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ASSOCIATION OF SERUM VITAMIN D LEVELS WITH SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS- A CASE CONTROL STUDY FROM TERTIARY CARE HOSPITAL.

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Abstract

Background

The incidence of vitamin D deficiency is rising, and it has been linked to a number of autoimmune and chronic diseases. The study's purpose is to analyse how vitamin D and SLE (systemic lupus erythematosus) are related.

Methodology

At the "Department of Dermatology and the Department of Medicine at Silchar Medical College and Hospital, a long-term case-control investigation has been carried out on 42 confirmed SLE patients, matched for age and gender with 50 healthy controls. Double-stranded DNA (dsDNA), anti-neutrophil cytoplasmic antibody (ANCA), anti-nuclear antibody (ANA), and vitamin-D levels were" measured by radioimmunoassay on all samples. Data was gathered and tabulated using various metrics, such as mean, standard deviation (SD), and percentage of values. Mean +/- SD was used to express quantitative data, while absolute numbers and percentages were used to express qualitative data. To compare the variables, the student's t-test had been utilized. P values </=0.05 (two-sided) will be regarded as statistically significant in all cases. MS Excel was used to compute these. Excel and Word were also utilized to create tables, graphs, and other documents.

Results

The SLE cases group's average "vitamin-D levels were determined to be 17.57 with an SD of 4.51. The SD was 2.01 and the mean was 52.23 for the control group. Vitamin D levels in SLE cases and control groups were compared in a paired test, and the outcomes demonstrated a statistically significant p-value of 0.001. After taking vitamin D supplements, the SLE cases group averaged 46.82 with an SD of 14.65. The data collected before and after Vitamin D supplementation in the SLE cases group were subjected to a paired sample t-test, which yielded a p-value of" less than 0.001, signifying statistical significance. P-values for the different ANA types show that there is no statistically significant relationship between them; they are 0.09 after supplementation and 0.21 before. Statistical insignificance was therefore determined.

Conclusion

Vitamin D levels had been low in SLE patients as compared to individuals in good health. Vitamin D did not display a major correlation with the different patterns of ANA in patients of SLE.

Recommendation

More longitudinal studies will be required with a larger study "population to establish the outcomes of the current research.

Keywords: *Vitamin D, Immune responses, Systemic Lupus Erythematosus*
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Introduction

SLE is a long-lasting autoimmune disorder where" antibodies attack various parts of the body, leading to skin, joint, blood, and organ symptoms. SLE is a multifactorial disease with a complex etiopathogenesis that has not been fully understood.[1]

Currently, there is sufficient data demonstrating varied SLE behavior in diverse populations. Environmental, hormonal, and genetic factors are related to the development and progression of this disease.[2] There is interest in determining how vitamin D contributes to the development of SLE because it is present in several cells in the innate as well as adaptive immune systems.[3] In addition to its many biological roles, vitamin D is

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involved in the maintenance of the phosphocalcic balance, particularly in the immune system. It modulates lymphocyte cells by reducing immunoglobulin production by B-lymphocytes, inhibiting T-lymphocyte proliferation, and reducing the killer cells' cytotoxic activity[4,7]. Research has shown that individuals with SLE have decreased 25-hydroxyvitamin D3 levels in comparison to individuals without the condition.[6] This research aims to identify any correlation among abnormal serum Vitamin-D levels & SLE onset.

Objectives

Investigate serum Vitamin-D levels in SLE patients and their relation with various ANA patterns.

Materials and methods

Study design

A long-term case-control analysis

Study setting

The research was carried out at the Central Composite Laboratory, the Department of Dermatology, the Department of Medicine, and the Multidisciplinary Research Unit at the Silchar Medical College and Hospital in Assam, Northeast India.

Study population

The study comprised 42 SLE patients who underwent treatment at the Department of Dermatology and Department of Medicine at Silchar Medical College and Hospital. These patients had a 95 percent confidence level, a five percent margin of error, and a 3.2 percent population proportion. The investigation also included fifty healthy controls who were of a similar age and gender.

Silchar Medical College conducted case-control research from January 2022 to December 2023. The study conducted in the departments of medicine and dermatology included 42 SLE patients, regardless of whether their condition was active at the time. All individuals have been diagnosed on the basis of the updated classification criteria established by the ACR (American College of Rheumatology) which was revised in 2018.[5] healthy controls, matched in gender and age, have been also included.

After providing their consent, patients who were 18 years of age or older and who satisfied the ACR guidelines (revised, 2018) as well as the SLICC for the classification of SLE criteria5 were included in the study.

Inclusion criteria

1. Minimum of four criteria which includes at "least 1 immunologic criterion & 1 clinical criterion
2. Lupus Nephritis can be the only clinical criterion if ANA or anti-ds DNA" antibodies are present.
3. Admitted to the hospital for a minimum of seven days.
4. Having the ability to communicate as well as understand.
5. Obtain their informed consent in writing.

Exclusion criteria

1. Patients "with other established chronic infectious and inflammatory conditions such as (HIV) Disease as well as acute malignancies.
2. Patients with sepsis or septic shock.
3. Individuals with disorders affecting consciousness as well as communication.
4. Patients utilize psychotropic medications like tranquilizers as well as antidepressants".
5. Individuals who were experiencing neuropsychiatric SLE.
6. Individuals with severe SLE-related dyslexia.

After obtaining informed consent, all participants underwent clinical evaluation which included systemic examination, local examination, and history taking. The following parameters have been documented: age, gender, SLE duration, skin alterations, photosensitivity, and active arthritis. The samples have been tested for dsDNA, ANA, and ANCA using immunofluorescence with double dilution ranging from 1:40 to "1:1280 on Hep 2 cells following evidence-based guidelines for the immunologic tests-Antinuclear antibody" testing[9]. Vitamin D samples were collected, centrifuged at 4000rpm for the time of 4 mins, and then computed as well as confirmed utilizing ELISA. It is generally accepted that vitamin-D levels below 30ng/ml indicate insufficiency and below 20ng/ml show deficiency.[6] We categorized any Vitamin D level below 30ng/ml for statistical analysis. Patients of SLE having inadequate Vitamin D levels have been given a 60000 IU supplement for 6weeks. Serum Vitamin-D levels were assessed after 6 weeks to determine if there was a notable improvement.[6]

Collection of data

Comprehensive demographic information, such as gender and age, was obtained from records of the hospital. Biochemical information has been collected from the institute's LIS ("Laboratory Information System"), including serum Vitamin-D levels and ANA.

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Statistical Analysis

After collecting the data, it was arranged in various formats, such as frequency, mean, median, percentage, and so on. A paired T-test was carried out in order "to compare the levels of serum vitamin-D in patients with SLE and healthy controls", as well as those of several ANA patterns both before as well as after the administration of vitamin-D supplementation. To determine the relations among vitamin-D levels in SLE instances as well as controls, as well as among several patterns of ANA both after & before vitamin D supplementation, the P value was computed. This was done in order to determine the relationship. For this particular task, the software programs SPSS and Microsoft Excel were utilized.

Bias

There were chances that selection bias would arise at the start of the study, but it was duly avoided by giving all participants identical information and the group allocation was hidden from the nursing staff who collected the data. Bias in matching was prevented by prompt selection of controls by matching their age and gender. Measurement bias was taken care of by running a proper quality control check before the measurement of the analytes for each sample.

Ethical consideration

This research had been approved by the institutional ethics committee vide no SMC/98/07/11864 dated 24/10/2019.

Results

Of the 42 confirmed SLE patients, 35 (83.33%) were female and 7 (16.66%) were male (Figure 1). Females in the reproductive age group, aged 20 to 30, made up 61.90 percent of the sample (n = 26) [Figure 2].

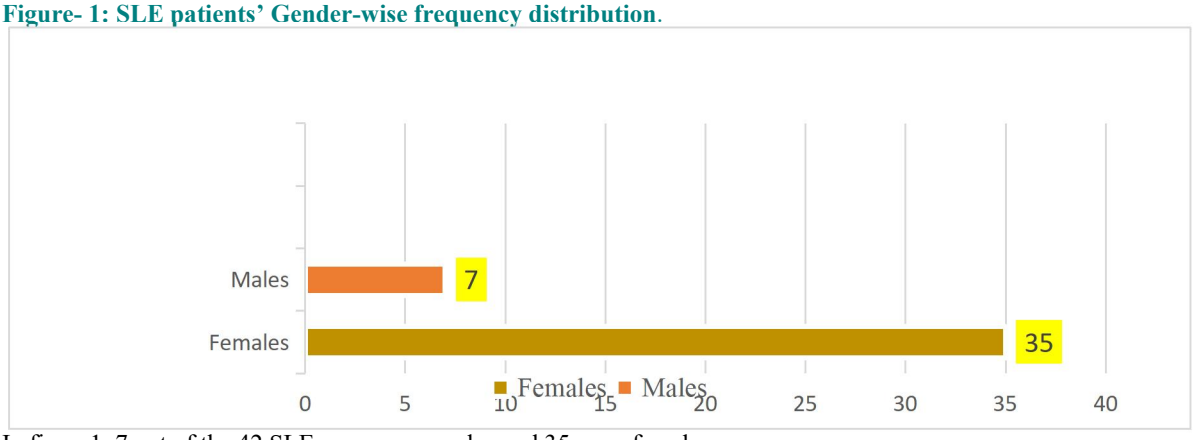
Disease severity was assessed and was evident that 47.61 percent (n=20) had mild disease, 47.61 percent (n=20) showed moderate disease and 0.047 percent (n=2) had severe disease [Figure 3]. The majority (71.42%) of the patients (n=30) presented with skin alterations (malar rash, discoid rash etc). 23.80% presented with photosensitivity (n=10). Only 4.76 percent had active arthritis (n=2) [Figure 4].

61.9% of the participants (n= 26) were deficient in Vitamin D with levels below 20.0ng/ml, 38.09% (n= 16) had inadequate levels between 20 to 30ng/ml, and none had sufficient levels above 30ng/ml [Figure 5].

It has been determined that "the SLE cases group's average vitamin-D levels are 17.57 with an SD of 4.51. The control group's mean was 52.23, SD 2.01. The results of a paired test comparing vitamin D levels in SLE cases and controls were statistically" significant (p=0.001). The SLE cases group's average score after receiving vitamin D supplementation was 46.82, with an SD of 14.65. In order to analyze the data collected in the SLE cases group both before and after the administration of vitamin D supplementation, a paired sample t-test has been carried out. The result was 0.001, indicating statistical significance.

42.85% (n=18) of the 42 SLE patients showed a homogenous pattern in ANA. With p-values of 0.21 prior to and 0.09 following supplementation, there hasn't been any statistically significant correlation observed among the various forms of ANA and vitamin-D levels. It has been determined that there is no statistically significant correlation among the levels of Vitamin D and the various ANA patterns.

Figure- 1: SLE patients' Gender-wise frequency distribution.



In figure1, 7 out of the 42 SLE cases were males and 35 were females

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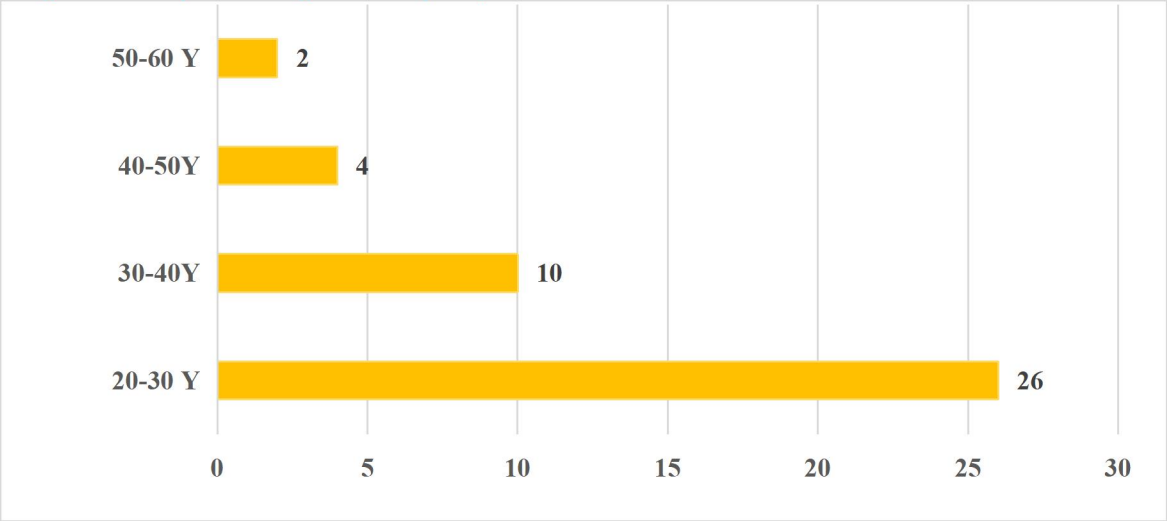
Figure 2: SLE patients' Age-wise frequency distribution.

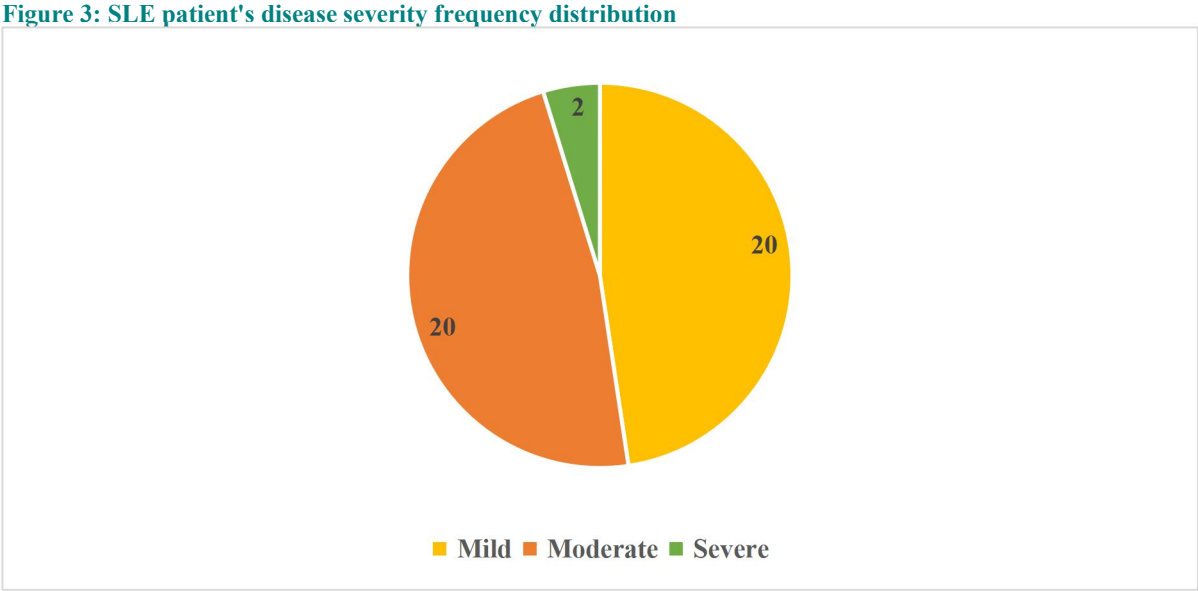


In figure 2, 10 SLE cases have been under the age group 30-40 y/o, 2 under 50-60 y/o, and 26 cases have been younger than the 20–30 age range.

Table1- Descriptives showing the demographic details of the SLE patients.

Demographic details	**Frequency (N)**
1. Gender	
Male	7
Female	35
2. Age Group (years)	
20 to 30	26
30 to 40	10
40 to 50	4
50 to 60	2

Figure 3: SLE patient's disease severity frequency distribution



20 SLE patients had mild, 20 had moderate and 2 had severe disease activity.

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Figure 4: SLE patient's clinical features frequency distribution.

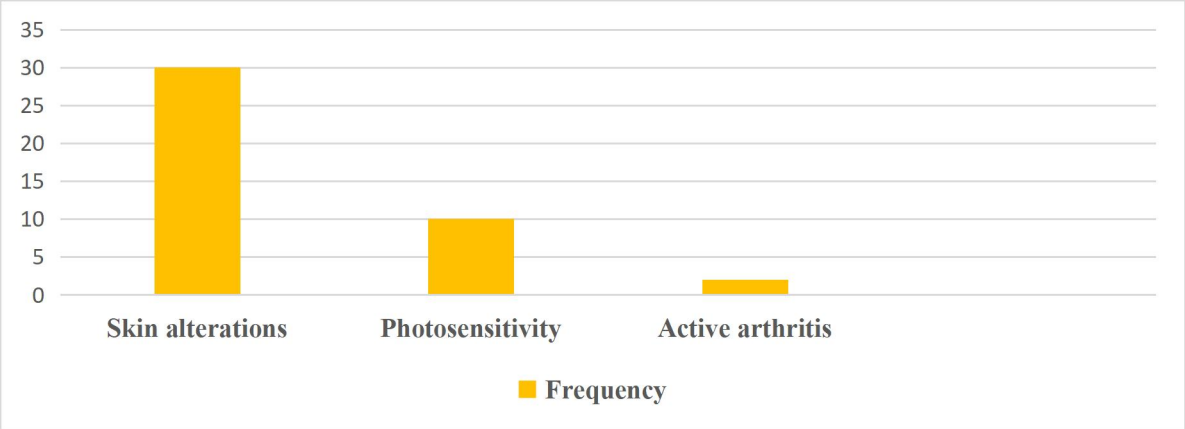
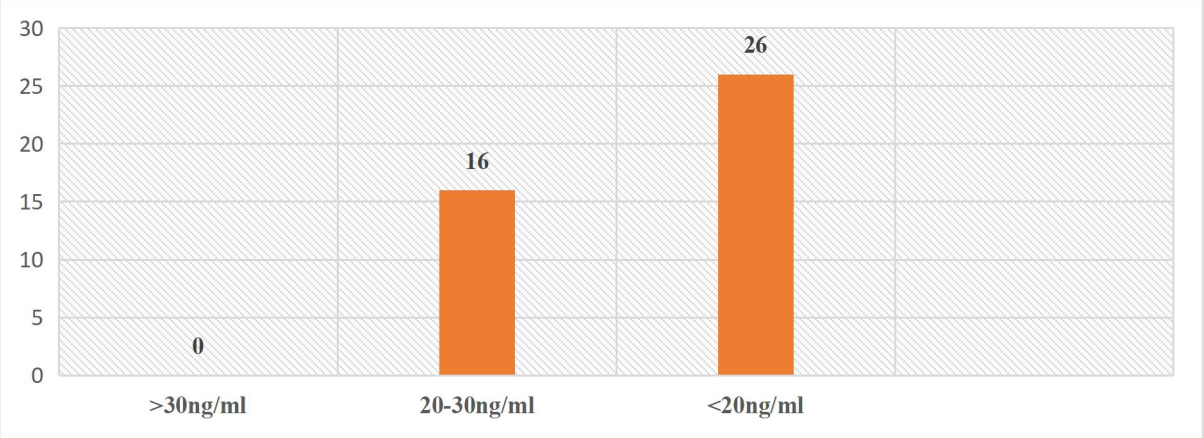


Table 2- Descriptives showing the clinical details of SLE patients

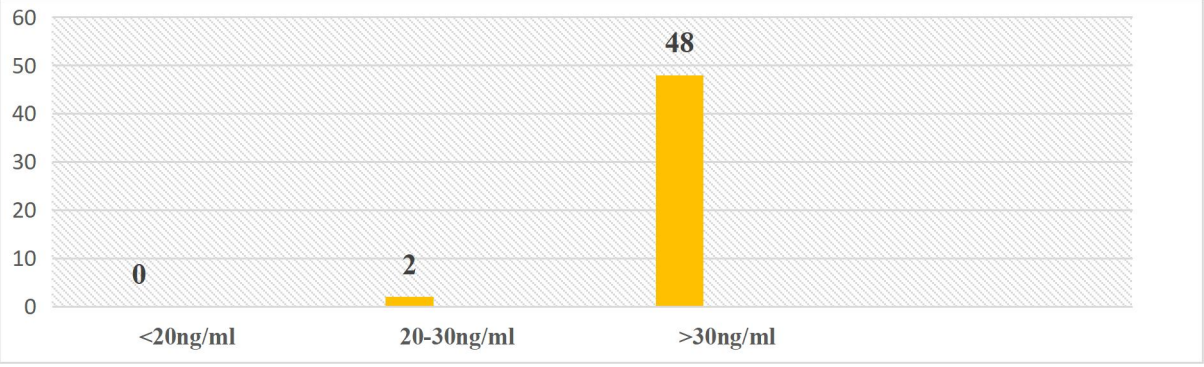
Clinical parameters	Frequency (N)
1. Disease severity	
Mild	20
Moderate	20
Severe	2
2. Photosensitivity	**10**
3. Skin alterations	**30**
4. Active arthritis	**2**

Figure: 5- Serum Vitamin-D levels' Frequency distribution among SLE patients.



Of the forty-two cases of SLE, 26 had levels <20 ng/ml (deficiency), none had levels >30 ng/ml (sufficient), and 16 had levels between 20 & 30ng/ml (insufficiency).

Figure: 6- Serum Vitamin D Levels' Frequency distribution among healthy controls.



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Of the 48 controls, two had levels between 20 & 30ng/ml of vitamin D, and none had levels <20ng/ml.

Table 3: Paired sample statistics

Pair 1	N	Mean	Deviation	Std. error mean
Vit- D (initial)	42	17.5695	4.51816	0.69717
Vit- D (after supplementation)	42	46.8245	14.65956	2.26202

Table 4: Paired samples test

Difference (Pre vs post)- t-test	Mean	SD	Std error mean	95% CI of difference	t	df	P value Sig (2-tailed)
Vit D initial- Vit D after supplementation	-29.25500	14.24802	2.19852	Lower: -33.69500 Upper: -21.81500	-13.307	41	0.001

Figure- 7: "Frequency distribution of ANA patterns among SLE patients.

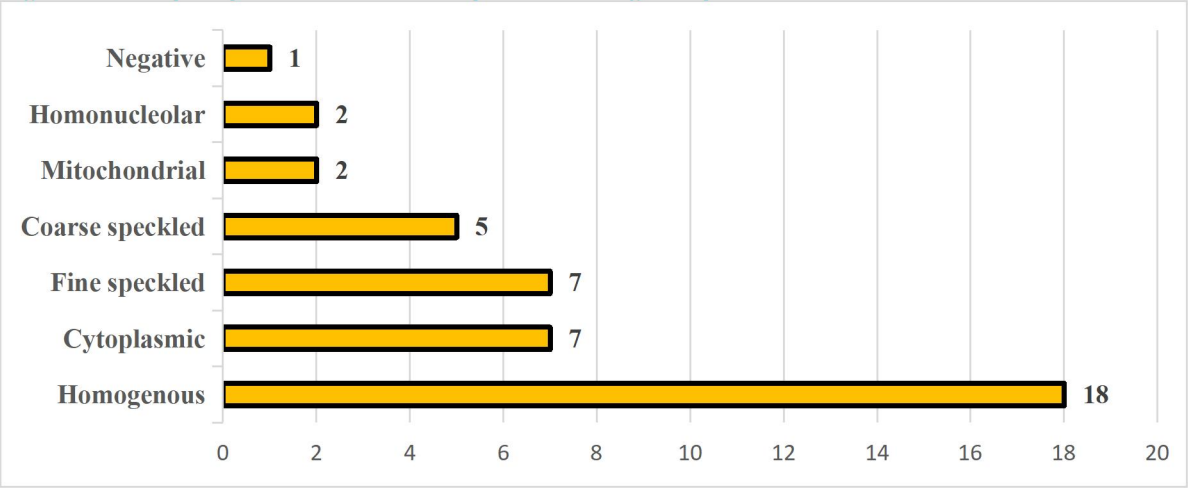

Table 5- "Descriptives for initial Vitamin D status among various ANA patterns in SLE patients.
N= Number of cases, SD= standard deviation (p= 0.21, considered statistically insignificant".

ANA pattern	N	Mean	Std error	SD	Maximum	Minimum
Homogenous	18	19.23	1.00	4.24	25.50	10.80
Negative	1	13.30	-	-	13.30	13.30
Fine speckled	7	18.46	1.82	4.83	23.10	8.13
Homo nucleolar	2	12.74	3.46	4.89	16.20	9.28
Coarse speckled	5	15.38	2.39	5.34	20.50	7.30
Cytoplasmic	7	15.70	1.41	3.72	19.70	10.30
Mitochondrial	2	18.52	0.02	0.03	18.54	18.50

Table 6- The characteristics of the "final vitamin D status after supplementation in patients with SLE who have a variety of ANA patterns
N= Number of cases, SD= standard deviation] (p= 0.09, considered statistically insignificant.

"ANA pattern	N	Mean	Std error	SD	Maximum	Minimum
Negative	1	61.10	-	-	61.10	61.10"
Homo nucleolar	2	33.63	2.40	3.39	36.03	31.23
Homogenous	18	52.72	3.25	13.79	76.20	21.90
Cytoplasmic	7	38.65	5.66	14.98	65.82	17.40
Fine speckled	7	50.26	6.05	16.02	75.50	34.40
Mitochondrial	2	36.95	0.95	1.34	37.90	36.00
Coarse speckled	5	38.63	4.58	10.24	55.92	30.87

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Discussion

The relationship between immune responses as well as autoimmune diseases and vitamin D has long piqued the interest of medical literature.[1,2] Patients with SLE have a complex association with vitamin D because the condition has a number of risk factors that can result in a deficiency and an increase in clinical symptoms.[3,5]

The current research purpose has been to analyze the relationship among serum vitamin-D levels and patients with SLE. There have been more female SLE patients in the "active reproductive age group.[8] This is consistent with Vasile M and coworkers' findings, who reviewed the evidence supporting the hypothesis that hormonal factors may be responsible for the greater incidence of autoimmune diseases in females. In patients with SLE, it has been discovered that serum vitamin D deficiency is extremely prevalent as the p-value obtained was 0.001. Hence, we arrived at the conclusion that there is an association between aberrant serum Vitamin D levels with SLE. Furthermore, the serum vitamin- D levels in" SLE cases have been observed to be majorly lower in contrast to those in the control group. Deficient levels were present in more than two-thirds of the patients, and more than 1/2 of them had insufficiency. This had been consistent with the findings of Ritterhouse and coworkers, who found a strong correlation among deficiency of vitamin D as well as disease activity in the "patients with SLE (N=32, 69percent) and a p-value of <0.001. Similar levels of prevalence, which could vary depending on a number of factors like geographical, genetic factors", and environmental, have been shown in other studies. These levels range from 16% to 96%.[5,6.] The research conducted by Mandal M and coworkers[5] yielded similar results, demonstrating an inverse relationship among levels of vitamin D & disease activity (p=<0.0001).

Our study's mean vitamin D level was 17.57+/-4.51ng/ml, which is nearly identical to research findings from Egypt (17.6+/-6.9ng/ml).[10] 1+/-9.5ng/ml, Serbia[11]. In comparison to the inactive control group, there has been a statistically major decline in the SLE cases group. This is in line with research by Cutillas and coworkers[2] on the sixty SLE patients in Cuttack, Odisha (the "odds ratio of serum vitamin-D deficiency in these cases was found to be 3.47), as well as research by Ritterhouse and coworkers[3] on 32 SLE patients. The rise in levels of vitamin D in SLE patients after receiving supplements was statistically significant (p=<0.001)" in the current study. Handor N and coworkers[6] obtained similar results.

The majority of the different ANA patterns have been observed to have a homogenous pattern. This matches the research findings on 49 SLE patients by Bogaczewicz J and coworkers(35.2percent)[12]. However, in our current study, which is consistent with Bogaczewicz and coworkers[12], there was no statistically major correlation found among levels of Vitamin D and different ANA patterns in the patients of SLE. This might be because of the smaller sample size in our analysis, and if more research is done, it might shed more light on this particular area.

Generalizability

Our study's generalizability may be limited by its regional context, the specific patient population, and the exclusion criteria. Further studies with larger, more diverse populations and in different settings are recommended to validate our findings and to amplify its applicability to broader contexts.

Conclusion

Statistically speaking, SLE patients have vitamin-D lesser levels in comparison to healthy people. Moreover, vitamin D levels among SLE patients improved statistically significantly following supplementation. However, among patients of SLE, there has been no discernible correlation between vitamin D and the different ANA patterns. Thus, vitamin D supplements should be given to patients of SLE whose vitamin serum levels have dropped.

Limitation

Other inflammatory parameters or markers that could affect how pathogenic SLE is are not measured in this study, which could have an impact on the findings. The range of genders and nationalities in our study population further restricts our ability to fully analyze the study's findings. More long-term research is necessary to fully generalize the findings of this study because of the smaller size of the sample.

Recommendation

More longitudinal studies will be required with a larger study population to establish the outcomes of the present research.

Acknowledgment

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List of abbreviations

SLE- Systemic "Lupus Erythematosus

dsDNA- double-stranded DNA

ANA- anti-nuclear antibody

ANCA - anti-neutrophil cytoplasmic antibody

Funding

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Conflict of interest

A conflict of interest didn't exist.

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