Materials and Methods

This is a cross-sectional study that comprised 30 patients – 21 (70%) females and 9 (30%) males – with NSV. Their ages ranged from 14 to 65 years, with a mean (±SD) of 30.23 ± 13.58 years. Seven (23%) patients were skin phototype (SPT) III while 23 (77%) were SPT IV. The clinical diagnosis was established on the existence of well-defined, depigmented areas, confirmed by Wood's lamp examination. Twenty-four (80%) patients presented clinically with vitiligo vulgaris, and 2 (7%) presented with the acrofacial type. The extent of the disease ranged from 5 to 80% of total surface body involvement (mean 35.67 ± 23.22). The disease was active in 12 (40%) patients and stable in 18 (60%) patients. Disease duration ranged from 1 to 31 years (mean 5.83 ± 5.92). Family history was positive in 4 (3%) patients while a relation of the disease to a stressful condition was reported in 13 (43%) patients. The study was performed during winter (November-December, 2015) to reduce the consequences of seasonal changes on vitamin D levels as there is a lower level of UV light exposure.

All patients recruited in this research were living in Cairo, Egypt, to prevent geographic diversity in vitamin D levels and sun exposure. We ruled out patients taking topical treatment for the last 2 weeks or systemic treatment for the last 2 months before the study; those receiving topical vitamin D, vitamin D supplements, or calcium-phosphate modifying drugs; patients suffering from malnutrition, dairy allergy or sensitivity, or any associated autoimmune or systemic disease such as liver or renal abnormalities; those with recent history of phototherapy or sunscreen usage; and pregnant or lactating females.

The control group included 40 age- and sex-matched ($p > 0.005$) healthy volunteers – 19 (47%) males and 21 (53%) females. Their ages ranged from 13 to 57

years (mean 35.38 ± 11.81). All individuals were taken from the dermatology out-patient clinics of Cairo University Hospital and the National Research Centre. All subjects (patients and controls) with a history of prolonged sun exposure (e.g., outdoor workers, excessive outdoor activities, …) were also excluded from the study in order to eliminate the possibility of lower sun exposure in vitiligo patients due to their fear of increasing the contrast of their lesions or burnt vitiliginous areas with prolonged sun exposure.

All individuals were subjected to detailed history taking, general and dermatological examination, along with the estimation of serum levels of vitamin D and IL-17. Disease activity was defined on the basis of the evolution of old lesions or the appearance of new lesions within the last 3 months [6]. Inactive disease was classified on the basis of the lack of progression of old lesions or the appearance of new lesions in the last 6 months [7]. The extent of vitiligo was assessed using the Rule of 9 according to Hamzavi et al. [8], which is the approximate percentage of the body surface area involved. SPT was determined according to the Fitzpatrick Scale, which is a numerical classification scheme for skin color [9].

Blood Sampling

Venous blood samples (10 mL) were withdrawn from each subject after overnight fasting, left to clot for 30 min, then centrifuged, and the separated sera were kept frozen at --70°C until analysis of the various parameters outlined below. To avoid seasonal variations of vitamin D, blood samples from the control group were taken at the same time as the patient group.

Assessment of Serum Levels of 1,25(OH)D

A total 1,25(OH)D assay was performed using an enzyme-linked immunosorbent assay (ELISA) kit (DRG GmbH, Germany). The experimental

procedures were performed according to the manufacturer's instructions. Serum 1,25(OH)D levels were divided into the following clinically acceptable cutoff values [10]: deficient: <10 ng/mL, insufficient: 10--30 ng/mL, and sufficient: ≥30 ng/mL.

Assessment of Serum Levels of IL-17

Quantitative determination of IL-17 was carried out using the commercially available ELISA kits (Biotech Clinical Laboratories, Inc., USA) [11].

A standard curve was made by plotting the absorbance for each reference standard against its concentration (in pg/mL) on a logarithmic scale. The absorbance was obtained by subtracting the negative control absorbance from the observed absorbance. The corresponding concentration of IL-17 (in pg/mL) in samples was determined by plotting the adjusted absorbance value for each sample on the standard curve.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows program. The χ^2 test was used to compare differences between the frequencies. The Mann-Whitney U test and the Student *t* test were used to compare mean values between groups. The Spearman rank correlation test was used for the assessment of correlation. A multivariate linear regression was performed to evaluate the relationship between serum IL-17 and vitamin D levels with the different demographic data of the patients. A p value ≤ 0.05 was considered statistically significant.