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Serum Midkine Levels in Systemic Lupus Erythematosus

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Abstract

Introduction: Systemic lupus erythematosus (SLE) is considered an autoimmune skin disorder in which the immune system attacks its own tissues, causing widespread inflammation and damage to the tissues.

Aim of the study: To compare and measure the serum level of midkine (MK) in patients with SLE and compare it with healthy controls, as well as analyze the relationship between disease activity in SLE and the level of MK in serum and to early diagnose whether patients were nephritis or nonnephritis SLE patients.

Subjects and Methods: Ninety subjects were included in this study, subdivided equally into three groups, 30 healthy-matched individuals and 60 SLE patients (Nephritis and non-Nephritis patients). For all groups, a 5mL blood sample for serum isolation was taken and analyzed by using the ELISA technique to measure the level of MK.

Results: The midkine levels were high in both nephritis and non-nephritis as compared to controls ($P= 0.001$ and 0.015, respectively). There was a positive correlation between midkine level and ESR in nephritis patients. There was no correlation between midkine and other clinical data among non-nephritis patients, $P>0.05$. Based on the disease severity classification, the serum level of midkine is higher in severe patients than in other groups. Regarding disease duration, we reported that midkine tended to be very high in patients with a duration of 12 months, 2021.76±813.57 then slightly decreased to 830.69±104.50 in duration between 12 and 24 months. The level of midkine increased again to 1357.86±436.42 for duration>24.

Conclusion: It can be concluded that MK can be used to early diagnose SLE patients and differentiate between nephritis versus non-nephritis patients.

Keywords: SLE; nephritis; Midkine; SELENA.

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1. Introduction

SLE is a prototypical systemic auto-immune skin disease that is mainly characterized by the formation of immune complexes, the production of multiple autoantibodies, and the inflammation of tissue in multiple organs [1]. Nephritis SLE is a form of SLE that may be caused by infection, inflammatory conditions, or certain genetic conditions. It is a condition in which the tissues in the kidney become

inflamed and have problems filtering waste from the blood [1–3].

The main reason for SLE is still unclear. However, the reasons could be hereditary, hormonal, or even an infection, which affects different organ systems in patients with lupus [4–6].

Midkine is involved in the pathogenesis of autoinflammatory and auto-immune diseases and may also serve as a positive biomarker in these conditions. Midkine is a heparin-binding growth factor that is markedly expressed during embryogenesis [6–8].

It promotes a number of functions in the target cells, like proliferation, migration, growth, survival, repair and reproduction, and gene expression. It has a vital role in the inflammation process [9].

In the present study, we aimed to evaluate the level of midkine in serum among SLE patients (with and; or without nephritis) to aid in early non-invasive diagnosis of renal com-plications among SLE patients.

2. Subjects and methods

2.1. Subjects

In the current case-control study, ninety subjects were enrolled and subdivided equally into two groups:

- Group 1: Including 45 healthy Controls
- Group 2: Including 45 SLE patients

In the present study, the global disease activity was used to assess patients with the SLE Disease Activity Index (SELDAI score) [10].

Inclusive criteria

Adult patients aged between 18 and 69 were included in the study. Patients with a history and disease activity of SLE were also included.

Exclusive criteria

Pregnant and lactating females were excluded. Patients with any other autoimmune or inflammatory skin diseases, the presence of hepatic derangement, or renal, preexisting hyperlipidemia were excluded from the study.

2.2. Methods

Laboratory assessment

From all participants, a 5 mL venous sample of blood was taken for serum isolation and then analyzed by using the ELISA technique to measure the serum level of midkine. Sera collected were stored at -20 °C, while samples of tissue were stored at -80°C until analysis. For the quantitation of midkine in serum using an ELISA kit provided (Shanghai Crystal day Biotech Co., Ltd., Shanghai, China).

2.3. Statistical analysis of data

The data were analyzed and encoded in an Excel sheet. Then, for the statistical analysis, Statistical Package for Social Science (SPSS) for Windows version 25 was used. For parametric quantitative data (between more than two groups), the analyses were done using the one-way

ANOVA test, followed by post hoc Tukey's analysis between each group. As for non-parametric quantitative data (between more than two groups), the Kruskal-Wallis test and Mann-Whitney test were used. Qualitative data analysis was performed using the Chi-square test. The receiver operator characteristic (ROC) curve was used for the nearly optimal cut-off point, AUC, sensitivity, and specificity, whereas the P-value was considered significant if it was less than 0.05.

3. Results

Serum samples were collected from 45 healthy controls (10 males, 35 females) with a mean age of 52.38±8.33 years. For the SLE group (3 males, 42 females) with a mean age of 30.76.9 years. A summary of the clinical and demographic characteristics of patients with SLE and controls is shown in Table 1. Results showed that there is a significant difference in age between the two groups (*P = 0.0001*). The sex ratio yielded no significant results (*P> 0.05*).

Table 1. Distribution of study subjects according to their demographic and clinical characteristics.

Clinical Data		SLE	Control	*P-value*
Age (years)		30.7 ± 6.9	52.38±8.33	0.0001*
Sex	Female	42(93.3%)	35 (77.7%)	>0.05[a]
	Male	3(6.7%)	10 (22.3%)	
Disease Duration (Months)		57.5±55.38	-	-
Age of Onset		26.61±6.79	-	-
ESR		57.9±35.9	33.80±22.02	0.014*
Score		11.35±6.27	-	-

Age (mean ±SD). [a] Chi-squared test is used, and gender is presented by number (percentage). *P values are significant at < 0.05.

The midkine level was examined in the serum for the nephritis and non-nephritis groups compared to healthy controls. Results revealed that midkine levels were high in both nephritis and non-nephritis as compared to controls, as shown in Table 2.

Table 2. Biomarkers level in serum samples for the studied groups.

Biomarker	SLE	Control	*P-value*

Midkine	1325.84±363.58	523.15±22.64	<0.0001*

SE is used for expression levels. *P values are significant at < 0.05.

After measuring the bivariate correlation using Pearson r, the results show that there is a positive correlation between midkine level and ESR (r = 0.436, *P = 0.016*) in patients with SLE. There was no correlation when comparing midkine level with other clinical data (*P> 0.05*) (Figure 1).

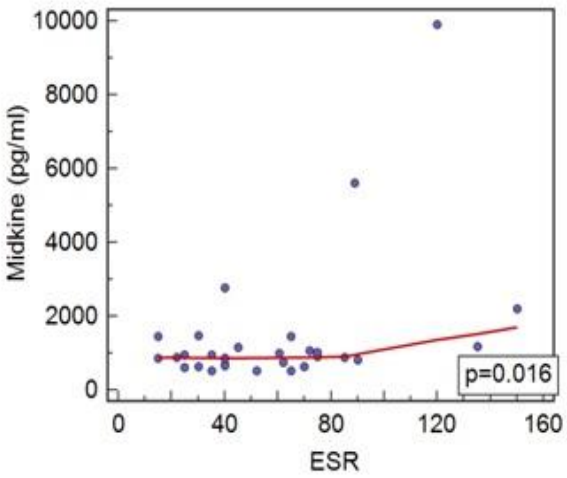

Figure 1. Correlation between serum level of midkine and ESR among SLE patients.

As for the ROC analysis among all groups, results revealed that the calculated sensitivity and specificity to discriminate SLE patients from the control group were 85% and 88.1%, respectively, with a cut-off value of 607.35 pg/ml (*P>0.05*). While for nephritis vs. controls, sensitivity was 86.7% and specificity was 88.1%, with a cut-off value of 623.6 pg/ml (*P<0.001*) (Figure 2).

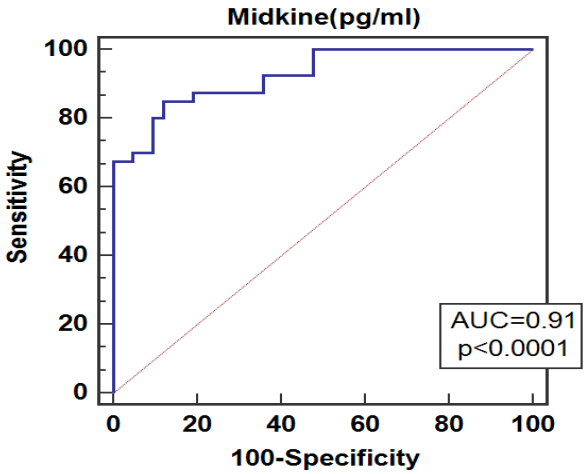

Figure 2. ROC curve analysis for serum level of midkine.

According to the SELENA index, the activity of the disease was deter-mined, and patients were classified into Nephritis (NSLE) and non-nephritis patients (N=25, N=20), respectively. The majority of NSLE had high activity, with a percentage of 78.6%. while 3.4% were mild and 20% were moderate. Based upon these classifications, results showed that the serum level of midkine is higher in severe patients than in other groups (Figure 3).

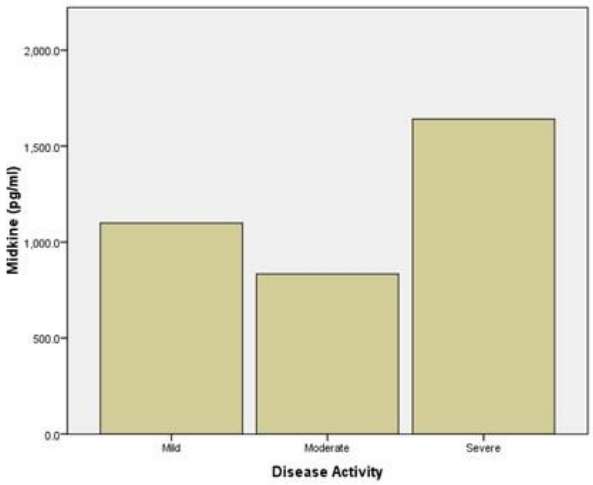

Figure 3. Serum level of midkine as regards disease activity among nephritis patients

There was no statistical significance between ESR, age of onset among the disease activities (*P>0.05*) as shown in Table 3. Regarding disease duration (DD), results showed that the level of midkine tended to be very high in patients with DD≤12 months (2021.76±813.57), then slightly decreased to 830.69±104.50 in

duration between 12 and 24 months. The level of midkine increased again to 1357.86±436.42 for duration > 24 (Figure 4).

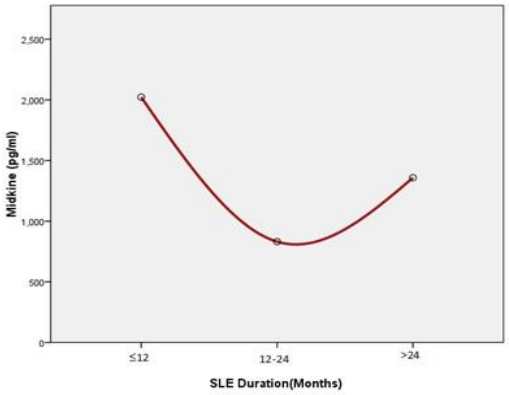

Figure 4. Serum level of midkine as disease duration among nephritis patients

Table 3. Comparison between disease activity and clinical data.

Clinical Data	Nephritis	Non- Nephritis	Control	P-value
ESR	84.250±38.7158	51.250±31.6168	59.34±37.22	0.612[a] 0.916[b] 0.636[c]
DD (Months)	94.50±81.737	68.00±59.479	39.39±37.07	0.811[a] **0.032[b]** **0.036[c]**
Age of onset (Months)	21.50±2.30	26.0±4.52	27.29±8.31	0.092[a] 0.085[b] 0.10[c]
Midkine (pg/ml)	1099.30±249.34	834.37±197.57	1641.12±438.61	**0.050[a]** 0.24[b] **0.050[c]**

SD is used for expression levels. P values in bold are significant at < 0.05. DD: disease duration, [a] between mild and moderate, [b] between mild and severe, [c] between moderate and severe

4. Discussion

In our present study, it was reported that the level of midkine in serum was higher in patients with SLE (statistically significantly higher in nephritis patients than non-nephritis patients). Also, our findings proved that midkine aids in determining the severity of SLE, where the serum level of SLE is much higher in severe SLE patients than in mild and moderate groups.

As for the correlation between midkine and clinical data in SLE patients, there was a positive correlation between midkine level and ESR in nephritis patients. While for non-nephritis patients, we reported that there was no correlation

between the midkine level and other clinical data (ESR, age of onset, disease duration).

A ROC curve analysis was performed in the study to measure the sensitivity and specificity of midkine in the early diagnosis of patients with lupus nephritis. This analysis showed that midkine sensitivity for discriminate SLE patients vs. controls was 23.3%, specificity was 86.7%, and specificity was 88.1% with a cut-off value of 623.6 pg/ml (*P=0.0001*).

Wu et al.,2017, evaluated in their study that the midkine level in plasma and pleiotrophin levels in patients with SLE can be considered biomarkers for the detection of SLE patients. They reported that the plasma level of midkine is elevated compared to healthy controls. They also reported that SLE patients with nephritis have higher levels of midkine than those with SLE without nephritis [11].

Marpaung and his team, 2018, reported that when comparing the midkine level in SLE patients and controls, a significant difference was shown. Also, when evaluating the level of midkine in the serum, it showed an elevation between active disease and remission [9].

To the best of our knowledge, this study can be considered the first in Egypt that evaluated the serum level of midkine in the differentiation of nephritis from non-nephritis SLE patients as regards disease activity and other clinical data.

Conclusions

Although kidney biopsy is used to diagnose lupus nephritis, it is the most commonly used invasive technique, but it is also the most expensive. Therefore, the presence of biomarkers such as midkine detection in the serum has a vital role in predicting and diagnosing this condition. Midkine in the serum is sensitive and specific in the diagnosis of nephritis lupus and can be considered a potential biomarker for the early diagnosis of nephritis.

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Ethical Approval Statement: In accordance with the Helsinki Declaration, the study was conducted. The Ethics Committee of the Faculty of Medicine, Fayoum University, approved this work (protocol code 64 with approval number M/393 and date of approval, April 14, 2019).

Informed Consent Statement: For all patients, written informed consent was obtained at the beginning of the study.

Conflicts of Interest: The authors declare no conflicts of interest.

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