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Correlation of Serum Calcium and Vitamin D on SLE Disease Activity Index among SLE (Systemic Lupus Erythematosus) Patients at a Tertiary Care Centre Structured

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Abstract: ***Background: Systemic Lupus Erythematosus is a prototypic autoimmune disease with diverse multisystem involvement and the production of an array of autoantibodies. The clinical presentation of this disease can be quite variable from mild skin or joint manifestations to life - threatening internal organ manifestation. The SLICC revised ACR criteria are used for the diagnosis of SLE. SLEDAI measures the disease activity based on 24 questions including physical examination, lab features and clinical manifestations. SLEDAI2K measures the disease activity in last 28 - 30 days. Objectives: To find the correlation of serum calcium and vitamin D on SLEDAI and to study the clinical profile of SLE patients. Methods: After obtaining institutional approval 50 SLE patients were enrolled for this study. Patients were interviewed; blood sample was taken for estimation of serum calcium and Vitamin D. Other relevant clinical history and details were collected using preformed questionnaire. The data was entered in to excel sheet and analysed using SPSS 27 software. Results: A negative correlation was associated between Serum calcium, Vitamin D and SLEDAI (Pearson correlation coefficient r= - 0.905 and - 0.50 (p<0.001)). The mean Vitamin D of SLE patients in this study was 11.56+ - 3.6. Cutaneous manifestation and joint manifestations were the predominant presentation. Conclusions: Majority of patients included in the study were Vitamin D deficient. There is a negative correlation between SLEDAI, Serum Calcium and Vitamin D.***

Keywords: Systemic Lupus erythematosus, SLEDAI, Calcium, Vitamin D, Correlation

1. Introduction

SLE (Systemic Lupus Erythematosus) is an autoimmune disease in which organs and cells undergo damage initially mediated by tissue binding antibodies and immune complexes. Systemic Lupus International Collaborating Clinics (SLICC) group revised and validated the American College of Rheumatology (ACR) systemic lupus erythematosus (SLE) classification criteria is used for identifying patients with the disease. The importance of serum calcium as an important signalling molecule in both intracellular and extracellular compartment has been well established. Many recent studies have pointed out the importance of serum calcium in the pathogenesis of dyslipidaemia, accelerated atherosclerosis, cell development and functioning, inflammation and immune tolerance. Calcium signalling through regulation of cAMP - cGMP is also involved in interferon generation and thereby controlling innate immunity. Hence this study aims at understanding the clinical significance of serum calcium and SLEDAI.

Vitamin D is a steroid hormone that has well - established roles in calcium and bone metabolism. Recent studies have elucidated the immunomodulatory role of this fat soluble vitamin and association between autoimmune diseases and chronic infectious diseases like tuberculosis. The study of serum Vitamin D and its association might be thus useful and extrapolation of these results may open new scopes for elucidation of the genetic link between Vitamin D and other autoimmune diseases.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease across the world among different ethnic and racial groups. Systemic lupus erythematosus develops predominantly in women of childbearing age and is more prevalent in non - Caucasians. Genetic predisposition, environmental susceptibility, and hormonal homeostasis contribute to disease development and activity. Clinical manifestations and the pattern of organ involvement are widely heterogeneous among patients with SLE, reflecting the complex pathogenic mechanisms of SLE. Notably, complement involvement and activation play an important role in the inflammatory response prompted by immune complex deposition in autoimmune - mediated tissue injury. According to the validated criteria published by Systemic Lupus International Collaborating Clinics (SLICC) group, low serum levels of complement C3 and C4 are included as one of the immunological classification criteria of SLE.2 The significance of complement to reflect SLE activity has been confirmed.

Low serum C3 is regarded as the most significant risk factor for cytopenia and muco cutaneous lesions and was negatively correlated with SLE disease activity. Calcium signaling participates in pathways involved in immune - tolerance and inflammation. Hence it has been well acknowledged that calcium by playing its central role in B cell development is involved in immune tolerance.

Calcium is involved in the regulation of cyclic GMP - AMP synthase/stimulator of interferon genes axis. Hence play its role in innate immunity modulation through type I interferons. Through its effect on the innate and adaptive immunity, abnormal calcium signaling was associated with several autoimmune diseases including SLE. The engagement of the T cell receptor results in increased production of inositol 1, 4, 5 - trisphosphate (IP3) and

increased release of calcium from endoplasmic reticulum. However, the clinical value of serum calcium level in patients with SLE has not been explored yet.

Calcium homeostasis is maintained and regulated by 1, 25 (OH) 2D3, which is the active form of vitamin D. It is reported that vitamin D deficiency is common in patients with SLE, and vitamin D supplementation is of benefit in modulating disease activity[1, 7]. Considering the important role of calcium signaling in autoimmune diseases and the clinical significance of vitamin D supplementation in SLE, the change of serum calcium level in patients with SLE and its clinical value should be investigated further.

It has been observed that the majority of SLE patients have low vitamin D levels. The important contributory factor is usually attributed to sun protection measures in order to avoid SLE flares. This elucidates the importance of assessing their vitamin D status and its association with clinical manifestations of SLE, cardiovascular risk factors, autoantibodies, and SLE disease activity.

Rationale of study

SLE is a common autoimmune disease with multiple systemic manifestations. The estimation of serum calcium and Vitamin D and finding its correlation with SLE Disease Activity Index will help in analysing the importance of modulating these factors in improving treatment of these patients. This study also aims at understanding the clinical spectrum of SLE patients.

2. Review of Literature

SLE is one of the most studied diseases in medicine with its protean clinical manifestations[21]. This is a disease predominantly of women in child bearing age group, which is associated with significant morbidity and mortality [53]. Hence is at the focus of new research that will generate insights into the pathogenesis of disease.

The term Lupus, describes the characteristic destructive nature of the rash of this disease which resemble "the gnawing of a wolf. "[21]Insights into the immunopathogenic mechanisms of autoimmunity resulting in tissue damage have evolved over decades. The lupus erythematosus (LE) cell, first described by Hargraves (1948)[20], suggest engulfment of cellular debris as a pathogenic mechanism at sites of active inflammation. The recognition of antibodies directed against cellular components, particularly cell nuclei in the sera of patients reflected the autoimmune nature of disease. Various defects in T cell function such as, defective production of IL - 2, altered activity of TLR (Toll Like Receptors) have been described.[19]

Various environmental triggers such as UV light mediated DNA damage and modification of DNA methylation can [73]render endogenous nucleic acid stimulatory to immune system.

Genetic factors

Observations of occurrence of several individuals with SLE within a family and a high frequency of concordance of SLE in identical twins The class II alleles *HLA DR2 (DRB1*1501*) and *HLA - DR3 (DRB1*0301*) are also very strong genetic risk factors associated with SLE and studies say that they confer an overall 2 - to - 3 - fold increased risk for SLE[22]. The homozygous deficiencies of early complement components (C2, C4, C1q) are associated with 5 - 10 fold increased disease susceptibility. C1q deficiency can augment IFN alfa and promote broad immune dysregulation.[23]CRP a member of pentraxin family also contribute to clearance of apoptotic debris [24].

Other recognized genetic variations include, TREX1, SAMHD1, UBE2L3, STAT4, TET3 and PTPN22.[22]

Gender Predisposition

SLE is a female preponderant disease. The extreme sex skewing is explained by various hormonal effects –estrogen modulating lymphocytes and pDC activation, and high level of Prolactin in their serum. X chromosome as a pathogenic factor is explained by the location of TLR7 and its role in type 1IFN production.[74]

The onset of SLE is in the childbearing years –after menarche and before menopause, with breastfeeding offering a protective action. Although not fully understood the carefully orchestrated demethylation re methylation of DNA, regulatory RNA production, RNA associated protein such as PIWI protein production in the maturing germ cells might provide a source for nucleic acid complexes[24]It was observed that testosterone suppresses IgG anti - dsDNA antibody and total IgG production from the various cell line of SLE patients and testosterone supplementation may improve disease activity (Kanda et al) [79]

Autoantibody in SLE

Virtually all lupus patients have appositive antinuclear antibody AntidsDNA and AntiSm antibodies are most specific for SLE Anti Ro, Anti La and Anti RNP antibodies are characteristic of SLE but are seen in other autoimmune disease as well[2]. Although some patients with characteristic clinical presentation don't have significant antibody titre it is highly likely that they have an unidentified autoimmunity target. Anti dsDNA is positive in 60 - 80% of patients with SLE, in study conducted by Compagno et al.[26]Anti - mitochondrial DNA antibodies are having a better correlation with LN than anti - dsDNA antibodies, according to study conducted by Wang et al[27.] Mitochondrial DNA is detected along with NETs in LN renal biopsies and additionally mitochondrial DNA/anti - mitochondrial DNA antibody complexes are described to induce plasmacytoid dendritic cells and IFNα production (important in the pathogenesis of the disease) greater than dsDNA/anti - dsDNA antibody complexes.

Criteria for diagnosis

The SLICC revised ACR criteria is used for diagnosis of SLE.

The SLICC (SLE International Collaborating Clinics) criteria[2]for SLE classification requires:

1) Fulfillment of at least four criteria, with at least one clinical criterion AND one immunologic criterion OR
2) Lupus nephritis as the sole clinical criterion in the presence of ANA or anti - dsDNA antibodies.

Clinical Criteria:

1) Acute cutaneous lupus
2) Chronic cutaneous lupus
3) Oral ulcers: palate
4) Non scarring alopecia (diffuse thinning or hair fragility with visible broken hairs) 5. Synovitis involving two or more joints, characterized by swelling or effusion OR tenderness in two or more joints and thirty minutes or more of morning stiffness.
5) Serositis
6) Renal involvement
7) Neurologic involvement
8) Hemolytic anemia
9) Leukopenia (< 4000/mm3 at least once)
10) Thrombocytopenia (<100, 000/mm^3) at least once.

Immunological Criteria:

1) ANA above laboratory reference range
2) Anti - dsDNA above laboratory reference range, except ELISA: twice above laboratory 3. Anti - Sm
3) Antiphospholipid antibody
4) Low complement
5) Direct Coombs test in the absence of hemolytic anemia

A systematic review of MEDLINE, EMBASE and the Cochrane database studied 13, 080 patients from 64 studies reporting ANA by immunofluorescence on HEp - 2 cells[3]. Meta regression of the operating characteristics found a sensitivity of 97.8% [95% confidence interval (CI) 96.8% – 98.5%] for ANA of ≥1: 80 which supported the use of ANA as an entry criterion.[28]

Mucocutaneous Manifestation

According to study conducted by Hassan et al [34]mucocutaneous manifestations the distribution of cutaneous manifestation were oral ulcers (69.8%); malar rash (65.6%); photosensitivity rash (53.1%), and discoid rash erythematous (21.9%). Non - specific LE cutaneous manifestations according to Gilliam classification like oral ulcers (69.8%), photosensitivity rash (53.1%), alopecia (86.5%), Raynaud's Phenomenon (39.6%), nail abnormalities (24.0%), periungual telangiectasia patients (13.5%), vasculitic lesions (12.5%), thrombophlebitis (44.8%), bullous lesion (5.2%) and erythema multiforme (5.2%) were also described [29]

Joint Manifestation

According to systematic meta - analysis by Beatrice et al the degree of articular manifestation can range from minor arthralgia without erosions or deformity to erosive articular involvement and severe functional disability.[34] Upto ninety percent of patients with SLE experience joint symptoms during their follow up visits. The concomitant presence of two autoimmune diseases – SLE and RA – in the same patient is called rhupus.[30]

Muscle Involvement

According to NIH study the prevalence of myositis among SLE patients was 8%.9Myositis usually involves proximal upper and lower extremities. The characteristic pathological changes include type 2 fibre atrophy and lymphocytic vasculitis.

Pleuropulmonary Manifestation

Pleuritis develop in 50% patients with SLE. Clinically apparent pleural effusion are usualy bilateral, exudative and small. Other Lung manifestations include inflammatory and fibrotic forms of interstitial lung disease, alveolar hemorrhage, shrinking lung syndrome, pulmonary hypertension, airway disease, and thromboembolic disease.[31]

Cardiovascular Manifestation

SLE can involve the pericardium, myocardium, or endocardium.25% of SLE patients at disease onset or during relapse can present with pericarditis which is the most common presentation. 40% of patients can present as asymptomatic pericardial effusion. Myocarditis even though rare, can lead to arrhythmias, ventricular dysfunction, dilated cardiomyopathy, or heart failure. Libman - Sacks endocarditis recognized by verrucous growth occurring on the valve leaflets, papillary muscles and endocardium can be seen in upto 6%. The incidence of atherosclerosis and CAD is 50% higher in women with SLE compared to heathy controls.[32]

Renal Manifestation

Although in all cases deposits of immunoglobulin are found in the glomeruli, only one half has clinical nephritis. Urine analysis of asymptomatic patients usually reveal hematuria and proteinuria. Renal failure and sepsis are two major causes of death in SLE patients. Glomerular disease develops within the first few years of disease onset and usually is asymptomatic.[34, 2] Acute or chronic renal failure present with uremia and fluid overload or electrolyte disturbances. Acute nephritic disease can manifest as hypertension and hematuria. Nephrotic syndrome may cause edema, weight gain, or hyperlipidemia. Lupus nephritis is caused by the deposition of immune complexes. Classification of Lupus nephritis is by performing biopsy.[34]

Secondary APS

The most common autoimmune disease associated with APS is SLE.3[6]40% of patients with SLE can have APS antibody positivity and 40% among them have thrombotic manifestations. APS is diagnosed according to Sydney revised Sapparo criteria.[34, 35]

Role of Complement in SLE

The pathogenic roles of complement activation in human SLE were indicated by various studies: low total complement hemolytic activity (CH50) and decreased C3 and C4 levels is seen in about 75% of SLE patients with focal nephritis and 90% in patients with diffuse nephritis [16]. Additionally, the co - localization of Ig isotypes IgG, IgA and IgM with C1q, C4 and C3 (and C5b - 9; the so called 'full house' pattern) in the glomeruli is present in glomeruli of patients with lupus nephritis. It has also been observed that, complement split products such as C3d and C5b - 9 can be detected in the urine of SLE patients

Calcium Homeostasis

Calcium is the fifth abundant element in human body, with total body iron stores ranging upto 1000 gm. It has important role in bone mineralisation and many other biological function.5 Current dietary calcium recommendations is 1000 to 1500 mg/d, depending on age. In elderly male and

postmenopausal female calcium supplementation is recommended to maintain the balance.5[2] The major factors that regulate body calcium are intestinal absorption, renal reabsorption, and bone turnover. These factors are in turn regulated by the complex interplay between parathyroid hormone (PTH), 1, 25 - dihydroxyvitamin D [1, 25 (OH) 2D], ionized calcium and corresponding receptors in the gut, kidney, and bone.[50.] Total body calcium exist in two forms ionised calcium and nonionised calcium. The serum protein, mainly albumin, provide short - term maintenance of a stable ionized calcium level by acting as a calcium buffer. Ionized calcium is in equilibrium with protein - bound calcium, and hence a transient drop in the ionized calcium level can be compensated by the concomitant release of calcium from the ~ 30 calcium binding sites on the albumin molecule, similar to the way weak acids and bases serve as pH buffers. Just like any any buffer system this regulatory system is highly pH sensitive. Changes in pH causes changes in the acidic amino acid residues on albumin which are charged, and subsequently the number of calcium ions bound, changing the number of free calcium ion Thus conditions that cause an increase in pH, like respiratory alkalosis caused by hyperventilation, lead to fall in ionised calcium, whereas decreases in pH cause the ionized calcium to rise.[54]

Normal serum calcium =8.6 - 10.2. Hypocalcemia refers to serum calcium value <8.6. Increased neural excitability is a common manifestation of hypocalcemia. The patient describes tingling of the tips of the fingers and perioral area. If unabated, they can progress in severity and extend to the limbs and face followed by pain and carpal spasm. They may have a positive Chvostek's and/or Trousseau's sign.[51]

Role of Calcium as a Second messenger

Calcium is acting as a second messenger in many cell types, including lymphocytes. At rest lymphocytes maintain a low internal concentration of Ca^{2+}[11].

However, engagement of antigen receptors will induce the influx of calcium from the extracellular space by several routes[12]. A major mechanism of Ca^{2+} entry into the lymphocytes is via the store - operated calcium (SOC) channels. The two important molecular components of SOC channels, that are identified include CRACM1 (the pore - forming subunit) and STIM1 (the sensor of stored calcium). This has paved ways into research that has allowed the genetic and molecular manipulations of the SOC[57, 14].

Within the lymphocytes, crosslinking of antigen receptors activates the phosphoinositide - specific phospholipase C. Phospholipase C breaks down phosphatidylinositol - 4, 5 - bisphosphate to liberate inositol - 1, 4, 5 - trisphosphate (Ins (1, 4, 5) P3) and diacylglycerol.9Ins (1, 4, 5) P3 binds its receptor located on the surface of internal Ca^{2+} stores, mainly the endoplasmic reticulum, and activates the release of Ca^{2+}into the cytoplasm.[15, 12] This event, is termed as 'store depletion', which in turn activates store - operated calcium (SOC) channels in the plasma membrane to recruit Ca^{2+}. High - throughput screens based on RNA - mediated interference have identified STIM1 (stromal interaction molecule 1) as the endoplasmic reticulum–resident Ca^{2+} sensor and CRACM1 (calcium release–activated calcium modulator 1; also called Orai1) as the pore - forming subunit of CRAC channels. Hence via a multitude of complex intracellular signalling pathways where calcium is acting as an important second messenger various immunological signals are decoded to the nucleus.[58]

According to study conducted by Yeqin Sha et al entitled [6]"Total Serum Calcium Level Is Negatively Correlated With Systemic Lupus Erythematosus Activity " showed that serum levels of calcium (P <.001), complement C3 (P <.001), complement C4 (P <.001), and albumin (P <.001) was lower in patients with SLE. A negative correlation was found between serum calcium level and systemic lupus erythematosus disease activity index (SLEDAI) rating. The role of Vitamin D on calcium metabolism and immune regulation was explored in journal entitled "Vitamin D and systemic lupus erythematosus: continued evolution"[7]. Calcium and vitamin D supplement intake may increase arterial stiffness in systemic lupus erythematosus patients.9 (Mellor –Pita *et' al).* According to another study by Xue du et al, there was a negative correlation between Serum Calcium and enhanced disease activity which was measured by an increase in serum IL - 2, IL - 10, IL - 6 and IFN - gamma in SLE patients.[75]

Vitamin D Metabolism

Vitamin D3 (cholecalciferol) is obtained primarily from in diet (fortified dairy products and fish oils) and is synthesized in the skin from 7 - dehydrocholesterol by UV B exposure[67]. The vitamin D synthesised from 7 - dehydrocholesterol depends on the duration of sunlight exposure, Latitude, Sunscreen use and Clothing. Vitamin D exerts numerous physiological functions such as inhibition of growth of tumor cells and protection against immune mediated disorders[70]. In the liver vitamin D is hydroxylated at C - 25 by the cytochrome P450 hydroxylases (including CYP2R1, CYP2D11 and CYP2D25), which results in the formation of 25 - hydroxyvitamin D3 (25 (OH) D3).25 (OH) D3, is transported to the kidney by DBP. Within the kidney, megalin, a member of the LDL receptor superfamily, helps in endocytic internalization of 25 (OH) D3. At the proximal renal tubule 25 (OH) D3 is hydroxylated at the position of carbon 1 of the A ring, which generates the physiologically active form of vitamin 1, 25 - dihydroxyvitamin D3 (1, 25 (OH) 2D3) mediated by the 25 (OH) D 1α hydroxylase (CYP27B1; 1α (OH) ase), predominantly present in the kidney. It is also found in extrarenal sites like placenta, monocytes and macrophages. The normal value of Vitamin D is 20 - 40 U/L. Vitamin D deficiency is characterised by growth retardation, hypocalcemia, elevated PTH and muscle weakness.[52]

Vitamin D –an immunomodulatory molecule

Vitamin D was long thought to play a role in calcium homeostasis and bone mineralization. But latest studies have unraveled its role in various immunomodulatory reactions. The incidence of vitamin D deficiency was found to be quite high in patients with COPD, Asthma, IBD, T1DM and cardiovascular diseases[70]. Consistently vitamin D supplementation in these group of patients seem to reduce the severity in these patients. At a molecular level, the hormonally active form of vitamin D which is - α1, 25 dihydroxyvitamin D3 - regulates the expression of vitamin D responsive genes which in turn mediate the various downstream signaling pathways of immune system

activation. For example, vitamin D is found to have a positive role in the regulation of iron homeostasis and erythropoiesis via the iron - hepcidin - ferroportin axis. Vitamin D deficiency is highly prevalent around the world including those countries with abundant sunshine.[71] Singh et al. reviewed the causes of vitamin D deficiency where they analyzed the role of genetic predisposition, gut microbiota, and immune system.[64] At the cellular level, vitamin D produces its anti - inflammatory effects on immune cells which express the vitamin D receptor (VDR) like the monocytes, macrophages, and T lymphocytes, via a cascade of immunological reactions. According to the study by Carlberg[62] Vitamin D mediated signaling results in chromatin remodelling as well as various epigenetic changes that modulates the release of various cytokines. The reduction of IL - 1β, which is induced via inflammasome activation results in suppression of immune activation. Vitamin D deficiency has been associated with B cell proliferation and various autoantibody generation in autoimmune diseases such as Rheumatoid Arthritis (RA), Multiple Sclerosis (MS), and Systemic Lupus

Erythematosus (SLE) [69]A systematic review conducted by Islam et al. on the immunomodulatory actions of diet and nutrients in SLE patients it was observed that supplementation of Vitamin D in SLE patients have found to alleviate symptoms such as fatigue and exercise intolerance. Meta - analysis conducted by Marjoire et al observed that SLE patients were found to have a low Vitamin D compared to healthy control subjects. Vitamin D inhibit cytokines like IL - 10, IL - 17, IFN - γ, and exert an inhibitory role in the proliferation of B - cells, Th1, Th17and CD4+ T helper cells.

Vitamin D deficiency has been also regarded as a risk factor for susceptibility to various infectious diseases. In patients with Tuberculosis, vitamin D induce the expression of cathelcidin (LL - 37), which is a defense peptide that activates bactericidal activity of macrophages, neutralizing bacterial endotoxins and activating TLR signaling augmenting host response.[68]

Effect of SLE on Vitamin D metabolism

According to National Health and Nutrition Examination Survey (NHANES) 2005 to 2006, 41.6% of adult participants (≥20 years) had 25 - hydroxyvitamin D (25 [OH]D) levels below 20 ng/ mL and it is estimated that the prevalence of hypovitaminosis D is on the rise globally. SLE patients can have renal involvement during course of the disease and subsequently 1 - Alpha hydroxylation of vitamin D into its active form is lost or significantly reduced. According to study by Sumethkul et al.[67]found that patients with lupus nephritis had significantly lower vitamin D levels compared to SLE patients without renal involvement, suggesting that nephritis is a significant predictor of vitamin D deficiency among SLE patients. Medications used for treatment of SLE can have an impact on Vitamin D levels. Data on hydroxychloroquine (HCQ) are rather inconclusive indicating the need for further studies to prove the association. Long term corticosteroid use decrease intestinal absorption and accelerates catabolism of 25 (OH) D and 1, 25 (OH) 2D through an increase in 24a - hydroxylase activity aggravating D deficiency status Sex hormones as already been described are pivotal in the pathogenesis of SLE. It is postulated that estrogen and prolactin affect maturation and selection of autoreactive B cells, thus acting as immune activators. Vitamin D by suppressing aromatase expression decreases peripheral synthesis of estrogen is thought to have a beneficial effect on disease activity. The role of Vitamin D in cardiovascular system is rather controversial. Some studies have postulated the beneficial role in cardiovascular mortality by complex array of mechanisms, improved endothelium dependent vasodilatation, reduced neo - angiogenesis, reduced NET (Neutrophil Extracellular Traps) and subsequent endothelial damage.[63]

According to study by McGhie et al Vitamin D deficiency has been linked with cognitive dysfunction in SLE patients[66]. However, review of previous study to establish an association between SLE disease activity and Vitamin D deficiency is arduous considering the heterogeneity in scaling systems used to quantify disease activity SLE patients. According to study by Gholamrezaei et al. vitamin D was found to have a role in sleep quality of SLE patients. Vitamin D supplementation in SLE Vitamin D supplementation is hence a good clinical practice in patients with rheumatic disease osteoporosis prevention[23]. However studies to understand the dose of Vitamin D to exert its immunomodulatory effects is yet to be conducted. According to the two randomised double blind placebo controlled trial by Abou rhai et al it was found that supplementation of the Vitamin is able to reduce disease activity, while other two cohort studies failed to observe any significant variation [70]. Some authors demonstrated that vitamin D supplementation have a role in reduction of inflammatory - haemostatic markers and in reducing urine protein - to - creatinine ratio.

According to Aranow et al. it was found that vitamin D3 supplementation up to 4000 IU day failed to reduce the IFN - alpha signature. As a rule, 100 IU/day of vitamin D intake is can increase 1 ng/ml of serum 25 (OH) D, within about 3 months of supplementation. It is already known that the use of sunscreen and sun avoidance are predictors of low serum levels of 25 (OH) D among SLE patients. In a study among 177 SLE patients [68], pericarditis, neuropsychiatric disease and deep - vein thrombosis were found to be predictors of lower serum 25 (OH) D. A study performed in India by Mandel et al showed a positive correlation between IFN levels and the severity of disease manifestations and a negative correlation between Vitamin D levels.[71] According to Shahin et al. hypovitaminosis D contributes to ANA antibody production subsequently high serum levels of IL - 23 and IL - 17 and disease activity.

Ding et al concluded that there were statistically significant associations between vitamin D status and ethnic group, lupus nephritis and hypertension after a large multicentric study[65]. In a prospective study of 60 SLE patients conducted by Ruiz - Irastorza et al, SLE patients were treated with vitamin D3 oraly for a median period of 24 months. They reported a beneficial effect of vitamin D supplementation on fatigue, but no change in disease activity[27]. Mice supplemented with vitamin D had less dermatologic lesions, less proteinuria and lower anti - DNA (Becker et al). In a

longitudinal prospective study (Michelle Petri et al) supplementation of Vitamin D to SLE patients and observing multiple parameters there was a statistically significant reduction in Urine Protein Creatinine ratio. Urine PC ratio is indeed a marker for Lupus Nephritis progression.

SLEDAI (SLE Disease Activity Index)

The SLEDAI measures disease activity within the last 10 days. A global index, it includes 24 clinical and laboratory variables that are weighted by the type of manifestation, but not by severity. Disease activity may range from 0 to 105. The SLEDAI includes scoring for the presence of autoantibodies (anti - dsDNA antibodies titres) and low complement, as well as for some renal and hematologic parameters.[31] The index has been validated, and demonstrated to be reliable and sensitive to change ratings were: 0 to 9 for the slight activity of patients with SLE, 10 to 14 for the moderate activity of patients with SLE, and 15 for high activity of patients with SLE. The weightage for the 9 different organ systems are as follows: 8 for central nervous system and vascular, 4 for renal and musculoskeletal, 2 for serosal, dermal, immunologic, and 1 for constitutional and hematologic. The maximum score theoretically is 105, but in clinical practice, few patients have scores greater than 45.[72]

SLEDAI-2K score	Descriptor	Definition
8	Seizure	Recent onset, exclude metabolic, infectious or drug causes.
8	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality.
8	Organic brain syndrome	Altered mental function with impaired orientation, memory or other intellectual function.
8	Visual disturbance	Retinal changes.
8	Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	Lupus headache	Severe, persistent headache which may be migrainous, but must be nonresponsive to narcotic analgesia.
8	Cerebrovascular accident	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter haemorrhages, or biopsy or angiogram proof of vasculitis.
4	Arthritis	≥2 joints with pain and signs of inflammation (i.e. tenderness, swelling or effusion).
4	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or biopsy showing myositis.
4	Urinary casts	Heme granular or red blood cell casts.
4	Haematuria	>5 red blood cells/high power field. Exclude stone, infection or other cause.
4	Proteinuria	>0.5 gram/24 hours.
4	Pyuria	>5 white blood cells/high power field. Exclude infection.
2	Rash	Inflammatory type rash.
2	Alopecia	Abnormal, patchy or diffuse loss of hair.
2	Mucosal ulcers	Oral or nasal ulcerations.
2	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation.
2	Low complement	Decrease in CH50, C3 or C4.
2	Increased DNA binding	Increased DNA binding by Farr assay.
1	Fever	>38°C. Exclude infectious cause.
1	Thrombocytopenia	<100 000 platelets / x10⁹/L, exclude drug causes.
1	Leukopenia	<3000 white blood cells / x10⁹/L, exclude drug causes.

C3 = Complement protein 3, C4 = Complement protein 4, CH50 = 50% haemolytic complement activity, DNA = deoxyribonuclease, SLEDAI-2K = SLE disease activity index 2000

Summarized from *Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. J Rheumatol. 2002;29:288-91* (99).

3. Objectives of the Study

Primary Objective

To study the correlation between serum Calcium and Vit D with SLE Disease Activity Index among SLE (Systemic Lupus Erythematosus) patients.

Secondary Objective

To study the clinical profile of SLE

4. Materials and Methods

Study Design: Hospital based cross - sectional study

Study Setting: Department of Internal medicine, Government Medical College, and Thiruvananthapuram.

Study Period: 1 year from date of IEC clearance, till required sample size is attained.

Study Population:

Inclusion Criteria:

All Patients with SLE who were diagnosed according to the revised criteria of the American College of Rheumatology are included in the study. Both newly diagnosed cases and patients under follow up will be studied.

Exclusion Criteria:

1) Patients who did not give consent for the study
2) Age <18 yrs
3) Oral supplemental calcium or Vitamin D intake
4) Malabsorption syndromes, Pre - existing Kidney unrelated to Disease

Sample Size

Sample size was calculated based on a study - ' Total Serum Calcium Level Is Negatively Correlated With Systemic Lupus Erythematosus Activity' (Yeqin Sha et al - Dose - Response: An International Journal April - June 2020: 1 - 7).[6]

The Formula used was[18]

Total sample size = N = $[(Z\alpha+Z\beta)/C]^2$ + 3 C = 0.5 * ln [(1+r) / (1 - r)] = 0.4165 N –sample size α (two - tailed) Type I error rate=0.05, β (Type II error rate) =0.20 Zα = 1.9600, Zβ = 0.8416 r= expected correlation coefficient=0.309

Substituting the minimal sample size required for the study =48

Study Variables

1) Sociodemographic data - Age and gender
2) Family history of Autoimmune diseases
3) Anthropometry –Height, Weight, BMI
4) Presence of Cutaneous manifestation - Rash, oral ulcer, non - scarring alopecia 5. Presence of joint manifestations –nonerosive arthritis, swelling or tenderness of joint.6. Clinical or X - ray / USG evidence of pleural effusion or ascites.
5) ECG or biochemical evidence of pericarditis.
6) Neurological evidence of SLE, seizure, psychosis
7) Smoking and alcohol consumption
8) Haematological parameters - Anaemia, Thrombocytopenia, Leukopenia - ESR, CRP - Direct Coombs Test, Serum LDH
9) Other relevant Investigation - RFT, LFT (Renal and Liver Function Tests) - Serum Calcium and Vit D levels
   - serum C3, C4
   - Urine Routine Examination
   - ANA, anti ds DNA, APLA Anti Sm antibody 12) SLEDAI (SLE Disease Activity Index)

Data collection tools:

A semi structured interviewer administered pro forma will be used for the study. SLE disease activity will be calculated according to SLE Disease Activity Index.

Data collection method:

All patients diagnosed with SLE and treated at Government medical college Thiruvananthapuram, fulfilling the inclusion criteria will be included in the study. An informed written consent in Malayalam will be read to study subjects for obtaining consent. All study questions will be explained to them and responses recorded. The relevant clinical details and laboratory data will be collected. The investigations pertaining to diagnosis include Complete Blood count, Renal function test, LFT, Urine Routine Examination, ANA, Anti ds DNA, Serum Calcium, Serum Vitamin D, Serum Complement component, Direct Coomb Test, LDH. SLE Disease Activity Index will be calculated using the collected details.

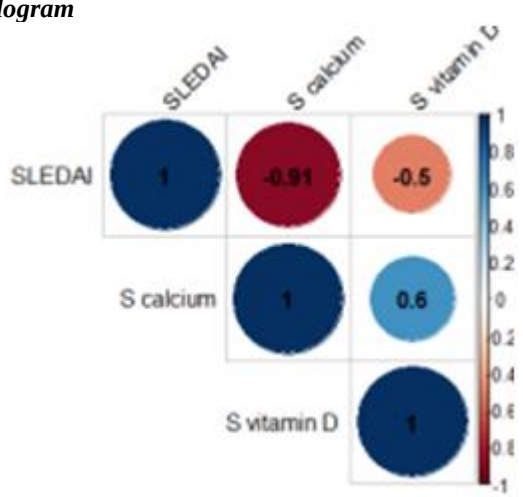
Data analysis

Data will be entered into excel sheets and analyzed using SPSS 27 Software. All quantitative variables will be expressed as mean and standard deviation and all categorical variables will be expressed as proportion. The correlation of total serum calcium level with SLEDAI rating, complement C3, complement C4 and albumin, will be analyzed by Pearson correlation coefficient/ Spearman rho correlation. The value of P <0.05 will be considered as statistically significant.

Ethical Concerns

The study will be started only after getting clearance from the Human Ethics Committee, Government Medical College, Thiruvananthapuram, and informed consent will be obtained from study participants, confidentiality of the participants will be maintained and no financial burden will be incurred upon study participants. All expenses including the laboratory investigations done other than routine investigations if any will be paid by the primary researcher.

Corellogram



The Pearson correlation coefficient for serum calcium and SLEDAI was - 0.905, with a p value of <0.001, indicating a statistically significant negative association. Vitamin D and SLEDAI was also negatively correlated with a correlation coefficient of - 0.5 (p value<0.001). SLEDAI and serum C3 also exhibited a negative correlation of - 0.69.

Correlation Analysis

Correlation between S Calcium Vitamin D and Sledai

S calcium – SLEDAI	Correlation	p value Upper limit	Lower limit
	- 0.905	<0.001 -0.838	- 0.945

S vitamin – SLEDAI	Correlation	t_value df p value Upper limit	Lower limit
	- 0.5	- 4.002 48 <0.001 - 0.258	- 0.683

5. Discussion

The study was conducted among 50 SLE patients who were either under treatment from Govt Medical college Trivandrum. Those patients fulfilling the SLICC revised

ACR diagnostic criteria were included in the study and data was collected over a one year period.

The mean age of the sample was 33 (SD - 8.14).50 % of the population was between age group of 30 - 40 years. According to study conducted by Singh et al [76], the onset of SLE is in the childbearing years – after menarche and before menopause, with breastfeeding offering a protective action.

100 % of study subjects were females. This data is in accordance with majority of studies conducted. The extreme sex preponderance is explained by various hormonal effects –estrogen modulating lymphocytes and plasma dendritic cell activation, and high level of Prolactin in their serum [24]. . It was also observed that many autoimmune diseases including lupus are gender biased, with females outnumbering males with a ratio of 9: 1.[77]

4 % of the sample had a family history of autoimmune disease. According to study by owen et al 30 % population had a positive family history of Lupus. Till recently over 100 SLE susceptibility loci have been identified, majority of which belong to alleles in the Major Histocompatibility Complex (MHC) region complex.[80] According to Raychaudari et al, SLE being a multifactorial disease an individual's risk for SLE development cannot be estimated using genetic factors alone[81]

5 % had a history of passive smoking. According to a cross sectional study conducted by Warren et al Among patients with SLE 35.4% (n=35 of 99) were smokers (with an average of 7 cigarettes/day for 24 years). Smokers had increased prevalence of malar rash (OR 3.40, 95% CI 1.23 to 9.34) and mucosal ulcers (OR 3.31, 95% CI 1.36 to 8.05). This is attributed to the raise in Interferon Gamma and B cell activating factors (BAFF) among them.[81]

The prevalence of hypothyroidism in the selected population was 2 %. According to Jing Yi et al, SLE patients had higher prevalence of hypothyroidism (9.10%) compared to control group and the prevalence decreases with age.[82]

The selected sample population had a mean height of 156cm (SD=5.95), mean weight of 64.4 kg (SD=5.66). The average BMI of the sample was 18.44kg/m^2 (SD=1.55). According to Sarah et al e it was observed that 85% significantly of SLE patients were obese.[85]reason attributed being long term steroid intake.

20% of patients had fever at the time of presentation The distribution of other manifestations were skin rash (10%), oral ulcer (22%), photosensitivity (7%) and Alopecia (11%). 26% of the study sample had joint involvement in the form of arthralgia (16%) and joint effusion (10%).

According to systematic meta - analysis by Beatrice et al the degree of articular manifestation can range from minor arthralgia without erosions or deformity to erosive articular involvement and severe functional disability.3[4] Upto 90 % of patients with SLE experience joint symptoms during their follow up visits. In a study conducted by Hassan et al [34]the distribution of cutaneous manifestation were oral ulcers (69.8%); malar rash (65.6%); photosensitivity rash (53.1%), and discoid rash erythematous (21.9%)

The distribution of patients with serositis were Pleural effusion (14%) and pericardial effusion (4%). Pleuritis develop in 50% patients with SLE.25% of SLE patients at disease onset or during relapse can present with pericarditis which is the most common cardiac manifestation.40 % of patients can present as asymptomatic pericardial effusion. According to study conducted at Hopkins Lupus Cohort 43% had pleurisy and 22% had pericarditis as their initial presentation. Factors which were associated with both pleurisy and pericarditis according to study was pulmonary hypertension, fever, pulmonary fibrosis, haemolytic anaemia, lymphadenopathy, Raynaud's syndrome and anaemia.[87]6 % of patients in our study had a history of coronary artery disease whereas 4 % had interstitial lung fibrosis. Age correlation was not studied. The risk ratio of CAD in patients with SLE was 3.39.8[9]According to Abrahamowicz, the risk for atherosclerosis and CAD is 9 - 50 times higher in SLE patients compared to general population. Another characteristic feature is the younger age of presentation.[90.]

10% of the patients had neurological manifestation at the time of presentation Cerebrovascular accident (2%), Depression (2%), Mononeuropathy (2%), Psychosis (2%), seizur e (2%) were seen. None of patients had ocular manifestations at the time of presentation. According to Bertsias et al 30 - 40% of patients with systemic lupus erythematosus (SLE) develop neuropsychiatric events during the course of their disease. Patients with SLE have 2 fold higher risk of developing stroke compared to general population.[88]. The pathogenic mechanisms can be quite varying ranging from vasculitis, accelerated atherosclerosis, amyloid angiopathy, venous stroke and intracerebral haemorrhage.

About 72 % of the population was having a Haemoglobin <10mg/dl. The mean Haemoglobin was 9.40 (SD=1.43).46 % of the study sample had Iron deficiency.6 % of the subjects had an elevated serum ferritin. According to National Family Health Survey (NFHS) and District Level Household Survey (DLHS) the prevalence of anemia among women in Kerala was around 30%.[86] According to study by Giannouli (2006) anemia is the most common haematological manifestation of SLE patients. Prevalence of Autoimmune haemolytic anemia, defined by DCT positivity was 38%.

According to Lang et al antierythrocyte antibodies against band 3 are present in SLE resulting in warm - type autoimmune haemolytic anemia with a prevalence of 30%[90] 8% of the study population had leukopenia, defined as a total WBC count <3000. According to Rivero et al leukopenia is common in SLE with a prevalence ranging 20% to 93%, however the WBC count can vary widely with fluctuating course owing to various modalities of drugs used in treatment of SLE.[89.]

36% of the population had thrombocytopenia, defined as Platelet count < 1 lakh. According to study by Reville et al, thrombocytopenia alone is an independent risk factor for prediction of mortality renal manifestation, secondary APS

and neuropsychiatric manifestations. According to Howe et al serum platelet - binding IgG and platelet - associated IgG are increased in SLE patients with thrombocytopenia. Overall 10 - 15% of patients may manifest as thrombocytopenia alone. 22 % had APLA antibody positivity, 18% had history of venous thrombosis at the time of presentation and 12 percent had a prior history of DVT. According to study conducted by Cervera et al the prevalence of secondary APS among SLE patients is 36.8% and screening of them for antibody is necessary.[36] 40% of patients with SLE can have APS antibody positivity and 40% among them have thrombotic manifestations.

The most common renal manifestation according to the study was proteinuria (23%). The other renal manifestation were - pyuria (2%), urinarycast (9%), haematuria (2%), deranged RFT (8%).4% of the population had ESRD, requiring haemodialysis. According to Lawrence et al Lupus nephritis (LN) affects approximately 40% of patients with systemic lupus erythematous (SLE).[86]The study subjects included were having a SLEDAI < 5 with disease well under control.

The distribution of population according to elevated inflammatory markers were –CRP (13%), ESR (50%) and LDH (13%). The level of CRP was found to be higher in SLE patients compared to general population. (Karen et al) [87]Inflammatory markers like LDH, Ferritin and ESR was higher among SLE patients with high disease activity. This finding was similar to our study.

About 10% of the study population had vasculitis at the time of presentation of which 2 were angiogram proven.6 % of the study sample had myositis at the time of presentation. 36% were anti ds DNA positive, whereas 74% were Anti Sm Antibody positive.32% of the study subjects presented with low serum C3 and 4% with low serum C4. . Majority of patients had hypoalbuminemia 60%.

The disease activity of SLE patients were measured using SLEDAI score.44 % of study population had a score <5, indicating the disease activity was well under control.8 % of the population under study had a SLEDAI score of >20, presented as acute flare during the time of sample collection. Further grading of the population via SLEDAI score – Mild (54%), Moderate (20%), severe (26%).[31] The SLEDAI score has been validated, and demonstrated to be reliable and sensitive marker. Ratings range from 0 to 9 for the slight activity of patients with SLE, 10 to 14 for the moderate activity of patients with SLE, and 15 for high activity of patients with SLE.

The mean serum calcium level at the time of presentation was 8.57 (SD=1.67). The prevalence of hypocalcemia in the study was 48%. The pearson correlation coefficient for serum calcium and SLEDAI was - 0.905, with a p value of <0.001, indicating a statistically significant negative association. According to study conducted by Yeqin Sha et al entitled [6]"Total Serum Calcium Level Is Negatively Correlated With Systemic Lupus Erythematosus Activity " showed that serum levels of calcium (P <.001), complement C3 (P <.001), complement C4 (P <.001), and albumin (P <.001) was lower in patients with SLE. A negative correlation was found between serum calcium level and systemic lupus erythematosus disease activity index (SLEDAI) rating. According to Xen qu et al %s of $CD8^+$ T in SLE patients were increased, and SLE patients with hypocalcaemia tend to have an enhanced cellular immunity. This is explained by the elevated levels of serum IL - 2, IL - 10, IL - 6 and IFN - γ in SLE patients with hypocalcemia.[89] Concomitanly several other factors tend to have a role in development of hypocalcaemia like low vitamin D, use of steroids, HCQ and reduced dietary absorption of calcium. Hence further studies are necessary to explore the effect of modulating these factors in changing the disease activity of these patients.

The mean Vitamin D level of the study population was 11.46 (SD=3.68). Majority of the study subjects were vitamin D deficient. According to study by Suzan et al (2007) 57 patients with active SLE were vitamin D deficient. Vitamin D and SLEDAI was also negatively correlated with a correlation coefficient of - 0.5 (p value<0.001). SLEDAI and serum C3 also exhibited a negative correlation of - 0.69.

Vitamin D deficiency is highly prevalent around the world including those countries with abundant sunshine.[71]. According to the study by Carlberg[62] Vitamin D mediated signalling results in chromatin remodelling as well as various epigenetic changes that modulates the release of various cytokines. The reduction of IL - 1β, which is induced via inflammasome activation results in suppression of immune activation. Vitamin D deficiency has been associated with B cell proliferation and various autoantibody generation in autoimmune diseases such as Rheumatoid Arthritis (RA), Multiple Sclerosis (MS), and Systemic Lupus Erythematosus (SLE) [69]In a systematic review conducted by Islam et al. on the immunomodulatory actions of diet and nutrients in SLE patients it was observed that supplementation of Vitamin D in SLE patients have found to alleviate symptoms such as fatigue and exercise intolerance. Meta - analysis conducted by Marjoire et al observed that SLE patients were found to have a low Vitamin D compared to healthy control subjects. Vitamin D inhibit cytokines like IL - 10, IL - 17, IFN - γ, and exert an inhibitory role in the proliferation of B - cells, Th1, Th17and CD4+ T helper cells.

According to National Health and Nutrition Examination Survey (NHANES) 2005 to 2006, 41.6% of adult participants (≥20 years) had 25 - hydroxyvitamin D (25 [OH]D) levels below 20 ng/ mL and it is estimated that the prevalence of hypovitaminosis D is on the rise globally. SLE patients can have renal involvement during course of the disease and subsequently 1 - Alpha hydroxylation of vitamin D into its active form is lost or significantly reduced. According to study by Sumethkul et al.6[7]found that patients with lupus nephritis had significantly lower vitamin D levels compared to SLE patients without renal involvement, suggesting that nephritis is a significant predictor of vitamin D deficiency among SLE patients. Medications used for treatment of SLE can have an impact on Vitamin D levels. Data on hydroxychloroquine (HCQ) are rather inconclusive indicating the need for further studies to prove the association. Long term corticosteroid use decrease intestinal absorption and accelerates catabolism of 25 (OH) D and 1,

25 (OH) 2D through an increase in 24a - hydroxylase activity aggravating D deficiency status.

According to study by Gholamrezaei et al. vitamin D was found to have a role in sleep quality of SLE patients. Vitamin D supplementation in SLE Vitamin D supplementation is hence a good clinical practice in patients with rheumatic disease, osteoporosis prevention[23]. However studies to understand the dose of Vitamin D to exert its immunomodulatory effects is yet to be conducted. According to the two randomised double blind placebo controlled trial by Abou rhai et al it was found that supplementation of the Vitamin is able to reduce disease activity, while other two cohort studies failed to observe any significant variation [70]. According to Aranow et al. it was found that vitamin D3 supplementation up to 4000 IU day failed to reduce the IFN - alpha signature.[71] According to Shahin et al. hypovitaminosis D contributes to ANA antibody production subsequently high serum levels of IL - 23 and IL - 17 and disease activity. Hence further studies are necessary to explore the role of routine supplementation of Vitamin D and change in SLEDAI score.

The total subjects studied were only 50, hence clinical profile may vary. We could not study the age related correlation of clinical profile. Majority of patients had hypocalcemia and all the patients in our study group had low Vitamin D levels hence further studies are required to study the effect of supplements on cellular immunity and disease flare.

6. Conclusion

1) Hypocalcaemia was seen in 48% of study subjects.
2) Vitamin D deficiency was seen in all the study subjects.
3) There was a significant negative correlation between serum calcium and SLEDAI score.
4) There was significant negative correlation between Serum Vitamin D and SLEDAI score
5) Family history of autoimmune disease was seen in 4 % of the study subjects.
6) All the patients in the study were females 7.22 % of the population had secondary APS, with 18% having a history of deep vein thrombosis.
7) 44% of the study population had a SLEDAI score <5, indicating the disease was well under control.26% of the population under study had a SLEDAI >15.
8) The most common haematological manifestation was anaemia.
9) 38% of the population was found to have autoimmune haemolytic anaemia.

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