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ORIGINAL ARTICLE

The behavior of neutrophil extracellular traps and NADPH oxidative activity in pediatric systemic lupus erythematosus: relation to disease activity and lupus nephritis

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Abstract

Objectives To evaluate the neutrophil extracellular traps (NETs) assay and NADPH oxidase (Nox2) activity in pediatric systemic lupus erythematosus (pSLE) in relation to each other and SLE characteristics.

Methods This cross-sectional study included 50 children and adolescents with pSLE who were clinically evaluated and underwent routine laboratory work up of SLE (CBC, ESR, 24 hrs urinary proteins, serum creatinine, complement-3 (C3), anti-dsDNA, and antiphospholipid antibodies). NETs assay and dihydrorhodamine (DHR) test were done for patient group and 50 age- and sex-matched control group.

Results The level of NETs was found significantly elevated among the patients (median 74.6 mU/ml) as compared to the controls (median 8.9 mU/ml) ($p < 0.001$), while values of DHR test were comparable between patients (median 95.5%) and controls (median 96.1%) ($P = 0.55$). There was a significant negative correlation between levels of NETs and DHR ($p < 0.001$). A significant positive correlation was noted between the 24 hrs urinary protein and NETs level ($p < 0.001$), but a significant negative correlation with DHR ($p < 0.0001$). Both NETs and DHR test values did not differ significantly between classes of lupus nephritis. NETs showed a significant positive correlation with anti-dsDNA titer ($p = 0.004$) and SLEDAI ($p < 0.001$), but a negative correlation with C3 ($p < 0.001$). DHR test was positively correlated with C3 levels ($p = 0.003$), but negatively correlated with anti-dsDNA titers ($p = 0.008$) and SLEDAI ($p < 0.001$).

Conclusion NETs seem to have strong association with biomarkers of pSLE activity. On the other hand, Nox2 activity of the neutrophils was noted to be linked to quiescent state of SLE.

Key Points

- *Neutrophils have displayed different actions in pSLE through the NETs and Nox2 activity.*
- *The inverse correlation between NETs and Nox2 activity makes the later a non-fundamental pathway for NETs formation.*
- *NETs are associated with pSLE flare and LN activity, while neutrophil Nox2 activity is related to disease remission.*

Keywords Dihydrorhodamine flow cytometry · NETosis · Neutrophil extracellular traps (NETs) · Neutrophil Nox2 activity · Pediatric systemic lupus erythematosus

Introduction

Activated neutrophils attack and destroy pathogens by phagocytosis, along with releasing bactericidal peptides, proteolytic enzymes, reactive oxygen species (ROS), and the neutrophil extracellular traps (NETs) [1–4]. NETs are composed of decondensed chromatin DNA in association with histones, granular proteins, and a few cytoplasmic proteins [5, 6]. They are formed through a unique cell death process, termed "NETosis" that facilitates efficient, non-inflammatory clearance of neutrophils. NETs release

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relies mainly on activity of the phagocyte NADPH oxidase (Nox2) in neutrophils [7].

A previous study reported that neutrophils from adult patients with systemic lupus erythematosus (SLE) are more prone to release NETs and that NETs can potently activate plamacytoid dendritic cells to produce type I interferons (IFN-α) [8]. The persistently exposed NETs components may directly damage tissues and may also serve as a novel source of autoantigens to augment the autoimmune response that could perpetuate the inflammation and exacerbate the disease progression in SLE [9].

However, Nox2-deficient mice have markedly exacerbated lupus, including increased renal disease, and elevated and altered autoantibody profiles suggesting that failure to undergo normal Nox2-dependent cell death may result in release of immunogenic self-constituents that stimulate lupus [10].

In chronic granulomatous disease (CGD) patients who have mutations in the phagocyte NADPH oxidase, neutrophils from these patients are unable to generate ROS upon PMA (phorbol 12-myristate 13-acetate) activation. Interestingly, neutrophils isolated from CGD patients activated with PMA did not show NETs. CGD patients are at significant risk for the development of autoimmune diseases [11]. Discoid lupus is most commonly reported in female carriers of X-linked CGD, although it has been described in CGD patients as well [12].

Therefore, the conflicting results about the relation of NETs and NADPH oxidase activity to pSLE activity stimulated us to assess neutrophil Nox2 activity and NETs in patients with pediatric SLE in comparison to apparently healthy subjects aiming to explore their relationship to the biomarkers and indices of pSLE activity and lupus nephritis.

Patients and methods

Study design and population

This study was a cross-sectional case-control study that was carried out at the Pediatric Allergy and Immunology Unit, Children Hospital, Ain Shams University. A written informed consent was taken from all parents or the legal guardians of the patients before enrollment. The study protocol gained approval of the Medical Research Ethics Committee of the Pediatric Department, Ain Shams University, approval number: FMASU 3099/2014.

Study population

Patients

This was a consecutive non-random sample that comprised 50 patients with pediatric SLE, who fulfilled at least four of the SLICC classification criteria of systemic lupus erythematosus [13].

Inclusion criteria:

- Age at onset of SLE is less than 18 years old
- Normal neutrophil count (age-referenced)

Exclusion criteria:

- Neutropenia
- Presence of infections whether viral, bacterial, or fungal infections at time of enrollment or during the last 2 weeks before enrollment in the study
- Allergic diseases

Control group

- Fifty age- and sex-matched apparently healthy children and adolescents recruited as controls from the Outpatient Orthopedic and Pediatric Clinics, Children's Hospital, Ain Shams University. Their ages ranged from 9 to 15 years with a mean ± SD of 12.2 ± 2.0 years. They were 45 females (90%) and 5 males (10%).

Inclusion criteria:

- Apparently healthy subjects without clinical manifestations or family history of allergic or autoimmune disorders in first degree relatives

Exclusion criteria:

- Neutropenia in the screening complete blood picture
- Acute infection or history of recent infections

Study measurements

All included patients were subjected to the following:

1. Clinical evaluation:
   History taking including name, sex, age, and age at presentation. Detailed history of organ involvement was taken with special stress on history of renal manifestations and history of neuropsychiatric manifestations. Calculation of the average daily dose of the received immunosuppressive therapy (steroids, azathioprine, mycophenolate mofetil, and cyclophosphamide) in the last month before enrollment in the study. Complete physical examination was done to all patients to assess the SLE activity status and different organ involvement at the time of enrollment in the study. Assessment of global disease activity was done using Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [14, 15]. Renal activity was assessed using

Renal SLEDAI. A SLEDAI score of ≥4 was taken as an indicator of active lupus nephritis [16]. Systemic Lupus Collaborating Clinic/American College of Rheumatology (SLICC/ACR) Damage Index (SDI) was used to assess SLE-related damage [17].

2. Laboratory investigations:

(a) Routine laboratory investigations were done for all studied patients including complete blood counts, erythrocyte sedimentation rate (ESR), serum creatinine, and blood urea nitrogen (BUN), 24 hrs urinary protein; collagen disease panel including anti-nuclear antibody (ANA), anti-double stranded deoxyribonucleic acid (Anti-ds DNA), complement-3 (C3), lupus anticoagulant, anticardiolipin antibodies IgM and IgG, and anti-neutrophil cytoplasmic antibody (ANCA).

Dihydrorhodamine (DHR) flow cytometric assay and neutrophil extracellular traps (NETs) assay were done for both patients and control groups as follows:

(b) DHR flow cytometric assay:

– This test is based on the measurement of respiratory burst of neutrophil granulocytes after their stimulation with *E. coli* bacteria. During the process of bacteria ingestion, phagocytes activate the NADPH oxidase producing reactive oxidative intermediates (respiratory burst). Resulting hypochlorite ions inside phagocytes strongly oxidize dihydrorhodamine 123 (DHR123) into fluorescent rhodamine 123 which is detected by a flow cytometer. A positive control sample is stimulated using PMA (Phorbol 12-myristate 13-acetate) which activates respiratory burst of granulocytes without adhesion and ingestion of the pathogen.
– In the neutrophil oxidative burst test, heparinized whole blood is incubated at 37 °C with *E. coli* bacteria and PMA in the presence of dihydrorhodamine 123. Formation of the reactive oxidants during the oxidative burst can be monitored by the addition and enzymatic oxidation of a fluorogenic substrate, DHR 123. The level of reactive oxygen radicals is determined by flow cytometry.
– Results of DHR are expressed as neutrophil oxidative index (NOI) which is the ratio of mean peak channel fluorescence (MPC-FL) of phorbol 12-myristate 13-acetate (PMA) stimulated over MPC-FL of unstimulated cells.
– Interpretation: Normal range of respiratory burst activity of granulocytes: 90–100%.

c-NETs assay

This NETs assay employs a specific elastase substrate, N-methoxysuccinyl-Ala-Ala-Pro-Val *p*-Nitroanilide, which is selectively cleaved by elastase to yield a 4-nitroaniline product that adsorbs light at 405 nm. PMA was used to stimulate NET formation and subsequently release NET-associated elastase.

– NET-forming cells (human peripheral blood neutrophils) were suspended in pre-warmed NET assay buffer. A concentration of at least 1×10^6 cells/ml was recommended. Nine hundred microliters of cells was added per well on a 24-well plate. Two wells containing culture medium were included only for background controls. One hundred microliters of the PMA working stock was added and incubated for 2 h at 37 °C.
– After stimulation and NETs formation was complete, NET assay buffer was gently aspirated from the wells, washed twice to remove soluble neutrophil elastase that is not NET-associated.
– A 500 μl of the diluted S7 nuclease was added to each well and incubated for 15 min at 37 °C to disrupt the NETs.
– The supernatants were transferred to polypropylene microfuge tubes. Ten microliters of the NETs assay EDTA solution was added to inactivate the nuclease and then centrifuged at 300×*g* for 5 min to pellet any cellular debris.
– Supernatant was transferred to a new polypropylene tube or other appropriate storage container and assayed for released neutrophil elastase immediately.

Statistical analysis

Data were analyzed using SPSS© Statistics version 21 (IBM© Corp., Armonk, NY, USA). Normality of numerical data distribution was examined using the Shapiro-Wilk test. Categorical variables were presented as number and percentage, and numerical data as median (IQR) as appropriate. Intergroup differences were compared using the Mann-Whitney test (for two-group comparison) or the Jonckheere-Terpstra trend test (for comparison of multiple ranked groups). The Conover test was used for post hoc comparisons whenever the Jonckheere-Terpstra test revealed a statistically significant difference among the groups. The Bonferroni method was used to adjust the level of significance for the number of post hoc comparisons. Correlations were tested using the Spearman rank correlation. A probability (p) < 0.05 was considered statistically significant. Receiver-operating characteristic (ROC) curve analysis was used to determine cutoff point for NETs assay values in SLE patients as compared to the controls. Areas under ROC curves were compared using the DeLong method.

Results

Demographic, disease characteristics, and routine laboratory data of the studied patients

The studied population comprised 50 children and adolescents with SLE, Their ages ranged from 8 to 18 years with a mean ± SD of 13 ± 2.2. They were 47 female patients (94%) and 3 male patients (6%). Their ages at onset of SLE ranged from 7 to 13 years with a mean ± SD of 10 ± 2.6 years. Major organ involvement included biopsy-proven lupus nephritis (LN) in 35 patients (70%); CNS lupus without clinical or laboratory evidence of LN in 9 patients (18%); and multiple-organ affection, including LN, CNS lupus, myocarditis and/or pericarditis, and autoimmune hemolytic anemia, was observed in 6 patients (12%). Anti-dsDNA was positive in 36 patients (72%), and C3 was consumed in 17 patients (34%). P-ANCA showed positivity in only two patients (4%). Also, 13 patients (26%) were anti-cardiolipin IgM positive and 4 patients (8%) were lupus anticoagulant positive, but none of them had history of any thrombotic events (Table 1).

Levels of NET and DHR assay among the studied patients and healthy controls

The level of NETs was found highly significantly elevated among the patients as compared to the controls, while values of DHR assay were comparable among patients and controls (Table 2). There was no significant correlation between NETs and DHR test values and ages of the patients ($p > 0.05$).

The sensitivity and specificity of NETs for SLE by ROC curve

At the level of 9.5 mU/ml, NETs assay was found to be an excellent discriminator between lupus patients and healthy controls, with AUC = 1 and 100% sensitivity and specificity (positive predictive value 100%, negative predictive value 98%) (Fig. 1).

Levels of NETs and DHR test in different major organ involvement

The levels of NETs and DHR test did not differ significantly between patients with LN and those without clinical or laboratory evidence of LN, as well as between different histological classes of LN where patients with proliferative LN (class III and class IV LN) had comparable values of both tests to those with non-proliferative LN (class II and class V LN) [($Z = -0.23$, $p > 0.05$), ($Z = -0.08$, $p > 0.05$) respectively]. Also, both tests were comparable between patients with LN and those with CNS lupus. On the other hand, patients with multiple organs involved have higher NETs levels than those with either CNS lupus or LN ($Z = 2.34$, $p = 0.034$), but both groups had comparable levels of DHR test.

The relationship between NETs and DHR test and the studied laboratory markers

There was a significant negative correlation between levels of NETs and DHR test values. ESR and 24 h urinary proteins had a significant positive correlation with levels of NETs, yet they were significantly negatively correlated with DHR test values. Absolute neutrophil count (ANC) and serum creatinine did not correlate significantly with either NETs or DHR. Anti-dsDNA levels had significant positive correlation with levels of NETs, yet a significant negative correlation with DHR test values. Also, there was a significant negative correlation between levels of NETs and C3, and a significant positive correlation between DHR test values and levels of C3. Anticardiolipin IgM and IgG did not correlate significantly with either NETs or DHR (Table 3).

The variation of NETs and DHR test with SLEDAI and the treatment given

SLEDAI had a significant positive correlation with levels of NETs, yet, a significant negative correlation with DHR test values ($p < 0.001$). The dose of corticosteroids dose had significant positive correlation with levels of NETs but showed a significant negative correlation with DHR test values ($p < 0.001$). On the other hand, other immunosuppressive drugs did not correlate significantly with either NETs or DHR ($p > 0.05$).

Levels of NETs and DHR test in patients with active renal disease and lupus related damage

Patients with higher renal SLEDAI had significantly higher median value of NETs ($Z = 2.85$, $p = 0.004$) but significantly lower median value of DHR test ($Z = 2.86$, $p = 0.004$) as compared to those with inactive renal disease. In addition, patients with evidence of lupus-related damage in terms of mild SDI have significantly higher median value of NETs but lower median value of DHR test with border line significance as compared to those with no damage [($Z = 2.08$, $p = 0.03$); ($Z = -1.96$, $p = 0.05$), respectively].

Discussion

This study revealed that NETs level was significantly higher in the patient group in comparison to controls ($p = < 0.001$). A cutoff value of 9.5 mU/ml was found to differentiate between patients and controls, with 100% sensitivity and specificity suggesting a strong relationship between NETs and SLE.

Table 1 The Demographic, characteristics and laboratory data of the studied patients

Variable	*N*	%		
Gender				
Female	47	94.0%		
Male	3	6.0%		
Lupus nephritis (LN) ($n = 35$)				
Proliferative LN ($n = 13$)				
Class III LN	7	14.0%		
Class IV LN	6	12.0%		
Non-proliferative LN ($n = 22$)				
Class II LN	16	32%		
Class V LN	6	12%		
CNS lupus ($n = 9$)				
Lupus headache	3	6.0%		
Seizures	7	14.0%		
Psychosis	4	8.0%		
Cognitive deficit	2	4.0%		
Multi-organ affection	6	12.0%		
Variable	Mean	SD	Minimum	Maximum
TLC (k/mm^3)	8.1	3.6	2.9	17.5
Hb (g/dl)	11.5	1.7	5.9	15.6
PLTs (k/mm^3)	292	121	93	795
ANC (k/mm^3)	5.0	2.9	1.7	13.6
ALC (k/mm^3)	2.4	1.2	0.2	5.5
ESR (mm/hr)	47	31	3	105
Serum creatinine (mg/dl)	0.7	1.4	0.2	10.3
BUN (mg/dl)	13	8	5	44
Urinary protein excretion (mg/m2/24 hrs)	228	394	10	2771
C3 level (mg/dl)	120	59	27	298
Anti-ds DNA level (IU/ml)	501	408	50	1700
Anti-cardiolipin IgM (MPL)	11.8	14.1	2.0	66.0
Anti-cardiolipin IgG (GPL)	11.3	12.9	3.0	66.0
Lupus anticoagulant (IU/dl)	38.5	14.9	27.5	120.0
NETs (mU/ml)	**66.0**	**29.3**	**10.0**	**105.6**
DHR (%)	**93.6**	**5.6**	**74.0**	**99.5**
SLEDAI score	5.9	5.3	1	21
SDI score	0.26	0.487	0	2
Steroids dose[a] (mg/kg/day)	0.62	0.53	0.10	2.00
Azathioprine dose[a] (mg/kg/day)	2.4	1.0	1.0	2.5
Cyclophosphamide dose (mg/m^2)[a]	585.7	234.2	500	750
Mycophenolate mofetil dose[a] (mg/m^2)	823	230	500	1250

CNS central nervous system, *LN* lupus nephritis, *N* number, *ALC* absolute lymphocyte count, *ANC* absolute neutrophil count, *Anti-dsDNA level* anti-double stranded deoxyribonucleic acid, *BUN* blood urea nitrogen, *C3* complement 3, *DHR* dihydrorhodamine, *ESR* erythrocyte sedimentation rate, *Hb* hemoglobin, *NET* neutrophil extracellular traps, *PLTs* platelets, *TLC* total leucocytic count

[a] Average daily dose over the last month before enrolment

Previous studies reported that neutrophils from adult SLE patients are more prone to release NETs as a result of increased neutrophil apoptosis and aggregation, leading to impaired phagocytosis and subsequently delayed clearance of apoptotic cell debris together with the presence of a special neutrophil subtype low-density granulocytes (LDGs) which have increased tendency toward NETs formation and release [18–21]. Among patients with pSLE, it has been reported that there were persistently increased NETs components which were related to the impaired NETs clearance ability [9].

Regarding the DHR test values, no significant difference was found between patients and controls. In contradiction to

Table 2 Levels of NETs and DHR assay among the studied patients and healthy controls

	Patients ($n = 50$)			Controls ($n = 50$)				
Variable	Median	IQR	Range	Median	IQR	Range	Z	p value
NETs (mU/ml)	74.6	89.5	10–105.6	8.9	9.4	8–9.9	− 4.76	< 0.001
DHR (%)	95.5	97.6	74–99.5	96.1	98.1	95.3–99.5	− 1.92	0.055

DHR dihydrorhodamine, *NETs* neutrophil extracellular traps

our findings, another study found that neutrophils from adult SLE patients exhibited increased reactive oxygen species (ROS) production and accordingly increased oxidative burst response [22].

Several studies have previously reported that the inability to form ROS in defective NADPH-oxidase patients prevents NETs formation [2, 23–25]. However, in our study, a strong negative correlation was found between levels of NETs and DHR test among the studied patients. Our finding suggests that NETs formation was independent of neutrophils Nox2 activity; moreover, it seems that they acted differently in pSLE.

The current study revealed that NETs had a significant positive correlation with anti-dsDNA but was inversely correlating with C3. This might support the role of immune complexes in excessive stimulation of NETs formation and the effect of active SLE on impaired degradation of NETs. The persistence of non-degraded NETs may lead to the production of autoantibodies against, which in turn block NETs degradation, thus forming a vicious circle [9].

Previous studies reported that adult patients with active SLE had low-degrading NETs displayed significantly higher levels of antibodies against dsDNA and also had low complement levels in comparison to those with high-degrading NET sera [18, 26].

On the other hand, DHR test was noted to have a significant negative correlation with ESR and anti-dsDNA but a significant positive correlation with C3. Moreover, we found that SLEDAI had a significant positive correlation with the level of NETs, but a significant negative correlation with DHR test. Taken together, we suggested that neutrophils behave differently in pSLE where neutrophil Nox2 activity seems to favor disease quiescence, while NETs appear to be related to biomarkers of SLE activity and disease flare. A previous study has reported that NETs were increased during SLE flare and that the majority of patients with low-degrading sera in flare were high-degrading in remission indicating that the ability to degrade NETs in the same patient is related to the immunological status of the disease [20].

We did not find significant correlation between the tested antiphospholipid antibodies (aPL) (lupus anticoagulant and anticardiolipis) and NETs. Worth to note that previous studies have reported that NETs can contribute to both arterial and venous thrombosis regardless the positivity of antiphospholipid antibodies, through the following mechanisms: the ability of NETs to bind and activate platelets, the DNA component of NETs activates factor XII, initiating contact pathway coagulation, leading to fibrin formation. Finally, NETs suppress fibrinolysis by intercalating into the fibrin clots [27–30]. None of our enrolled patients had history of thrombotic events even those with high NETs levels.

Similar to our findings, a previous study has reported that DNase-mediated NETs degradation did not correlate with the presence of aPL antibodies, such as anti-beta-2-glycoprotein 1

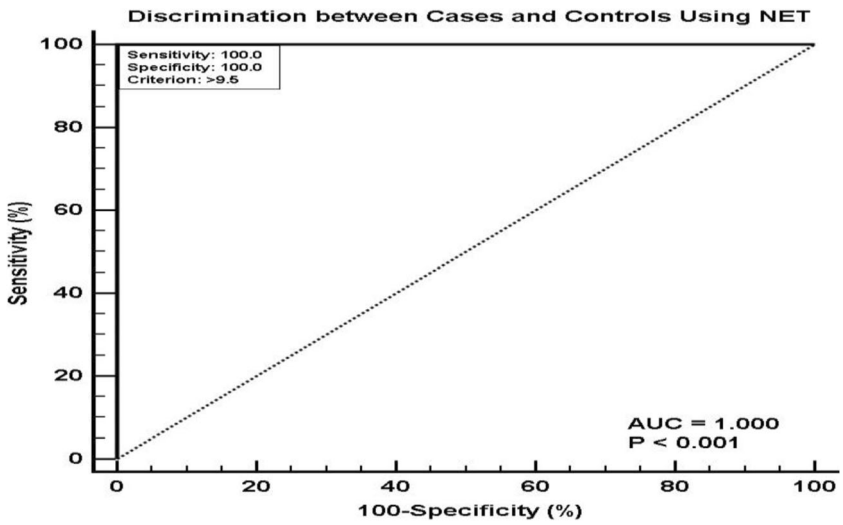

Fig. 1 Receiver-operating characteristic (ROC) curve to define the best cutoff value of NETs to differentiate the studied children and adolescents with SLE from healthy controls

Table 3 The relationship between levels of NETs and DHR test and the studied routine and immunological laboratory data

Variable	NET		DHR	
	Correlation coefficient	*p* value	Correlation coefficient	*p* value
NETs	–	–	− .690**	< .001
DHR	− .690**	< .001	–	–
TLC	− .036	.803	.068	.641
ANC	.086	.552	− .084	.560
ALC	− .240	.093	.339*	.016
ESR	.511**	< .001	− .523**	< .001
Serum creatinine	.134	.355	− .164	.256
BUN	.344*	.014	− .445**	.001
24 hrs urinary protein	.498**	< .001	− .599**	.000
C3 level	− .504**	< .001	.417**	.003
Anti-dsDNA level	.396**	.004	− .373**	.008
Anti-cardiolipin IgM	− .026	.856	− .129	.373
Anti-cardiolipin IgG	− .140	.331	− .024	.866

ALC absolute lymphocyte count, *ANC* absolute neutrophil count, *Anti-dsDNA* anti-double stranded deoxyribonucleic acid, *BUN* blood urea nitrogen, *C3* complement 3, *DHR* dihydrorhodamine, *ESR* erythrocyte sedimentation rate, *NET* neutrophil extracellular traps, *TLC* total leucocytic count

(anti-B2GP1), anti-cardiolipins, or lupus anticoagulant. Moreover, they found no evidence that aPL antibodies coincide with or cause failed NETs degradation [26].

On the other hand, a previous study described that the release of NETs was promoted by anticardiolipin antibodies and that isolated neutrophils of APS patients enhanced spontaneous NETs release, when compared with controls [31].

With respect to the relation between NETs and organ involvement in pSLE, NETs did not appear to be significantly related to the presence of lupus nephritis (LN) where comparable NETs values were found between patients without clinical or laboratory evidence of LN and those with different classes of LN. Also, no difference was found in patients with proliferative and non-proliferative LN. These findings need to be further evaluated as small sample size, and lack of studying in situ NETs in renal biopsy might interfere with the possible relation between NETs and renal involvement in pSLE. Interestingly, NETs level was found to be significantly higher in patients with renal activity in comparison to those in remission as assessed by renal SLEDAI; this was also supported by the direct positive correlation between NETs and 24-hrs urinary protein excretion ($p < 0.001$).

Some previous studies reported that adult SLE patients with decreased ability to degrade NETs and increased NETosis developed LN more frequently than high degraders. These studies have documented deposition of NETs in tubules and glomeruli in the kidney of SLE patient who degraded NETs poorly [18, 19, 26]. NETosis have been correlated with more severe LN and more levels of antidsDNA and antihistones antibodies as well as urinary cellular casts [19, 26]. However, one study reported no significant difference in NETs degradation between different classes of LN [18].

On the other hand, DHR test had a significant negative correlation with proteinuria and was found to be significantly decreased in patients with renal activity measured by renal SLEDAI compared to those with inactive LN. However, no significant difference was found in DHR results in patients with LN compared to those without. Similarly, Nox2-deficient lupus-prone mice were found to have markedly exacerbated lupus and markedly elevated proteinuria with more severe histologic glomerular disease compared to controls [10, 32].

CNS lupus was diagnosed in nine patients who had comparable NETs to those without such disorder. However, a contribution of NETs to CNS lupus through stimulation of IFN-α production by pDCs has been previously demonstrated [33].

Corticosteroids and immunosuppressive therapy affect functions and number of the immune cells. As none of our patients had neutropenia, we assessed the relationship between these medications and the studied neutrophil functions. We found a positive direct correlation between NETs and corticosteroid dose, whether this is a direct effect of steroid through steroids-induced neutrophilia or a reflection of higher disease activity. This finding needs further studies for more conclusive results, as no relation was found between NETs and other immunosuppressive drugs. Also, a previous study did not find a significant correlation between NETs-degrading ability and different immunosuppressive medications used [20].

In this study, we found that only corticosteroids had a significant negative correlation with DHR test values, whether the reduced DHR test is related to high SLE activity and therefore higher corticosteroids dose or this is direct inhibitory

effect of steroids on neutrophil Nox2 activity. This finding comes in accordance with what was previously reported that patients receiving corticosteroids had dose-dependent inhibition of ROS production by polymorphonuclear leukocytes [34]. On the contrary, another recent study did not find any correlation between corticosteroid dose and the amount of intracellular ROS produced; however, this was explained by relatively low doses of corticosteroids received that were likely too weak to affect the function of neutrophils. Moreover, other forms of immunosuppressive drugs neither seem to affect ROS production [35].

Our study had some limitations including small sample size, lack of adequate male representation in the sample, and inability to study the NETs in renal biopsies and relating it to activity and chronicity indices of renal biopsy.

In conclusion, neutrophils seem to behave differently in pSLE through NETs and its Nox2 activity where NETs were significantly increased in patients and were related to biomarkers of SLE and LN activity, but Nox2 activity was associated with disease remission. Wider scale longitudinal studies of NETs in pSLE and in respect to different organs involvement will help to give more clear role of NETs in pathogenesis of pSLE and its relation to disease activity and remission in same patient. This might make NETs a future therapeutic target for SLE.

Author contributions Dalia El-Ghoneimy: Study concept and design, analyzed the data, interpreted the results, and wrote the manuscript.

Mohamed Hesham: Contributed to analysis of the data, interpretation of the results, and drafting the manuscript.

Rasha Hasan: Contributed to analysis of the data, interpretation of the results, and drafting the manuscript.

Mohamed Tarif: Perform the laboratory work up of the study and contributed to drafting the manuscript.

Sally Gouda: Patients assessment and recruitment, contributed to interpretation of the results and drafting the manuscript.

Compliance with ethical standards

Disclosures None.

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