanocytes (UVB).

7. Conclusion

Our data indicate that vitamin D levels in Vitiligo patients were substantially lower than in controls; however, we did not observe a significant effect of vitamin D on the advancement of Vitiligo lesions. Other notable results that can be taken from our study include the following:

1. Vitamin D insufficiency is more widespread in young populations;
2. Non segmental Vitiligo is the most prevalent form of Vitiligo; and
3. Vitamin D deficiency affects females disproportionately,
4. Dietary pattern influences the onset of Vitiligo due to its multivariate incidence pattern.

It is necessary to do additional research to understand the problem better, including larger sample numbers and more extended periods on Vitiligo subtypes with varying degrees of severity. We propose using vitamin D to treat Vitiligo, and additional research is necessary for a more exhaustive understanding.

8. Source of Funding

None.

9. Conflict of Interest

None.

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