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FULL PAPER

Correlation between tumor necrosis factor receptor 1 and neutrophil extracellular traps in systemic lupus erythematosus patients

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Neutrophil extracellular traps (NETs) were released from apoptotic neutrophils to capture and kill pathogens. Tumor Necrosis Factor Receptor 1 (TNFR1), the receptor for tumor necrosis factor (TNF)-α, has been shown to stimulate NET release. In addition, NETs can bolster proinflammatory cytokines' output; including TNF-α, establishing a positive feedback loop that perpetuates inflammation and immune dysregulation in systemic lupus erythematosus (SLE). This cross-sectional study was conducted on SLE patients at two hospitals in Denpasar, Bali. TNFR-1 and NET serum levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit. Correlation, Odd Ratio (OR), and 95% Confidence interval (CI) were calculated. 44 patients with SLE were included in this study. Spearman Correlation test showed that TNFR1 had a positive association with NETs ($r=0.621$; $p<0.001$). Chi-Square test confirmed the positive association between TNFR1 and NETS (OR=7.11; 95% CI 1.88-26.80; $p=0.003$). Our study revealed a positive correlation between TNFR-1 and NETs. When TNFR1 levels are elevated, NET levels also increase.

KEYWORDS

Neutrophil extracellular traps; systemic lupus erythematosus; tumor necrosis factor receptor 1.

Introduction

Systemic lupus erythematosus (SLE) is an illness marked by abnormal immunological responses, resulting in inflammation and damage to several organs. In the pathogenesis of SLE, apoptosis or programmed cell death plays a crucial role by contributing to the release of autoantigens, immune complexes creation, and the activation of inflammatory pathways, ultimately resulting in tissue damage and the clinical manifestations of the disease [1]. In SLE, there is often impaired clearance of apoptotic cells, resulting in the buildup of cellular debris. Normally, phagocytes swiftly envelop and clear apoptotic cells without provoking an immune response. However, in SLE, this process is dysregulated, leading to secondary necrosis and the release of autoantigens. When apoptotic cells are not efficiently cleared, they undergo secondary necrosis, releasing intracellular contents, including self-antigens. These autoantigens can then stimulate the

immune system, producing autoantibodies. The autoantibodies generated against self-antigens form immune complexes with their targets. These immune complexes can deposit in various tissues, particularly in the kidneys, skin, and joints, triggering inflammation and tissue damage [2,3].

Apoptotic cells and immune complexes can activate inflammatory pathways, including the complement system and NET. This leads to the production of inflammatory cytokines, including TNF-α, perpetuating the inflammatory response and tissue damage. Chronic exposure to self-antigens and the sustained inflammatory response in SLE can cause a breakdown in self-tolerance, leading to the activation of T cells that respond against the body's cells and the manufacture of antibodies by B cells that target the body's tissues. This perpetuates the autoimmune response and contributes to the systemic nature of the disease [4].

Several studies showed an elevated level of neutrophil extracellular traps (NETs) in SLE patients compared with non-SLE [5,6] and in SLE patients with complications such as lupus nephritis [7,8]. Apoptotic neutrophils released NET to capture and kill pathogens [9]. The NETosis event is a host defence mechanism to create NETs to trap and kill microorganisms. NET is the origin of antigens in the SLE, stimulating the continued production of B lymphocyte autoantibodies against the body. NETs facilitate inflammation, causing endothelial damage. In addition, SLE patients experience impaired NET degradation, further elevating the NETs level [4,10-11].

Tumor Necrosis Factor Receptor 1 (TNFR1) is a cell surface receptor protein of TNF-α [12,13]. When TNF-α binds to TNFR1, it triggers a cascade of signalling events within the cell, leading to various biological responses such as inflammation, apoptosis (programmed cell death), cell proliferation, and immune regulation. The activation of TNFR1 can influence diverse physiological processes, including host defence against pathogens. TNFR1 is implicated in various pathological conditions, including SLE [14,17]. Understanding the signalling pathways mediated by TNFR1 has led to the development of therapeutic agents targeting TNF-α or its receptor to treat inflammatory and autoimmune diseases.

In NET formation, TNF-α, the ligand for TNFR1, has been shown to stimulate NET release from neutrophils. In addition, NETs can stimulate the production of proinflammatory cytokines, including TNF-α, establishing a positive feedback loop that perpetuates inflammation and immune dysregulation in SLE [3-4,12]. There has been little research into the relationship between TNFR1 and NETs in humans. Therefore, this study aimed to investigate the correlation between TNFR1 and NETs in SLE patients.

Materials and methods

study design and population

This cross-sectional study was conducted on SLE patients treated at two hospitals in Denpasar, Bali, Indonesia. We included individuals who were at least 18 years old and diagnosed with SLE according to the American College of Rheumatology 1997. Patients with SLE who were pregnant, had diabetes mellitus, malignancy, infections, or terminal renal failure on dialysis were excluded.

data and sample collection

The minimum sample calculation using 90% power and α =0.05 was 40 samples. To avoid dropout, we added 10% of the minimum samples, so 44 participants were recruited in this study. Study subjects were consecutively enrolled and signed a written informed consent form. Serum TNFR1 was measured using an enzyme-linked immunosorbent assay (ELISA) kit from Bioassay Technology Laboratory. TNFR1 was carried out at a dilution of 1 in 40. Serum NETs were also

measured using the ELISA Assay Kit from the Bioassay Technology Laboratory.

Statistical analysis

All data were analyzed using SPSS version 21.0 software (SPSS Inc, Chicago, IL). The Kolmogorov-Smirnov test showed that the data were not normally distributed, so the Spearman correlation test was used to analyze the correlation between TNFR1 and NET. Next, the 95% Confidence Interval (CI) and Odds Ratio (OR) were determined using the Chi-Square test. P-values less than 0.05 are regarded as statistically significant.

Results

This study included 44 patients with SLE. All patients were between 20 and 60 years old and dominated by females (95.4%). The clinical characteristics of all study subjects are presented in Table 1.

TABLE 1 Characteristics of study subjects

Variables	n (%)
Sex	
Male	2 (4.6)
Female	42 (95.4)
ANA immunofluorescence	
1: 80 homogenous	1 (2.3)
1: 100 speckled	9 (20.5)
1: 100 homogenous	4 (9.1)
1: 320 speckled	8 (18.1)
1: 320 nuclear homogenous	5 (11.4)
1: 1000 Speckled	9 (20.5)
1: 1000 Nuclear Homogenous	8 (18.1)
SLE Duration (years)	
1-5	22 (50.0)
6-10	17 (38.6)
11-15	4 (9.1)
16-20	1 (2.3)
Hematology Result	
Anemia	14 (31.8)
Leukopenia	4 (9.1)
Lymphopenia	4 (9.1)
Medication during study	
Corticosteroid	33 (75.0)
Antimalaria	39 (88.6)
Mycophenolate	26 (59.0)
Azathioprine	6 (13.6)
Methotrexate	2 (4.5)
Leflunomide	3 (6.8)

Correlation analysis using the Spearman Correlation test showed that TNFR1 had a positive association with NETs (r=0.621; p<0.001) (Table 2). Chi-Square test confirmed the positive association between TNFR1 and NETS (OR=7.11; 95% CI 1.88-26.80; p=0.003) (Table 3).

TABLE 2 Correlation between TNFR1 and NETs

Variable	Variable	Correlation coefficient	Direction of correlation	*P*-value
TNFR1	NETs	0.621	Positive	<0.001*

*Spearman Correlation Test

TABLE 3 Association between TNFR1 and NETs

Variable	Category	NETs		Total	OR	95% CI	*P*-value
		Low	High				
TNFR1	Low	16 (72.7)	6 (27.3)	22 (100)	7.11	1.88-26.80	0.003*
	High	6 (27.3)	16 (72.7)	22 (100)			

*Chi-Square Test

Discussion

Our study revealed a positive correlation between TNFR1 and NETs. The correlation between TNFR1 and NETs may arise from the complex interaction between inflammation, immune dysregulation, and neutrophil activation. Several factors may contribute to this correlation. TNFR1 is known to activate signalling cascades that result in the recruitment and activation of neutrophils. Upon binding of TNF-α, the ligand for TNFR1, downstream signalling events can stimulate the production of adhesion molecules and chemokines, promoting the migration of neutrophils to sites of inflammation. Once activated, neutrophils can release NETs as a component of their immune system against microorganisms. During the process, stimulation by TNF-α causes the euchromatin and heterochromatin to homogenize and neutrophil nuclei to lose shape. Subsequently, the granule membranes and nuclear envelope separate, facilitating the blending of NET constituents. At last, the cell membrane ruptures, releasing the NETs [18].

In addition to direct signalling by TNF-α, the TNF-α also stimulates the generation of reactive oxygen species (ROS) via a Cytosolic Phospholipase A2-linked Cascade and activates protein kinases, which are critical steps in the formation of NETosis [19,20]. Reactive oxygen species are produced by NADPH oxidases in mitochondria. The mitochondrial reactive oxygen species (mtROS) activated NADPH oxidase with the involvement of PKC, which possesses two sulfur-zinc clusters that are sensitive to redox reactions within its diacylglycerol-binding domain. Furthermore, several members of the Src kinase family, including Lyn kinase, can activate PKC by phosphorylating its tyrosine residues, a process that is facilitated by ROS. A further redox-sensitive method for the activation of NADPH oxidase in neutrophils involves the connection of the disulphide isomerase protein with the p47phox subunit, which is dependent on ROS. his association results in the translocation of the p47phox subunit to the membrane, where it subsequently assembles with NADPH oxidase [21].

Neutrophil extracellular traps released by activated neutrophils can, in turn, stimulate the production of TNF-α and other cytokines such as IL-1β, IL-8, and B-cell-activating factor (BAFF) [22,23]. NETs also may induce the formation of ROS by NADPH oxidase. The mechanism involves the induction of significant DNA damage and subsequent activation of the DNA repair pathway, resulting in the decondensation of chromatin [24]. NETs contain various immune-stimulatory molecules, including DNA, histones, and granule proteins, which can stimulate the activation of immunological cells to produce TNF-α. This creates a positive feedback loop where TNF-α promotes NET formation, and NETs further amplify TNF-α production, exacerbating inflammation [22,23].

Inflammatory conditions such as SLE create an environment conducive to both TNF-α signalling and NET formation [25]. Elevated levels of TNF-α and other pro-inflammatory cytokines characteristic of SLE contribute to sustained activation of TNFR1 on immune cells, including neutrophils. Concurrently, the presence of autoantibodies and immune complexes in SLE can stimulate neutrophils to release NETs [3,11].

An advantage of this study is its conducted in humans. To our knowledge, there have been no studies examining the correlation between TNFR1 and NETs in SLE patients, therefore our study is the first. However, the limitation of this study is that we did not do multivariate analysis to control the confounding variable. In addition, the sample size was limited because of the rarity of SLE.

Conclusion

There is a positive correlation between TNFR-1 and NETs. When TNFR1 levels are elevated, NET levels also increase. The correlation between TNFR1 and NETs in SLE provides an engaging pathway for further research into disease mechanisms and therapeutic interventions. By elucidating the complex interaction between these two factors, we can advance our understanding of SLE pathogenesis and identify novel strategies for disease management.

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Authors' Contributions

IARWM contributed to the design and implementation of the research, analysis of the results and writing of the manuscript. KS, IMB, IMS contributed to supervision of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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