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Correlation Between Renal Activity Index and C3 Complement Expression in Mouse Lupus Nephritis Model

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ABSTRACT

Background: Lupus nephritis is a disease which currently has no definitive therapy. For lupus nephritis, histological examination remains the best option to assess the degree of disease activity, which is essential for therapy evaluation and monitoring. At present, there is no single laboratory that can be used to assess disease activity. Therefore, this study aims to determine the correlation between renal activity index and C3 complement expression in mouse lupus nephritis model. Research methods: The study was conducted on male mice. Lupus was induced using intraperitoneal pristane injection at 0.5 cc, then left for four months. At the end of the 4th month, the mice were sacrificed, and histological examination of mice's kidneys and renal C3 complement expressions were evaluated. Statistical tests were done using t-test, Mann-Whitney test, and Spearman correlation test, with a significance level of $P < 0.05$. Results: activity index on histological examination of mouse kidney in such aspects as glomerular proliferation, karyorrhexis, and fibrinoid necrosis, cellular crescents, hyaline deposits, and interstitial inflammation were associated with C3 complement expression in kidney tissue ($r = 0.680$; $p = 0.001$) Conclusion: The study demonstrateds that the activity index in kidney histological examination correlates with C3 complement expressions in the lupus nephritis model.

Keywords: *Renal Activity of Lupus, C3 Complement, Lupus Nephritis Model*

1. INTRODUCTION

Lupus is a chronic inflammatory autoimmune disease with a vast clinical spectrum and effect on many organs, including the kidneys.[1] Lupus nephritis is a severe complication of systemic lupus erythematosus for which there is no definitive therapy. Lupus nephritis predominantly affects women with SLE. Recently, it was found that abnormal pathology of the kidneys were present in some SLE patients with normal urinalysis examinations, and this is associated with severe organ damage if left untreated [2].

The pathogenesis of lupus nephritis involves many factors, namely, epigenetic, and environmental factors. Lupus nephritis is characterized by a loss of immunologic self-tolerance, which results in the formation of polyclonal autoantibodies, and a positive immunofluorescent pattern in kidney biopsy. In the early stages of the disease, the innate immune system will activate T cells and B cells, causing activation of the adaptive immune system. T cells, including T helper cells 1 and 17, causes activation of B cells. Activation of B cells leads to the production of autoantibodies and cytokines.[1,3]

Biopsy is the only standard examination for lupus nephritis. In human examination, class 4 lupus nephritis is the most common pathology found in kidney examination of patients with lupus nephritis.[2] To perform a lupus nephritis therapy, it is essential to distinguish the degree of activity of the disease, whether it is currently active or has just occurred. At present, examination of disease activity and organ damage is performed on renal histology after renal biopsy and clinical status assessment, renal laboratory test, and proteinuria test. It is essential that kidney biopsy is performed only once at the disease's onset, rather than, routinely in all patients. Therefore, it becomes essential, too, to find the right marker to define active disease in lupus nephritis, thus Identification of new markers for lupus nephritis activity could prove to be useful in the management of lupus nephritis. [4]

The complement system plays a vital role in the pathogenesis of lupus nephritis. A decrease in the circulating levels of C3 and C4 indicates that complement activation will increase lupus nephritis activity and low complement C3 and C4 have been included in the diagnosis of lupus. The OHIO study examined complement C3 and C4 for SLE in a flare in one village where 70 patients had a flare or Lupus relapse. The study found that complement C3 and C4 decreased during a flare but not at two months before ending, with specificity and sensitivity below 75%.4 The complement system is part of the innate immunity, which is vital in the defense of the host against infectious micro-organisms, to clean immune complexes and dead cells and act as a bridge between the inert and adaptive

immune systems. However, it also has a role in the pathogenesis of lupus, in which case complement deficiency predisposes to lupus, while complement activation with immune complexes is a prominent feature of lupus.[5] Research on markers in urine to predict and assess lupus nephritis activity has not been satisfactory as of now.[6] Pristane has long been used as a tool for the induction of lupus nephritis in mice through the interferon pathway mechanism.[7] This study aims to evaluate the renal complement C3 expression pattern along with the degree of activity of the renal histology.

2. METHODS

This research was conducted in the histology laboratory of the Universitas Sebelas Maret Faculty of Medicine, from March to August 2020. Pristan to induce lupus was obtained from Sigma-Aldrich, and C3 complement expression examination was done using a kit from Sigma Aldrich. Histology examination was conducted by two anatomical pathology specialists. This research has been cleared by the ethical committee of the Universitas Sebelas Maret Faculty of Medicine with ethical clearance number 059 /UN27.06.6.1/ KEPK/EC/2020.

The study used 16 mice:, eight mice were assigned to the control group, while the other eight in the experimental group with pristane administration to induce lupus nephritis. Lupus induction was performed by intraperitoneal injection of pristane at a dose of 0.5 cc.7 After four months, the mice were sacrificed, the right kidney was taken and a comparison analysis of the kidney lupus activity index with complement C3 was done. Examination of complement C3 used immunohistochemistry methods by looking at the expression of complement C3 in the kidney tissue every 100 lymphocytes. The kidney tissue used was that of the right kidney

Lupus nephritis occurs when antibodies and complement build up in the kidneys, causing an inflammatory process in the kidney tissue. The parameters assessed from the kidney biopsy were the degree of kidney damage, based on a scale of 0-3. 0 = no abnormality, 1 = minimal abnormality, 2 = moderate abnormality, and 3 = severe abnormality. Assessment of the lupus activity index of the kidney, which reflects the degree of kidney damage, includes the following. [7]

Glomerular proliferation indicates that the hypercellularity of the endocapillary causes a decrease in the glomerular capillary loops (mesangial, endothelial, and possibly infiltrating monocytes). The lesions were scored by loss of circulatory space due to segmental proliferative changes in less than 25% (1+), 25–50% (2+), or greater than 50% (3+) of glomeruli.

Karyorrhexis is known for the presence of split and picnotic cells, whereas fibrinoid necrosis is known on Mason's stain examination, usually with karyorrhexis in the affected glomerulus. This condition is known by the eosinophil material in the solid segment of the glomerulus. The following scale of severity was used: karyorrhexis if greater than 50% (3+) of glomeruli, fibrinoid necrosis if 25 to 50% (2+), and fibrinoid necrosis if less than 25% (1+).

Inflammatory cells are infiltratin of more than two polymorphonuclear leucocytes per glomerulus was considered abnormal. Exudation was scored as extensive (3+), moderate (2+), or mild (1+).

Eosinophilic material of a homogenous consistency along the circumference of the glomerular capillaries luminal surface constituted the classical wire 1oop lesion. The hyaline material was considered to represent a massive accumulation of immune complexes. Hyaline lesions were scored as extensive (3+), moderate (2+), or few (1+).

Infiltration of lymphocytes, plasma cells, and macrophages into interstitial spaces was categorized mild (1+), moderate (2+), or extensive (3+).

Statistical analysis was done using t-test, Mann-Whitney test, and Spearman correlation test, with a significance level of P less than 0.05.

3. RESULTS

The results showed that in the control group and the lupus nephritis group, there were differences in the examination of glomerular cell proliferation, karyorrhexis, fibrinoid necrosis, Inflammatory cells, hyaline deposits, interstitial inflammation, and C3 complement.

Table 1. Assesment of kidney damage between the control group and the lupus nephritis group

	C3 Complement (corelation score/r)	p value
Glomerular cell proliferation	0,789	0,001
Karyorrhexis and fibrinoid necrosis	0,666	0,001
Inflammatory cells	0,587	0,001
Hyaline deposits	0,681	0,001
Interstitial inflammation	0,565	0,001
Total score (renal activity index)	0,68	0,001

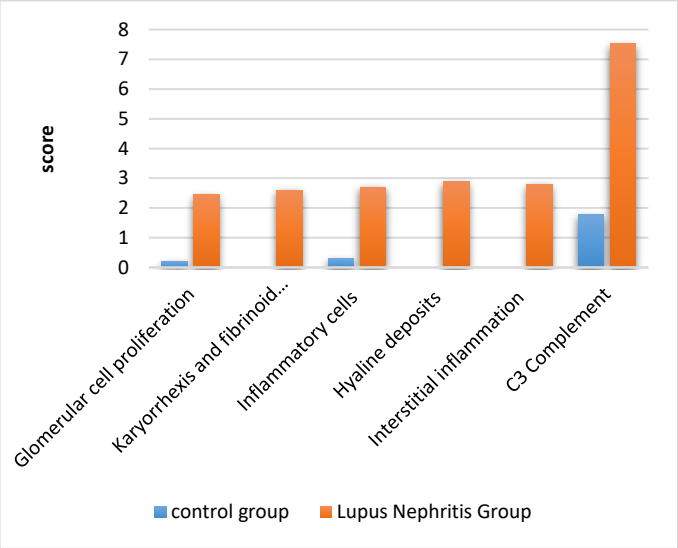

Fig.1. Assesment of kidney damage between the control group and the lupus nephritis group

In the lupus nephritis group, an analysis of the relationship between complement C3, glomerular cell proliferation, karyorrhexis, and fibrinoid necrosis was carried out. There was a significant positive correlation between complement C3 and the renal activity index. C3 complement would increase glomerular cell proliferation, karyorrhexis, fibrinoid necrosis, inflammatory cells, hyaline deposits, interstitial inflammation, and total score of renal activity index in the lupus nephritis group.

Table 2. Correlation between C3 complement and histology variables in the lupus nephritis group

Variable	Control group	Lupus Nephritis Group	P value
Glomerular cell proliferation	0.2 ± 0.23	2.45 ± 0.58	0.001
Karyorrhexis and fibrinoid necrosis	0	2.6 ± 0.67	0.001
Inflammatory cells	0.3 ± 0.12	2.7 ± 0.33	0.001
Hyaline deposits	0	2.9 ± 0.21	0.001
Interstitial inflammation	0	2.8 ± 0.12	0.001
C3 Complement	1.78 ± 3.45	7.53 ± 7.34	0.001

There was a significant correlation between C3 complement and renal activity, where the former would increase the total amount of the latter, as showed in the histological examination (r = 0.680; p = 0.001)

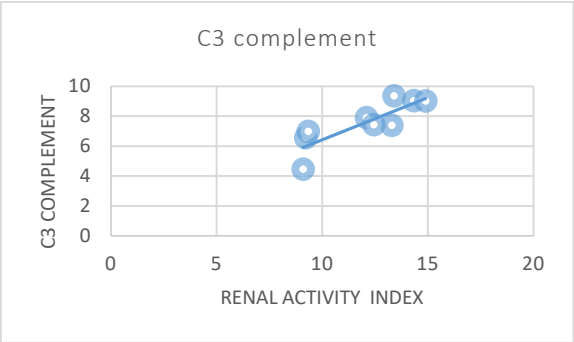

Fig.2. C3 complement increased the total amount of renal activity in the histological examination (r = 0.680; p = 0.001).

4. DISCUSSION

Lupus is a chronic autoimmune disease that can attack various organs such as the skin, joints, nerves, heart, and kidneys. Renal involvement in lupus or lupus nephritis is the leading cause of morbidity and mortality in lupus patients, with the most significant predisposition to women and young people. Up to 50% of lupus patients will develop lupus nephritis. During the disease, kidney involvement can affect up to 75% of patients, showing that lupus nephritis is indeed very large, or, in other words, that most of lupus patients will suffer from kidney involvement.[8] Laboratory tests are not very specific, especially on creatinine levels, which sometimes would still be categorized as normal, and on urine, which, too, would be considered as normal despite the presence of protein or the case of hematuria.[8]

The histologic features of the kidneys of patients with lupus nephritis vary in the glomerular and vascular intestinal tubules' appearance. The classification of lupus nephritis itself has evolved with more renal lesions identified in the histological examination, and this poses new challenges to correlating these biopsy features with the pathogenesis of clinical conditions, treatment options, and prognosis in patients with lupus nephritis.[9]

Damage to the tubules and interstitials is typical in lupus nephritis, which can be seen in glomerular damage. For instance, the study of Lan Ting et al. found that patients with neutrophil infiltration, tubular lesions, and arteriosclerosis had twice the chance of tubulointerstitial damage, and inflammation of the tubulointerstitial indicated progressive kidney disease.[10]

Clinical manifestations usually consist of three significant predictions based on the topography and character of the glomerular damage to the messenger pathology point, causing a syndrome of microscopic hematuria and nephrotic proteinuria and a minimal decrease in the estimated glomerular filtration rate. The endocapillary damage pattern is characterized by a rapid decrease in the glomerular filtration rate of hematuria and mild to moderate proteinuria. In contrast, the pattern of damage to the membrane is associated with significant urine protein and is often accompanied by nephrotic syndrome and a gradual decrease in glomerular filtration rate.[9]

There are 3 activation of complement pathways, namely, the alternative route of the classical pathway and the lectin pathway. Activation of this pathway causes the C3 convertase assembly, and this will be followed by the C5 convertase formation and, finally, the C5b-9 membrane attack complex. In general, the complement system has three prominent roles: the opsonization of C3b from cells or microbes by phagocytosis, through terminal and C5b-9; lysis of cells or microbes that occur; and anaphylaxis through C3a and C5a, which causes the collection of neutrophils and macrophages at the injury site.[11]

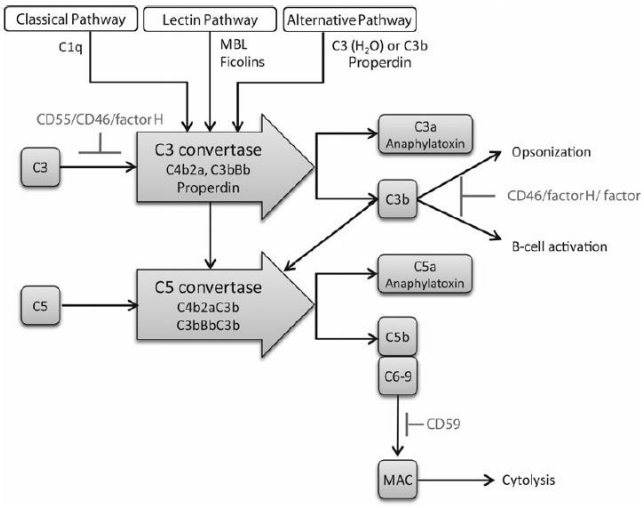

Fig 3. Overview of the complement cascade[11]

This study showed that complement would increase in the kidney tissue of mice with lupus. This study was consistent with other studies. Another study stated that the complement pathway not only responds to pathogens, but its activation can be due to necrotic, apoptotic autoantibodies, or ischemic cells and tissues. Activation of complement causes inflammatory fibrosis and tissue damage.[12]

A significant correlation was found between increased C3 complement in kidney tissue and kidney disease activity, as seen by histological examination. The hypothesis supported this theory because it was consistent with the theory that the role of complement is not only in the damage to the glomerulus, but also in the process of scar formation and progressive kidney disease, which causes proteinuria and activation of components that cause damage to the tubular lumen.[13]

The formation of immune complexes is the first step in activating the classical pathway of the complement system. In this pathway, C1q causes stimulation of the complement system activation to form immune complexes. Furthermore, this bridges the innate and adaptive immune systems, when antigens meet selfie with the presence of complement and the limit activation of B cells decreases. Clearing the immune complexes from circulating complement system points can also lead to bindings to apoptotic cells and play a role in helping to eliminate these cells from the tissue. If the complement system fails, this waste material can accumulate and cause an autoimmune response.[14]

Immune complexes can be deposited in the glomerulus through passive storage from the circulation or formation of immune complexes through antibody binding to local antigens. Local formation of immune complexes occurs when circulating antigens are recognized by antibodies and after deposits on the glomerulus. Subepithelial complement deposits are found in membranous nephropathy, which causes damage due to anaphylactic. Sub-endothelial deposits of complement cause inflammatory response due to anaphylactic production. The subendothelial immune complex is a marker of lupus nephritis.[15]

Activation of complement via alternative pathway causes tissue damage exacerbation in Lupus. An increase in the C3 pathway causes the formation of inflammatory mediators in the glomerular immune complex. There is a balance between complement activation via the classical pathway, which causes clearance of the immune complex, and alternative pathways, causing exacerbation.[5]

By direct immunofluorescent staining, kidney biopsy showed some complement and immunoglobulins. Also, certain complement products such as C3 and C5d9 were also detected in the urine of patients with lupus nephritis. The leading cause of complement activation in lupus is the formation of immune complexes, which will lead to complement activation via the classical route. Previous studies have shown an association between the complement of C3 and C4 and lupus nephritis.[16]

Several factors play a role in decreasing serum complement levels, including total disease activity versus catabolism and the presence of autoantibodies that directly

attack complement proteins such as the C1q antibody.[17] Based on experimental research models on autoimmune diseases, it is now well known that immune complexes in the kidney cause tissue damage and are related to specific immunoglobulin antigens, the capacity to adhere to antigen, and activation of complement that causes inflammation.[9]

5. CONCLUSION

There is a positive correlation between renal complement C3 levels and the renal activity index. The presence of C3 complement in the kidney tissue is associated with glomerular cell proliferation, karyorrhexis, fibrinoid necrosis, inflammatory cells, hyaline deposits, interstitial inflammation, and total score of renal activity index in lupus nephritis.

AUTHORS' CONTRIBUTIONS

Data gathering and idea owner of this study, Study design, Data gathering, Writing and submitting manuscript, Editing and approval of final draft, all events done by all the authors. All authors read and approved the final manuscript.

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