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Detection of Macrophage Colony Stimulating Factor in Systemic Lupus Erythematosus Patients and its Relation to Disease Activity and Severity

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Abstract:

Introduction: Systemic lupus erythematosus (SLE) is a polyorgan persistent autoimmune syndrome that typically affects women throughout their reproductive years. Its etiopathogenesis remains obscure and complicated, and its clinical symptoms, severity, and responsiveness to therapy vary.

Aim of the study: To monitor the circulating concentrations of M-CSF (macrophage colony-stimulating factor) in SLE subjects and investigate its correlation to disease progress, renal inclusion, peripheral vascular affection, and other disease characteristics.

Subjects and methods: Fifty SLE patients and fifty healthy controls were included. All patients were subjected to a complete history, clinical examination, and laboratory investigations; a nailfold capillaroscopy assessment was done; and a renal biopsy was done when indicated. the Safety of Estrogens in Lupus Erythematosus National Assessment (SLEDAI-SELENA), the SLE Disease Activity Index was used to examine disease activity. M-CSF levels were measured by enzyme-linked immunosorbent assay in both SLE patients and controls.

Results: The SLE patients had a mean age of 33. 9±10.8 years; 47 were females (94%), and 3 were males (6%); the disease duration was 5.9±5. 4 years. The most prevalent clinical symptoms were musculoskeletal and cutaneous. In patients with lupus nephritis, Class IV had the highest frequency. 92% of SLE patients had abnormal nailfold capillaroscopy findings. M. CSF levels were shown to be greater in SLE patients compared to healthy controls (p-value <0.05). Additionally, there was a strong correlation between the levels of M. CSF and the renal biopsy classes, with patients in classes V, IV, and III having higher levels.

Conclusion: The M. CSF level was high in SLE patients and it was substantially correlated with various renal biopsy classes. Keywords:

Keywords: Systemic Lupus Erythematosus (SLE); Macrophage Colony-Stimulating Factor (M-CSF); Nailfold Capillaroscopy (NFC).

1. Introduction

Persistent inflammation and the generation of autoantibodies stand out as prominent characteristics of systemic lupus erythematosus (SLE) [1, 2]. The induction of tissue damage and concurrent health conditions, particularly those related to kidney participation, can be attributed to either the treatment for SLE or the condition itself [3]. Individuals with SLE may encounter signs such as microvascular involvement, which emerges as a notable feature [4].

Employing nailfold capillaroscopy (NFC) may be advantageous in evaluating the changes in microvascular patterns evident in individuals diagnosed with SLE [5]. The involvement of the kidneys presents as a significant manifestation of SLE, potentially resulting in an unfavorable prognosis and the development of end-stage kidney failure [6]. Renal biopsy continues to serve as the primary method for diagnosing lupus nephritis, while the availability of validated biomarkers for tracking the progression of SLE remains limited [7].

Consequently, there exists a pressing necessity for the recognition of innovative biomarkers capable of evaluating renal engagement and disease activity. The sequence of inflammatory reactions sparked by the disruption of the immune system's equilibrium and the identification of self-antigens sets in motion a recurrent cycle of immune cell activation in SLE. Individuals affected by SLE are demonstrating a growing prevalence of abnormalities in both the composition and operation of monocytes and macrophages [8, 9].

When in an adequate state, macrophages and monocytes are fundamental segments of the innate immune system. They perform a variety of immunological tasks, including presenting antigens, phagocytosing particles, and generating cytokines [10]. Activated by GM-CSF and TNF-α, macrophages—also referred to as activated macrophages—show dual roles in the germ defensive framework and inflammation. The cytokines released by these activated macrophages include IL-6 and TNF-α. On the other hand, IL-4 and M-CSF cause a specific subgroup of macrophages—referred to as M2 macrophages—to polarize. The capacity of these M2 macrophages to produce cytokines that reduce inflammation, such as IL-10, sets them apart [11, 12]. Additionally, there exists a connection between macrophages showcasing the soluble urokinase plasminogen activator receptor (uPAR) and those characterized by the M2 phenotype [13]. Moreover, M-CSF and Interleukine-34 operate through a shared receptor, guiding macrophages toward a phenotype responsible for regulating M2 [14].

This study aims to analyze the concentrations of M-CSF in SLE patients' serum, exploring its correlation with disease progress, renal involvement, effects on peripheral blood vessels, and other related factors.

2. Subjects and Methods

2.1. Subjects

The research was carried out within the inpatient and outpatient departments of the rheumatology department at Fayoum University Hospital. Fifty individuals diagnosed with SLE, meeting the 2019 EULAR/ACR criteria, were enrolled in the study. Exclusions comprised individuals with cancer, infections, or alternative autoimmune conditions [15].

Furthermore, the study incorporated 50 healthy individuals without any previous record of systemic or chronic autoimmune conditions as control subjects.

2.2. Methods

A comprehensive set of laboratory assessments, clinical evaluations, and meticulous history gathering were conducted. Renal biopsies were carried out as required, adhering to the guidelines outlined in the 2004 classification set forth by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) [16]. Employing the Safety of Estrogens in Lupus Erythematosus National Assessment modification (SLEDAI-SELENA) for gauging the progression of the illness [17].

The assessment of peripheral vascular affection and its relationship to serum M-CSF levels was done using nailfold capillaroscopy. An ×200 magnification DinoCapture 2.0 multipurpose digital microscope (version 1. 5. 28. D) was used to capture the images. under the Nailfold Capillaroscopy unit of the Fayoum University Hospital's Rheumatology and Rehabilitation department. The thumbs were excluded from the study due to their frequent microtrauma and poorly observable capillaries. NFC was conducted in nailfold cuticles, where capillary loops can be seen in the last row at full length. The assessment included the second through fifth fingers on each hand, and blood was drawn in addition to nailfold capillaroscopy pictures. The Pan-American League of Associations for Rheumatology (PANLAR) capillaroscopy research team of 2019 outlined consensus guidelines for the investigation of nailfold capillaries and the exegesis of the capillaroscopy findings [18].

Detecting M-CSF levels

For detecting M-CSF levels, samples were procured from individuals under medical scrutiny and those serving as reference points garnered, and allowed to coagulate for a quarter of an hour within unadorned receptacles. Subsequently, they were subjected to a period of gravitational separation lasting ten minutes, at a force multiplier of four thousand times the Earth's gravitational pull. Until requisitioned for use, the distinct serious elements were maintained at a temperature of negative twenty degrees Celsius. Employing a kit reliant on enzyme-linked immunosorption, a methodology

hailing from the laboratories of BT located in Zhejiang, China, the quantification of serum Macrophage-Colony Stimulating Factor (M-CSF) was undertaken. Following the guidelines issued by the fabricator, they bore the serial number E0127Hu. To forestall discrepancies among the assays, all specimens were scrutinized on the very day of their extraction.

2.3. Statistical Analysis

For analyzing data, the Statistical Package of Social Sciences (SPSS) software version 22 was utilized on a Windows 7 platform. After collection and coding, the data was formatted to facilitate handling previous to being inputted twice into Microsoft Access (SPSS Inc., Chicago, IL, USA). The arithmetic mean values serve to gauge the central tendency, while standard deviations serve to gauge the spread of numerical parametric data. Qualitative data is quantified through numerical representations and proportions. In the case of numerical parametric data, a test involving independent samples (t-test) was employed to compare quantitative measurements across two distinct groups.

The Bonferroni posthoc method was utilized to explore the differentiation among every pair of sets, while the one-way ANOVA examination was employed to assess quantitative assessments across multiple distinct groups. In the assessment of multiple separate groups with quantitative nonparametric data, Kruskal-Walli's analysis was utilized, while the comparison of two independent groups was conducted using the Mann-Whitney test. For the investigation of qualitative data, the Bivariate Pearson correlation test was utilized to establish connections between variables, while the Chi-square test was employed to compare multiple qualitative groups. A *P*-value below 0.05 was deemed to indicate statistical significance.

3. Results

This study comprised thirty-seven female participants (accounting for 94%) and three male participants (representing 6%) diagnosed with SLE. The age range among the study's participants spanned from 18 to 56 years, with a mean age of 33.9 ±10.8 years. In contrast, the control group exhibited an age range of 21 to 52 years, with a mean age of 31.1 ±8.7 years.

Table 1 shows the distribution frequency of various clinical symptoms observed in individuals diagnosed with systemic lupus erythematosus.

Table 1: Frequency of different clinical manifestations among systemic lupus erythematosus cases.

Variables		Frequency
Disease duration (years)		5. 9 ±5. 4, Median (4/7. 5)
Family history	Negative	44 (88%)
	Positive	6 (12%)
Clinical manifestations	Joint manifestations	48 (96%)
	Cutaneous manifestations	46 (92%)
	Mucosal ulcers	41 (82%)
	Alopecia	40 (80%)
	Raynaud's phenomenon	34 (68%)
	Fever	22 (44%)
	Hematological manifestations	33 (66%)
	Nephritis	20 (42%)
	Serositis	10 (20%)
	Neurological disorders	12 (24%)
	Thrombotic events	10 (20%)
	Vasculitis	3 (7%)
	Myositis	1 (2%)

Furthermore, the laboratory characteristics of the SLE patients involved in the study are delineated in **Table 2**. Among the twenty patients diagnosed with lupus nephritis who underwent renal biopsies, the results indicated that 6% were classified as class I, 10% as class II, 4% as class III, 12% as class IV, 6% as class V, and 2% as mixed class III/IV. The SLEDAI-SELENA Score, with a mean ±standard deviation of 7.5±4.9, ranged from 1 to 22. Notably, within the SLE cases, 22% experienced no flare, 62% experienced mild to moderate flare, and 16% experienced severe flare. Detailed insights into the nailfold capillaroscopy findings reveal that 92% of SLE patients exhibited abnormalities, as illustrated in **Table 3** and **Figure 1**.

Table 2: Frequency of laboratory investigations of systemic lupus erythematosus patients under the study.

Variables	Mean ± SD	Range
TLC (4. 5 to 11. 0 × 10^9/L)	5. 7 ± 2. 04	2-12. 8
HB (12 to 17 g/dl)	11. 5 ± 1. 6	6. 9-14. 7
PLT (150 to 450 × 10^9/L)	270. 7 ± 98. 2	9. 1-464
ALT (4 to 36 U/L)	21. 4 ±14. 6	2. 5-94
AST (8 to 33 U/L)	24. 6±12. 6	7-71
ESR (1–20 mm/hr)	39. 6±31. 3	5-135
CRP (less than 6 mg/L)	7. 6 ± 11. 2	0-68
24hr urine proteins (<0. 5 g/day)	0. 94 ± 1. 6	0. 01-8
Serum creatinine (0. 7 to 1. 3 mg/dL)	0. 74 ± 0. 20	0. 40-1. 3
Pyuria	5 (10%)	
Pyuria & Hematuria	5 (10%)	
Consumed C3	15 (30%)	
Consumed C4	7 (14%)	
Positive ANA	50 (100%)	
Positive Anti-ds DNA	42 (84%)	
Positive Anti-Phospholipids antibodies	15 (30%)	

SD, standard deviation; CBC, complete blood count; n, number; TLC, total leucocytic count; HB, hemoglobin; PLT, platelets; ALT, Alanine transaminase; AST, Aspartate transaminase; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; L, liter; dl, deciliter; g, gram; hr, hour; U, unit; mg, milligram; mm, millimeter; C3, complement 3; C4, complement 4; ANA, antinuclear antibody; Anti-dsDNA, anti-double stranded Deoxyribonucleic acid antibody.

Table 3: Nailfold capillaroscopy findings among systemic lupus erythematosus patients.

Variable	Number	Frequency
Abnormal	46	92 %
Tortuous	44	88 %
Crossed/Meandering	44	88 %
Micro-hemorrhage	32	64%
Dilated	24	48 %
Bushy/Branching	14	28 %
Avascular areas	9	18 %
Normal pattern	4	8 %
Scleroderma like pattern	3	6 %
Non-specific pattern	43	86 %
Very good density	8	16 %
Good density	33	66 %
Reduced density	6	12 %
Low density	3	6 %

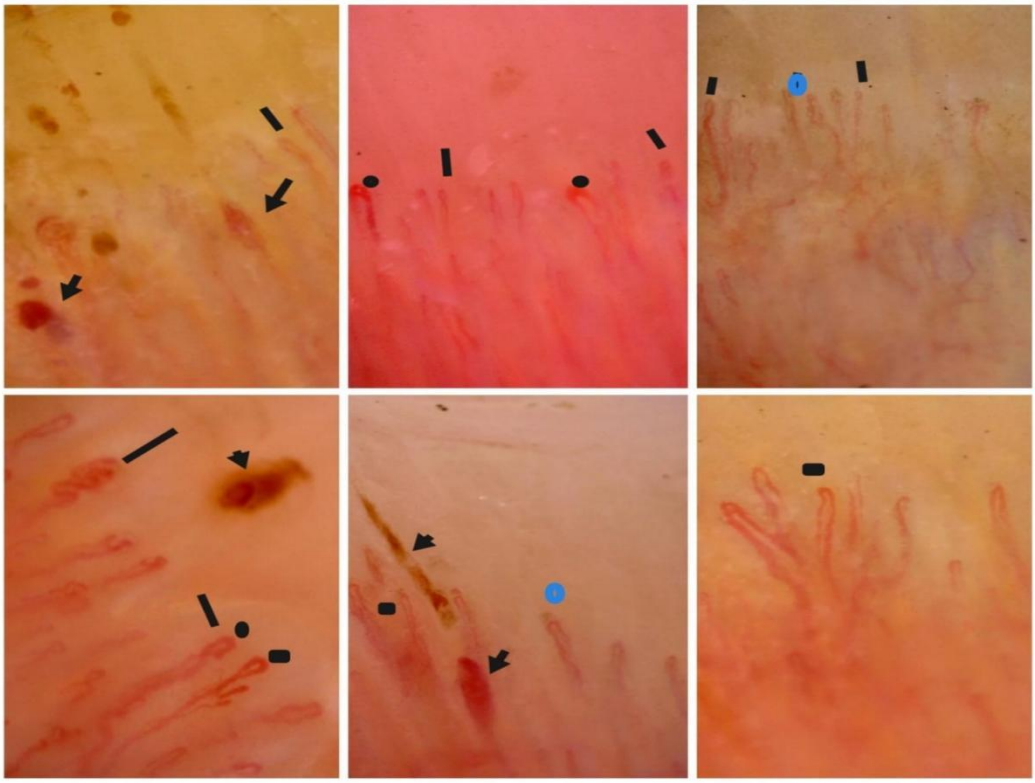

Figure 1: Nailfold capillaroscope findings among our SLE patients. Arrows refer to microhemorrhages, black circles refer to dilated capillaries, squares refer to branching capillaries, rods refer to crossed loops and blue circles refer to tortous loops.

A statistically meaningful contrast was noted between the sets concerning the sample concentration of M-CSF, with a median level of 1257.3 ng/L observed among SLE cases compared to a median level of 1170.4 ng/L among controls (p =0.01) (**Table 4)**.

Table 4: Macrophage colony-stimulating factor levels in systemic lupus erythematosus patients and controls.

Variables	Cases (N=50)		Control (N=50)		*P*-value
	Median	**IQR**	**Median**	**IQR**	
M. CSF concentrations	1257. 3	2409. 3	1170. 4	179. 9	**0. 01**

In the study group, the level of macrophage colony-stimulating factor did not exhibit any statistically significant variance concerning the clinical manifestations of SLE. Additionally, no statistically significant correlation was found between the M-CSF level and age, disease duration, SLEDAI-SELENA Score, or laboratory findings. The fluctuation of macrophage colony-stimulating factor levels exhibited noteworthy variations based on the categorization of renal biopsy classes. Notably, class V - LN cases demonstrated the most elevated concentrations, while class I cases presented the least values, as illustrated in **Table 5**.

Table 5: Relation of macrophage colony-stimulating factor levels and renal biopsy findings among SLE cases with lupus nephritis.

Variables	M. CSF concentration	P-value
Class I	1067. 9 ±0	**0.04**
Class II	963. 5 ±180.4	
Class III	1157. 5 ±0	
Class IV	1174. 1 ±2065.3	
Class V	1208. 7 ±0	

On the contrary, there was no notable contrast found ($p > 0.05$) in the concentrations of macrophage colony-stimulating factor, the degrees of SLEDAI-SELENA Score, or the findings from Nailfold Capillaroscopy among SLE cases as illustrated in **Table 6.**

Table 6: Comparison of macrophage colony stimulating factor levels with nailfold capillaroscopy findings and SLEDAI-SELENA Score among SLE cases.

Variables		M.CSF concentration	*P*-value
Nail fold Capillaroscopy Findings	Normal	1151.1 ±277.5	0.9
	Abnormal	1116.5 ±713.2	
SLEDAI-SELENA Score	No flare ≤ 3	1099.9 ±379.9	0.5
	Mild / moderate flare >3-12	1151.1 ±279.1	
	Sever flare >12	1126.1 ±2233.8	

4. Discussion

Systemic lupus erythematosus (SLE) is a persistent autoimmune disorder that affects numerous organ systems. It is perceived by repeated trends and a broad assortment of clinical manifestations spanning from mild to potentially life-threatening [19].

The origins and progression of the disease remain a mystery, with every day uncovering a new aspect of the enigma behind SLE. Both the innate and adaptive immune systems can be compromised by environmental stimuli in those who are genetically prone to them. Due to this disruption, the immune system becomes less tolerant to self-antigens, and the IFN-I pathway is activated for a longer period. This results in the development of antibodies against nuclear antigens, the initiation of inflammatory processes, and the synthesis of immunological complexes. After that, these complexes are placed inside tissues [20].

A wealth of documentation brings to light the impact that innate immune pathways have on the pathophysiology of SLE. Many studies have found abnormalities in macrophage recruitment, polarization, phagocytic efficiency, and excretion that are exacerbated by a high cytokine load [21]. The cytokine known as M. CSF/CSF-1 plays an important role in the advancement, sustenance, activation, and function of the monocyte-macrophage lineage [22].

There is a wealth of supporting data showing that innate immune pathways serve a key involvement in the pathogenesis of SLE. According to reports, dysfunctional cytokine synthesis exacerbates discrepancies in macrophage recruitment, polarization, phagocytic capacities, and secretory activities [21]. The cytokine called M-CSF/CSF-1 holds a crucial role in the progression, maintenance,

activation, and functionality of the monocyte-macrophage lineage [22].

This study examined the relationship between circulating M-CSF concentrations and other components of SLE as well as disease progress in patients with the illness. The study involved fifty individuals diagnosed with SLE, with an average age of 33.9 years and a female-to-male ratio of 15.6:1. The findings from a prior study examining the illness characteristics and epidemiology among Egyptian patients align with the predominance of females and the higher occurrence of middle-aged individuals observed in this study. This study encompassed 3,661 participants, comprising 3,296 females and 365 males, resulting in a female-to-male ratio of 9.03:1. The median age of participants in that study was thirty [23].

In the current study, 96% of SLE patients exhibited joint symptoms, 92% presented cutaneous manifestations, 80% had mucosal ulcers, 80% experienced alopecia, 68% showed Raynaud's phenomenon, 44% reported fever, 66% displayed hematological manifestations, 42% had nephritis, 20% exhibited serositis, 24% suffered from neuro-psychiatric disorders, 20% experienced thrombotic events, 7% had vasculitis, and 2% were diagnosed with myositis.

Our study supports every other study by emphasizing the range of clinical symptoms and system affection in SLE. Thrombosis, vasculitis lesions, and myositis are fewer common presentations; mucocutaneous, joint, and constitutional symptoms are the most prevalent. These are followed by renal, hematological, neuropsychiatric, and serositis symptoms. In a cross-sectional study comprising 211 SLE patients, 30.5% exhibited serositis, 38% experienced Raynaud's phenomenon, 34% were diagnosed with nephritis, 8.9% presented neuropsychiatric disorders, 66% displayed skin rash, 70% showed signs of lymphopenia, 18% suffered from thrombosis, and 19% had cutaneous vasculitis [24].

Furthermore, in a retrospective multicenter and hospital-based investigation that included 896 SLE patients from Nigeria, the prevalence rates were as follows: Joint symptoms were observed in 61.6% of cases, serositis in 32.6%, neurologic symptoms in 33.8%, mouth ulcers in 47.7%, alopecia in 45%, cutaneous rashes in 70.8%, and renal involvement in 30.1% [25].

The fluctuation in clinical symptoms can be ascribed to a range of variables, encompassing disparities in case definitions, inclusion criteria, and sample sizes among studies. Additionally, alterations in environmental factors and variations in racial/ethnic features further contribute to the variability observed.

Our examination unveiled remarkable revelations concerning the outcomes of nailfold capillaroscopy in individuals suffering from SLE. Among the cohort, a staggering 92%

exhibited aberrant findings. These anomalies encompassed a spectrum of abnormalities, including the proliferation of bushy or branched capillaries (observed in 28% of cases), micro-hemorrhages (present in 64% of cases), dilation of capillaries (encountered in 48% of cases), as well as the presence of twisted and intersecting capillaries (noted in 88% of cases), and areas devoid of vascularization (detected in 18% of cases).

Conversely, a mere 8% of subjects displayed a conventional pattern, while 6% manifested a pattern reminiscent of scleroderma, leaving a substantial 86% with patterns that defied categorization. Furthermore, within this group, 6% exhibited a decline in capillary density, 3% demonstrated markedly reduced density, whereas 16% showcased an extraordinarily high density, with the majority, constituting 66%, displaying an exemplary capillary density beyond comparison.

In their 2020 study, Bernardino and colleagues discovered that 91.4% of the 58 patients diagnosed with SLE exhibited abnormal NFC results. The prevalent aberrations predominantly consisted of minor variations such as tortuosities (detected in 98% of cases), crossing capillaries (present in 93% of cases), and non-scleroderma patterns (found in 79% of cases). In comparison, a scleroderma pattern was identified in 12% of instances.

Patients had hemorrhages in 52% of the instances, and 29% had expanded capillaries with a high density. Neo-angiogenesis was also seen in 8.6% of the patient sample [26]. 54 SLE sufferers participated in cross-sectional case-control research, and it was demonstrated that 88.8% of them had tortuous capillaries, 50% had bushy capillaries, 18.5% had hemorrhages, 46.2% had dilated/enlarged capillaries, and 75.9% had avascular areas [27].

Major alterations are infrequent, and atypical nailfold capillaroscopy (NFC) outcomes are apparent in all prior studies, including ours, with similar or varying frequencies. The majority of SLE patients typically exhibit non-scleroderma patterns. Overall, the findings display only minor discrepancies. These variations in frequencies may be attributed to differences in patient demographics, disease duration, and treatment protocols. In our study, we discovered that SLE patients had much higher serum levels of M-CSF—the median was 1257. 3 ng/L. Consistent with our research, a 2008 study conducted in China by Yang et al. discovered elevated M. CSF serum levels in 32 SLE patients [28]. In addition, Wang et al. found the same results in 100 SLE patients [29]. Moreover, Menke et al.'s study revealed an elevation in the levels of M. CSF in both serum and urine among 263 SLE patients across two separate cohorts from Germany and Italy [30]. Furthermore, a substantial correlation was shown between the M-CSF level and SLEDAI by Yang et al. [28].

The statistically insignificant difference we observed between M. CSF levels and disease activity in comparison to other research findings may stem from various factors. These include discrepancies in patient demographics, environmental variables, variations in clinical features, and the influence of pharmaceutical treatments. Additionally, significant heterogeneity in qualitative clinical data among different studies could also contribute to these differences.

The findings of our investigation did not reveal a statistically noteworthy association between M. CSF concentrations and the progress of lupus nephritis or its accompanying indices. As well, there was no observed association between M. CSF levels and any other clinical complaints in the individuals under examination. However, according to Yang et al., no correlations were identified in the inactive group, and there were no connotations found between M. CSF and anti-dsDNA, C3, or C4 levels. Nevertheless, they did observe a relationship between proteinuria, ESR, and CRP levels in individuals with active SLE [28].

Additionally, the investigation by Menke and colleagues suggested that higher amounts of M. CSF were found in the bloodstream and urine of people diagnosed with SLE in two separate groups with LN. Furthermore, increased levels of M. CSF were observed in individuals with lymph node involvement. However, similar heightened concentrations were also noted in those exhibiting symptoms related to the skin, serositis, and musculoskeletal issues [30].

The discrepancies between the findings of our study and other research on the relation between M. CSF and LN could be attributed to various treatment approaches, when they were implemented, how the patient subgroups were constructed, and variations in patient demographics. Additionally, the studies by Menke et al., and Tian et al., used serial sampling of M. CSF levels in LN patients and longitudinal follow-up, which our study did not, to both detect and predict LN flares [30, 31].

Based on our findings, a statistically notable contrast emerges in the concentration levels of M-CSF among distinct renal biopsy groups. Notably, Class I and Class II exhibited diminished M-CSF levels, whereas higher concentrations were evident in Classes V, IV, and III. The inconsistencies allude to a conceivable link between the extent of kidney degradation and the harshness of nephritis. In line with our discoveries, a separate inspection noted that levels of M-CSF in the serum and urine of SLE patients were most preeminent in class IV, reasonably eminent in class III, and eminent to the lowest degree in class II. These findings were congruent with the notion that M-CSF expression is heightened in class IV renal tubules compared to class II renal tubules in SLE patients [30].

Conclusion

Our preliminary inquiry, marking the inaugural utilization of NFC in this context, failed to reveal any link between the levels of M-CSF in circulation and the occurrence of peripheral vascular impediments. The limited sample size in our study underscores the need for further research to confirm the relationship between M. CSF levels and other clinical features of the disease. Additionally, due to the cross-sectional research design, only enrolled patients had access to follow-up data, highlighting the necessity for longitudinal studies to assess the predictive efficacy of M-CSF in the early detection of renal flare-ups. Furthermore, although the effects of medications were not examined in this investigation, the use of immunosuppressive and anti-inflammatory medications in SLE patients may have influenced M-CSF levels.

Eventually, our findings revealed greater levels of M. CSF in SLE patients. Besides, patients in classes V and IV had greater M. CSF levels than those in classes I and II, suggesting a possible link to renal disease severity.

Ethical approval: Following the provision of informed written consent by both patients and controls at the outset, the project received approval from the Ethics Committee (Reference: M588) of the Faculty of Medicine's Biomedical Research at Fayoum University.

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Conflicts of Interest: All authors declare they have no conflicts of interest.

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