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The Relationship between Nerve Growth Factor and Vitamin B12 as a Predictive Marker for Nerve Damage in Iraqi Patients with Systemic Lupus Erythematosus

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Abstract

Determination of serum nerve growth factor (NGF) and vitamin B12 in Iraqi female patients with systemic lupus erythematosus (SLE) and assessment of the effect of disease duration on the development of nerve damage. In addition, NGF may be a predictive marker for nerve damage in SEL patients. The current study included 130 individuals whose ages ranged from 18 to 48 years old, 100 of whom were female patients with SLE and 30 individuals as controls. Each patient was diagnosed after undergoing a thorough clinical evaluation backed by laboratory testing by a board-certified rheumatologist. The patients were classified according to the duration of disease into 15 patients with a disease duration of less than 3 months (G1), 29 patients with a disease duration of 1 year (G2), and 56 patients with a disease duration of more than one year (G3). This study showed a significant increase in serum NGF and a significant decrease in vitamin B12 levels in SLE patient groups when compared with control, as well as their level in SLE patients compared with control. Also, there was an essential negative correlation between NGF and vitamin B12 in SLE patients and controls. The present results found that the serum NGF levels in SLE patients considerably increased with the duration of the disease. Therefore, NGF may be a diagnostic and follow-up marker of the development of disease. In addition, the significant negative correlation between NGF and vitamin B12 indicates that SLE patients may be prone to nerve damage, and NGF may be a predictive biochemical marker for the development of nerve damage in SLE patients.

Keywords: Systemic lupus erythematosus, nerve damage, nerve growth factor, vitamin B12.

1. Introduction

Lupus is an autoimmune disorder in which the body's immune system mistakenly attacks and kills healthy cells and tissues. Types of lupus include systemic lupus erythematosus (SLE), which is a chronic inflammatory disorder that has a disastrous impact on almost every organ and tissue in the body [1]. Due to mistaking good tissue for harmful invaders, the body's immune system causes the autoimmune disease lupus. Several physiological processes are negatively impacted [2].

Since lupus affects every part of the body, prompt diagnosis and treatment are essential for reducing tissue damage and enhancing health outcomes [3]. SLE is influenced by both environmental and genetic factors [4]. Pregnant women and young women are more vulnerable to developing SLE. An increased risk of developing SLE may be traced back to hormones in a variety of ways, not only the disruption of homeostasis of a particular hormone. According to the findings of McMurray RW et al. meta-analysis [5]. Most patients in community-based caucasian registries are middle-aged women, and almost half of all cases are considered to be relatively mild when first diagnosed [6].

Chronic multi-organ autoimmune illness, also known as SLE. The immune system's sensitivity to self-antigens is broken, causing the production of excessive auto-antibodies. When this happens, immune complexes are released into the bloodstream, and tissue damage occurs as a result of an immune response [7]. One of the most typical indicators of lupus is the presence of abnormal antibodies in the blood. SLE has unknown causes; however, genetics, infections, sun exposure, and medicines are all possible contributors [8]. Antinuclear (ANA) and anti-double-stranded (ds) DNA antibodies, which are currently used for diagnosis, lack specificity and sensitivity, respectively, and add to the difficulties in the diagnosis of SEL [9]. The central nervous system (CNS) may be affected by SLE, a systemic autoimmune illness with various immunologic abnormalities. Both the CNS and peripheral nervous system (PNS) are involved in the effects of SLE. The involvement of the nervous system in SLE continues to be a leading cause of morbidity and death [10]. Nerve growth factor (NGF) is a neurotrophic protein thought to be critical for the development of sympathetic and sensory afferent neurons. Synaptic plasticity, neurotransmitter release, axonal tract formation, and gene transcription are all affected by nerve growth factors [11]. NGF, the first neurotrophic factor discovered, plays a significant role in the development of the brain's neural systems. Adult endogenous NGF secretion is involved in a wide range of physiologic functions, including the genetic and functional maintenance of sympathetic and sensory fibers in the peripheral nervous system [12]. Vitamin B12 (Vit. B12), or cobalamin, is a water-soluble vitamin essential for normal cellular metabolism and for maintaining a healthy nervous system [13]. It is crucial for healthy nerves and vital in DNA synthesis, meaning it helps cells divide. Clinical symptoms of Vit. B12 deficiency mainly include anemia and demyelination of the neurological system [14].

2. Materials and Methods

2.1. Study design

A cross-sectional sample was analyzed on patients at the Rheumatology Unit of Baghdad Teaching Hospital, Medical City (Baghdad) between September 2022 and February 2023. The present research comprised 130 participants, ranging in age from 18 to 48. One hundred of them were female patients with SLE, and 30 additional participants served as healthy controls. A rheumatologist diagnosed each case of SLE after thorough clinical evaluations backed up by laboratory findings.

The patients were classified according to the duration of the disease into:

1- G1= 15 patients with a disease duration of less than 3 months.

2- G2= 29 patients with a disease duration of more than 3 months to 1 year.

3- G3= 56 patients with a disease duration of more than one year.

2.2 Sample collection

Human venous blood was collected from both patients and healthy subjects. The serum was then frozen for use in future laboratory evaluations, which included dsDNA, ANA, NGF,

and Vit. B12 in serum and white blood cells (WBC), red blood cells (RBC), platelets, blood hemoglobin (HGB), platelets (PLT), and erythrocyte sedimentation rate (ESR) in whole blood. The ethics of scientific research apply to the collection of samples and data. All participants gave their informed permission before participating.

2.3 Exclusion criteria

All patients were free from other autoimmune diseases such as (rheumatoid arthritis and scleroderma), thyroid diseases, hematological diseases, pregnant women, and tumors.

2.4 Methods

The blood samples, which were drawn from all of the patients and the control group, were divided into whole blood and were used to measure the WBC, RBC, HGB, and PLT using an automated CBC analyzer. The serum was then frozen until it was needed for the analysis. According to the prescribed procedure, the Westergren technique was used to measure the ESR. After obtaining the patients' serum using the Alegria device, ANA and dsDNA determinations were made. The results were provided in IU/ml for ANA and dsDNA. The NGF was determined by an enzyme-linked immune-sorbent assay (ELISA) kit purchased from (Fine Test, China). This kit was based on a sandwich enzyme-linked immune-sorbent assay reader and then the concentration technology. The serum of Vit. B12 was assayed by the enzyme-linked immune-sorbent assay (ELISA) kit from My Biosource, USA. This kit was based on the competitive ELISA detection method.

2.5 Statistical analysis

The data were expressed as the mean ± standard error of the mean (SEM). Several statistical tests, including the student t-test, the LSD test, and the correlation coefficient, were used to examine the differences between the patient and control groups. As a rule of thumb, P-values of ≥ 0.05 were considered not statistically significant, whereas P-values of 0.05 were supposed to be so. The receiver operating characteristics (ROC) curve was used to determine the cutoff value, sensitivity, and specificity.

3. Results

The clinical parameters of the study groups are shown in **Table** 1. The measurement of dsDNA, ANA, NGF, and Vit. B12. ESR, HBG, WBC, RBC, and platelet levels were detected in SLE patients with a disease duration of less than 3 months (G1), SLE patients with a disease duration of more than 3 months (1 year) (G2), SLE patients with a disease duration of more than one year (G3), as well as control (C). The results in the current study display a significant ($P \leq 0.05$) increase in dsDNA and ANA levels in G1, G2, and G3 patient groups compared with the control (C) group, as well as no significant ($P > 0.05$) differences in dsDNA and ANA levels between patient groups when comparing between them.

The data presented in Table 1 elucidate a notable significance. ($P \leq 0.05$) increase in serum NGF and a significant ($p \leq 0.05$) decrease in Vit. B12 levels among patient groups (G1, G2, and G3) compared to the control group (C). Additionally, there was no significant difference ($P > 0.05$) in NGF levels between patients in groups G1 and G2, as well as between G1 and G3. In contrast, a significant difference in NGF level was found between G2 and G3. In the current study, the level of NGF in the patient group (G2) was higher than in G1 and G3. There were no significant ($P > 0.05$) differences in vit. B12 levels between patient groups (G1, G2, and G3) when comparing them. The results in this study showed a significant ($P \leq 0.05$) increase in ESR levels in G1, G2, and G3 when compared to the control group (C), and there were significant variations

in ESR levels between patient groups (G1, G2) and (G1, G3). The current study showed a significant ($p \leq 0.05$) decrease in HBG and WBC levels in patient groups (G1, G2, and G3) compared to the control group (C) and no significant ($P > 0.05$) differences in HBG value between patient groups. At the same time, there were significant variations in WBC levels between patient groups (G1 and G2) and (G2 and G3). In **Table 1**, there were no remarkable ($P > 0.05$) differences in RBC and PLT levels in patient groups G1, G2, and G3 when compared with the control group (C).

Table 1. Mean ± SEM of levels of studied parameters in control (C) , and SLE patients groups G1, G2, and G3.

Parameters	**C No.(30)**	**G1 No.(15)**	**G2 No.(29)**	**G3 No.(56)**
dsDNA (IU/mL)	7.0±0.61	80.52±5.85*a*	81.79±5.39*a*	74.83±1.70*a*
ANA (U\mL)	0.32±0.03	1.92±0.16*a*	1.64±0.13*a*	2.14±0.36*a*
NGF (pg\mL)	87.06±2.52	210.83±20.1*a*	239.96±20.95*a*	201.39±10.69*ad*
Vit.B12 (ng\mL)	31.12±1.21	4.73±0.24*a*	4.69±0.14*a*	4.91±0.11*a*
ESR (mm\h)	5.38±0.35	40.66±2.76*a*	26.95±2.89*ab*	31.37±8.38*ac*
HGB (10^6\uL)	13.1±0.20	11.66±0.92*a*	12.23±0.23*a*	11.77±0.20*a*
WBC (10^3\uL)	10.19±0.246	6.43±0.85*a*	8.46±0.50*ab*	6.64±0.33*ad*
RBC (10^3\uL)	4.37±0.48	4.36±0.11	4.41±0.10	4.40±0.12
PLT (10^3\uL)	254.60±5.04	273.66±27.93	243.95±11.26	243.69±8.09

^{a} LSD test between four groups and control, ^{b} LSD test between G1 and G2 patients, ^{C} LSD test between G1 and G3 patients, ^{d} LSD test between G2 and G3 patients. *Significant: * $P \leq 0.05$; No significant: $P > 0.05$.*

Table 2 shows a notable inverse correlation between serum NGF levels and dsDNA, Vitamin B12, and WBC count. Additionally, a meaningful positive correlation was observed between NGF levels and ANA, RBC count, and PLT count in the control group. NGF was correlated with ESR by no significant positive relationship in the control group. In patients' groups, there was a significant positive correlation between serum NGF and dsDNA and ANA, and there was also a significant negative correlation between NGF and Vit. B12 in SLE patients' groups. The current study shows no remarkable negative relationship between NGF and ESR, HGB, RBC, and PLT, in addition to no significant positive correlation with WBC in SLE patient groups.

Table 3 and **Figure 1** show the results of an ROC analysis for the NGF marker, which demonstrates that it is an excellent diagnostic marker for SLE disease. The test indicated a cut-off value of 99% sensitivity and 100% specificity.

Table 2. Pearson correlation coefficient (r) and P-value between NGF and all studied parameters in control and SLE patients groups.

Parameters	Control		Patients groups	
	r	P-value	r	P-value
dsDNA(IU\ml)	-0.234	.000	0.110	.000
ANA(U\ml)	0.173	.000	0.066	.000
Vit.B12(ng\ml)	-0.671	.000	-0.295	.003
ESR(mm\h)	-0.040	.382	-0.087	.396
HGB(10^6\ul)	0.267	.154	-0.018	.859
WBC(10^3\ul)	-0.355	.055	0.052	.610
RBC(10^3\ul)	0.728	.000	-0.114	.263
PLT(10^3\ul)	0.000	.000	-0.140	.167

*Significant: * $P \leq 0.05$; No significant: $P>0.05$*

Table 3. Sensitivity, specificity & cut-off value of NGF for diagnosis of SLE.

Test results	Area under curve%	Sensitivity %	Specificity %	Cut-off value	Asymptotic Sig.	Accuracy	
						Lower Bound	Upper Bound
NGF	99.3%	99%	100%	105.9	.000	0.98	1.0

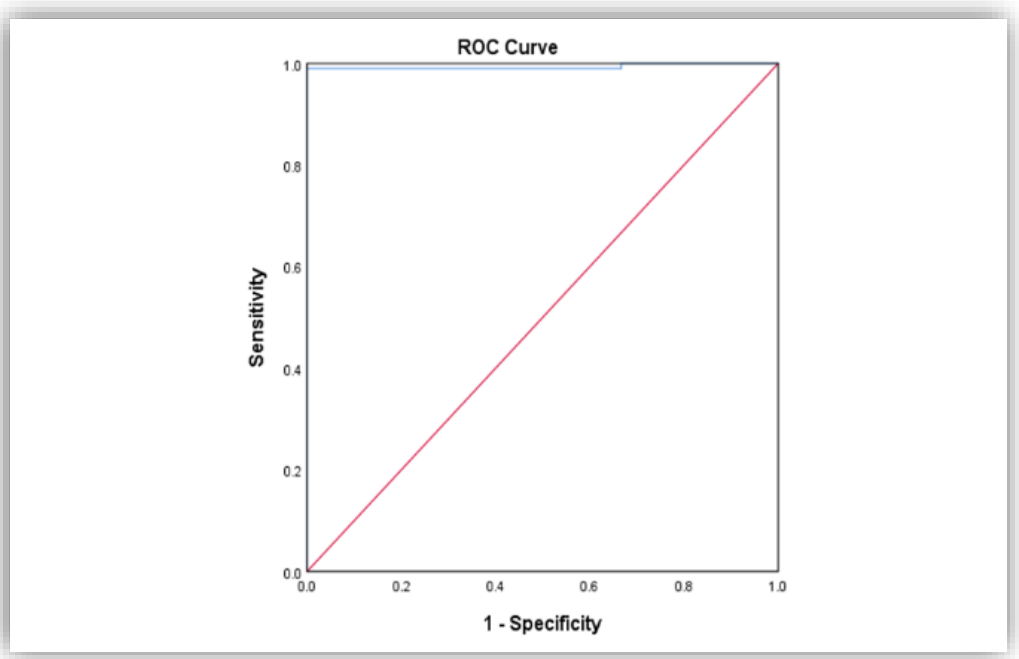

Figure 1. The Roc curve of NGF in SLE and control groups

4. Discussions

Systemic lupus erythematosus is one of the most gender-differentiated autoimmune illnesses, affecting primarily females between the ages of 15 and 44 (the childbearing years)

[15]. The results suggest that both women's age and gender have a role in the development of SLE. While the incidence of ANA positivity is often lower in cross-sectional research, the current study confirms previous findings that ANA positivity in SLE patients surpass 90%. Although some studies have claimed a 100% frequency, the majority show a lower percentage, from 95% to 99% [16]. Previous Iraqi studies showed a significant variation in ANA, with a wide range of frequency variations of 98% [17] and 93% [18].

The present study showed a significant increase in ANA and dsDNA levels in patients compared to the control. This result agrees with a study by Noor Ahuda et al. (2020) that found a significant increase in ANA, Anti-ds DNA, ESR, and h-CRP in groups of Iraqi SLE patients in comparison with control [19]. Anti-dsDNA antibodies are the most heavily weighted criteria in the immunologic domain of the 2019 EULAR/ACR classification. They are one of the most distinctive kinds of ANA, with a high specificity (96%) for SLE. The levels of anti-dsDNA antibodies are highly linked with disease activity and may change over time [20]. This means that levels might be undetectable throughout therapy but rise dramatically after a flare, especially for patients with active nephritis. Anti-dsDNA antibodies have limited diagnostic sensitivity (52% to 70%) because of their fleeting presence [21]. Anti- dsDNA and ANA are two examples of autoantibodies often seen in patients with SLE. As a result, SLE is thought to be a B lymphocyte-mediated disease [22]. Although circulating autoantibodies, immune complex deposition, and complement protein activation are all characteristics of SLE, it is clear that an imbalance in cytokines is also a significant contributing factor [23]. In addition, autoantibodies are usually present before symptoms appear [24]. The term "damage" refers to the long-term effects of SLE and related factors such as illness and treatment. The pattern of damage shows a linear increase over time [25]. Definitions and criteria for the classification of 19 CNS and peripheral nerve syndromes (PNS) have been suggested by the American College of Rheumatology in the context of SLE. SLE is often thought to present itself in the PNS [26]. The clinical manifestation of PN is determined by the nerve's diameter, the nature of the demyelinating or axonal damage, and whether or not it is an acute or chronic occurrence [27]. Pathological studies of the peripheral nerves in SLE have revealed axonal pathological changes, inflammatory changes, and vasculitis, which may suggest the likelihood of various pathogenetic components for the different types of SLE-related neuropathy but do not shed light on the pathogenesis of SLE-related neuropathy [28].

The primary inflammatory mediators secreted by immune cells act on sensory neurons, causing peripheral sensitization and hyperalgesia. In addition to the ischemic vascular mechanism, such as vasa nervorum vascularity or microthrombi connected to antiphospholipid antibodies, this normal inflammatory response following injury may promote the pathogenetic activity of antineutral autoantibodies. The other acceptable processes include immunologic damage caused by a direct attack of antibodies and entraining obliteration of the peripheral nerve component [29]. The term "neuropathic pain" refers to a kind of pain that occurs as a result of injury or malfunction to the neurological system, and it may appear on its own or in response to external stimuli. With nerve damage, neuropathic pain may develop, but the CNS may shift, leading to another symptom. Nerve growth factors are a kind of neurotrophic factor that promotes neuronal survival and growth throughout development, preserves the structural and functional integrity of the adult nervous system, and governs the plasticity of the damaged or diseased adult nervous system [30]. Peripheral sensory nerve fibers produce signaling molecules to regulate immune response. When this happens, it's called neurogenic

inflammation (NI), and NGF has an essential function as a mediator. It raises levels of inflammatory peptides such as substance P and calcitonin gene-related peptides. There are two receptors for nerve growth factor: a high-affinity tyrosine kinase A and a low-affinity p75 receptor, which is frequently thought of as a co-receptor for tropomyosin-related kinase A. TrkA, a neurotrophic factor known as NGF has been investigated as a treatment for diabetic peripheral neuropathy (DPN) [31]. Changes in NGF levels are implicated in the pathophysiology of chronic pain syndromes, including neuropathic pain. NGF is known to impact the phenotype of adult nociceptors receptors as well as the proper development of the embryonic nervous system [32]. The nerve growth factor is a biomarker reflecting damage to different types of nerves [33]. It has also been linked to hyperalgesia across a wide range of painful conditions, as well as the genesis of acute and chronic cases. High levels of NGF are seen in inflamed or injured tissues, where it facilitates the transmission of pain signals through nociceptive nerve fiber. Congenital insensitivity to pain or an impaired capacity to feel pain is the result of mutations in NGF or its tyrosine kinase receptor TrkA [34]. This study showed that there is a significant increase in the level of NGF in SLE patients with the duration of the disease, and this explains the exacerbation of pain in these patients with the length of the disease.

The B-complex vitamins, especially thiamine (B1), pyridoxine (B6), and cobalamin (B12), are discussed. Certain vitamins are also referred to as "neurotropic" vitamins because of their beneficial effects on the nervous system. Certain B vitamins are thought to play a role in promoting neuron regeneration. On the other hand, Vit. B12 plays a crucial role in remyelination and the upkeep of myelin sheaths, which greatly increases the likelihood of nerve cell survival [35]. In the current study, there is a significant decrease in the level of Vit. B12 in patients with SLE, and these patients suffer from neuralgia, especially arthritis. However, to elucidate the mechanism of the role of Vit. B12 and NGF in maintaining neuroprotective functions, more studies are needed in this field.

Non-specific indicators of systemic inflammation like C-reactive protein (CRP) and ESR might be effective biomarkers in this familiar clinical setting. Inflammation, whether caused by infection, cancer, or an autoimmune disorder, may raise both CRP and ESR. At the same time, SLE patients have been reported to have a greater propensity for ESR elevations than CRP elevations when compared to rheumatoid arthritis patients [36] since ESR increases with both lupus activity and infection. It lacks specificity in distinguishing between the two. However, elevated ESR levels are closely linked to disease flare-ups in SLE. In active SLE, an increased ESR has been recognized to occur on a regular basis for quite some time. Both alterations in serum proteins and changes in ESR might theoretically contribute to elevations in ESR. Common examples of the former group include hypergammaglobulinemia, monoclonal gammopathy, and elevated fibrinogen. The primary latter results from smaller and fewer red blood cells. Some of these ESR-increasing variables are inflammatory, while others are not [37].

Another crucial inflammatory component of ESR is anemia. Patients with active lupus often have low amounts of HGB and ESR. This is seldom attributable to hemolytic anemia despite being a common symptom of SLE. Hemolytic anemia, as was previously indicated, would also fall under the category of a direct autoimmune characteristic since it is brought on by antibodies to erythrocytes rather than being a sign of inflammation [38]. Autoimmune cytopenias are frequent in SLE, as are hematological diseases and new haematologic abnormalities. Patients

with SLE may have immune thrombocytopenia and hemolytic anemia, both of which need immunosuppressive treatment. Cytopenias are widespread throughout SLE, and the most common causes include medications, infections, and immune-mediated disorders. Lymphopenia, which affected 40.3% of patients, was the most frequent kind of white cell abnormality, with leukopenia affecting almost 30% of patients. Only about 11% of current patients had a very uncommon incidence of thrombocytopenia associated with SLE. In most cases, the immune system is to blame for irregularities in WBC and thrombocytes because antibodies kill off the cells that produce them. Patients with SLE who had high hematological involvement were also more likely to have significant disease in the renal, central neurological, and general systems but not in the other systems, according to a study by Sultan The majority of SLE patients have hematological abnormalities at the time of diagnosis, and many of these patients continue to have abnormalities throughout their follow-up care for many years following diagnosis. With 63% of patients suffering from anemia, it was the most prevalent condition. Anemia in SLE may have a variety of reasons, both immunological and non-immune. The abnormalities, such as anemia, leukopenia, and thrombocytopenia, are linked to specific illness symptoms and organ involvement. This knowledge might improve the management strategy for patients with SLE [39].

5. Conclusions

Compared to prior studies, this is the first study to investigate the relationship between NGF and Vit. B12 in patients with SLE. The present results found that serum NGF levels in SLE patients were considerably increased with the length of disease duration. Therefore, NGF may be a diagnostic and follow-up marker of the development of the disease. In addition, the significant negative correlation between NGF and Vit. B12 indicates that SLE patients may be prone to nerve damage, and NGF may be a predictive biochemical marker for the development of nerve damage in SLE patients. The ROC test for the NGF marker in SLE patients and control groups showed a perfect cutoff value with 99% sensitivity and 100% specificity, which indicates it is considered a good diagnostic marker for SLE patients.

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Conflict of Interest

The authors declare that they have no conflicts of interes.

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Ethical Clearance

The samples were gained according to Local Research Ethics Committee approval in Iraqi Ministry of Health No.37755 11\9 \2022.

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