Immunopathologia Persa
http www.immunopathol.com
DOI:10.34172/ipp.2022.xx

Ki-67 proliferative index in kidney biopsies of lupus nephritis patients: a preliminary study

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Received 9 Nov. 2021
Accepted 18 Jan. 2022
Published online 12 Feb. 2022

Keywords: Systemic lupus erythematosus, Lupus nephritis, Ki-67

Abstract

Introduction: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that involves multiple organs including the kidneys, skin, joints and serous membranes. Previous studies have shown that elevated Ki-67 indices are correlated with the cellular proliferation and clinical findings in lupus nephritis (LN).
Objectives: The aim of this study was to examine the relationship between glomerular, tubular and interstitial expression of Ki-67 in kidney biopsy specimens of different classes of LN and and various clinicopathological features.
Patients and Methods: This cross-sectional study was conducted on 16 biopsy-proven LN patients. The diagnosis of LN was based on renal biopsy findings, particularly by immunofluorescence (IF) study. The diagnosis of LN with IF was concluded by the deposition of C1q in association with prominent IgG and C3 deposits and the deposition of IgM and IgA (full-house pattern). The morphologic variables on light microscopy were also examined. In this study, the glomerular (gKi-67), interstitial (iKi-67) and tubular (tKi-67) expressions of Ki-67 were assessed.
Results: This study comprised of 16 cases of biopsy-proven LN which were stained for Ki-67 with immunohistochemistry. Of the 16 patients, 13 (81.2%) were females. The mean ± SD of age, quantity of proteinuria and serum creatinine in all patients were 37±11.6 years, 1844±582 mg/d and 1.5±0.93 mg/dL, respectively. Our study showed that the association between gKi-67, iKi-67, and tKi-67 with age, gender, level of proteinuria and serum creatinine was not significant ($P>0.05$). The association between Ki-67 with interstitial fibrosis, the number of crescents, and the activity and chronicity percentages was also not significant ($P>0.05$). Moreover, the relationship of gKi-67 with global versus segmental involvement of the glomeruli was not significant ($P>0.05$). Furthermore, the correlation of gKi-67 with IgA, IgG, IgM, C3 and C1q deposits was not significant ($P>0.05$).
The association of iKi-67 with age, gender, level of proteinuria and serum creatinine was not significant as well. However, the correlation of iKi-67 with C1q deposits was inversely significant (r=-0.544, $P=0.029$); however this correlation was not significant with IgA, IgG, IgM and C3 deposits ($P>0.05$).
Conclusion: In this study, the relationship of iKi-67 with C1q deposits suggests that C1q has a significant role in the inflammatory process of LN. Since our study was conducted on a relatively small sample size, it, therefore, requires further investigations on larger samples.

Citation: Mowla K, Emami E, Biglari Abhari M, Zununi Vahed S, Mubarak M, Bahadoram M, Maghsoudi AR, Nasri H, Hooshyar N. Ki-67 proliferative index in kidney biopsies of lupus nephritis patients: a preliminary study. Immunopathol Persa. 2022;x(x):e0x. DOI:10.34172/ipp.2022.xx.

Introduction

Systemic lupus erythematosus (SLE), a systemic autoimmune disease, involves multiple organs. It mostly involves the kidneys, skin, joints and serous membranes. Lupus nephritis (LN) denotes the renal involvement in this disease, which consists of various features of renal diseases involving the tubulointerstitial, glomerular and vascular areas (1). Different factors are implicated in causing lupus kidney injury including complements, auto-antibodies, genetic factors and the environment (2). Kidney disease in SLE is detected in about 60% of adults, while 25–50% of the individuals present with clinical kidney disease at the time of SLE

Key point

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that involves multiple organs including the kidneys, skin, joints, and serous membranes. Kidney involvement is one of the most serious complications and causes significant morbidity and mortality in SLE patients. Previous studies have shown that elevated Ki-67 indices are correlated with the cellular proliferation and clinical findings in lupus nephritis (LN). In a cross-sectional study on 16 biopsy-proven LN cases, we found a positive relationship between interstitial Ki-67 stain percent and C1q deposits on immunofluorescence microscopy. If corroborated in further large-scale studies, this marker may prove to be useful for the early detection the management and prognosis.

presentation (3). Kidney involvement is one of the main predictors of poor prognosis in SLE patients (4). The majority of SLE patients are younger than 50 years of age and are female. Nevertheless, male individuals with SLE tend to have more serious kidney disease (5,6). Numerous studies show that LN is the main risk factor for mortality and morbidity in SLE patients (1). Despite a wide choice of treatment options, consisting of immunosuppressive and anti-inflammatory treatments, some individuals progress to chronic kidney disease (CKD) and eventually, end-stage renal disease (ESRD) (7). Various laboratory, morphological and clinical parameters, such as a panel of urinary proteins and auto-antibodies have been explored as indices for LN activity, which are required for the prognosis and prediction of the disease's progression. In fact, an appropriate assessment of LN activity depends on integrated analysis of various indices rather than a single factor (8). The morphologic diagnosis of LN is based on the morphological classification of lesions into six classes by assessing the activity and chronicity of the indices according to the ISN/RPS (International Society of Nephrology/Renal Pathology Society) LN classification (9). In this classification, class I comprises minimal mesangial LN, class II is pure mesangial proliferative LN, while classes III and IV refer to focal and diffuse proliferative LN, respectively. Class III is focal and segmental, while class IV is a diffuse segmental or global lesion in LN. In addition, Class V is membranous nephropathy and class VI is the final stage of LN demonstrating an advanced sclerosing phase (10). In LN, particularly classes III and IV, there are various parameters for assessing the disease activity. In this regard, markers of cellular proliferation could facilitate the interpretation of the disease activity. Ki-67 belongs to a group of monoclonal antibodies which is detectable in the proliferating phase of cells during tubular regeneration, glomerular proliferation and also interstitial inflammation. Ki-67 is a protein that is encoded by the *MKI67* gene (antigen identified by monoclonal antibody Ki-67) (11). Ki-67 protein has been extensively used as a proliferation marker of malignant cells for many years (4-6). Immunohistochemically, an elevated Ki-67 proliferating index is correlated with the proliferation of cellular tissue (4-6).

Objectives

The aim of this study was to examine the relationship between glomerular, tubular and interstitial expressions of Ki-67 in the kidney biopsy specimens of SLE patients with various classes of LN and various clinicopathological features.

Patients and Methods

Patients and specimens

This cross-sectional study was conducted on biopsy-proven LN cases between January 2020 to January 2021. In this study, we selected 16 kidney biopsies of patients with LN that were eligible for investigation. Kidney biopsies with fewer than 10 glomeruli were excluded from the study. The diagnosis of SLE was defined by the revised criteria of the American College of Rheumatology (ACR) for SLE (12). The relevant data elements consisting of age, gender, amount of 24-hour proteinuria and serum creatinine level were also documented and were correlated with various clinicopathological features.

Definition of LN

The diagnosis of LN was based on the renal biopsy findings, particularly by immunofluorescence (IF) study. The diagnosis was defined with significant positivity of C1q in association with prominent IgG and C3 and also deposition of IgM and IgA in variable intensity (full-house pattern), which was semi-quantitatively classified from 0 to 3+. The results of the kidney biopsies were reported according to the 2003 ISN/RPS classification of LN (13). Renal biopsies were divided into two fragments for assessment by light and IF microscopies. The fragments which were fixed in 10% formalin were processed for histopathologic examination by performing staining with periodic acid Schiff (PAS), Jones silver, Masson's trichrome and hematoxylin and eosin (H&E). The specimens used for IF were stained with IgA, IgM, IgG, C1q and C3 antibodies.

Immunohistochemical analysis for Ki-67

Immunohistochemical staining of Ki-67 was carried out on 4-µm-thick sections using rabbit monoclonal antibody (Zytomed Systems GmbH, Berlin, Germany). In our study, the glomerular (gKi-67), interstitial (iKi-67) and tubular (tKi-67) expressions of Ki-67 were assessed.

Statistical analysis

Statistical analysis was performed through the SPSS software version 24. We reported mean, mode, and range for some variables due to the skewed distribution of data. We tested the relationships between qualitative variables by chi-square test. The correlations between quantitative variables were tested through Pearson's or Spearman's correlation tests.

Differences between quantitative means of variables were investigated through independent *t* test or one-way

ANOVA. The level of statistical significance was defined as $P \leq 0.05$.

Results

This study included 16 cases of kidney biopsies diagnosed with LN, on which Ki-67 immunostaining was conducted. Of the 16 patients, 13 (81.2%) were females and the mean ± SD of age, quantity of proteinuria, and serum creatinine in all patients were 37 ± 11.6 years, 1844 ± 582 mg/d and 1.5±0.93 mg/dL, respectively. Eight patients had class IV, five had class III; two had class II, and one patient had class I LN. The activity percents in the classes III and IV were 19 ± 15% (median = 15, range = 40) and 57.5 ± 33 (median = 55, range = 80), respectively. The chronicity percent in classes III and IV were 17 ± 10% (median = 20, range = 30) and 23.25 ± 3010 (median = 5, range = 80), respectively.

We found that the association between gKi-67 and age, gender, level of proteinuria and serum creatinine level was not significant ($P > 0.05$). The association between gKi-67 and interstitial fibrosis (as a percent), the number of crescents, and the activity and chronicity percentages was also not significant ($P > 0.05$). Moreover, the relationship of gKi-67 with global versus segmental involvement of glomeruli was also not significant ($P > 0.05$). Furthermore, the correlation of gKi-67 with IgA, IgG, IgM, C3 and C1q deposits was not significant as well ($P > 0.05$). Analysis regarding the association of iKi-67 with, age, gender, level of proteinuria and serum creatinine level was not significant ($P > 0.05$). Similarly, the association between iKi-67 and interstitial fibrosis, the number of crescents and the activity and chronicity percentages was not significant ($P > 0.05$). Moreover, the relationship of iKi-67 with global versus segmental involvement of the glomeruli was not significant ($P > 0.05$). However, the correlation of iKi-67 with C1q deposits was inversely significant (r=-0.544, p=0.029) while the correlation of iKi-67 with IgA, IgG, IgM and C3 was not significant ($P > 0.05$). Studies regarding the association between tKi-67 and age, gender, level of proteinuria and serum creatinine level were not significant too ($P > 0.05$). The association between tKi-67 and interstitial fibrosis, the number of crescents, and the activity and chronicity percentages was not meaningful too ($P > 0.05$). Moreover, the relationship of tKi-67 with global versus segmental involvement of the glomeruli was not significant as well ($P > 0.05$). Furthermore, the correlation of tKi-67 with IgA, IgG, IgM, C3 and C1q deposits was not significant ($P > 0.05$).

Discussion

The proliferation of glomerular mesangial and other cells is a significant histologic finding in kidney disease. In SLE, kidney biopsy findings have an important role in the treatment selection and disease prognosis, since the histopathological changes can be seen even in patients without clinical evidence of LN. All the components of renal parenchyma including renal interstitium, tubules and glomeruli can be targeted in SLE. The proliferation of the mesangial cells is a common pathological feature of LN (1). Under pathological circumstances, inflammation or cell injury initiates the unusual proliferation of these cells resulting in the release/secretion of different inflammatory mediators that lead to glomerular hypercellularity, sclerosis, and fibrosis, ultimately, resulting in ESRD (2). The role of cell proliferation in glomerular hypercellularity is confirmed by an increased level of Ki-67 expression in the biopsy samples of the cases that have WHO class IV LN (3). The Ki-67, a proliferation marker, is expressed in all proliferating cells and is involved in the mitotic and interphase stages of the cells. Its cellular distribution dramatically alters during the cell cycle progression (4). For decades, Ki-67 has been used extensively in human tumor cells; however, there are limited data regarding its application in non-neoplastic pathologies. In the realm of kidney diseases, it is reported that Ki-67 can indicate cellular proliferation in biopsy samples. Antibodies against Ki-67 have been used to visualize the proliferation of cells in glomerulopathies and normal kidneys (5, 6). An increased expression of Ki-67 was observed in patients with membranoproliferative glomerulonephritis and IgA nephropathy (IgAN), as well as post-infectious endocapillary glomerulonephritis. The results indicated that constant injury and diffuse activation could even be seen in normal-appearing mesangial cells in IgAN (5). Normal and mature podocytes do not express the nuclear Ki-67 marker; however, increased expression of this marker is seen in hyperplastic podocytes (7).

Since an association between the proliferation and apoptotic indices was detected, it is suggested that apoptosis and proliferative activity have essential roles in the pathogenesis of LN (6, 8). The co-expression of P53 and Ki-67 can serve as a potential biomarker for the management and remission of childhood LN in the proliferative and active phases of LN before progressing to ESRD (6). Likewise, higher expression levels of Ki-67 and Bcl-2 were seen in biopsy samples of patients with LN (class IV) and IgAN (9). Ki-67 expression and activated complement C3 were also studied by Jeruc et al in LN (8). These results imply that Ki-67 can be used as a proliferation biomarker in LN.

In a study, high P53 and Ki-67 expressions were observed in all children with class IV LN and they had significant correlations with LN activity indices and its subclasses (II, III and IV), anticardiolipin antibodies, proteinuria and serum creatinine (6). The same results were found in adult patients with LN. The results of a study conducted on 29 adults demonstrated that the proliferation index was associated with disease activity in LN (classes III and IV). Moreover, the results found that Ki-67 staining was high in some patients with class II LN, indicating a subclinical injury (10). Ki-67 proliferation and indices of LN activity had a rising trend in patients with class III and IV LN (10).

Likewise, in our study, high numbers of Ki-67$^+$-stained cells were observed in the kidney tubules, glomeruli and interstitium of patients with LN classes III and IV. It has been reported that the Ki-67 proliferative index was associated with the laboratory features, disease activity, and class of LN in adult patients (3,10). However, in our study, we could not find any association between glomerular, interstitial and tubular expressions of Ki-67 and proteinuria, serum creatinine and demographic data. In the aforementioned studies, no associations were found between the Ki-67 proliferation index and the serum levels of C3 and C4 complements in adults (10). Similar to these results, we could not find statistically significant correlations between the glomerular, interstitial and tubular expressions of Ki-67 and IgA, IgG, IgM and C3 deposits, interstitial fibrosis, the number of crescents and the activity and chronicity percentages as well as the global versus segmental involvement of the glomeruli. However, in pediatric LN, significant correlations were found between co-expression of P53 and Ki-67 and also C3/C4 (6). In the present study, only iKi-67 was significantly associated with C1q deposits.

In addition to kidney cells, the expression of Ki-67 by natural killer (NK) cells is linked remarkably with severe clinical features and active nephritis in SLE. Proteomic results specify that the stimulation of Ki-67 in NK cells is promoted by interleukin-15, suggesting a role for these cells in modulating the immune-derived pathology of SLE (11).

Conclusion

Our results suggest that Ki-67 may be an early biomarker in LN and its diagnostic value requires further exploration in large-scale studies.

Limitations of the study

There are certain limitations in this study as well. It is a single center based, cross-sectional study with no follow-up data. The sample size and the number of cases in each class of LN are small. Nevertheless, it is a significant contribution to existing literature on this subject from Iran.

We conducted this study in the COVID-19 pandemic. We suggest additional studies on this feature of patients with lupus nephropathy.

Authors' contribution

HN and NH conducted the investigation. MBA conducted the statistical analysis. HN and EM prepared the primary draft. SZV prepared the discussion and edited the manuscript. SZV and HN prepared the final manuscript. MM critically reviewed and edited the final manuscript with intellectual input. All authors read and signed the final paper.

Conflicts of interest

The authors declare no conflict of interest.

Ethical issues

This investigation was in accordance with the Declaration of Helsinki. This study was conducted on paraffin embedded blocks of kidney biopsies to assess Ki-67 positivity in the interstitial, glomeruli and tubule areas. Written informed consents were obtained from the patients at the time of renal biopsy. The ethical board committee of National Institute for Medical Research Development (NIMAD) has approved this study (NIMAD; http://nimad.ac.ir, ethical code #IR.NIMAD.REC.1398.068). Besides ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/Support

This research project was conducted by a grant from NIMAD from Iran (Grant #982578).

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