Association of urine Interleukin-8 (IL-8) with renal impairment in lupus patients

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with clinical manifestations that vary between individuals with progressive and irreversible SLE organ damage. Recently, it has become known that Interleukin-8 (IL-8) levels in the blood rise in SLE patients compared to healthy people. The increase of IL-8 causes kidney inflammation by promoting the release of the Neutrophil Extracellular Traps (NET) complex, which supports further renal damage. This study aims to evaluate the association of urine interleukin-8 with renal impairment in SLE patients.

Methods: A total of 45 lupus participants—22 with and 23 without renal abnormalities—had their urine collected. The interleukin-8 concentration was measured using an enzyme-linked immunosorbent assay (ELISA)—renal impairment criteria between individuals with progressive and irreversible SLE organ damage. Recently, it has become known that Interleukin-8 (IL-8) levels in the blood rise in SLE patients compared to healthy people. The increase of IL-8 causes kidney inflammation by promoting the release of the Neutrophil Extracellular Traps (NET) complex, which supports further renal damage. This study aims to evaluate the association of urine interleukin-8 with renal impairment in SLE patients.

Methods: A total of 45 lupus participants—22 with and 23 without renal abnormalities—had their urine collected. The interleukin-8 concentration was measured using an enzyme-linked immunosorbent assay (ELISA)—renal impairment criteria based on renal biopsy or ACR 1997 criteria. Mann-Whitney and Spearman correlation tests were employed in the statistical analysis. Data were analyzed using SPSS version 25.0 for Windows.

Results: There was no significant difference in IL-8 urine levels between the SLE group with renal impairment (111.27±59.03 pg/ml) and the SLE group without renal impairment (125.76±66.62 pg/ml) was not significant (p=0.67). Urine IL-8 also did not significantly correlate with blood neutrophil count, leukocyte count and leukocyturia (p>0.05).

Conclusion: In lupus patients, urine Interleukin-8 levels are not associated with renal impairment.

Keywords: Interleukin-8, Lupus, Renal Impairment.


INTRODUCTION

Renal impairment in patients with Systemic Lupus Erythematosus (SLE) occurs in more than 50% of patients during the disease and strongly predicts SLE morbidity and mortality.¹ The deterioration of the renal that causes chronic renal failure is estimated to occur in 25% of patients within ten years after renal abnormality that requires dialysis or transplantation.² Several factors influence renal damage in SLE, including chronic inflammation, activation of intrinsic renal cells (endothelial cells, podocytes and mesangial), cell hypoxia, abnormal metabolism, impaired tissue repair, and tissue fibrosis.³

The one cause of inflammation in renal impairment is an increase in the number of neutrophils due to the activation of Interleukin-8 (IL-8) or CXCL8 (C-X-C motif chemokine ligand 8). Interleukin-8 is a chemotactic cytokine that functions as a chemotaxis to recruit immune cells to the site of inflammation. Macrophages, endothelial cells and epithelial cells all express interleukin-8. Infection or tissue damage will induce IL-8 to recruit granulocytes and neutrophils to these sites. Based on research by Mao et al. (2018), IL-8 levels in the blood rise in SLE patients compared to healthy people.³

IL-8 activation will affect neutrophil activation and trigger the release of the neutrophil extracellular trap (NET), which is associated with the pathogenesis of SLE and leads to renal damage.⁴ The release of NETs that contain many self-antigens in individuals susceptible to autoimmune diseases will trigger the formation of autoantibodies that will destroy the immune system's tolerance.⁵ NETs can also trigger inflammation, leading to endothelial damage and production of IFN-α by dendritic cells, further exacerbating inflammation and autoimmune conditions. Furthermore, the complement system can promote inflammation by luring leukocytes to the renal or directly damaging it by forming its membrane attack complex (MAC).⁷

The study by Aragón et al. (2020) showed that IL-8 increased SLE activity and progression, but the direct correlation of IL-8 to renal impairment was still unclear, making it interesting to be studied.⁸ Based on the abovementioned, this study aims to evaluate the association of urine IL-8 with renal impairment in SLE patients.

METHODS

In this study, we conducted a cross-sectional design using a random purposive sampling technique. The Systemic Lupus International Collaborating Clinics (SLICC) criteria were used to determine the SLE patients. The participants should fulfill at least four SLICC criteria, including one clinical and one laboratory criterion or with a renal biopsy indicating lupus nephritis and a positive antinuclear antibody test (ANA) or anti-ds DNA.⁹ Determining renal impairment in lupus patients was based on the ACR criteria, namely persistent proteinuria >0.5 g per
RESULTS

This study had 45 participants, consisting of 23 SLE patients without renal abnormalities and 22 SLE patients with renal abnormalities. The characteristics of the participants are shown in Table 1.

The correlation between IL-8 levels with leukocytes, neutrophils, leukocyturia and erythrocyturia can be seen in Table 2 and Figure 2.

DISCUSSION

The participants included in this study had a mean age of 25.87±7.80 years in the lupus group without renal impairment and 32.64±10.28 years in the lupus group with renal impairment. These results are in accordance with several studies, which state that lupus occurs in women of childbearing age due to the possible influence of hormonal factors.

This study showed that the SLE group without renal impairment showed Interleukin-8 expression with a mean of 125.76±66.62 pg/ml, while the lupus nephritis group scored 111.27±59.03 pg/ml, and there was no difference between the two groups. (p>0.05). The results of our study differ from the study of Mao et al., which stated that IL-8 caused an increase in blood circulation in SLE patients compared to healthy individuals. This increase has an impact on the recruitment and activation of neutrophils, which are known to play an important role in the pathogenesis of SLE.

Our study results indicated no significant difference in urinary IL-8 levels between lupus and nephritis patients. It is possible that increased IL-8 still occurs in renal tissue because IL-8 can last up to days in an inflammatory situation to recruit the immune system against pathogens, for example, in pneumonia and peritonitis, where most of the cytokines are usually made and broken down in a matter of hours. There is a possibility that IL-8 expression in tissues is high, but IL-8 levels in urine are low, so further research is needed to evaluate IL-8 expression in renal tissue by immunohistochemistry.

In addition, related to regulating IL-8 in the body’s homeostatic conditions, IL-8 gene expression is very sensitive to the presence of oxidants. So, these cytokines can be reduced in ischemia-reperfusion conditions that produce excessive reactive oxygen intermediates (strong oxidants). Activation of IL-8 secretion is also influenced by lipopolysaccharide

Table 1. Characteristics of Research Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Lupus without Renal Impairment</th>
<th>Lupus with Renal Impairment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25.87±7.80</td>
<td>32.64±10.28</td>
<td>0.005*</td>
</tr>
<tr>
<td>Leukocytes Counts (10³/μl)</td>
<td>6.88±2.60</td>
<td>8.30±3.45</td>
<td>0.159</td>
</tr>
<tr>
<td>Absolute Neutrophil Count (10³/μl)</td>
<td>4.81±2.22</td>
<td>6.24±4.11</td>
<td>0.351</td>
</tr>
<tr>
<td>Absolute Lymphocyte Count (10³/μl)</td>
<td>1.55±0.74</td>
<td>1.94±1.02</td>
<td>0.192</td>
</tr>
<tr>
<td>Absolute Monocyte Count (10³/μl)</td>
<td>0.63±0.65</td>
<td>0.69±0.49</td>
<td>0.555</td>
</tr>
<tr>
<td>Urine Erythrocyte Count (10³/μl)</td>
<td>5.27±14.90</td>
<td>26.63±68.23</td>
<td>0.004*</td>
</tr>
<tr>
<td>Urine Leukocyte Count (10³/μl)</td>
<td>10.97±37.87</td>
<td>11.38±16.52</td>
<td>0.048*</td>
</tr>
</tbody>
</table>

*Note: Age, urine erythrocyte count and urine leukocyte count show a significant median difference between the groups of SLE without renal abnormality and SLE with renal abnormality (p<0.05)
stimulation, cell bonds with bacteria and various proinflammatory cytokines such as TNF and IL-1. Other factors affect the production of IL-8, such as microRNA 146a (MiRNA146a/b-5p indirectly suppress IL-8 expression by suppressing IRAK1 production). The 3'UTR part of IL-8 contains a segment rich in A/U bases, making it unstable under certain conditions such as high glucose levels, obesity and others. Genetic factors, namely IL-8 gene polymorphisms, are also thought to play a role in IL-8 production. Based on this, it is necessary to evaluate other factors that affect urine IL-8 levels through further research.

This study showed no significant difference in IL-8 levels between groups with mild, moderate and severe lupus disease activity. This study’s results differ from research by Aragón et al., in 2020, which showed that IL-8 increased SLE activity. It is estimated that an increase in IL-8 will trigger inflammation in the renal which encourages further renal damage and triggers the development of SLE through the release of its Neutrophil Extracellular Traps (NET) complex through IL-8’s binding to neutrophils as its potent activator.

There are several limitations to the study, namely that the examination of IL-8 was only assessed based on its level in the urine and did not examine the expression of IL-8 in renal tissue. Confounding factors for the results of the IL-8 examination, such as obesity, excessive oxidants and genetic predisposition, were not examined. We suggest further studies examining IL-8 expression in kidney tissue using immunohistochemistry and considering the previously mentioned confounding factors.

**CONCLUSION**

Based on the results of our study, it can be concluded that IL-8 levels are not associated with renal impairment in lupus patients.

**CONFLICT OF INTERESTS**

The authors declare that there is no conflict of interest in this publication.
ETHICS CONSIDERATION
The ethical committee of Dr. Saiful Anwar General Hospital, Malang, Indonesia, approved this study with ethical number 400/247/K.3/302/2020. All patients included in this study signed informed consent.

FUNDING
None

AUTHOR CONTRIBUTIONS
EH, UK, HS and K conceptualized the idea and study design. EH and HS performed the literature search. EH, LC, and DPS managed the patient and data collection. EH, UK, HS and K performed data analysis, interpretation and drafted the manuscript. The authors would like to thank all participants of this study.

REFERENCES