Effects of Vitamin-D 3 Supplement on the Cognitive Status in Patient with Systemic Lupus Erythematosus with Neuropsychiatric Phenomenon

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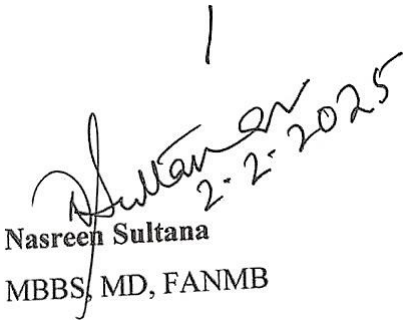
Declaration

I, **Nasreen Sultana**, hereby sincerely declare that the thesis entitled **"Effects of Vitamin-D 3 Supplement on the Cognitive Status in Patient with Systemic Lupus Erythematosus with Neuropsychiatric Phenomenon"** is based on a research work carried out by me under the supervision of **Professor Dr. Md. Saidul Arefin**, Director, Institute of Nutrition and Food Science, University of Dhaka, Dhaka and no part of it has been presented previously for any academic degree.



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Certification

The thesis entitled **"Effects of Vitamin-D 3 Supplement on the Cognitive Status in Patient with Systemic Lupus Erythematosus with Neuropsychiatric Phenomenon"** has been completed sincerely and satisfactory by Dr. Nasreen Sultana, Session: 2020-2021, Re-registration no: 6., enrolled in the Institute of Nutrition and Food Science, University of Dhaka, Bangladesh, for the degree of Doctor of Philosophy (Ph.D), is an original research work and record and was supervised by us can be submitted to the examination committee for evaluation.

To the best of our knowledge, no part of the work has been submitted for any other degree or qualification in any other institute.

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Nasreen Sultana

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Dedicated To

My Father Professor Md. Amir Hossain &

My Mother Dr. Nurun Nahar Begum

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LIST OF ABBREVIATION

SLE: Systemic lupus erthythematosus

NPSLE: Neuropsychiatric Systemic lupus

erthythematosus

ECD: Ethyle cystinate dimer

MMSE: mini mental state examination

NINMAS: National Institute of Nuclear Medicine &

Allied Sciences

e ZIS Eazy Z score imaging systemsm Ci Milli curie

SPECT: Single Photon Emission Computed

Tomography

INFS: Institute of Nutrition & Food Sciences

BS DICOM data: Brain SPECT DICOM Data

CONSORT: Patients allocation following Consolidated Standards of Reporting Trials

ABSTRACT

Background

Systemic Lupus Erythematosus (SLE) is a complicated autoimmune illness that affects several organ systems, including the central nervous system. Neuropsychiatric (NP) SLE (NPSLE) is characterized by neurological and psychiatric symptoms such as cognitive dysfunction, mood problems, and seizures, and has been linked to severe impairments in memory, attention, and executive functioning can lead to altered brain perfusion. Vitamin D3 has shown promise in neuro-protection and cognitive improvement, which may benefit NPSLE patients. This study investigates the effects of Vitamin D3 supplementation on cognitive function and brain perfusion in NPSLE patients.

Material & Methods

This randomized controlled trial (RCT) included NPSLE patients (N=72), divided into an intervention group (n=34) receiving Vitamin D3 supplementation, and a control group (n=38) without supplementation. Baseline and six-month evaluations of serum Vitamin D levels, Mini-Mental State Examination (MMSE) scores, and brain perfusion using Single-photon emission computed tomography (SPECT) imaging (Z-scores) were conducted at National Institute of Nuclear Medicine & Allied Science (NINMAS), Dhaka. The intervention group received 40,000 IU of Vitamin D3/week for six weeks, followed by a maintenance dose of 2000 IU/day for three months, along with standard SLE management. Both verbal and written informed consent was obtained, and adherence was encouraged through weekly reminders via text or calls. After six months, outcomes were analyzed to assess the impact of Vitamin D3 on cognitive function and brain perfusion in NPSLE patients.

Results

Baseline characteristics (age, gender, BMI, and waist-to-hip ratio/WHR) were indifferent (P>0.05) between groups (Control vs. cases). No significant differences (P>0.05) were found between groups in age (28.24±7.18 vs. 26.32±7.94,), gender distribution (predominantly female), BMI (19.0±1.4 vs.19.2±2.0,), and waist-to-hip ratio (0.78±0.11 vs. 0.76±0.10). Moreover, at baseline, vitamin D levels were low and similar across both groups (14.5±5.3 ng/ml vs. 16.2±4.9, p=0.173). After the study period, a significant (p<0.001) increase in vitamin D3 levels (28.3±5.3 ng/ml) was observed in the intervention group as compared to controls (15.1±3.4 ng/ml), indicating effective supplementation. Moreover, at baseline, MMSE scores were similar (24.1±1.7 vs. 24.3±1.5) between groups (P=0.677) while at the end line, the intervention group (26.5±1.4) showed a significant improvement in MMSE scores than control (23.8±2.25; p<0.001). This suggests a positive effect of Vitamin D3 supplementation on cognitive function. Brain perfusion was analyzed using SPECT imaging techniques (Z-scores), and at baseline, abnormal perfusion was prevalent in both groups (81.6% vs. 88.2%, P=.522), primarily in the frontal, parietal lobes, and precuneus regions (z-score: 1.4 vs. 1.46, P=0.549). This baseline homogeneity indicates effective randomization and establishes comparable initial conditions between the study groups. However, at the end line, mean z-scores were insignificantly (P=.457) higher in the control group (n=13, z-scores=1.84) than intervention group (n=21, z- scores=1.66) while 65.8% (n=25) of the Brain SPECT of the controls were not available at end line, as compared to cases (n=13). On top of that, significant between-group differences emerged in performing perfusion test results (Fisher's exact test= 6.997, p = 0.028) when considered comparison in missing cases between groups, which indicates among available controls (n=13), eleven

showed abnormal brain perfusion. Furthermore, the intervention group demonstrated distinct patterns of perfusion compared to controls, suggesting a potential effect of Vitamin D3 supplementation on cerebral perfusion parameters. These findings suggest that despite the study groups (intervention vs. control) exhibited indifferent (all P>0.05) vitamin D3 levels, MMSE scores, and perfusion characteristics at the baseline, significant differences emerged between groups especially in the MMSE-scores (small effect size, Partial eta squared=.460 for MMSE between case and. control) attributable to the effective vitamin D3 supplementation (High effect size, Partial eta squared=.714 for Vitamin D, Case vs. control) following the intervention period (6-months).

Conclusion

Vitamin D3 supplementation led to substantial improvements in serum vitamin D levels, cognitive function (MMSE scores), and brain perfusion in the intervention group. These findings support the potential role of Vitamin D3 as an adjunctive therapy in NPSLE, enhancing cognitive performance and cerebral perfusion. Further studies are recommended to validate these results and assess the long-term benefits of Vitamin D3 in managing NPSLE-related cognitive impairment.

Chapter One
Introduction

1.1 Background

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease affecting multiple organ systems, including the central nervous system (CNS). Neuropsychiatric systemic lupus erythematosus (NPSLE) refers to the neurological and psychiatric manifestations of SLE, affecting 15–75% of patients and causing cognitive dysfunction, mood disorders, seizures, and impairments in memory, attention, and executive functions (Schwartz et al., 2019). It affects mostly women of child-bearing age and the clinical courses are unpredictable with periods of remission and flares (Dema & Charles, 2016).

Anti-double stranded DNA (Anti-ds DNA) antibodies, a subset of antinuclear antibodies targeting double-stranded DNA, are linked to hippocampal injury and cognitive dysfunction in SLE patients (Stock et al., 2013). Vitamin D3 deficiency, common in SLE due to reduced sunlight exposure, medication, and renal issues, may exacerbate inflammatory pathways and neuropsychiatric symptoms (Amital et al., 2010; Lima et al., 2016). Low vitamin D3 levels correlate with cognitive dysfunction severity in NPSLE, highlighting its potential as a modifiable factor (Tay et al., 2015).

Vitamin D3, beyond its role in bone health, exhibits neuro-protective properties by supporting neurogenesis and synaptic plasticity via brain vitamin D receptors (VDRs) (Eyles et al., 2014). Vitamin D3 supports cognitive function in NPSLE by reducing inflammation and protecting neuronal health (Cui et al., 2015). It also boosts neurotrophic factors like brain derived neurotrophic factors, BDNF, important for 20 cognitive health (DeLuca et al., 2013). A randomized trial found that SLE patients receiving vitamin D3 experienced improved mood, reduced fatigue, and cognitive improvements due to decreased inflammation (Lima et al., 2016). Additionally, it aids

in producing neurotransmitters like acetylcholine and serotonin, vital for cognitive processes (Pertile et al., 2016).

SPECT brain perfusion imaging helps detect cerebral blood flow abnormalities in SLE patients, showing reduced blood flow in areas like the frontal, temporal, and parietal lobes, which are linked to cognitive and neuropsychiatric symptoms (Zhang et al., 2005). This suggests that SPECT can help assess the effects of interventions, such as Vitamin D3, by tracking changes in brain perfusion. Preliminary evidence suggests that Vitamin D3 supplementation may improve cerebral perfusion in NPSLE patients, enhancing blood flow in affected regions and reducing neuropsychiatric symptoms (DeLuca et al., 2013).

1.2 Rationale of the Study

Vitamin D deficiency is a recognized risk factor for cognitive impairment in the general population and is associated with conditions such as Alzheimer's disease, multiple sclerosis, and systemic lupus erythematosus (SLE). Among SLE patients, those with cognitive dysfunction exhibit significantly lower serum Vitamin D levels compared to those without cognitive issues (Stock et al., 2013). Neuropsychiatric SLE (NPSLE), a severe neurological manifestation, affects approximately 39% of SLE patients (Unterman et al., 2011) and is linked to Vitamin D insufficiency in 16–95% of cases. Previous studies have explored the relationship between Vitamin D levels and SLE-related cognitive dysfunction using case-control, cohort, and retrospective observational designs. However, interventional research addressing the role of Vitamin D supplementation in cognitive dysfunction among NPSLE patients remains limited, especially in Bangladesh. Advanced imaging techniques like brain single-photon emission computed tomography (SPECT) have not been utilized in such contexts. NPSLE leads to significant disability, diminished quality of life, and socio-economic burdens for patients and families. Current therapeutic approaches have limited effectiveness, underscoring the need for innovative strategies. This study aims to evaluate the impact of Vitamin D supplementation on cognitive performance and cerebral perfusion in NPSLE patients using brain SPECT imaging as an objective tool.

If successful, the findings will provide robust clinical and imaging evidence supporting the use of Vitamin D as an adjunct therapy for cognitive dysfunction in NPSLE. This breakthrough could transform patient management, improving outcomes and quality of life while reducing the socio-economic impact. Additionally, it may establish brain SPECT as a valuable modality for monitoring therapeutic responses, contributing to both local and global advancements in managing SLE-associated cognitive dysfunction.

1.3 Objectives

General Objective

To evaluate the effect of Vitamin D3 supplementation on the cognitive status in SLE patients with neuropsychiatric phenomena.

In order to achieve general objective, the study was designed to:

- Collect socio-demographic data of the participants
- Analysis of anti-ds-DNA and anti-phospholipid antibody
- Determine baseline serum Vitamin D status and baseline cognitive impairment status by Neuropsychological tests (NP tests) with MMSE (Mini mental state examination)
- Find out the area of brain perfusion deficit by brain SPECT imaging through the evaluation of Z-score for severity of cognitive impairment both at baseline and end line
- Evaluate the effect of Vitamin D3 intervention on cognitive function after 6-months, by end-line serum Vitamin D status, MMSE and brain SPECT (Z-scores) study.

1.4 Hypothesis

In NPSLE patients with low vitamin D status, a randomized controlled parallel arm study using vitamin D supplements might improve cognitive impairment in the intervention group after around six months of supplementation as compared to the control group.

Chapter Two

Literature Review

2. Literature Review

2.1 Systemic Lupus Erythematosus (SLE)

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disorder that can affect multiple organ systems, including the central nervous system (CNS). Neuropsychiatric manifestations of SLE (NPSLE) are common and contribute significantly to the disease's morbidity and poor quality of life. Symptoms of NPSLE include cognitive dysfunction, memory loss, mood disorders, seizures, and psychosis, which severely impair daily functioning (Hanly et al., 2010). Among these symptoms, cognitive dysfunction is particularly concerning, as it affects memory, attention, executive function, and information processing speed. Given the limited effective treatments available for NPSLE, there is growing interest in exploring potential therapeutic interventions, such as vitamin D3 supplementation, to improve cognitive function and overall neuropsychiatric symptoms in SLE patients. Systemic Lupus Erythematosus (SLE) is significantly more common in women than in men. The female-to-male ratio for SLE is approximately 9:1, with women being disproportionately affected, especially during their reproductive years. Several factors contribute to this disparity, including hormonal, genetic, and immune system differences. Estrogen, a hormone that is more prevalent in women, has been shown to enhance immune responses and may predispose women to autoimmune diseases like SLE. This is supported by studies that have observed higher disease prevalence during periods of high estrogen levels, such as in the reproductive years and during pregnancy (Mok et al., 2012). Additionally, the genetic component plays a role, with women carrying two X chromosomes, which are thought to increase the likelihood of developing autoimmune conditions due to the presence of immune-related genes on

the X chromosome. In contrast, men have only one X chromosome, which may offer some protection against the development of SLE (Aggarwal et al., 2010). The immune system in women also tends to be more active than in men, which can lead to an overactive immune response, increasing the risk of autoimmune diseases. Environmental factors, including hormonal fluctuations triggered by pregnancy and menstrual cycles, are also believed to influence the onset and exacerbation of SLE in women. Research has consistently shown that the majority of SLE cases occur in women of childbearing age, and flare-ups often coincide with hormonal changes, further supporting the link between hormones and disease onset (Bertsias et al., 2012)

2.1.1 Factors behind Systemic Lupus Erythematosus (SLE)

Systemic Lupus Erythematosus (SLE) is a complex, chronic autoimmune disease that involves various mechanisms in its pathogenesis. It is characterized by a hyperactive immune system that produces autoantibodies against self-antigens, leading to inflammation and damage in multiple organ systems. The exact cause of SLE remains unclear, but it is believed to result from a combination of genetic, environmental, and hormonal factors.

Genetic Factors*:* A genetic predisposition plays a significant role in the development of SLE. Studies have shown that several genes, particularly those associated with immune regulation, such as the human leukocyte antigen (HLA) complex, have been linked to SLE susceptibility. For instance, HLA-DR2 and HLA-DR3 alleles are more commonly found in SLE patients, suggesting that these genetic variations increase susceptibility to the disease (Graham et al., 2007). Additionally, mutations in genes responsible for regulating the immune system, such as the complement system and various cytokine pathways, contribute to the pathogenesis of SLE (Lahita et al., 2010)

Environmental Triggers: Environmental factors such as ultraviolet (UV) light, infections, and drugs can act as triggers for SLE in genetically predisposed individuals. UV light, for example, can induce the release of autoantigens from the skin, promoting an autoimmune response. Infections, particularly viral infections like Epstein-Barr virus (EBV), have been implicated in triggering SLE flares due to their ability to stimulate B-cell activity and increase (Bertsias et al., 2012) the production of autoantibodies (Bertsias et al., 2012).

Immune System Dysregulation: In SLE, there is a loss of immune tolerance, resulting in the production of autoantibodies against self-antigens. These autoantibodies, such as anti-dsDNA, anti-Sm, and anti-nucleosome antibodies, form immune complexes that deposit in various tissues, leading to inflammation and tissue damage. B-cells, T-cells, and dendritic cells play central roles in this dysregulated immune response, with a notable increase in type I interferon (IFN) activity, which contributes to the activation of the immune system (Lahita et al., 2010). Additionally, impaired clearance of apoptotic cells leads to the accumulation of autoantigens, further promoting immune activation.

2.2 Pathogenesis of Neuropsychiatric Systemic Lupus Erythematosus (NPSLE)

Neuropsychiatric (NP) Systemic Lupus Erythematosus (NPSLE) refers to the neurological and psychiatric manifestations observed in SLE patients, including cognitive dysfunction, seizures, psychosis, and mood disorders. These symptoms, seen in approximately 25–75% of SLE patients, arise from a combination of systemic autoimmune activity and central nervous system (CNS) involvement. The pathogenesis of NPSLE is multifactorial, involving immune-mediated damage, vascular abnormalities, blood-brain barrier dysfunction, neuroinflammation, and cerebral perfusion deficits.

***Immune-mediated Damage**:* In NPSLE, autoantibodies, including anti-ribosomal P antibodies, anti-NMDA receptor antibodies, and anti-phospholipid antibodies, have been implicated in the pathogenesis of neurological manifestations. These autoantibodies can target specific receptors and proteins in the brain, leading to neuronal damage. For example, anti-NMDA receptor antibodies can interfere with neurotransmitter signaling, resulting in cognitive and psychiatric symptoms (Hughes et al.,2010).

***Cerebral Vascular Involvement**:* Anti-phospholipid antibodies, which are commonly present in SLE patients, increase the risk of thrombosis, which may lead to cerebral ischemia or infarction. This can result in cognitive dysfunction, seizures, and stroke like symptoms, which are common in NPSLE (Bertsias et al., 2012). The presence of these antibodies disrupts the normal clotting cascade and promotes endothelial damage, leading to thrombotic events in the CNS.

***Blood-Brain Barrier Dysfunction**:* Inflammation and immune complex deposition in the CNS can lead to dysfunction of the blood-brain barrier (BBB). The BBB typically protects the brain from immune cell infiltration, but in NPSLE, this protective barrier is compromised. This allows immune cells and inflammatory mediators, such as cytokines, to enter the brain and cause neuronal damage and neuroinflammation, contributing to NPSLE symptoms (Duarte-Delgado et al., 2019).

***Non-inflammatory and Neuro-degeneration**:* Chronic inflammation, particularly due to the increased production of type I interferons, plays a role in the neurodegenerative processes seen in NPSLE. Elevated levels of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), are found in the cerebrospinal fluid (CSF) of NPSLE patients, suggesting that neuro-inflammation is a

key factor in the development of neuropsychiatric symptoms (Hirohata & Kikuchi, 2021). This inflammation can lead to neuronal death, synaptic dysfunction, and cognitive decline.

2.3 Cognitive dysfunction

Cognitive dysfunction in Neuropsychiatric Systemic Lupus Erythematosus (NPSLE) is a significant and often debilitating manifestation of the disease. It can affect various aspects of cognitive functioning, including memory, attention, executive function, and processing speed. These cognitive impairments are common in NPSLE patients and can severely impact their quality of life. The key areas of cognitive dysfunction seen in NPSLE are described below.

2.3.1 Memory Dysfunction: Memory impairment is one of the most common cognitive disturbances in NPSLE. Patients often experience difficulties with both short-term and long-term memory, which can manifest as forgetfulness, difficulty in recalling recent events, and problems with learning new information. Studies have shown that impaired verbal memory is particularly prevalent in NPSLE patients, and it is thought to be associated with cerebral ischemia, neuro-inflammation, and hippocampal dysfunction (Shapira-Lichter et al., 2013). Memory deficits can significantly interfere with daily life and the ability to manage routine tasks.

2.3.2 Attention Deficits: Attention deficits are another hallmark of cognitive dysfunction in NPSLE. Patients may experience difficulties in focusing, maintaining attention, and shifting attention between tasks. This can lead to poor task performance, difficulty concentrating, and decreased ability to multitask. Attention deficits have been found to correlate with structural changes in the brain, including reduced gray matter volume in areas like the prefrontal cortex, which is responsible

for attention regulation (Jung et al., 2010). These impairments may result from both autoimmune-mediated damage and vascular changes in the brain.

2.3.3 Executive Functioning Impairment: Executive functions, such as planning, decision-making, problem-solving, and cognitive flexibility, are often impaired in patients with NPSLE. These functions are mediated by the prefrontal cortex, and dysfunction in these regions can result in difficulties in organizing tasks, initiating activities, and regulating emotions. Cognitive flexibility, the ability to adapt to new situations, is frequently affected in NPSLE, leading to inflexible thinking and difficulties in adjusting to changes in the environment (Wu et al., 2018). These impairments can contribute to the overall cognitive decline observed in NPSLE.

***2.3.4 Processing Speed*:** Cognitive processing speed refers to the ability to quickly and accurately process information. In NPSLE, patients may experience slowed cognitive processing, which can affect tasks that require rapid decision-making or the ability to complete tasks in a timely manner. Processing speed is often assessed through tasks like timed recall or response time in reaction tests, and it is typically found to be impaired in NPSLE patients. Slower processing speeds are associated with disruptions in brain areas such as the frontal lobes, which are integral to higher cognitive functions (Shucard et al., 2004)

***2.3.5 Mood and Psychiatric Symptoms*:** Cognitive dysfunction in NPSLE is often accompanied by psychiatric symptoms, including depression, anxiety, and psychosis. These mood disturbances can further exacerbate cognitive decline by affecting concentration and memory. Depression, in particular, is commonly observed in NPSLE patients, and it has been shown to be associated with poor cognitive performance, especially in tasks involving executive function and memory (Antypa et

al., 2021). Psychiatric symptoms may also mask or complicate the assessment of cognitive dysfunction.

2.4 Vitamin D and Neuropsychiatric Systemic Lupus Erythematosus (NPSLE): A Summary

Vitamin D, a vital fat-soluble vitamin and pro-hormone steroid, plays essential roles in bone health, immune regulation, and cardiovascular health. Recent research highlights its significance in autoimmune diseases, particularly Neuropsychiatric Systemic Lupus Erythematosus (NPSLE). NPSLE encompasses a range of neurological and psychiatric symptoms associated with systemic lupus erythematosus (SLE), a chronic autoimmune condition. These symptoms include cognitive dysfunction, mood disorders, seizures, and psychosis, often attributed to immune dysregulation, neuro-inflammation, and vascular abnormalities (Sultana et al., 2022).

2.4.1 Role of Vitamin D in Autoimmune Disorders and NPSLE

Vitamin D deficiency is linked to autoimmune diseases, including SLE. Mechanisms by which Vitamin D may mitigate NPSLE symptoms include:

2.4.1.1 Immune Regulation: Vitamin D enhances anti-inflammatory cytokines (e.g., IL-10) and inhibits pro-inflammatory cytokines (e.g., IL-17, IL-6), reducing autoimmune neuro-inflammation in NPSLE

2.4.1.2 Neuro-protection: By regulating calcium homeostasis and promoting neural cell survival, Vitamin D helps counter neuronal damage associated with NPSLE.

2.4.1.3 Endothelial Health: Vitamin D promotes endothelial function, enhancing nitric oxide production and reducing vascular inflammation, potentially improving

cerebral blood flow.

2.4.1.4 Reduction of Oxidative Stress: Its antioxidant properties counteract oxidative stress, which contributes to neuronal damage in NPSLE.

2.4.1.5 Impact on Neurotransmitters: Vitamin D influences serotonin and dopamine synthesis, addressing mood and cognitive disturbances common in NPSLE.

2.4.2 Vitamin D3 Supplementation and Cognitive Function in NPSLE

Cognitive impairment, characterized by deficits in memory, attention, and executive function, is common in patients with neuropsychiatric systemic lupus erythematosus (NPSLE) (Hussein et al., 2018). Vitamin D deficiency has been linked to cognitive dysfunction in lupus patients (Tay et al., 2015), as well as being associated with the neuropsychiatric manifestations of lupus (Sultana et al., 2022). Some study observed in animal models of lupus; supplementation with vitamin D improved cognitive function and increased VDR expression in the hippocampus (Yan et al., 2019) . Additionally, vitamin D may delay immune cell infiltration in the choroid plexus and reduce biomarkers linked to cognitive decline in MRL/l pr mice (Li et al., 2023). Sultana et al., (2022) suggest that reduced vitamin D-binding protein expression in patients' serum and hypo-vitaminosis D may precede cerebral hypo-perfusion in NPSLE, indicating potential prophylactic implications. These benefits are attributed to vitamin D3’s anti-inflammatory effects, its role in reducing oxidative stress, improving vascular health, and regulating calcium homeostasis- all of which are critical in mitigating NPSLE related cognitive dysfunction.

2.5 Diagnosis of Vitamin D Deficiency in NPSLE

Vitamin D deficiency in NPSLE is diagnosed by measuring serum 25-hydroxyvitamin D [25(OH) D] levels. The chemiluminescence immunoassay (CLIA) is a reliable diagnostic tool, with reference ranges categorized as normal **(**>30 ng/mL), insufficient (20–30 ng/ml), and deficient (<20 ng/mL). Vitamin D deficiency is prevalent in NPSLE and associated with worsened neuropsychiatric symptoms, immune dysregulation, and cardiovascular complications. Vitamin D plays a pivotal role in mitigating NPSLE symptoms by modulating immune responses, protecting neural cells, and improving cerebral perfusion. Addressing vitamin D deficiency through supplementation and lifestyle changes may serve as a therapeutic adjunct for managing NPSLE. Further research is essential to fully elucidate its benefits and establish evidence-based guidelines for treatment.

2.6 Neuroimaging Findings

Neuroimaging studies, including brain MRI and SPECT scans, have shown abnormalities in patients with NPSLE. These studies often reveal white matter lesions, cerebral atrophy, and reduced perfusion in areas associated with cognition, such as the frontal and temporal lobes. These changes are thought to reflect the underlying neuro-inflammation and vasculopathy that contribute to cognitive dysfunction in NPSLE (Vacca et al., 2024). Brain SPECT, for instance, often shows abnormal perfusion patterns in NPSLE patients, which can correlate with cognitive deficits, particularly in the frontal and parietal lobes.

Chapter Three

Methodology

3. Methodology

3.1 Study design: Randomized controlled parallel arm trial

3.2 Place of the study: National Institute of Nuclear Medicine & Allied Sciences (NINMAS), Dhaka.

3.3 Study period: March 2021 – November 2024

3.4 Study population:
Confirmed diagnosed cases of NPSLE (according to American College of Rheumatology Guideline).

3.5 Study sample: Confirmed diagnosed cases of NPSLE (according to the American College of Rheumatology nomenclature and case definition for NPSLE, 1999).

3.6 Sampling technique: Purposive

3.7 Sample Size: Using the following formula (Altman et al., 2000) the calculated sample size was N=100 (50+50), a total of 50 random numbers, with a minimum value of 1 and a maximum value of 100, were determined using an online random number generator. All patients whose serial numbers matched the predetermined random numbers were included in case group. The remaining patients were included in the control group.

3.7.1 Sample size calculation:

$$n = \left(\frac{2(z_{power} + z_{1-\alpha})}{\frac{2(\mu_1 - \mu_2)}{\delta}} \right)^2$$

Sample size (n): two equal sized subgroups (n1 and n2),

$n^1 = n^2$. Post treatment Z score between the two

populations is taken as μ1- μ2.

Mean difference of post treatment brain perfusion Z score among two subgroups is assumed to be 0.5.

Standard deviations for brain perfusion Z score assumed to be same (δ1= δ2= δ) with an assumed value of 0.63.

The power is assumed 80% i.e. 0.8, thus z 0.8 = 0.8416.

Level of significance α is assumed 2.5% thus Z1- α i.e. Z0.975 = 1.96.

$$\text{The total sample size} = \left(\frac{2(0.8416 + 1.96)}{\frac{0.5}{0.63}} \right)^2 = 49.78 \approx \mathbf{50}$$

Thus, the each group shall consist of 50 patients.

3.8 Ethical Permission:

Ethical approval has been taken from Biological science department of University of Dhaka (**Reference No: 119/Biol. Scs. February 04, 2021**). The study also registered as a clinical trial with—University hospital Medical Information Network (UMIN) Center, Japan‖ which is the largest and most versatile academic network information center for biomedical sciences in the world (**UMIN Clinical Trial Registry No: UMIN0000 56299**).

3.9 Selection Criteria

The inclusion criteria for an NPSLE study are adults aged 18-50 with a confirmed diagnosis of Systemic Lupus Erythematosus (SLE) based on American College of Rheumatology (ACR) guidelines (The American College of Rheumatology, 1999) exhibiting neuropsychiatric symptoms like cognitive dysfunction, mood disorders, seizures, psychosis, or other neurological issues linked to SLE. Diagnosis should be supported by relevant autoantibodies (e.g., anti-dsDNA, anti-phospholipid). Participants must have had SLE for at least six months and provide informed consent.

3.9.1 Exclusion Criteria

- Current use of vitamin D3 or calcium supplement.
- Current or past diagnosis of cognitive impairment or dementia due to known brain disorders
- Use of certain drugs such as corticosteroid, hormone replacement, psychoactive drugs
- Pregnancy and Lactation state

3.10 Study variables

Independent Variables	**Dependent Variables**
● Gender ● Age ● BMI (kg/m^2) ● Waist to Hip ratio (WHR)	● Lab Variables : ◆ Aniti-ds DNA antibody ◆ Anti-Phospholipid antibody ◆ Vitamin D level (ng/ml) ● MMSE-score Values ● Imagining Variables ◆ Cerebral blood flow at imaging areas ◆ Z-score values

3.11 Feasibility of the study

This study is feasible to be done with existing facilities and human resources available in National Institute of Nuclear Medicine and Allied Science (NINMAS), Bangladesh Medical University Campus, Shahbag, Dhaka, Bangladesh.

3.12 Data Collection:

- Clinical record forms were used to collect participant information.
- The objectives and procedures of the study were thoroughly explained to the study subjects, and informed written consent was obtained from each participant. Confidentiality of participant information was strictly maintained throughout the study, and participants were assured of their right to withdraw from the study at any time without any consequences.

3.13 Patients allocation following Consolidated Standards of Reporting Trials (CONSORT)

We set up a list of items from existing quality assessment and reporting tools, with Consolidated Standards of Reporting Trials guidelines. CONSORT flow diagram (Figure 1) of the progress through the phases of a parallel randomized trial of a few groups (i.e. enrolment, intervention allocation, follow-up, and data analysis) (**URL: Consort- statement.org/consort-statement/flow-diagram**).

3.14 CONSORT-Steps (Figure 1)

- Patients were included according to inclusion and exclusion criteria and assigned serial numbers of regular counting orders from 1 to 100.
- A total of 100 random counting numbers with a minimum value of through a maximum value of 100 are determined (13, 29, 48) from an online random number generator **(URL: calculator.com/statistics/random number generator 1-100).**
- All those patients (n=34) with their serial number matched with *a* pre-determined random number are included in (Intervention group case). The remaining 38 patients are included in control group.

Diagrammatic presentation of sampling procedure and technique

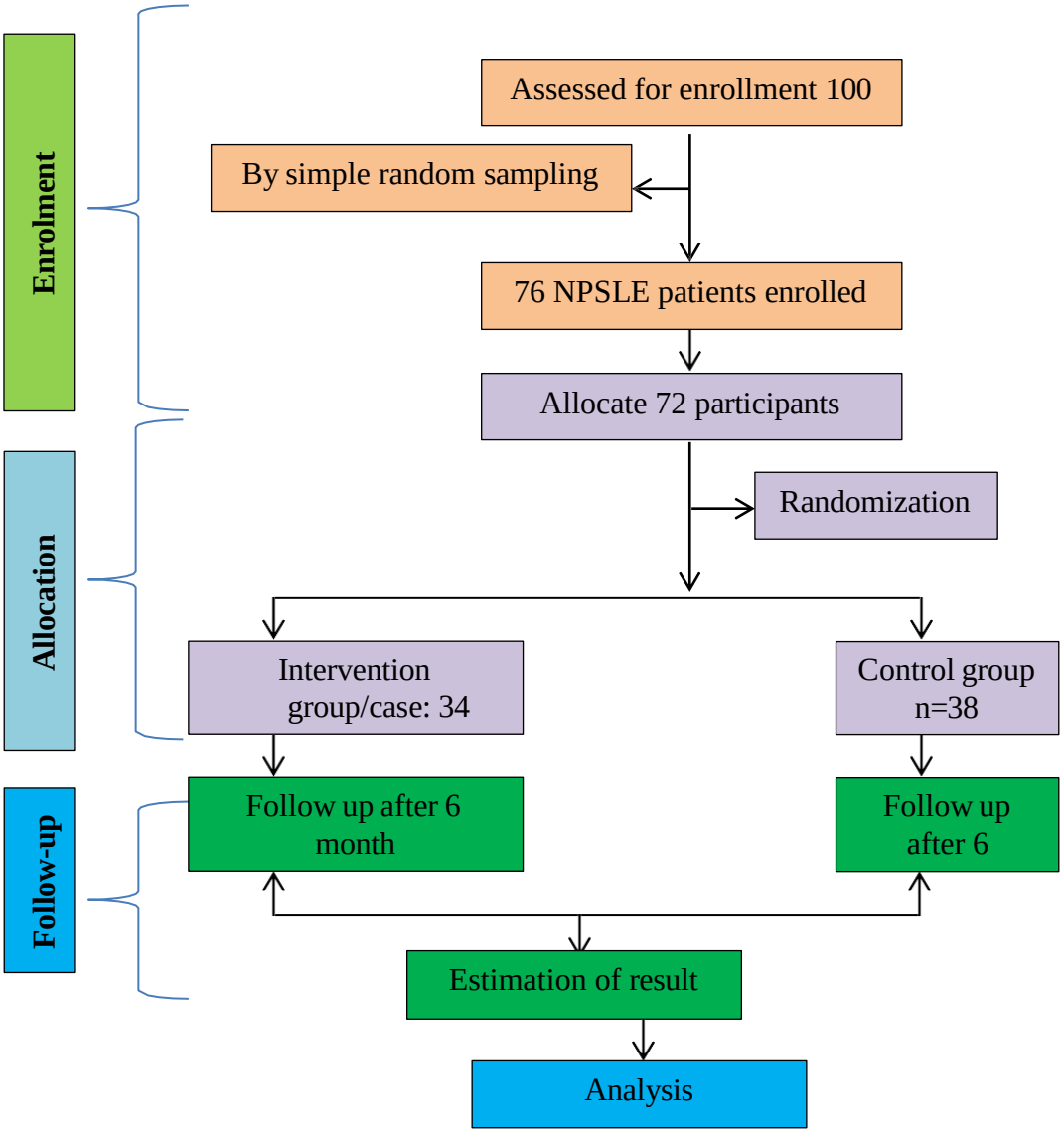

Figure 1: CONSORT flow diagram

3.15 Study Procedure

- Random selection of NPSLE, as per sampling frame (n= n1+ n2).
- Verbal/written invitation of study participant with trial information.
- Baseline evaluation by physical examination, laboratory investigations (vitamin D, anti-ds-DNA antibody, and anti-phospholipid antibody), neuroNP tests (MMSE score) and Brain SPECT with Z scores.
- Intervention Group/Case: Participants received 40,000 IU Cholecalciferol weekly for 6 weeks, then 2,000 IU daily for 3 months, along with standard SLE treatment.
- Instructions: Take the tablet on a fixed day with a main meal.
- Adherence: Weekly reminders via text or phone.
- Post-6 Months: Reassessment of vitamin D levels, MMSE score, and Brain SPECT with Z scores among the study population (Case & control).

3.16 Assessment of Serum Vitamin D

The serum level of Vitamin D (Vit-D) was determined in each patient within one month of undergoing Brain SPECT (BS). The Quantitative chemiluminescent immunoassay (CLIA) method was used for non-specific detection of both the free forms of Vit-D in the serum as well as the bound forms after they got dissociated from the binding protein during the incubation. According to the laboratory reference in NINMAS, a level of 30-100 ng/ml was categorized as sufficient, less than 30 up to 20 ng/ml as insufficient, and less than 20 ng/ml as deficient.

3.17 Assessment of cognitive function by the clinician

All patients also had undergone an assessment of cognitive function (Folstein et al., 1975; Kurlowichz and Wallace, 1999) within a month before undergoing the BS with the mini-mental state examination (MMSE), a clinician-administered 30-point questionnaire for the assessment of cognitive impairment (CI). A score of 30 to 25 was clinically categorized as No CI, 24 to 20 as mild CI, 19 to 10 as moderate CI, and lower than 10 as severe CI. The neuropsychiatric symptoms or a diagnosis in each patient were available from the rheumatologists' note which was done by some other physicians belonging to the specialty of neurology or psychiatry. The operational criteria for neuropsychiatric evaluation were unknown to the authors.

SPECT data acquisition and reconstruction

A pre-imaging consultation session was attended by each patient along with his or her caregiver. The purpose was an assessment of the patient's ability to comply and cooperate during the BS imaging procedure, verbal communication regarding patient preparation and imaging procedure, documentation of clinical data, and obtaining the patient's consent. The requirement to refrain from caffeine and alcohol on the day of the test was explained and consumption of other drugs with known effects on cerebral blood flow was checked. On the day of the test, the patient was instructed to void, and shortly thereafter they had an intravenous line in place about 10 to 20 minutes before the administration of radiotracer. Each patient got an intravenous injection of 600 MBq of Tc-99m-ethyl cysteinate dimer (ECD) while reclining or sitting comfortably in a dimly lit, quiet, well ventilated space with indoor air temperature around 25ºC. The patients received specific instructions to keep their eyes and ears open and refrain from speaking or reading. The resting phase or uptake phase lasted for 30 minutes

from the injection of the radiotracer. Thereafter, BS was performed with dual-headed gamma camera (Symbia Evo Excel, Siemens, USA) equipped with low-energy and high-resolution parallel-hole collimators. For each camera, projection data were obtained in a 128 x 128 matrix through 360° rotations at steps of 2.8° for 20 seconds per view. Filtered back-projection and Butterworth and Ramp filter was used for SPECT image reconstruction.

3.18 Derivation of z-score from BS DICOM data

The easy z-score imaging system (eZIS) (Matsuda et al., 2007) is free software published under the Asia Oceania Research Initiative Network (AORIN). Brain was utilized to generate 3D surface images of the brain as well as z-scores from each patient's BS DICOM data. The 3D surface images were visually evaluated to determine the locations of hypo-perfusion.

4.0 Statistical Analysis

Data were analyzed by Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA, version 27). Descriptive statistics were employed and values were expressed as frequency, percentage, mean and standard deviation as and where necessary for qualitative and quantitative variables. Moreover, cross-classification-analysis (א2-test or Fisher's exact Test) have been used to examine possible associations between categorical variables. Fisher's exact test, which is more robust for small sample size, verified the results found from Chi-Square tests. Moreover, in this study, advanced statistical quantitative analysis like two-ways repeated measure ANOVA (GLM/general linear model) was performed to assess the variation over 'Time' between 'Intervention and the Control group'. Additionally, for the measurement of 'Effect sizes' and significant 'Mean changes' of some parameters (Vitamin D3, MMSE-scores, and SPECT Imaging Z-scores) within timelines (within group

comparison for either baseline or end line), and between timelines (Intervention Versus Control) following 6-months of Vitamin D3 supplementation. 'Pair sample t-tests' were also used where necessary for sub-group analysis or for the assessment of Time*Group interaction. P-value <0.05 was set as significance.

5.0 Operational Definition of Terms

- **Neuropsychiatric Systemic Lupus Erythematosus (NPSLE)**

Neuropsychiatric systemic lupus erythematosus (NPSLE) refers to a set of neurological and psychiatric manifestations observed in patients with systemic lupus erythematosus (SLE), attributed directly to immune-mediated damage. NPSLE encompasses a broad spectrum of clinical presentations that affect the central and peripheral nervous systems, including cognitive dysfunction, seizures, psychosis, mood disorders, cerebrovascular disease, peripheral neuropathy, and headaches. The American College of Rheumatology (ACR) (The American College of Rheumatology, 1999) has classified these into 19 syndromes, covering conditions such as acute confusional states, anxiety, depression, cerebrovascular disease, and others. Diagnosis of NPSLE is based on clinical evaluation and supported by evidence from imaging modalities (e.g., magnetic resonance imaging/MRI or single-photon emission computed tomography/SPECT), laboratory markers (e.g., elevated anti-dsDNA, anti-phospholipid antibodies, or low complement levels), and cerebrospinal fluid (CSF) analysis. Symptoms must persist for at least 24 hours and cannot be explained by other etiologies such as infections, metabolic abnormalities, or medication side effects.

- **Vitamin D Deficiency in NPSLE**

Vitamin D deficiency in the context of Neuropsychiatric Systemic Lupus Erythematosus (NPSLE) refers to the insufficient levels of 25-hydroxyvitamin D (25(OH)D) in the bloodstream, which can influence the pathogenesis and clinical manifestations of NPSLE. Vitamin D deficiency is typically defined as a serum concentration of 25 (OH)D lower than 20 ng/mL, while levels between 20-30 ng/mL 41 are considered insufficient, and levels greater than 30 ng/mL are considered normal. This definition is commonly assessed through blood tests using chemiluminescence immunoassay (CLIA) or other reliable methods. In patients with NPSLE, low levels of vitamin D may contribute to exacerbation of neuropsychiatric symptoms such as cognitive dysfunction, depression, fatigue, and even psychosis, through various mechanisms including immune dysregulation, inflammation, and impaired calcium homeostasis (DeLuca et al., 2013).

Vitamin D deficiency is considered a contributing factor in the onset and progression of neuropsychiatric manifestations of lupus due to its involvement in immune modulation, neuronal function, and regulation of inflammatory pathways. Vitamin D deficiency in NPSLE patients is often linked to poor clinical outcomes and may serve as an indicator for potential therapeutic interventions to manage neuropsychiatric symptoms (Schwartz et al., 2019).

Vitamin D is an essential pro-hormone steroid and a fat-soluble vitamin. It is acquired from sunlight exposure, dietary sources, and supplements in biologically inactive forms, necessitating two hydroxylations within the body for activation. Sun exposure accounts for 50% to 90% of vitamin D absorption through the skin, while the remaining portion is obtained from food. Vitamin D exists in two forms: D2

(ergocalciferol) and D3 (cholecalciferol). Ergocalciferol (D2) and cholecalciferol (D3) are primarily obtained through dermal synthesis and diet (fatty fish livers, fortified foods), respectively. In the liver, the hepatic enzyme 25- hydroxylase converts both of these substances into 25-hydroxy-vitamin D2 (25-OHD2) and 25-hydroxy-vitamin D3 (25-OH-D3). The kidney's 1-alpha-hydroxylase enzyme then changes both 25-OH-D2 and 25-OH-D3 into vitamin D's most active 42 form, 1, 25 dihydroxyvitamin D. This 1,25-dihydroxyvitamin D, also known as—calcitriol. Which is active, reduces renal excretion of calcium and phosphate while increasing intestine absorption of calcium and bone resorption (Holick, 2007).

- **CLIA method**

To determine vitamin D levels, a variety of commercially available 25-hydroxyvitamin D assays are utilized. Chemiluminescence immunoassay (CLIA) has been a widely applied test because of its high sensitivity, good specificity, wide range of applications, wide linear range. rapid and low-cost detection methods. Chemiluminescence Immunoassay (CLIA) is a technique used to quantify sample concentrations based on the luminescence intensity emitted by the chemical reaction. The measurement of Vitamin D was conducted using the ADVIA Centaur 25OH Vitamin D total assay, a precise and validated CLIA method.

- **Brain Single-photon emission computed tomography (SPECT) Imaging**

Single-photon emission computed tomography (SPECT) is a technique that generates tomographic images of the three-dimensional distribution of a radiopharmaceutical. When applied to the brain, SPECT can effectively measure regional cerebral perfusion. To be suitable for this purpose, radiopharmaceuticals must possess three key physiological properties:

1. **Blood-Brain Barrier Penetration**: The compounds must efficiently cross the blood-brain barrier.

2. **High and Flow-Independent Extraction:** Their extraction should be nearly complete and independent of blood flow, ensuring their initial distribution reflects regional cerebral blood flow (rCBF).

3. **Stable Retention**: The radiopharmaceuticals must remain fixed in the brain in their initial distribution long enough to allow the acquisition of diagnostic tomographic images.

Ideally, tracer uptake should show minimal redistribution, allowing the initial uptake—reflecting rCBF within a short time after injection—to remain stable for several hours. This creates a "frozen" image that is independent of rCBF changes occurring after the fixation period. Several radiopharmaceuticals are commercially available for brain perfusion SPECT, with the most commonly used being technetium-99m (99mTc)-labelled compounds, such as ethyl cysteine dimer (ECD, Neurolite) and hexa methyl propylene amine oxime (HMPAO, Ceretec). Both ECD and HMPAO share similar properties in normal brain tissue, including their ability to cross the blood-brain barrier due to their lipophilic nature and their retention in brain cells following conversion to hydrophilic compounds. However, there are distinctions between the two in terms of:

a. In Vitro Stability: Differences in how stable the compounds are outside the body.

b. Uptake Mechanisms: Variations in how the agents interact with brain cells.

c. Cerebral Distribution: Differences in how they spread across brain regions.

d. Dosimetry: The radiation dose delivered to the patient.

For ECD, retention occurs through de-esterification, which converts the compound to a hydrophilic form. In the case of HMPAO, retention is associated with the instability of the lipophilic form and interactions with glutathione. These characteristics 44 influence their diagnostic utility and clinical applications in measuring rCBF using SPECT.

- **99m Tc ECD Brain SPECT in NPSLE**

In NPSLE, SPECT is employed to assess the presence of abnormalities such as hypo-perfusion or altered blood flow in specific regions of the brain, which are often associated with cognitive dysfunction, seizures, mood disorders, and other neuropsychiatric symptoms. These changes can include reduced blood flow in areas such as the frontal cortex, temporal lobes, hippocampus, and other brain regions linked to cognitive and mood disturbances. SPECT imaging is particularly useful in identifying early or subclinical brain abnormalities that might not be detectable with conventional structural imaging techniques. SPECT is considered a non-invasive, functional imaging tool that provides valuable insights into the pathophysiology of NPSLE by revealing alterations in regional brain function related to lupus-induced neurovascular damage (Sahebari et al., 2018; Zaher, 2013).

- **Mini-Mental State Examination** (**MMSE) in NPSLE**

The Mini-Mental State Examination (MMSE) is a widely used cognitive screening tool for assessing cognitive function in individuals with suspected neuropsychiatric conditions, including patients with Neuropsychiatric Systemic Lupus Erythematosus (NPSLE). In the context of NPSLE, the MMSE is employed to evaluate various cognitive domains such as orientation, memory, attention, language,

and visuospatial skills. It consists of 30 questions or tasks, scored on a scale from 0 to 30, with lower scores indicating greater cognitive impairment. A score of 24 or lower typically suggests cognitive dysfunction, which is common in NPSLE patients and may reflect deficits in memory, attention, or executive function associated with the neuropsychiatric manifestations of lupus (Hossain et al., 2021; Monahan et al., 2023).

This cognitive impairment is often linked to neurological damage, which can be evaluated through clinical symptoms and brain imaging techniques. The MMSE score is used as part of a comprehensive assessment to gauge the extent of cognitive dysfunction, which may aid in monitoring the progression of NPSLE and assessing the impact of therapeutic interventions. MMSE is commonly used in clinical settings to differentiate between cognitive deficits caused by lupus and those resulting from other conditions like depression or vascular disease (Borba et al., 2023).

Chapter Four

Results

4.1 Result

The study enrolled a total of 72 patients, comprising 38 participants in the control group and 34 in the intervention (case) group. At baseline, there were no significant differences between the groups in terms of demographic and clinical characteristics, including age, gender, body mass index (BMI), waist-to-hip ratio (WHR), and baseline Vitamin D levels. The control group had a mean age of 28.24 ± 7.18 years, while the intervention group had a mean age of 26.32 ± 7.94 years ($P > 0.05$). Both groups were predominantly female, with no significant difference in gender distribution ($P > 0.05$). The BMI was 19.0 ± 1.4 for the control group and 19.2 ± 2.0 for the intervention group, while the WHR values were 0.78 ± 0.11 for the control group and 0.76 ± 0.10 for the intervention group ($P > 0.05$). Baseline Vitamin D levels were similar between the groups, with the control group showing a mean of 14.5 ± 5.3 ng/ml and the intervention group 16.2 ± 4.9 ng/ml ($P = 0.173$). Mini-Mental State Examination (MMSE) scores at baseline were also comparable, with the control group scoring 24.1 ± 1.7 and the intervention group 24.3 ± 1.5 ($P = 0.677$). Z-score imaging of brain perfusion revealed abnormal perfusion in both groups, with 81.6% of the control group and 88.2% of the intervention group demonstrating abnormal brain perfusion, primarily in the frontal, parietal lobes, and precuneus regions (Z-scores: 1.4 vs. 1.46, $P = 0.549$). After the 6-month intervention period, the intervention group showed significant improvements compared to the control group. Vitamin D3 levels in the intervention group increased significantly, with a mean of 28.3 ± 5.3 ng/ml, compared to 15.1 ± 3.4 ng/ml in the control group ($P< 0.001$). MMSE scores also improved significantly in the intervention group, rising from 24.3 ± 1.5 to 26.5±1. ($P<0.001$), whereas the control group showed no significant change, with an end-line MMSE score of 23.8 ± 2.25 ($P< 0.001$). These findings suggest

that Vitamin D3 supplementation had significant positive effect on cognitive function as measured by MMSE scores. For brain perfusion, no significant difference in baseline values was observed between the groups (P = 0.522). At the study's conclusion, the mean Z-scores for brain perfusion were slightly higher in the control group (1.84) compared to the intervention group (1.66), but this difference was not statistically significant (P = 0.457). Notably, 65.8% of the brain SPECT data was unavailable from the control group at the study's end, while no missing data was reported in the intervention group. Among the available data, 11 out of 13 control participants demonstrated abnormal brain perfusion, while fewer participants in the intervention group exhibited abnormal perfusion. Fisher's exact test revealed a significant between-group difference in brain perfusion (Fisher's exact test = 6.997, p = 0.028), suggesting that Vitamin D3 supplementation may have a positive impact on cerebral perfusion, especially in areas associated with neuropsychiatric symptoms in Systemic Lupus Erythematosus (SLE) patients. Regarding statistical analysis of brain perfusion patterns, no significant differences were found between the groups at baseline (Pearson χ^2 = 2.152, p = 0.905; Fisher's exact test = 2.393, p = 0.913), indicating effective randomization and comparable initial conditions. However, significant between-group differences emerged in perfusion results at the conclusion of the study (Pearson χ^2 = 7.114, p = 0.029; Fisher's exact test= 6.997, p = 0.029), with the intervention group demonstrating distinct patterns of brain perfusion compared to controls, supporting the potential effect of Vitamin D3 supplementation on cerebral perfusion.

The use of both Pearson's chi-square and Fisher's exact test was necessary due to the presence of cells with expected frequencies less than 5 in 50% of the contingency table for baseline measurements. The Fisher's exact test, being more robust for small

sample sizes and sparse contingency tables, corroborated the results of the traditional chi-square analysis, enhancing the statistical validity of the findings.

Statistical analysis using two-way repeated measures ANOVA revealed a significant interaction between the groups across time points, with a moderate to large effect size observed for MMSE scores (Partial eta squared = 0.460), indicating substantial improvement in cognitive function in the intervention group. The effect size for Vitamin D levels was even larger (Partial eta squared = 0.714), further emphasizing the effectiveness of Vitamin D supplementation. These results suggest that Vitamin D3 may serve as an adjunctive therapy to improve cognitive function in NPSLE patients.

While baseline Vitamin D levels, MMSE scores, and brain perfusion characteristics were similar between the groups (all $P > 0.05$), significant differences emerged by the end of the study, particularly in MMSE scores, potentially due to the effective Vitamin D3 supplementation. Further studies with complete end-line brain perfusion data would be valuable in confirming the potential effects of Vitamin D3 on cerebral perfusion in NPSLE patients.

These findings suggest that Vitamin D3 supplementation may be a promising adjunctive therapy for improving cognitive function and brain perfusion in patients with NPSLE, though further investigation is needed to confirm these effects, especially concerning brain perfusion.

Table 1. Age distribution between Cases & Controls

Variables (N=72)	Control (n=38) n (%)	Cases (n=34) n (%)	P-values for between groups (Cases Vs. Controls)
Age (years) (***Mean ± SD***)	28.24 ± 7.18	26.32 ± 7.94	*P=.289
Age Categories			
18	03 (7.9)	04 (11.8)	**P>0.05
19-25	14 (36.8)	15 (44.1)	
26-30	11 (28.9)	18 (52.9)	
>31	10 (26.3)	07 (20.6)	

*Student's t-test; **Fisher's exact test

Table 1 showing the age distribution among study population. The age distribution between case and control groups showed no significant differences overall. The mean age was slightly higher in the control group (28.24 ± 7.18 years) compared to the case group (26.32 ±7.94 years), but the difference was not statistically significant (*P=.289, Student's t-test*). Within specific age categories, 7.9% of controls and 11.8% of cases were 18 years old, while the majority of participants fell into the 19–25 and 26– 30 age groups, with cases having a higher proportion in the latter category (52.9% vs. 28.9%). For participants aged >31 years, controls had a slightly higher percentage (26.3% vs. 20.6%). Fisher's exact test confirmed that these categorical differences were not statistically significant.

Table 2. Gender distribution among the study population

Gender (N=72)	Control (n=38) n (%)	Cases (n=34) n (%)	P-value
Female	35 (92.1)	30 (88.2)	P=.700*
Male	03 (7.9)	04 (11.8)	

*Fisher's exact test

Table 2 showing the gender distribution among the study population. The gender distribution among the study population showed no significant differences between the case and control groups (*P = .700, Fisher's exact test*). Females constituted the majority in both groups, with 92.1% in the control group and 88.2% in the case group. Males made up a small proportion, accounting for 7.9% of the control group and 11.8% of the case group. This indicates a predominantly female representation in the study population with similar gender distributions across cases and controls.

Table 3. Biochemical tests for SLE confirmation

SLE Conformation (N=72)	Control (n=38) n (%)	Cases (n=34) n (%)	P-Value
Anti-ds DNA-Ab			
Yes	**38 (100)**	**34 (100)**	-
No	00 (0.0)	00 (0.0)	
Anti-Phospholipid-Ab			
Yes	**35 (100)**	**33 (100)**	**P=.617***
No	03 (7.9)	01 (2.9)	

*Fisher's exact test

Table 3 shows Biochemical test results for SLE confirmation showed complete agreement for Anti-ds DNA antibodies, with 100% positivity in both the control and case groups. For Anti-phospholipid antibodies, positivity was also high, at 100% in controls and 97.1% in cases. However, a small proportion tested negative: 7.9% in the control group and 2.9% in the case group. These differences were not statistically significant (*P=.617, Fisher's exact test*), indicating similar biochemical profiles for SLE confirmation across the groups.

Table 4. Anthropometric Distribution

Anthropometry (N=72)	Control (n=38) n (%)	Cases (n=34) n (%)	P-values
BMI (Kg/m^2) (Mean± SD)	19.0± 1.4	19.2 ± 2.0	*P=.741
<18	16 (42.1)	17 (50.0)	
18.5-23.0	22 (57.9)	16 (47.1)	
23.1-25	00 (00)	01 (2.9)	
Waist to Hip ratio (WHR)(Mean± SD)	0.78 ±.11	0.76 ±.10	*P=.596

*Student's t-test

Table 4 describes the anthropometric distribution of the study population revealed in table 4 and there was no significant differences between case and control groups. The mean BMI was similar, at 19.0 ± 1.4 kg/m² for controls and 19.2 ± 2.0 kg/m² for cases (*P=.741, Fisher's exact test*). A higher proportion of cases (50.0%) had a BMI below 18 compared to controls (42.1%), while most participants in both groups fell within the 18.5–23.0 BMI range (57.9% in controls and 47.1% in cases). Only 2.9% of cases had BMI between 23.1–25 while none in the control group. The waist-to-hip ratio (WHR) was also comparable, with mean values of 0.78 ± 0.11 for controls and 0.76 ±0.10 for cases (P=.596, Student's t-test).

Table 5. Categories of Vitamin D deficiency between groups across timelines

Vitamin D (ng/ml)	Baseline (N=72)			End line (N= 72)		
	n (%) Control (n=38)	n (%) Cases (n=34)	P-trends (Cases Vs. Controls)	n (%) Control (n=38)	n (%) Cases (n=34)	P-trends (Cases Vs. Controls)
≤9 ***(severe deficiency)***	4 (10.5)	06 (17.6)	P=.615	08 (21.1)	00 (00)	
10-19 (***Deficiency***)	26 (68.4)	20 (58.8)		27 (71.1)	01 (2.9)	*P<0.001
20-29 (***Insufficiency***)	08 (21.1)	08 (23.5)		03 (7.9)	18 (52.9)	
≥30 (***Sufficient***)	00 (00)	00 (00)		00 (00)	15 (44.1)	

*Fisher's exact test

Vitamin D status between case and control groups from baseline to end line revealed in Table 5 and shown statistically significant improvements among cases. At baseline, severe deficiency (≤9 ng/ml) was present in 10.5% of controls and 17.6% of cases (*P = .615, Fisher's exact test*), with most participants in both groups exhibiting deficiency (10–19 ng/ml; 68.4% controls, 58.8% cases). Insufficiency (20– 29 ng/ml) was comparable between controls (21.1%) and cases (23.5%), and no participants were sufficient (≥30 ng/ml). By the end line, severe deficiency persisted in 21.1% of controls but was eliminated in cases. Deficiency remained high in controls (71.1%) but dropped significantly in cases to 2.9% (*P < 0.001*). Cases demonstrated a marked increase in insufficiency (52.9%) and sufficiency (44.1%), compared to controls (7.9% and 0.0%, respectively). These findings highlight a significant improvement in vitamin D levels in the case group, as evidenced by Fisher's exact test.

Table 6. Mean Vitamin D (ng/ml) as a time-dependent variable between groups

levels	Baseline (n %) (N=72)			End line (n %) (N= 72)			@Baseline Versus. End line
	Control (n=38)	Cases (n=34)	Cases Vs. Controls	Control (n=38)	Cases (n=34)	Cases Vs. Control	
Vitamin D (ng/ml) (***Mean ±SD***)	14.5±5.3	16.2±4.9	#P=.173 ***ES@=.026	15.1±3.4	28.3±5.3	#P<0.001 ***ES@=.695	#P<0.001 ***ES@=.714

***Effect size=Partial *Eta* squared; @2-ways Repeated measure ANOVA; #Pair sample t-test

Mean vitamin D levels as a time-dependent variable demonstrated significant improvements in the case group compared to the control group. At baseline, the mean vitamin D levels were 14.5 ± 5.3 ng/ml in controls and 16.2 ± 4.9 ng/ml in cases. By the end line, controls showed a slight, non-significant increase to 5.1 ± 3.4 ng/ml (*P=.179*), whereas cases exhibited a statistically significant increase to 28.3 ± 5.3 ng/ml (*P<0.001*). Two-way repeated measures ANOVA revealed significant group-by-time interaction effects for vitamin D levels (*P<0.001*, partial eta squared= .714), with a very large effect size indicating the substantial impact of the intervention on the case group. These results confirm a robust and statistically significant improvement in vitamin D levels in the case group over time, compared to controls (Table 6).

Table 7. Categories of MMSE-score between groups from baseline to the end line

levels	Baseline (n %) (N=72)			End line (n %) (N= 72)		
	Control (n=38) n %	**Cases (n=34)** n %	**Cases Vs. Controls** n %	**Control (n=38)** n %	**Cases (n=34)** n %	**Cases Vs. Control**
Mini-Mental state examination (MMSE) scores						
MMSE Categories						
1-19 (***Severe***[**])	Nil	Nil		Nil	Nil	
20-24 (***Mild CI***)	25 (65.8)	24 (70.6)	[*]P=.801	22 (57.9)	03 (8.8)	[*]P<0.001
24.1-25 (***Questionable CI***)	00 (00)	00 (00)		04 (10.5)	04 (11.8)	
≥26 (***Normal***)	13 (34.2)	10 (29.4)		12 (31.6)	27 (39.4)	

[*]Fisher's exact test; [**]No severe case was observed;

The categories of MMSE scores between groups showed significant improvements in cognitive function in the case group from baseline to the end line. At baseline, mild cognitive impairment (CI) scores (20–24) were predominant, observed in 65.8% of controls and 70.6% of cases (*P = .801, Fisher's exact test*). By the end line, the prevalence of mild CI decreased significantly in the case group (8.8%) compared to controls (57.9%; *P < 0.001*). Notably, the proportion of participants with normal MMSE scores (≥26) increased significantly in the case group, from 29.4% at baseline to 39.4% at the end line, whereas in controls, it slightly declined from 34.2% to 31.6%. These results, supported by Fisher's exact test, indicate a statistically significant improvement in cognitive function in the case group over time (Table 7).

Table 8. Time-dependent mean MMSE-scores between groups across timelines

levels	Baseline (n %) (N=72)			End line (n %) (N= 72)			[@]Baseline Versus. End line
	Control (n=38)	Cases (n=34)	Cases Vs. Controls	Control (n=38)	Cases (n=34)	Cases Vs. Control	
Mini-Mental state examination (MMSE) scores							
(***Mean ± SD***)	24.1±1.7	24.3±1.5	[#]P=.677 [***]ES[@]=.002	23.8±2.25	26.5±1.4	[#]P<0.001 [@]P<0.001 [***]ES[@]=.335 [***]ES[@]=.460	

[***]Effect size=Partial *Eta* squared; [@]2-ways Repeated measure ANOVA; [#]Pair sample t-test

The time-dependent analysis of MMSE scores revealed significant cognitive improvements in the case group compared to the control group. At baseline, the mean MMSE scores were similar between groups, with controls at 24.1 ± 1.7 and cases at 24.3 ± 1.5. By the end line, the control group showed no significant change, with a mean score of 23.8 ± 2.25 ($P = .777$), whereas the case group demonstrated a statistically significant increase to 26.5 ± 1.4 ($P < 0.001$). Two-way repeated measures ANOVA confirmed a significant group-by-time interaction effect for MMSE scores ($P < 0.001$, partial eta squared = .347), with a large effect size, indicating meaningful cognitive improvement in the case group. The comparison between cases and controls also showed a significant difference over time ($P < 0.001$, partial eta squared = .460). These results underscore the significant and clinically relevant impact of the intervention on cognitive outcomes in the case group (Table 8).

Table 9. Percent of the patients according to the SPECT image of Brain hypo-perfusion

Brain Perfusion	Base Line (N= 72)			End Line (N=34)		
	Control (n=38) %	Cases (n=34) %	P-trends (Cases Vs. Controls)	Control (n=13) %	Cases (n=21) %	P-trends (Cases Vs. Controls)
Not Done	Nil	Nil	*P=.522	25 (65.8)	13 (38.2)	*P=.028
Normal	07 (18.4)	04 (11.8)		02 (5.3)	01 (2.9)	
Abnormal	31 (81.6)	30 (88.2)		11 (28.9)	20 (58.8)	

*Fisher's exact test

The analysis of brain perfusion status using SPECT imaging showed significant changes between the intervention (cases) and control groups from baseline to end line. At baseline, brain hypo-perfusion was more prevalent in both groups, with 81.6% of controls and 88.2% of cases showing abnormal results. At the end line, the percentage of abnormal perfusion decreased significantly in the control group (28.9%) but increased in the case group (58.8%), with a significant difference between the groups (*P = .028, Fisher's exact test*). Normal brain perfusion was rare at both time points, with only 18.4% of controls and 11.8% of cases showing normal perfusion at baseline, and 5.3% and 2.9%, respectively, at the end line. These findings indicate a significant shift in brain perfusion patterns between the groups, particularly in the case group, suggesting a marked difference in brain health over the course of the intervention (Table 9).

Table 10. Distribution of the areas of Brain's hypo-perfusion between groups

Area of Brain Hypo-perfusion	Base Line (N= 72)			End Line (N=34)		
	Control (n= 38) n %	Case (n=34) n %	P-trends (Cases Vs. Controls)	Control (n= 13) n %	Case (n= 21) n %	P-trends (Cases Vs. Controls)
Hypo-perfusion Absent	06 (15.8)	04 (11.8)	*P=.912	01 (7.7)	01 (4.8)	*P=.916
Frontal Lobe	07 (18.4)	06 (17.6)		02 (15.4)	06 (19.0)	
Frontal Lobe +Precuneous	05 (13.2)	03 (8.8)		01 (7.7)	02 (4.8)	
Frontal Lobe + Parietal Lobe	08 (21.1)	11 (32.4)		07 (30.8)	11 (47.6)	
Frontal Lobe +Precuneous+Basal Ganglia	01 (2.6)	02 (5.9)		01 (7.7)	02 (9.5)	
Frontal Lobe +Temporal Lobe + precuneus	02 (5.3)	01 (2.9)		01 (7.7)	01 (00)	

*Fisher's exact test

The distribution of brain hypo-perfusion areas between the control and case groups showed no significant differences at both time points. At baseline, hypo-perfusion was absent in 15.8% of controls and 11.8% of cases (*P = .912, Fisher's exact test*), and at the end line, the percentages were 7.7% in controls and 4.8% in cases (*P =.916*). The most common areas of hypo-perfusion at baseline included the frontal lobe (18.4% in controls, 17.6% in cases), and the combination of frontal and parietal lobes (21.1% in controls, 32.4% in cases). At the end line, the frontal lobe and parietal lobe combination remained the most affected area, with 30.8% in controls and 47.6% in cases. Other regions, such as the precuneus and basal ganglia, showed minor changes over time. These findings suggest that although brain hypo-perfusion was observed in both groups, no significant differences were found in the distribution of hypo- perfused areas between controls and cases over time (Table 10).

Table 11. Percent of the patients according to the categories of Brain perfusion

SPECT Imaging or Brain perfusion	Base Line (N= 72)			**End Line (N=34)			@Baseline Versus. End line
	Control (n=38) (n %)	Cases (n=34) (n %)	Control Vs. Cases	Control (n=13) (n %)	Cases (n=21) (n %)	Control Vs. Cases	
Categories of Perfusion (Z-scores)							
00 to 1 (***Normal***)	13 (34.2)	10 (29.4)	*P=.947	02 (15.4)	03 (14.3)	*P=.094	
1.1 to 2 (***Mild Deficient***)	19 (50.0)	19 (55.9)		05 (38.5)	15 (71.4)		
2.1 to 3 (***Moderate Deficient***)	06 (15.8)	05 (14.7)		06 (46.2)	03 (14.3)		

*Fisher's exact test; **Missing controls, n=25 & Cases=13

The categorization of brain perfusion based on Z-scores between the control and case groups revealed some shifts over time, although statistical significance was limited. At baseline, the majority of both groups had mild deficient perfusion (1.1 to 2), with 50.0% of controls and 55.9% of cases in this category (*P = .947, Chi-square test*). A smaller proportion had normal perfusion (00 to 1), with 34.2% of controls and 29.4% of cases, and moderate deficiency (2.1 to 3) was seen in 15.8% of controls and 14.7% of cases. By the end line, normal brain perfusion (00 to 1) slightly decreased in both groups (15.4% in controls, 14.3% in cases), with no significant change (*P = .094, Fisher's exact test*). Mild deficiency (1.1 to 2) increased notably in the case group (71.4%) compared to controls (38.5%). Moderate deficiency (2.1 to 3) showed a higher proportion in controls (46.2%) than in cases (14.3%). Overall, these results suggest a trend towards more mild deficiency and a decrease in moderate deficiency in the case group over time, but the changes were not statistically significant in most comparisons (Table 11).

Table 12. Brain perfusion (Z-score) between groups from baseline to the end line

Brain perfusion	Base Line (N= 72)			**End Line (N=34)			@Baseline
	Control (n=38) (n %)	Cases (n=34) (n %)	Control Vs. Cases	Control (n=13) (n %)	Cases (n=21) (n %)	Control Vs. Cases	Versus. End line
Z-scores of Brain perfusion (Mean± SD)	1.4±.71	1.46±.67	#P=.549 ***ES@=.026	1.84±.701	1.66±.65	#P=.457 ***ES@=.017	@P=.966 ***ES@=.000

The analysis of brain perfusion Z-scores between the control and case groups from baseline to end line showed no significant changes in either group. At baseline, the mean Z-scores for brain perfusion were 1.4 ± 0.71 in the control group and 1.46 ± 0.67 in the case group. By the end line, these scores increased slightly to 1.84 ± 0.70 in the control group and 1.66 ± 0.65 in the case group, but these changes were not statistically significant (*P = .749* for controls and *P = .457* for cases). Two-way repeated measures ANOVA revealed no significant group-by-time interaction effect (*P = .549*, partial eta squared = .021), and no significant effect for Z-scores over time for either group (*P = 0.966*, partial eta squared = .000). These findings suggest that brain perfusion, as measured by Z-scores, did not show meaningful changes across time in either group, with small effect sizes observed.

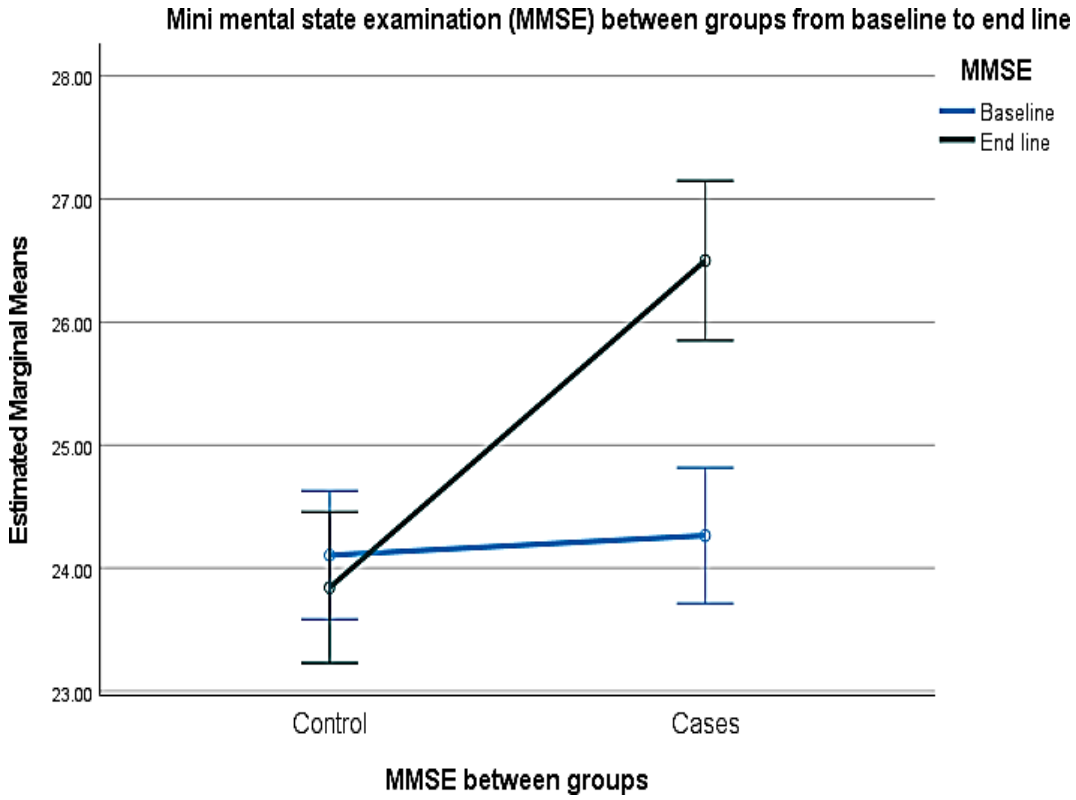

Figure 2: Mean MMSE-scores between Intervention and Control groups

The Figure 2 represents a graph comparing the Mini Mental State Examination (MMSE) scores between two groups (Control and Cases) from baseline to end line. The x-axis represents the groups (Control and Cases), while the y-axis shows the estimated marginal means of MMSE scores. Two lines are plotted: one for the baseline scores (shown in blue) and one for the end line scores (shown in green). The graph clearly indicates that the MMSE scores for the control group remained relatively stable between baseline and end line, while the case group showed a marked increase in MMSE scores from baseline to end line. Error bars are included, indicating the variability in the data, and the increase in MMSE scores for the case group is statistically significant.

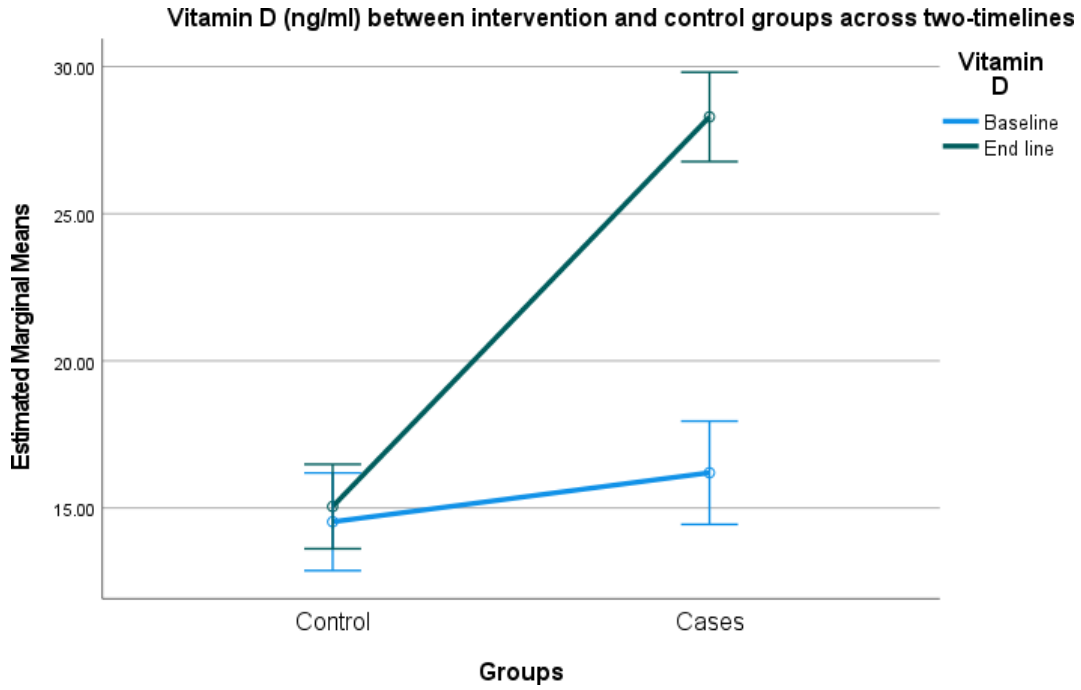

Figure 3: Vitamin D3 (ng/ml) levels between Intervention and Control groups

The image displays a graph comparing the levels of Vitamin D (in ng/ml) between the intervention (Cases) and control groups across two timelines (baseline and end line). The x-axis shows the two groups (Control and Cases), while the y-axis represents the estimated marginal means of Vitamin D levels. The baseline values, shown in blue, are nearly the same for both groups, with the control group around 15 ng/ml and the cases slightly higher. However, at the end line (shown in green), the Vitamin D levels for the case group significantly increased, reaching approximately 30 ng/ml, while the control group's levels remained almost unchanged at around 15 ng/ml. Error bars indicate the variability in the data for both timelines, and the results suggest a significant improvement in Vitamin D levels in the case group compared to the control group (Figure 3).

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Chapter Five

Discussion

5. Discussion

This randomized controlled trial (RCT) aimed to evaluate the effects of Vitamin D3 supplementation on cognitive performance and cerebral perfusion in patients with neuropsychiatric (NP) systemic lupus erythematosus (SLE) or NPSLE. Advanced statistical analysis revealed significant group-by-time interaction effects (Sullivan & Feinn, 2012) for vitamin D levels ($P<0.001$) with a large effect size (Table 6), indicating the substantial impact of the Vitamin D3 supplementation on the intervention group for the improvement of cognitive function/neuropsychiatric symptoms in NPSLE patients. Evidence suggests that cognitive/neurogenic dysfunction of systemic lupus erythematosus (SLE) or NPSLE can be treated with Vitamin D (Cui et al., 2015; DeLuca et al., 2013; Tay et al., 2015; Lima et al., 2016; Vacca et al., 2024; Zaher et al, 2013). Improved disease activity and fatigue have been noted when Vitamin D has been supplemented in vitamin D-deficient/insufficient SLE patients. One possible mechanism could be the suppression of the interferon signature gene expression (Magro et al, 2021). Vitamin D3 supports cognitive function in NPSLE by reducing inflammation and protecting neuronal health (Cui et al., 2015). It also boosts neurotrophic factors like brain-derived neurotrophic factor (BDNF), valuable for cognitive health (DeLuca et al., 2013). Some studies observed in animal models of lupus, supplementation with vitamin D improved cognitive function and increased VDR expression in the hippocampus (Yan et al., 2019). Additionally, vitamin D may delay immune cell infiltration in the choroid plexus and reduce biomarkers linked to cognitive decline in MRL/lpr mice (Li et al., 2023).

Anti-double-stranded DNA (Anti-dsDNA) antibodies, a subset of antinuclear antibodies targeting double-stranded DNA, are linked to hippocampal injury and

cognitive dysfunction in SLE patients (Stock et al., 2013). Low vitamin D3 levels correlate with cognitive dysfunction severity in NPSLE, highlighting its potential as a modifiable factor (Tay et al., 2015). A randomized trial found that SLE patients receiving vitamin D3 experienced improved mood, reduced fatigue, and cognitive improvements due to decreased inflammation (Lima et al., 2016). Additionally, it aids in producing neurotransmitters like acetylcholine and serotonin, vital for cognitive processes (Pertile et al., 2016).

Several observational (Hussain et al, 2018; Tay et al, 2019; Vacca et al., 2024), longitudinal/interventional (Yang et al., 2020; Lima et al., 2016), and even animal models (Yan et al, 2019) showed that Vitamin D deficiency could have a significant impact on cognitive performance in NPSLE, even in neuropsychiatric disorder in people without SLE/NPSLE (Sultan et al, 2020). Rat model of SLE (Yan et al, 2019) concluded that vitamin D treatment alleviated neurobehavioral deficits in the mice with SLE, activated the expression of VDR and reduced the number of dead cells in the CA1 region of the hippocampus as well as regulated caspase-3 and Bcl-2 expression, thus playing a protective role by suppressing inflammatory cytokines, thereby ultimately inhibiting the progression of apoptosis in a mouse model of SLE. Deficiency of 25(OH) D3, a potentially modifiable risk factor, independently predicted cognitive impairment in SLE patients as compared to healthy controls (Tay et al, 2015). Meta-analysis also echoed these findings for SLE (Islam et al, 2019) and adult population (Chen et al, 2024).

On top of that, mean cognitive performance (± SD), assessed with the help of mini mental state examination (MMSE) scores significantly improved (Medium effect) from baseline (24.1±1.5) to end line (26.5±1.4) in the ‘Intervention group’ than ‘Control’ (Table 3).

Recent research showed cerebral perfusion and corresponding brain functional networks altered in the NPSLE patients (Liu et al, 2025). The analysis of the categories of brain perfusion status using SPECT imaging Z-scores (Table 3) revealed some insignificant (P>0.05) shifts of brain hypo-perfusion level from moderate to mild across timelines between the control and case groups. Moreover, the present study showed insignificant differences in areas of brain hypo-perfusion imaging (SPECT) between intervention and non-intervention group, a remarkable amount of missing samples (Control=25, Cases=13) can be a logical reason. PET and SPECT studies have identified several brain regions with altered perfusion, such as the hippocampus and frontal cortex, which are associated with NPSLE9. Additionally, the study reflected that brain Perfusion SPECT is a sensitive noninvasive diagnostic imaging modality with significant correlation between SPECT findings and disease activity for detection of cerebral blood flow/CBF abnormalities in SLE patients, reflecting sequelae of vasculitis (Zaher et al, 2013). Different brain perfusion patterns were observed between NPSLE and non-NPSLE patients in a recent study (Zhuo et al, 2020), using MR perfusion imaging, and reported that the cerebral blood volume (CBV) and cerebral blood flow (CBF) of certain specific brain regions were highly related to NPSLE.

Perfusion MRI (DSC-MRI) conducted among primary NPSLE Patients had significantly lower CBF and CBV in several normal-appearing white matter areas compared with controls (P<0.0001), and lower CBF in the semioval centre bilaterally, compared with non-NPSLE and patients with secondary NPSLE (P<0.001) (Papadaki et al., 2017).

Neuroimaging like brain MRI and single-photon emission computed tomography (SPECT) scans have shown white matter lesions, cerebral atrophy, and reduced

perfusion especially in the frontal and temporal lobes (cognition areas) of NPSLE patients, reflected underlying neuro-inflammation and vasculopathy that contribute to cognitive dysfunction in NPSLE (Vacca et al., 2024). Brain perfusion imaging or SPECT helps detect cerebral blood flow abnormalities in SLE patients, showing reduced blood flow in areas like the frontal, temporal, and parietal lobes, which are linked to cognitive and neuropsychiatric symptoms (Zhang et al., 2005). This suggests that SPECT can help assess the effects of interventions, such as Vitamin D3, by tracking changes in brain perfusion. Preliminary evidence suggests that Vitamin D3 supplementation may improve cerebral perfusion in NPSLE patients, enhancing blood flow in affected regions and reducing neuropsychiatric symptoms (DeLuca et al., 2013).

In this study, the most common areas of hypo-perfusion at both timelines included the combination of frontal and parietal lobes (*Baseline*: Controls=21.1% versus Case=32.4%; *End line*: 30.8% versus 47.6%), and the frontal lobe (18.4% versus 17.6%). Other regions, such as the precuneus and basal ganglia, showed minor changes over time. These findings suggest that although insignificant (P>0.05) improved changes (a trend towards more 'Mild deficiency' from 'Moderate deficiency') of brain hypo-perfusion was observed mainly in intervention group after 6-months of vitamin D supplementation, Cerebral blood flow abnormalities may be helpful for the early diagnosis of neurological lesions in NPSLE. (Jia et al., 2019). In the 35 patients with SLE, decreases in blood perfusion were seen in some areas, and were unilateral and asymmetrically distributed. There was obvious asymmetry between sides in areas including the frontal lobe, temporal lobe, parietal lobe, and occipital lobe. The incidence of perfusion decreases in the frontal lobe in the NPSLE group was significantly higher than in the SLE group.

Conclusion

Vitamin D3 supplementation markedly enhanced cognitive function in NPSLE patients, likely due to its neuro-protective and anti-inflammatory effects. Correcting Vitamin D deficiency should be an integral part of managing SLE to improve both neurological and overall health outcomes. These results suggest that Vitamin D3 may serve as an adjunctive therapy to improve cognitive function in NPSLE patients. Additionally, enhancements in vitamin D levels and improvements in MMSE scores were noted, emphasizing the dual cognitive and psychological benefits of vitamin D3 supplementation. In conclusion, the study suggests that targeted interventions, including Vitamin D supplementation and regular cognitive and brain perfusion monitoring, may have significant therapeutic potential for improving the quality of life and clinical outcomes in patients with neuropsychiatric SLE

Study Strengths and Limitations

The baseline homogeneity between the groups (Tables 1-4) suggests that randomization was effectively carried out, ensuring comparable initial conditions between study cohorts across timelines. Additionally, the study received funding from the International Atomic Energy Agency (IAEA), which provided valuable support for the research.

One limitation of the study was the unavailability of certain radiopharmaceuticals, which could have impacted the assessment of brain perfusion or other diagnostic outcomes. Furthermore, participant drop-out, particularly in the control group (n=25/38) at the end of the study for brain perfusion scan, may have introduced bias and reduced the power of the findings, affecting the generalizability of the results.

Recommendation

Based on the findings of this NPSLE (Neuropsychiatric Systemic Lupus Erythematosus) study, several recommendations can be made:

1. Vitamin D Supplementation in SLE Patients: The significant increase in Vitamin D levels in the case group (intervention) compared to the control group over the study period suggests that Vitamin D supplementation could play a crucial role in improving the overall health of SLE patients. Therefore, clinicians should consider incorporating Vitamin D supplementation as part of the management plan for patients with SLE, especially those who are Vitamin D deficient, to enhance their immune and mental health status.
2. Improved Cognitive Function Monitoring: The increase in MMSE scores in the case group highlights the potential cognitive benefits of the intervention. This suggests that early intervention with Vitamin D or other therapeutic strategies may help prevent or reduce cognitive impairment in SLE patients. Regular cognitive screening through tools like the MMSE should be considered for patients with SLE to detect early signs of cognitive dysfunction and take corrective action.
3. Brain Perfusion Monitoring: Although there was no significant difference in brain perfusion z-scores between groups, brain hypo-perfusion was noted more often in SLE patients. This emphasizes the need for regular brain imaging and perfusion assessments in patients with neuropsychiatric manifestations of SLE to better understand and monitor cerebral involvement in the disease.

4. **Individualized Treatment Approaches**: Given that the study shows significant differences in outcomes between the case and control groups, individualized treatment plans tailored to each patient's specific needs (e.g., Vitamin D levels, cognitive function status) should be implemented. This could involve adjusting therapy based on the patient's baseline and end-line parameters, with a focus on improving cognitive and physical health.
5. **Further Research and Longitudinal Studies**: Since the results are promising, further longitudinal studies with larger sample sizes and extended follow-up periods should be conducted to confirm the long-term effects of Vitamin D supplementation and other interventions on cognitive function, brain perfusion, and overall disease progression in NPSLE.

Chapter Six

References

6. References

Altman, D.G., Machin, D., Bryant, T.N., Grdner, M.J. (2000). Statistics with confidence.2nd edition. BMJ Books 2000. ISBN: 978-0-727- 91375-3

Amital, H., Szekanecz, Z., Szücs, G., Dankó, K., Nagy, E., Csépány, T., Kiss, E.,Rovensky, J., Tuchynova, A., Kozakova, D., Doria, A., Corocher, N., Agmon-Levin, N., Barak, V., Orbach, H., Zandman-Goddard, G., & Shoenfeld, Y. (2010). Serum concentrations of 25-OH vitamin D in patients with systemic lupus erythematosus (SLE) are inversely related to disease activity: Is it time to routinely supplement patients with SLE with vitamin D? *Annals of the Rheumatic Diseases*, *69*(6), 1155–1157. https://doi.org/10.1136/ard. 2009.120329.

Aggarwal, R., Namjou, B., Li, S., D'Souza, A., Tsao, B. P., Bruner, B., James, J. A., & Scofield, R. H. (2010). Male-only Systemic Lupus. *The Journal of Rheumatology*, *37*(7), 1480–1487. https://doi.org/10.3899/jrheum.090726

American College of Rheumatology (ACR) guidelines: ACR nomenclature and case definitions for NPSLE (1999).

Antypa, D., Simos, N. J., Kavroulakis, E., Bertsias, G., Fanouriakis, A., Sidiropoulos, P., Boumpas, D., & Papadaki, E. (2021). Anxiety and depression severity in neuropsychiatric SLE are associated with perfusion and functional connectivity changes of the frontolimbic neural circuit: A resting-state (functional) MRI study. *Lupus Science & Medicine*, *8*(1), e000473. https://doi.org/10.1136/ lupus-2020-000473

Bertsias, G., Cervera, R., & Boumpas, D. T. (2012). Systemic lupus erythematosus: Pathogenesis and clinical features. *EULAR Textbook on Rheumatic Diseases*, *5*, 476–505.

Borba, E. A., Scoto Dias, E., Terczіany Vanzin, J. H., Ferreira de Queiroz Junior, N., Dos Santos, T. A. F., Skare, T., & Nisihara, R. (2023). Cognitive dysfunction in patients with systemic lupus erythematosus. A cross-sectional study in a Brazilian sample. *Lupus*, *32*(7), 900–909. https://doi.org/10.1177/ 09612033231176794.

Chen, W. Y., Cheng, Y. C., Chiu, C. C., Liu, H. C., Huang, M. C., Tu, Y. K., & Kuo, P. H. (2024). Effects of Vitamin D Supplementation on Cognitive Outcomes: A Systematic Review and Meta-Analysis. *Neuropsychology review*, *34*(2), 568–580. https://doi.org/10.1007/s11065-023-09598-z

Cui, X., Gooch, H., Groves, N. J., Sah, P., Burne, T. H., Eyles, D. W., & McGrath, J. J. (2015). Vitamin D and the brain: Key questions for future research. *The Journal of Steroid Biochemistry and Molecular Biology*, *148*, 305–309. https://doi.org/10.1016/j.jsbmb.2014.11.004

DeLuca, G. C., Kimball, S. M., Kolasinski, J., Ramagopalan, S. V., & Ebers, G. C. (2013). Review: The role of vitamin D in nervous system health and disease. *Neuropathology and Applied Neurobiology*, *39*(5), 458–484. https://doi.org/10.1111/nan.12020

Dema, B., & Charles, N. (2016). Autoantibodies in SLE: Specificities, Isotypes and Receptors. *Antibodies*, *5*(1), 2. https://doi.org/10.3390/antib5010002

Duarte-Delgado, N. P., Vásquez, G., & Ortiz-Reyes, B. L. (2019). Blood-brain barrier disruption and neuroinflammation as pathophysiological mechanisms of the diffuse manifestations of neuropsychiatric systemic lupus erythematosus. *Autoimmunity Reviews*, *18*(4), 426–432. https://doi.org/10.1016/j.autrev. 2018.12.004

Eyles, D. W., Liu, P. Y., Josh, P., & Cui, X. (2014). Intracellular distribution of the vitamin D receptor in the brain: Comparison with classic target tissues and redistribution with development. *Neuroscience*, *268*, 1–9. https://doi.org/10.1016/j.neuroscience.2014.02.042

Folstein, M., Folstein, S.E., McHugh, P.R. (1975). "Mini-Mental State" a Practical Method for Grading the Cognitive State of Patients for the Clinician. *Journal of Psychiatric Research,* 12(3); 189-198.

Graham, R. R., Ortmann, W., Rodine, P., Espe, K., Langefeld, C., Lange, E., Williams, A., Beck, S., Kyogoku, C., Moser, K., Gaffney, P., Gregersen, P. K., Criswell, L. A., Harley, J. B., & Behrens, T. W. (2007). Specific combinations of HLA-DR2 and DR3 class II haplotypes contribute graded risk for disease susceptibility and autoantibodies in human SLE. *European Journal of Human Genetics*, *15*(8), 823–830. https://doi.org/10.1038/sj.ejhg.5201827

Hanly, J. G., Omisade, A., Su, L., Farewell, V., & Fisk, J. D. (2010). Assessment of cognitive function in systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis by computerized neuropsychological tests. *Arthritis & Rheumatism*, *62*(5), 1478–1486. https://doi.org/10.1002/art.27404

Hirohata, S., & Kikuchi, H. (2021). Role of Serum IL-6 in Neuropsychiatric Systemic lupus Erythematosus. *ACR Open Rheumatology*, *3*(1), 42–49. https://doi.org/10.1002/acr2.11217

Holick, M. F. (2007). Vitamin D Deficiency. *New England Journal of Medicine*, *357*(3), 266–281. https://doi.org/10.1056/NEJMra070553

Hossain, F., Hawlader, M. D. H., Mitra, D. K., Nabi, M. H., & Rahman, Md. M. (2021). Pattern and prevalence of neuropsychiatric lupus: A retrospective study from a tertiary level hospital in Bangladesh. *The Egyptian Journal of Neurology, Psychiatry and Neurosurgery*, *57*(1), 77. https://doi.org/10.1186/s41983-021-00334-z

Hughes, E. G., Peng, X., Gleichman, A. J., Lai, M., Zhou, L., Tsou, R., Parsons, T. D., Lynch, D. R., Dalmau, J., & Balice-Gordon, R. J. (2010). Cellular and Synaptic Mechanisms of Anti-NMDA Receptor Encephalitis. *Journal of Neuroscience*, *30*(17), 5866–5875. https://doi.org/10.1523/JNEUROSCI.0167-10.2010

Hussein, H. A., Daker, L. I., Fouad, N. A., Elamir, A., & Mohamed, S. R. (2018). Does Vitamin D Deficiency Contribute to Cognitive Dysfunction in Patients with Systemic Lupus Erythematosus? *Innovations in Clinical Neuroscience*, *15*(09–10), 25–29.

Islam, M. A., Khandker, S. S., Alam, S. S., Kotyla, P., & Hassan, R. (2019). Vitamin D status in patients with systemic lupus erythematosus (SLE): A systematic review and meta-analysis. *Autoimmunity reviews*, *18*(11), 102392. https://doi.org/10.1016/j.autrev.2019.102392

Jia, J., Xie, J., Li, H., Wei, H., Li, X., Hu, J., Meng, D., Y Zhang, Y., et al (2019). Cerebral blood flow abnormalities in neuropsychiatric systemic lupus erythematosus. *Lupus* **28**, 1128–1133.
http s://doi.rg /10.1177/0961203319861677(2019).

Jung, R. E., Segall, J. M., Grazioplene, R. G., Qualls, C., Sibbitt, W. L., & Roldan, C. A. (2010). Cortical thickness and subcortical gray matter reductions in neuropsychiatric systemic lupus erythematosus. *PloS One*, *5*(3), e9302. https://doi.org/10.1371/journal.pone.0009302

Kurlowicz, L. & Wallace, M. (1999). The Mini Mental State Examination (MMSE). Try This: best practices in nursing care to older adults, from The Hartford Institute for Geriatric Nursing, Division of Nursing, New York University. Available on the internet at www.hartfordign.org.

Lahita, R. G., Tsokos, G. C., Buyon, J. P., & Koike, T. (2010). *Systemic Lupus Erythematosus*. Academic Press.

Li, X., Xu, S., Liu, J., Zhao, Y., Han, H., Li, X., & Wang, Y. (2023). Treatment with 1,25-Dihydroxyvitamin D3 Delays Choroid Plexus Infiltration and BCSFB Injury in MRL/lpr Mice Coinciding with Activation of the PPARγ/NF-κB/TNF-α Pathway and Suppression of TGF-β/Smad Signaling. *Inflammation*, *46*(2), 556–572. https://doi.org/10.1007/s10753-022-01755-5

Liu, H., Liu, H., Tian, B., Yang, P., & Fan, G. (2025). Alterations in cerebral perfusion and corresponding brain functional networks in systemic lupus erythematosus with cognitive impairment. *Scientific Reports* 15:1310 | https://doi.org/10.1038/s41598-025-85648-1

Lima, G. L., Paupitz, J., Aikawa, N. E., Takayama, L., Bonfa, E., & Pereira, R. M. R. (2016). Vitamin D Supplementation in Adolescents and Young Adults With Juvenile Systemic Lupus Erythematosus for Improvement in Disease Activity and Fatigue Scores: A Randomized, Double-Blind, Placebo-Controlled Trial. *Arthritis Care & Research*, *68*(1), 91–98. https://doi.org/10.1002/acr.22621

Magro, R., Saliba, C., Camilleri, L., Scerri, C., & Borg, A. A. (2021). Vitamin D supplementation in systemic lupus erythematosus: relationship to disease activity, fatigue and the interferon signature gene expression. *BMC rheumatology*, *5*(1), 53. https://doi.org/10.1186/s41927-021-00223-1

Matsuda, H., Mizumura, S., Nagao, T., Ota, T., Iizuka, T., Nemoto, K. et al (2007). Automated discrimination between very early Alzheimer disease and controls using an easy Z-score imaging system for multicenter brain perfusion single-photon emission tomography. AJNR Am J Neuroradiol. 2007; 28:731-6.

Mok, C., Birmingham, D., Ho, L., Hebert, L., Song, H., & Rovin, B. (2012). Vitamin D deficiency as marker for disease activity and damage in systemic lupus erythematosus: A comparison with anti-dsDNA and anti-C1q. *Lupus*, *21*(1), 36–42. https://doi.org/10.1177/0961203311422094

Monahan, R. C., Middelkoop, H. A. M., Beaart-van de Voorde, L. J. J., Fronczek, R., Groenwold, R. H. H., Kloppenburg, M., Huizinga, T. W. J., & Steup-Beekman, G. M. (2023). High Prevalence but Low Impact of Cognitive Dysfunction on Quality of Life in Patients with Lupus and Neuropsychiatric Symptoms. *Arthritis Care & Research*, *75*(5), 1017–1025. https://doi.org/10.1002/acr.24904

Papadaki, E., Fanouriakis, A., Kavroulakis, E., et al. (2017). Neuropsychiatric lupus or not? Cerebral hypoperfusion by perfusion-weighted MRI in normal appearing white matter in primary neuropsychiatric lupus erythematosus. *Ann Rheum Dis*. doi:10.1136/annrheumdis-2017-212285

Pertile, R. A. N., Cui, X., & Eyles, D. W. (2016). Vitamin D signaling and the differentiation of developing dopamine systems. *Neuroscience*, *333*, 193–203. https://doi.org/10.1016/j.neuroscience.2016.07.020

Sahebari, M., Rezaieyazdi, Z., Khodashahi, M., Abbasi, B., & Ayatollahi, F. (2018). Brain Single Photon Emission Computed Tomography Scan (SPECT) and functional MRI in Systemic Lupus Erythematosus Patients with Cognitive Dysfunction: A Systematic Review. *Asia Oceania Journal of Nuclear Medicine and Biology*, *6*(2), 97–107. https://doi.org/10.22038/aojnmb.2018.26381.1184

Schwartz, N., Stock, A. D., & Putterman, C. (2019). Neuropsychiatric lupus: New mechanistic insights and future treatment directions. *Nature Reviews. Rheumatology*, *15*(3), 137–152. https://doi.org/10.1038/s41584-018-0156-8

Stock, A. D., Wen, J., & Putterman, C. (2013). Neuropsychiatric Lupus, the Blood Brain Barrier, and the TWEAK/Fn14 Pathway. *Frontiers in Immunology*, *4*, 484. https://doi.org/10.3389/fimmu.2013.00484

Shapira-Lichter, I., Vakil, E., Litinsky, I., Oren, N., Glikmann-Johnston, Y., Caspi, D., Hendler, T., & Paran, D. (2013). Learning and memory-related brain activity dynamics are altered in systemic lupus erythematosus: A functional magnetic resonance imaging study. *Lupus*, *22*(6), 562–573. https://doi.org/10.1177/0961203313480399

Shucard, J. L., Parrish, J., Shucard, D. W., McCABE, D. C., Benedict, R. H. B., & Ambrus, J. (2004). Working memory and processing speed deficits in systemic lupus erythematosus as measured by the paced auditory serial addition test. *Journal of the International Neuropsychological Society*, *10*(1), 35–45. https://doi.org/10.1017/S1355617704101057

Sullivan, G. M., & Feinn, R. (2012). Using effect size—Or why the p value is not enough. Journal of Graduate Medical Education, 4(3), 279-282. https://doi.org/10.4300/JGME-D-12-00156.1

Sultana, N., Sarkar, A. K., Matsuda, H., Ihsan, M. A., Haq, S. A., Arefin, M. S., & Islam, S. N. (2022). Relationship Between Vitamin D Status and Brain Perfusion in Neuropsychiatric Lupus. *Nuclear Medicine and Molecular Imaging*, *56*(3), 158–168. https://doi.org/10.1007/s13139-022-00741-x

Tay, S. H., Ho, C. S., Ho, R. C.-M., & Mak, A. (2015). 25-Hydroxyvitamin D3 Deficiency Independently Predicts Cognitive Impairment in Patients with Systemic Lupus Erythematosus. *PLOS ONE*, *10*(12), e0144149. https://doi.org/10.1371/journal.pone.0144149

The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. (1999). *Arthritis and rheumatism*, *42*(4), 599-608. https://doi.org/10.1002/1529-0131(199904)42:4<599::AID-ANR2>3.0.CO;2-F

Unterman, A., Nolte, J. E. S., Boaz, M., Abady, M., Shoenfeld, Y., & Zandman-Goddard, G. (2011). Neuropsychiatric Syndromes in Systemic Lupus Erythematosus: A Meta-Analysis. *Seminars in Arthritis and Rheumatism*, *41*(1), 1–11. https://doi.org/10.1016/j.semarthrit.2010.08.001

Vacca, S., Porcu, M., Piga, M., Mannelli, L., Chessa, E., Suri, J. S., Balestrieri, A., Cauli, A., & Saba, L. (2024). Structural Brain MR Imaging Alterations in Patients with Systemic Lupus Erythematous with and without Neuropsychiatric Events.*American Journal of Neuroradiology*. https://doi.org/10.3174/ajnr.A8200

Wu, B.-B., Ma, Y., Xie, L., Huang, J.-Z., Sun, Z.-B., Hou, Z.-D., Guo, R.-W., Lin, Z.-R., Duan, S.-X., Zhao, S.-S., Yao-Xie, Sun, D.-M., Zhu, C.-M., & Ma, S.-H. (2018). Impaired decision-making and functional neuronal network activity in systemic lupus erythematosus. *Journal of Magnetic Resonance Imaging*, *48*(6), 1508–1517. https://doi.org/10.1002/jmri.26006

Yan, L.-J., Wu, P., Gao, D.-M., Hu, J., Wang, Q., Chen, N.-F., Tong, S.-Q., Rao, L., & Liu, J. (2019). The Impact of Vitamin D on Cognitive Dysfunction in Mice with Systemic Lupus Erythematosus. *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research*, *25*, 4716–4722. https://doi.org/10.12659/MSM.915355

Yang, T.; Wang, H.; Xiong, Y.; Chen, C.; Duan, K.; Jia, J.; Ma, F (2020). Vitamin D Supplementation Improves Cognitive Function through Reducing Oxidative Stress Regulated by Telomere Length in Older Adults with Mild Cognitive Impairment: A 12-Month Randomized Controlled Trial. *J. Alzheimer's Dis*. 78, 1509–1518.

Zaher, A. (2013). Role of Brain Perfusion SPECT in the Assessment OF Neuropsychiatric Systemic Lupus Erythematosus. *The Egyptian Journal Nuclear Medicine*, *7*(7), 48–54. https://doi.org/10.21608/egyjnm.2013.5467

Zhuo, Z. et al. Different patterns of cerebral perfusion in SLE patients with and without neuropsychiatric manifestations (2020). *Hum. Brain Mapp.* 41, 755–766. https://doi.org/10.1002/hbm.24837.

Zhang, X., Zhu, Z., Zhang, F., Shu, H., Li, F., & Dong, Y. (2005). Diagnostic value of single-photon–emission computed tomography in severe central nervous system involvement of systemic lupus erythematosus: A case-control study. *Arthritis Care & Research*, *53*(6), 845–849. https://doi.org/10.1002/art.21591

Appendices

APPENDIX-I

Ethical Clearance Certificate

ডিন অফিস
জীববিজ্ঞান অনুষদ
ঢাকা বিশ্ববিদ্যালয়, ঢাকা-১০০০, বাংলাদেশ

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Ref. No. 119/Biol. Scs.

February 04, 2021

Ethical Review Committee

Professor Dr. Sheikh Nazrul Islam
Institute of Nutrition and Food Science
University of Dhaka

Sub: Ethical Clearance.

Dear Dr. Sheikh Nazrul Islam,

With reference to your application on the above subject, this is to inform you that your research proposal entitled "Effect of vitamin D3 supplement on the cognitive status in patient with systemic lupus erythematosus with neuropsychiatric phenomena" has been reviewed and approved by the Ethical Review Committee of the Faculty of Biological Sciences, University of Dhaka.

I wish for the success of your research project.

Professor Dr. Md. Imdadul Hoque
Dean, Faculty of Biological Sciences
University of Dhaka

APPENDIX-II

Data Collection Sheet

Effects of Vitamin-D 3 Supplement on the Cognitive Status in Patient with Systemic Lupus Erythematosus with Neuropsychiatric Phenomenon

1. Enrollment number: _____ Date of Enrollment:____/____/_____
2. Contact number: _____, _____
3. Name:
4. Age: ---------- ☐
5. Gender: Male=1, Female=2 ---------- ☐
6. Address: Village: _____Pourashava / City corporation:_____

   Upazila: _____District: _____
7. Residence: Rural=1, Urban (Pourashava & City corporation)=2 ---------- ☐
8. Level of education: Illiterate=1, Primary=2, Secondary=3

   Higher secondary=4, Graduate and above=5---------- ☐
9. Occupation: Student=1, Housewife=2, Self-employed=3, Agricultural work=4 Service

   holder=5, Retired=6, Business=7, Unemployed=8, others=9 ---------- ☐
10. Group: control =1, case =2 ---------- ☐
11. Age at onset of neuropsychiatric manifestation_____ ☐
12. Disease duration (Months): _____ ☐

Investigation Data

Base line		**End line**	
Vitamin D		Vitamin D	
MMSE Score		MMSE Score	
Brain Perfusion imaging findings (Areas)		Brain Perfusion imaging findings (Areas)	
Z score from eZIS		Z score from eZIS	

Base line		**End line**	
Brain Perfusion imaging findings (Areas)		**Brain Perfusion imaging findings (Areas)**	
Frontal Lobe		Frontal Lobe	
Frontal Lobe +Precuneous		Frontal Lobe +Precuneous	
Frontal Lobe + Parietal Lobe		Frontal Lobe + Parietal Lobe	
Frontal Lobe +Precuneous + Basal Ganglia		Frontal Lobe +Precuneous + Basal Ganglia	
Frontal Lobe + Temporal Lobe + precuneus		Frontal Lobe + Temporal Lobe + precuneus	

APPENDIX-III

Informed consent form

Study Number…….

Informed Consent Letter

Title of the Study: Effects of Vitamin-D 3 Supplement on the Cognitive Status in Patient with Systemic Lupus Erythematosus with Neuropsychiatric Phenomenon (NPSLE)

Investigator: Dr. Nasreen Sultana

Institution: National Institute of Nuclear Medicine and Allied Sciences, Dhaka (BSMMU Campus)

Purpose of the Study:

This study aims to evaluate the effects of Vitamin D3 supplementation on cognitive function and neuropsychiatric symptoms in patients with Systemic Lupus Erythematosus (SLE), particularly those exhibiting neuropsychiatric manifestations such as cognitive dysfunction, seizures, or mood disorders. The study will assess brain perfusion using SPECT, cognitive status through the Mini-Mental State Examination (MMSE), and Vitamin D serum levels. The goal is to determine the efficacy of Vitamin D supplementation in improving neuropsychiatric symptoms and overall disease management in NPSLE patients.

Selection Criteria:

You have been selected for this study as you are diagnosed with SLE and are experiencing neuropsychiatric symptoms. Your participation will help assess the impact of Vitamin D supplementation on these symptoms.

What is Expected from Participants:

If you agree to participate, you will undergo an evaluation that includes:

- Serum Vitamin D level testing
- MMSE (Mini-Mental State Examination) to assess cognitive function
- SPECT brain perfusion scan

Risks and Benefits:

You will continue receiving your regular treatment and follow-up care during the study. The potential benefit is the assessment of Vitamin D supplementation's impact on your cognitive function and neuropsychiatric symptoms.

Privacy, Anonymity, and Confidentiality:

Your personal and medical information will remain confidential. Data will be securely stored and accessible only to the investigators and authorized study personnel. Any published results will ensure anonymity, with no identifying information shared.

Future Use of Information:

In the future, anonymous or abstracted data from this study may be used for further research. This will not compromise your privacy or confidentiality.

Right to Withdraw:

Participation is voluntary. You may withdraw from the study at any time without penalty, and this will not affect your medical care in any way.

Compensation:

Your participation is voluntary, and there will be no financial compensation. However, you will be provided with information regarding your medical condition and the results of the tests.

If you have any questions, please contact Dr. Nasreen Sultana at the National Institute of Nuclear Medicine and Allied Sciences, Dhaka (BSMMU Campus).
Phone: +88 01711481442

If you agree to participate, please sign below or provide your left thumb impression.

Signature of Participant: _____

Date: _____

Signature of Witness: _____

Date: _____

Thank you for your cooperation.

গবেষণা অবহিতক্রমে সম্মতিপত্র

অংশগ্রহণকারীর আই, ডি: | | | |

এই সম্মতিপত্রের উদ্দেশ্য হলো আপনাকে প্রয়োজনীয় তথ্য প্রদান করা, যে তথ্যগুলো আপনাকে সিদ্ধান্ত নিতে সাহায্য করবে, আপনি এই গবেষণায় অংশগ্রহণ করবেন কি না ?

উদ্দেশ্য পদ্ধতি ঃ Vitamin D and Brain Perfusion Scan test ব্যবহার করে আপনার শরীরে অসুখের **বিস্তার** কতটু ঘটেছে, আপনার কি ধরনের চিকিৎসা পদ্দতি দরকার এবং আপনার রোগের ভবিষৎ কি তা নির্ণয় করা।

গবেষণার ঝুঁকি ঃ

এই গবেষণায় অংশগ্রহণে আপনার/রোগীর কোন ধরনের ঝুঁকির সম্ভাবনা নাই।

গবেষণায় অংশগ্রহণের সুবিধাদি ঃ

এই গবেষণায় অংশগ্রহণ করলে আপনি ব্যক্তিগতভাবে সরাসরি লাভবান হতে পারেন। এই গবেষণা বাংলাদেশে চিকিৎসকদের এই রোগ সম্পর্কে আরো জানতে সহায়তা করবে।

বিকল্প ঃ

এই গবেষণায় অংশগ্রহণ করা কিংবা না করার ব্যাপারে বা অংশগ্রহণ করার পর যে কোন সময় আপনি/আপনার রোগীকে গবেষণা থেকে সরিয়ে নিতে পারেন।

খরচ ঃ

এই গবেষণায় অংশগ্রহণের জন্য আপনার কোন খরচ নাই বা আপনাকে কোন টাকা পয়সা দেয়া হবে না।

গোপনীয়তা ঃ

গবেষণা চলাকালীন ও পরবর্তীতে সকল তথ্য কঠোরভাবে গোপন রাখা হবে। পরবর্তীতে ফলোআপ ও অনুসরণ প্রক্রিয়ার জন্য আপনাকে একটি আইডি নম্বর দেওয়া হবে। আপনার আইডি নম্বর সম্বলিত সব ধরণের কাগজপত্রে আপনার/রোগীর নাম ও ঠিকানা বসিয়ে অফিসের ফাইলিং কেবিনেটে তালাবদ্ধ থাকবে ব্যক্তিগত বিষয়াদি তথ্য বিশ্লেষণ, প্রতিবেদন তৈরিতে এবং প্রকাশনার কাজে ব্যবহার হবে না এবং গবেষণার পরীক্ষক ব্যতীত কারো প্রকাশ করা হবে না। ফলে আপনার/রোগীর কোন তথ্য অন্য কেউ জানতে পারবে না।

স্বেচ্ছামূলক অংশগ্রহণ ঃ

এই গবেষণায় আপনার অংশগ্রহণ সম্পূর্ণ স্বেচ্ছামূলক। আপনি গবেষণায় অংশগ্রহণে অস্বীকৃতি জানাতে পারেন অথবা গবেষণা চলাকালীন যে কোন সময় গবেষণা থেকে আপনি/আপনার রোগীকে প্রত্যাহার করে নিতে পারেন। তাতে আপনার চিকিৎসার কোন তারতম্য হবে না। এই ফরমে স্বাক্ষর করলে আপনার আইনগত কোন অধিকার খর্ব হবে না।

প্রশ্নবলী ঃ

যদি আপনার কোন প্রশ্ন থাকে তবে দয়া করে জিজ্ঞাসা করুন। আমরা তার উত্তর প্রাদন করার যথাসাধ্য চেষ্টা করবো। যদি ভবিষ্যতে আপনার অতিরিক্ত কোন প্রশ্ন থাকে তাহলে গবেষণারত ডাক্তারের সাথে যোগাযোগ করতে পারেন।

সম্মতি স্বীকারোক্তি ঃ

আমি গবেষণায় নিয়োজিত চিকিৎসক-এর সাথে (যিনি আমার/রোগীর শারীরিক পরীক্ষা করবেন) এই গবেষণায় নিয়ে আলোচনায় সন্তুষ্টি প্রকাশ করছি। আমি এটা বুঝেছি যে গবেষণায় অংশগ্রহণ স্বেচ্ছামূলক এবং আমি যে কোন সময় কোন বাধ্যবাধকতা ছাড়াই গবেষণা থেকে আমাকে/রোগীকে বিরত রাখতে পারি। আমি উপরোক্ত শর্তগুলো পড়িছে/আমার সম্মুখে পঠিত হয়েছে এবং স্বেচ্ছায় গবেষণায় অংশগ্রহণ করতে সম্মতি জ্ঞাপন করছি।

সাক্ষাৎকার গ্রহণকারীর স্বাক্ষর ঃ

তারিখ ঃ

অংশগ্রহণকারীর স্বাক্ষর

অংশগ্রহণকারীর বৃদ্ধাঙ্গুলির ছাপ

APPENDIX-IV

Illustration

Image 1: Symbia Evo Dual head Gamma Camera at NINMAS .

Image 2: Vitamin D analyzer , ADVIA Centaur at NINMAS

Image 3: BD tube with EDTA

Image 6: EDTA stands for Ethylenediaminetetraacetic acid. EDTA functions by binding calcium in the blood and keeping the blood from clotting used in serum Vitamin D.

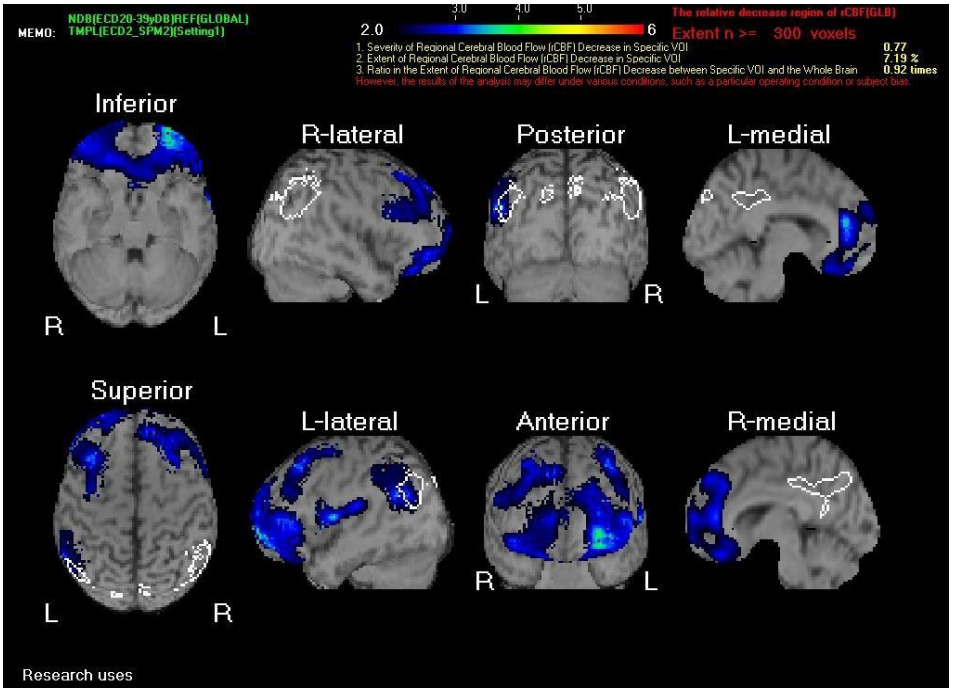

Image A: Base line image of a case-1

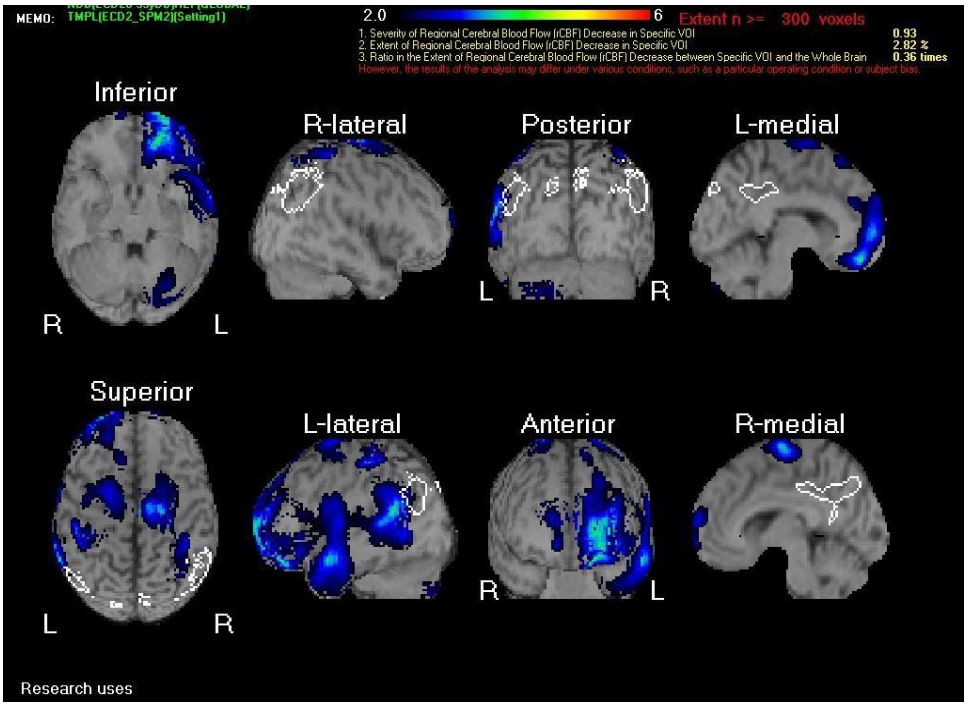

Image B: End line image of case-1

Figure A & Figure B represent a 22-year-old female with NPSLE underwent brain SPECT imaging, with DICOM data analyzed after eZIS application. The 3D surface view shows a significant improvement in perfusion status from baseline to end line. At baseline, hypoperfusion was observed in both frontal lobes and the left parietal lobe. However, at the end line, there is a notable improvement in perfusion, particularly in the right frontal lobe.

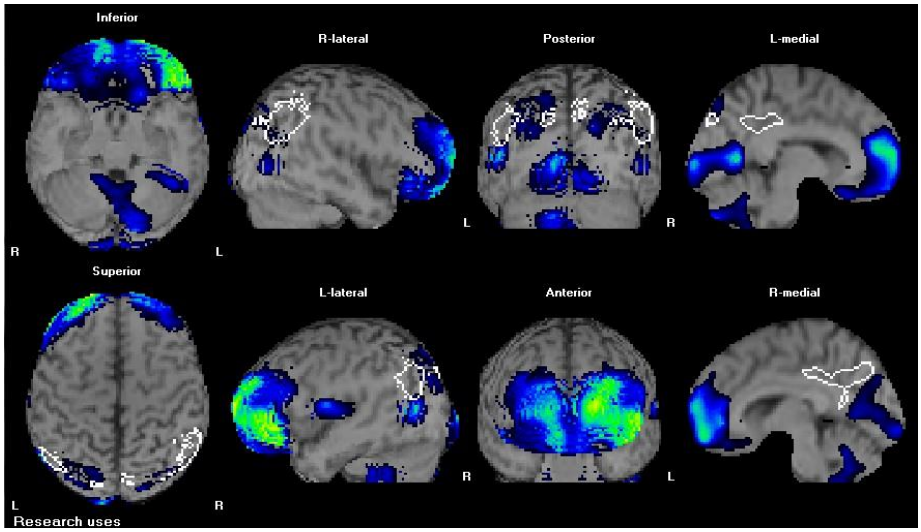

Image C: Base line image of a case-2

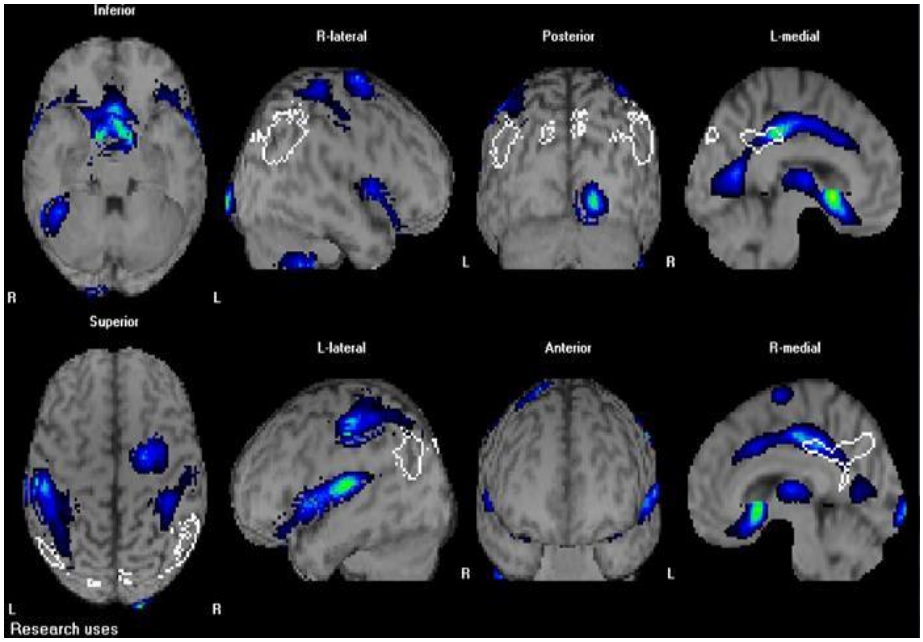

Image D: End line image of a case-2

Figure C & D represent a 20-year-old female with NPSLE underwent brain SPECT imaging, with DICOM data analyzed using eZIS application. The 3D surface view reveals a significant improvement in perfusion from baseline to end line. At baseline, hypoperfusion was noted in both frontal lobes and the both parietal lobes. By the end line, there was a notable improvement in perfusion in both frontal lobes.

Sum/Parliament

"দু'টি সন্তানের বেশী নয় একটি থাকলে ভাল হয়"
বাংলাদেশ পরমাণু শক্তি কমিশন
"পরমাণু ভবন"
ই-১২/এ, আগারগাঁও, শেরে বাংলা নগর, ঢাকা-১২০৭
Website : www.baec.org.bd Fax : ৮৮-০২-৮১৮১৮৪২

নং-৩৯.২১৮.০৩১.০১.০০.৬৪৬.২০১৩-২১৬(৩)

তারিখ ঃ ২২ মাঘ ১৪২০ / ০৪ ফেব্রুয়ারী ২০১৪

বিষয় ঃ উচ্চতর শিক্ষা গ্রহণের জন্য দেশি/বিদেশী বিশ্ববিদ্যালয় সমূহ যোগাযোগের পূর্বানুমতি প্রদান প্রসংগে।

বাংলাদেশ পরমাণু শক্তি কমিশনের ন্যাশনাল ইনস্টিটিউট অব নিউক্লিয়ার মেডিসিন এন্ড এ্যালায়েড সায়েন্সেস, শাহবাগ, ঢাকার মুখ্য চিকিৎসা কর্মকর্তা ডাঃ নাসরিন সুলতানা-এর ০৭/০১/২০১৪ খ্রি. তারিখের আবেদনসূত্রে কর্তৃপক্ষের সিদ্ধান্তক্রমে জানানো যাচ্ছে যে, উচ্চতর শিক্ষা গ্রহণের জন্য দেশি/বিদেশী বিশ্ববিদ্যালয় সমূহে যোগাযোগের বিষয়ে তাঁকে কর্তৃপক্ষ কর্তৃক অনুমতি প্রদান করা হয়েছে।

(শামছুজ্জোহা আহমদ)
উর্ধতন প্রশাসনিক কর্মকর্তা
সংস্থাপন-১ শাখা

ডাঃ নাসরিন সুলতানা
মুখ্য চিকিৎসা কর্মকর্তা
ন্যাশনাল ইনস্টিটিউট অব নিউক্লিয়ার মেডিসিন এন্ড এ্যালায়েড সায়েন্সেস
শাহবাগ, ঢাকা।

অনুলিপি ঃ

০১। পরিচালক, ন্যাশনাল ইনস্টিটিউট অব নিউক্লিয়ার মেডিসিন এন্ড এ্যালায়েড সায়েন্সেস, শাহবাগ, ঢাকা।

Effects of Vitamin-D 3 Supplement on the Cognitive Status in Patient with Systemic Lupus Erythematosus with Neuropsychiatric Phenomenon

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15 DEC 2024

Prof. Dr. Md. Saiful Alam
Librarian (In-charge)
University of Dhaka

15.12.24