DISCUSSION

The SLE is a multiorgan autoimmune disease with a broad range of circulating autoantibodies, which after forming immune complexes with antigens are deposited in different organs as kidneys, joints, and skin, and cause damage after activating the complement. This typically multiorgan SLE manifestation makes the unambiguous assessment of SLE activity difficult. [195] Numerous disease activity indices for SLE have been developed for better disease assessment, proper estimation of the cumulative organ damage and adequate determination of the management plan according to the degree of activity of the disease. [52] Despite all these efforts, lupus nephritis remains a major health problem that limits survival rate among all populations. [196]

The exact etiology of SLE and LN remains obscure although many factors are believed to be implicated. Several pathogenic mechanisms are postulated in LN that results in either renal regeneration or fibrosis. [197] Different drugs are being developed which target the various pathogenic abnormalities associated with this disease. [198]

The TLR are a group of innate pattern recognition receptors that recognize multiple classes of microbes through conserved molecular patterns. [199]

In recent years, it has become apparent that TLR-7 and TLR-9, which sense single-stranded RNA and unmethylated DNA, respectively, contribute to the development of autoimmune diseases such as rheumatoid arthritis, SLE, and psoriasis.[200, 201]

The TLR7 is one of the intracellularly expressed TLRs. They are mainly expressed in pDCs, macrophages and B lymphocytes and play crucial roles in activation of innate and adaptive immunity. The activation of TLR can lead to the production of many cytokines as IFN-α, and IL-12. The released IFNs produce powerful modulation of cell physiology including cell proliferation, survival, differentiation and protein translation. [157]

IFN-λ1 (IL-29) is one of Type III interferons (IFNs-III/ IFN lambdas) that represent a newly identified group of the class II cytokine family and are produced from a limited group of cells and signal through a different receptor IFN-λ receptor (IFNLR). Binding of TLRs to their respective ligands result in a downstream signalling cascade that leads to induction of IFN-λ genes. The released IFN-λ binds to IFNLR and activates various ISGs which encode synthesis of proteins that mediate antiviral, immunomodulatory and antitumor activities. Therefore, it may participate in the pathogenesis of systemic autoimmune diseases such as SLE and LN. [170]

So, the aim of the present work was to determine TLR7 in peripheral blood monocytes and serum IL-29 in patients with SLE, and to estimate their correlation to disease activity and lupus nephritis.

To achieve these goals, 15 patients with SLE without laboratory evidence of LN (Group I), 15 patients with LN (Group II), and 15 normal subjects of matched age and sex were included as controls (Group III). All patients and controls were sero-negative for hepatitis B and C virus, CMV and HIV. Patients with chronic liver disease, diabetes mellitus, systemic infection, malignancy, cardiac, respiratory or other autoimmune diseases were excluded. Also, patients who have received previous anti-viral drugs were excluded from the study.

Using flow cytometric analysis, the present study demonstrated that the number TLR7 in peripheral blood monocytes were significantly higher in both groups of patients than healthy subjects and especially in patients with LN. This means that there was upregulation of TLR7 in peripheral blood monocytes in all patients with SLE and higher in patients who developed LN. This may indicate an ongoing inflammation and stimulation by the disease process which is more evident in patients with end organ affection as patients with LN, which can lead to progressive kidney dysfunction and failure. This was evidenced by the presence of positive correlation between TLR7 and both the inflammatory and disease activity markers; CRP, ANA, anti-ds.DNA antibody and SLEDAI, with markers of renal function; S.Cr, urinary albumin/urinary creatinine ratio and was negatively correlated with C3 and C4, and with e-GFR in patients with LN. So, TLR7 has a potential influence on the disease and could play a role in the pathogenesis of LN and could be a marker for diagnosis and follow up of patients with SLE and LN. Also, it could provide a biochemical basis for the development of block of TLR and targeting therapy for treatment and even prevention of the diseases.

Komatsuda et al, [202] studied the expression of TLRs messenger RNAs in peripheral blood mononuclear cells (PBMCs) from patients with SLE. They found that the expression levels of TLR2, TLR7, TLR9, IFN-α and LY6E mRNAs (a type I IFN inducible gene) in SLE patients were significantly higher than those in healthy controls. Expression levels of TLR7 and TLR9 mRNAs correlated with that of IFN-α mRNA in SLE patients. They concluded that these results suggest that up-regulated expression of TLR7 and TLR9 mRNAs together with increased expression of IFN-α mRNA in PBMCs may also contribute to the pathogenesis of human lupus.

In animal models, Berland et al. [203] reported TLR-7- dependent loss of B cell tolerance in pathogenic autoantibody knock-out mice. Christensen et al. [204] demonstrated that TLR-7-deficient lupus-prone mice had ameliorated disease, decreased lymphocyte activation and decreased serum IgG. Furthermore, mice with the Y-linked autoimmune accelerating locus mutation, leading a twofold increase in the expression of several genes including TLR-7, develop a severe form of murine lupus. [205] These findings suggested strongly that TLR-7 had an important role in the pathogenesis of murine lupus models.

However, it remains to be determined whether the upregulation of TLR9 and TLR7 would lead to altered B cell responses to the cognate ligands. Zhu et al [206] characterized the responses of B cells from SLE patients to TLR7 and TLR9 stimulation and explored the potential role of single immunoglobulin interleukin-1 receptor related molecule (SIGIRR) in the regulation of TLR-mediated responses of SLE B cells. The SIGIRR is one of the negative regulators of TLR-IL-1 signaling that interferes with the downstream signaling pathway, or facilitates the degradation of TLRs. [207] They demonstrated that, despite the reported upregualtion of TLR7 and TLR9 in SLE, B cells from SLE patients mounted a largely normal, if not diminished, response to the TLR signal in terms of cell proliferation and antibody secretion. This contradiction may be partly explained by the elevated expression of the negative regulators SIGIRR expression. Intervention targeting the TLRs in SLE should consider the complicated network regulating the signaling pathway.

On the contrary, Wong et al, [208] determined the differential protein expressions of TLR-1–9 of monocytes and different lymphocyte subsets from patients with SLE and normal control subjects by flow cytometry in Chinese women. Their results showed that the expression of intracellular TLRs (TLR-3, -8, -9) and extracellular TLRs (TLR-1, -2,

-4, -5, -6) were elevated in monocytes, CD4+ T lymphocytes, CD8+ lymphocytes and B lymphocytes of SLE patients compared to control subjects. Although the TLR-7 expressions in different immune cell subsets were not significantly different between SLE patients and control subjects, however, after TLR7 stimulation resulted in aberrant activation of monocytes, T and B lymphocytes for the production of cytokines and chemokines. They concluded that these results suggested that the innate immune response for extracellular pathogens and self originated DNA plays immunopathological roles via TLR activation in SLE.

Another study by Klonowska-Szymczyk et al, [209] recently showed a significantly higher percentage of TLR3- and TLR9-positive cells among all PBMCs and their subpopulations (CD3+, CD4+, CD8+, and CD19+ lymphocytes) as well as TLR7 in CD19+ B-lymphocytes in SLE patients compared to the control group, however there was no significant difference as regard to TLR7 expression in both subpopulations of T lymphocytes in SLE patients than in healthy subjects. They found a correlation between TLR and some clinical (joint lesions) and some laboratory features, which suggest that TLR3, 7, and 9 may play a role in the pathogenesis of SLE and have an impact on organ involvement in SLE. The difference of these results from the results of the present study with significantly increased TLR7 expression in PBMCs in patients with lupus than in healthy control and even in patients with LN than those with SLE without nephropathy, this can be attributed to the heterogeneous nature of the study groups used by the two studies and their different genetic background or may be due to the previous different intake of immunosuppressive treatment used.

There is a long and growing list of genes associated with this disease ranging from genes involved with innate immunity and HLA genes to genes involved in apoptosis and intracellular signaling. [210] Earlier studies showed that disease was initiated by a translocation of several genes, including TLR7, from the X-to-Y chromosome. [205, 211] In addition, autoantibody production was decreased in mice deficient in TLR7 signaling. [212] Moreover, mice deficient in TLR7 experienced reduced autoantibody levels and ameliorated renal disease. [204] Further studies using TLR7- and TLR9-deficient recipient animals showed that TLR7, but not TLR9, aided in the development of anti-ds DNA and antihistone antibodies. Moreover, it was suggested that deposition of immune complexes in the renal glomeruli of these mice was mediated by TLR7. These studies suggest an important role for TLR7 in the development of autoreactive antibodies leading to multiple organ damage including LN. [213] Moreover, injection of syngeneic late apoptotic thymocytes into MyD88-/- mice had no effect, while their injection into wild type B6 mice led to anti-ds DNA and anti-histone antibody production, suggesting a role for TLRs in the development of anti-ds DNA antibodies as a result of impaired clearance of apoptotic bodies. [198]

SNPs in the TLR7 gene in humans that are associated with lupus recently had been discovered in Asian populations, especially males and predisposed patients to increased TLR7 transcript levels as well as an enhanced IFN signature. [214] In addition, Garcia-Ortiz et al [215] reported an association between increased TLR7 copy numbers and childhood onset SLE. Furthermore, another two SNPs in TLR7 gene were associated with SLE in Japanese women. [216] Visentini et al [217] had reported a patient with SLE who had long-lasting remission of her autoimmune disease after development of an antibody deficiency after development of common variable immunodeficiency (CVID)-like disease. This patient maintained antinuclear antibody positivity for >10 years however, B cells were unable to proliferate in response to TLR7- and TLR9-targeted stimulus. [198, 217]

However, Guiducci et al [43] showed that stimulation of TLRs inhibits the anti-inflammatory action of glucocorticoids in SLE. Deng et al, [218] provided evidence that microRNA acts as a negative regulator to control TLR7 expression, suggesting the possibility of miRNA-based therapies for amelioration of autoimmune diseases such as SLE.

ELISA assays were used to detect serum IL-29. On comparing the three groups, Serum IL-29 was significantly higher in both groups of patients especially patients with organ affection; LN than the control subjects. There was a positive correlation between IL-29 with TLR7, inflammatory and disease activity markers; ESR, CRP, ANA, anti-ds.DNA antibody and SLEDAI, with markers of renal function; S.Cr, urinary albumin/urinary creatinine ratio and was negatively correlated with C3, C4 and e-GFR. These implied that IL-29 could play a powerful effect on the inflammatory process and probably involved in the disease activity of SLE. Also, IL-29 could trigger TLRs expression in patients with SLE. So, this suggesting a role for IL-29 in the pathogenesis of SLE and may involve in development of renal involvement in SLE. So, IL-29 may provide a novel research target for the pathogenesis and therapy of SLE.

Although, the ROC curve analysis revealed that the sensitivity and specificity of the TLR7 in the discrimination of patients with LN from patients with SLE were found to be 100% and 93.33%, respectively (cut-off value = 28 cell/μl). The latter values were better than those of IL-29, which were 93% and 40%, respectively (cut-off value = 29.8pg/ml). These results revealed that measurement of TLR7 in PBMC is more reliable than serum IL-29 in discriminating the SLE patients with the presence of renal affection than patients without renal affection and for the follow up the progression of kidney disease and renal dysfunction in these patients.

In agreement with the findings of the present study Wu et al, [191] found that IFN-λ1 protein expression was significantly higher in patients with SLE with renal involvement and arthritis in comparison to patients without nephritis or arthritis as well as normal controls and associated with disease activity. In addition, they reported significant positive correlations between serum IFN-λ1 levels and SLEDAI, anti-ds. DNA antibody and CRP, and there was also a negative relationship between IFN-λ1 levels and C3. Also, they observed that there was a synergic effect of IFN-λ1 and LPS on the chemokines expression. Thus, they inferred that, except for the various effects of single LPS on the development of SLE and LN, together with the synergic effect of IFN-λ1 and LPS on the chemokines secretion, they could play a powerful effect on the inflammatory process of SLE, and promote the disease aggravation in patients with SLE, especially with LN. Thus, they supposed that not only did IFN-λ1 participate in the renal involvement but also it played the pathogenic role by combining with the effect of LPS. Therefore, for patients with SLE accompanying LN, they should avoid bacterial infections in order to prevent disease progression.

Another family member of IFN-λ, the IL-28 was studied by Lin et al, [219] in patients with SLE. Also, the IL-28 and IL-28RA transcript levels in PBMCs and peripheral blood T cells were determined. Their results showed that patients with SLE more frequently had detectable IL-28 protein in serum than normal individuals, and the IL-28 transcript expression in activated CD4+ T cells was dysregulated in patients with SLE. They concluded that IL-28 appears to be able to act as an autocrine in T cells, their expression is dysregulated and biological functions of IL-28 may be enhanced in patients with SLE, so, IL-28 may contribute to some of the SLE pathogenesis.

In addition to the role of IFN-λ in autoimmune diseases, IFN-λ was found to induce antiviral activity that had been demonstrated against many different viruses, including encephalomyocarditis virus and vesicular stomatitis virus in several different cell types. [174, 220, 221] In addition, IFN-λ has been shown to inhibit HBV replication in a differentiated murine hepatocyte cell line. [175] Furthermore, IFN-λ also inhibits replication of subgenomic and full-length HCV replicons in the human hepatoma cell line. [175, 176, 221] Moreover, MHC class I antigen expression is also up-regulated on the surface of cells following exposure to either type I or type III IFNs that may enhance the ability of the immune system to recognize and destroy virally infected cells. [170, 174]

Jordan et al, [222] had previously demonstrated the ability of IFN-λ1, that produced by virus infection, to elicit a panel of cytokines, IL-6, -8 and -10, from human immune cells, particularly monocytes through morphological and motility changes.

However pattern of gene expression induced by either type I IFN or type III IFN is very similar, the relative magnitude of gene expression induced by type I IFN is often greater than that induced by type III IFN in many cell types. This may reflect a difference in the relative strength of signaling through type I IFN receptors versus type III IFN receptors and therefore the relative levels of expression of these receptors on the cell membrane. The more limited tissue expression of IFN-λ receptors may exhibit less hematopoietic toxicity than IFN-α when administered clinically as a therapeutic antiviral agent. [170]

It had been demonstrated that IFN-λs had antiproliferative activity, in addition to the antiviral, using several target cell types, including intestinal epithelial cells and the human glioblastoma cell line. [173, 223] The ability of IFN-λs to induce antiproliferative activity in target cells may depend on the relative levels of IFN-λR1 expression as IFN-λs can effectively inhibit proliferation of cells engineered to express high levels of IFN-λR1. The results from a number of published studies using murine tumor models support the hypothesis that IFN-l may have potential as a novel antitumor agent for treatment of at least some types of cancer. [224, 225]

IL-29 had been implicated in the pathogenesis of different immune diseases. Wang et al, [226] showed that IL-29 may contribute to synovial inflammation during rheumatoid arthritis pathogenesis as they found serum levels of IL-29 were greatly elevated in patients with rheumatoid arthritis than healthy control subjects and mRNA levels of IL-29 as well as its specific receptor IL-28Ra in PBMC were significantly higher in patients with rheumatoid arthritis than healthy control subjects. Furthermore, Xu et al [227] investigated the underlying molecular mechanism by which IL-29 contributes to synovial inflammation in rheumatoid arthritis by demonstration of involvement of IL-29 in an augmented TLR2, TLR3 and TLR4-mediated inflammatory cytokine production in patients with rheumatoid arthritis.

In the present study there was an increase in the markers of disease activity; the ANA, anti-dsDNA, ESR and CRP in patients with SLE especially patients with LN than the normal subjects. Also, there was a positive correlation between them and the TLR7 and IL-29. There was an increase in the disease activity index (SLEDAI) in patients with organ affection (LN) than patients without nephritis. This activity index was found to have a positive correlation with the TLR7 and serum creatinine in both groups of patients and with IL-29 in patients with LN. Also, there was a significant decrease of C3 and C4 in both groups of patients than the controls. There was a negative correlation with the other disease activity markers, SLEDAI, TLR7, IL-29 and serum creatinine. This showed that TLRs may play a key role in autoantibody responses in SLE and

especially in patients with renal dysfunction and that the kidney affection augments TLR expression further. So, the use of combination of these markers probably provides the most useful clinical information on SLE disease activity, in particular patients with lupus nephritis.

The relationship between the antibodies against dsDNA and SLE disease activity remains controversial. The problem is that SLE patients can show persistently elevated anti-dsDNA antibody levels with no evidence of disease activity or persistent clinical activity with normal anti-dsDNA antibody levels. [228]

Nucleic acid (NA)–sensing TLRs (NA-TLRs) promote the induction of anti-nuclear Abs in SLE. Using Unc93b13d lupus-prone mice that lack NA-TLR signaling, Koh et al, [229] recently documented that NA-TLRs promote the induction of antinuclear Abs in SLE. Their data indicates that the presence of NA-TLRs in B cells is necessary to drive the initial autoimmune response and to promote the activation and escape of tolerance of self-reactive B cells. Also, they suggested that targeting of NA-TLR signaling in B cells alone would be sufficient to specifically block production of a broad diversity of autoantibodies. In addition, over-expression of TLR7 within the B cell compartment was found to enhance B cell TLR7 expression, permit the specific development of anti-RNA autoantibody production, and exacerbate SLE disease in an animal model. [230, 231]

Lau et al, [232] demonstrated that two-receptor paradigm, the B cell antigen receptor (BCR) and TLR7 (BCR/TLR7) cause activation of autoreactive B cells by RNA and RNA-associated autoantigens. They found that autoimmune-prone mice, lacking the TLR adaptor protein MyD88, had markedly reduced chromatin, Sm, and rheumatoid factor autoantibody titers.

This was recently clarified by Chauhan et al, [233] who reported differential and unique TLR expression patterns associated with different autoantibody profiles in SLE. The presence of anti-extractable nuclear antigens, RNA-associated antigens (anti-ENA) and anti-dsDNA autoantibodies in SLE patients was associated with elevated levels of TLR7 and TLR9 respectively and was suggestive of differential immuno-regulatory pathways operating in different subsets of SLE patients.

TLR-7 has a highly selective function in the initiation and propagation of anti-snRNPs antibody production. This was shown by Savarese et al [234] who demonstrated that TLR-7 is specifically required for the production of RNA-reactive autoantibodies and the development of glomerulonephritis in pristane-induced murine lupus, a model of environmentally triggered SLE in the absence of genetic susceptibility to autoimmunity. In addition, they noticed that low or absent anti-snRNP/ Sm antibody production in TLR-7–deficient mice was associated with lower immunoglobulin deposition and less severe glomerulonephritis in the pristane-induced model of lupus. Therefore, they concluded that specific interference with TLR-7 activation by endogenous TLR-7 ligands may be a promising novel strategy for the treatment of SLE and LN.

Another study by Pawar et al [235] Showed that the use of TLR-7– antagonistic oligodeoxynucleotides attenuated glomerulonephritis and lung injury after the onset of autoimmunity in experimental lupus and therefore administration of TLR-7 antagonists may potentially prolong the disease-free time interval in predisposed individuals.

To assess whether ESR levels correlate with the level of disease activity in patients with SLE, Stojan et al, [236] showed that categorical levels of ESR (normal,

mild, moderate, severe) were strongly associated with disease activity in SLE measured by the Safety of Estrogens in Lupus Erythematosus National Assessment trial (SELENA) modification of SLEDAI (SELENA-SLEDAI), physician global assessment (PGA), and with organ specific activity including, rash, renal, joint, hematology, neurological, pulmonary, and serositis visual analogue scale, as well as with hematuria and proteinuria even in patients with no anti-dsDNA antibody positivity or low complement levels.

Similar data were obtained in a cross-sectional study from Iran, in which Nasiri et al, [237] showed that the increase in overall scores on the BILAG index were associated with increasing ESRs, decreasing C3 levels, decreasing C4 levels, elevated anti-dsDNA levels, and increasing SLEDAI-2K scores. They concluded that these findings showed that the ESR, C3, C4, and anti-DNA should be used in the evaluation and management of patients with SLE. Also the results show that the BILAG index has construct validity. A combination of anti-dsDNA, serum complements, C3 and C4, ESR, and CRP are most commonly used and probably provide the most useful clinical information on SLE disease activity, in particular patients with lupus nephritis. [238. 239] However, some SLE patients in clinical remission have persistently abnormal serological findings. Careful monitoring of specific organ functions, such as renal function, with the help of relevant tissue histology, remains an important part in the assessment of disease activity and response to treatment.

CRP is an acute-phase protein known as a biomarker for inflammation, and has been traditionally used to detect and predict the outcome of infections, inflammatory, necrotic processes as well as monitor clinical disease activity together with efficacy of treatment. [240, 241] Reports have displayed that CRP levels above 60 mg/l in febrile SLE patients without serositis almost always indicate infection; whereas in SLE alone, CRP levels are only moderately raised even in patients with very active disease. [242] In the present study, the highest CRP value was 45.9 mg/l, and most CRP values were less than 20 mg/l; therefore, in this study, CRP was a biomarker not indicating infection but monitoring disease activity.

Rezaieyazdi et al, [243] found that serum level of high sensitive CRP (hs-CRP) was significantly higher in SLE regardless the disease activity, laboratory markers and type of organ involvement. In another study, in which the CRP level was not as high as expected, Tan et al, [244] explained the low CRP level despite high disease activity in SLE as these patients had circulating autoantibodies against monomeric CRP (mCRP) which could be a possible explanation. The difference in these results was explained by the different origin of patients and the less presence of serositis in their patients which is the most common cause of increase of CRP. One must keep in mind suggestive evidence that CRP is associated with factors such as age, smoking, coronary artery disease, and increased cholesterol and glucose levels. In addition, the assay used for measuring CRP may not be as sensitive or specific compared to hs-CRP assays. [245]

Also, Firooz et al, [246] revealed that hs-CRP was significantly lower in SLE patients with active disease than in those with either documented or suspected infections and proposed that elevated hs-CRP levels could be used as a predictor of active infection with a specificity of 80% at levels greater than 5 mg/dl and 84% at levels greater than 6 mg/dl and assumed that serum hs-CRP level greater than 6 mg/dl in a patient with SLE could be a strong predictor of active infection.

Activation of complement cascade by autoantibodies or preformed immune complexes generates proteolytic fragments that are capable of attracting inflammatory

cells, inducing production and release of inflammatory mediators with recruitment of phagocytic neutrophils and monocytes leading to immune mediated tissue damage. [247] However, measuring serum complements level were used by many investigators as an indicator for assessing disease activity in SLE and was included as a separate parameter in all disease activity indices, some studies have found that C4 and C3 levels remain normal during SLE flares. [248, 249] This was explained by the measurement of the native form of complement proteins that can be affected by the increased synthesis during disease activity in order to balance the increased consumption. Moreover, a low baseline C4 level may reflect an underlying C4 deficiency that can be present even in normal healthy subjects rather than being consumed. [247]

The relationship of the clinical activity index and serum titers of anti-dsDNA antibodies, C3 and C4, in an 8-year prospective study conducted in patients with SLE was studied by Villegas-Zambrano et al. [250] Although they found a significant correlation between them but it was at a low level, and different levels of the serological tests was correlated with the clinical activity index. They concluded that the absence of abnormal biomarkers does not equate with clinical stability, but their presence may be predictive of disease exacerbation. There is no single serologic test that reliably measures disease activity in SLE. Anti-dsDNA antibodies and complement profiles have been classically considered to be useful in assessing disease activity in SLE patients. The highest association between anti-dsDNA antibody levels and activity has been demonstrated in the setting of hypocomplementemia. [250, 251]

In accordance with the results of the present study Andrejevic et al, [251] have recently assessed serum levels of anti-dsDNA antibodies using different assay methods, and calculate the clinical activity index SLEDAI-2K. They found that the high-avidity anti-dsDNA antibodies were found to be in the closest association to SLEDAI-2K and was significantly correlated with both C3 and C4 concentrations and anti-nucleosome antibodies. Patients with positive antibodies for C1q, nucleosomes and dsDNA had a higher risk of renal involvement and patients with low levels of C3 component were found to have higher risk of having cytopenia, mucocutaneous lesions and SLE-related central nervous system features. The SLEDAI-2K score was strongly negatively associated with concentration of both C3 and C4. So, they concluded that the presence of high-avidity anti-dsDNA antibodies represented a risk for renal, joint, and most importantly for serosal involvement and that the high-avidity anti-dsDNA antibodies was the test of good clinical utility for the assessment of global SLE activity.

Also, a positive correlation was found between anti-dsDNA antibodies and anti-nucleosome antibodies with the disease activity index (SLEDAI) and inversely correlated with C3 but not C4 in the study of Saisoong et al, [252] but they found that the prevalence of antinucleosome antibodies were higher than anti-ds DNA antibodies. So, they concluded that antinucleosome antibodies could be one of the earliest and most sensitive markers in diagnosis of SLE, particularly in anti-dsDNA antibodies-negative patients. Similar results were found by different studies. [253-256]

Dieker et al, [257] suggested that the presence of anti-nucleosome antibodies could serve as a better marker for SLE than anti-dsDNA antibodies. Also, the study of O'Flynn et al, [258] demonstrated a direct binding of both nucleosomes and C1q to glomerular endothelial cells in vitro. The subsequent binding of autoantibodies against nucleosomes in patients with systemic lupus erythematosus is potentially pathogenic and autoantibodies against C1q seem to have an additional effect.

Renal disease develops in more than half of SLE patients, and represents the first clinical manifestation of SLE in 15%–20%. [259, 260] Routine performance of a renal biopsy has been advocated by some nephrologists in SLE patients with any signs of kidney disease. [261, 262] However, the role of the renal biopsy in prediction of outcome and prognosis has been a matter of controversy.

Renal biopsy was done for 14 patients with LN and was classified according to ISN/RPS classification, consensus 2003. The activity index was positively correlated with serum creatinine, urinary albumin/urinary creatinine ratio, CRP, ANA, TLR7 and IL-29 and negatively correlated with C3 and C4, the chronicity index was only negatively correlated with IL-29. This indicates that the increase of auto-antibodies with the secondary decrease of complement were associated with the increase of activity index of the renal tissues with increase in protein excretion and renal dysfunction. Also, this study suggested an important role for TLR7 and IL-29 in the development of autoreactive antibodies and promotion of early events leading to renal pathogenesis with LN. As IFNλ has been shown to decrease the production of Th2-type cytokines (IL-4, IL-5, IL-13, IL-14, IL-15), potentially favoring a Th1 immune pathway, [178, 179] so**,** IL29 can be implicated as an antifibrotic substance but enhance the activity reaction in patients with LN.

Pawar et al, [235] showed that inhibition of TLR7 in experimental mice improved the activity index and chronicity index for LN. This was associated with significant reduction of renal glomerular and interstitial macrophage infiltrates and the number of interstitial T cells.

Several studies showed that early clinical and histologic diagnosis of LN was pivotal in order to minimize the risk of progression to ESRD. [76, 77] In this setting, a renal biopsy was generally indicated in any case with acute increase in serum creatinine, proteinuria >500 mg/24 h or urine protein/creatinine ratio >0.5 g protein/g creatinine, hematuria in presence of any level of proteinuria, and active sediment/cellular casts. [262]

However, histologic parameters of renal disease and the activity index, but not the chronicity index, on repeat biopsy after treatment reliably predicted renal outcome by logistic regression in a recent study. [263]

Podocytes injury with different degrees of proteinuria was found in patients with LN. The renal injury could be the result of immune complex deposition, with complement activation, along the GBM, or subendothelial immune deposits, endothelial cell swelling, podocyte damage, or scarring and remodeling of the GBM. Loss of podocyte regeneration is the predominant cause of chronic dysfunction, persistent proteinuria, progressive interstitial fibrosis and progression to chronic kidney disease.[264]

Wang et al, [265] recently showed that podocyte damage was common in LN and was correlated with the degree of proteinuria. Podocyte damage varied significantly with different types of LN, and the patients with combined LN presented with the most severe podocyte lesions.

The long-term course of patients with LN was assessed by Contreras et al. [266] Patients reaching the composite outcome of doubling serum creatinine, ESRD, or death had predominantly proliferative LN with higher activity, and higher chronicity index scores. In addition, patients reaching the composite outcome had higher baseline mean arterial pressure and serum creatinine or proteinuria, but lower baseline hematocrit and

C3 compared with controls. Taken together, these data support that histologic lesions, particularly proliferative and/or chronic, predict poor prognosis.

Tan et al, [244] found that positive correlations between levels of mCRP autoantibodies and semiquantitative scores of renal histologic features were first observed in lupus nephritis with interstitial inflammation, tubular atrophy, interstitial fibrosis, and chronicity index score. They concluded that autoantibodies against mCRP are prevalent in patients with lupus nephritis and are associated with disease activity and renal tubulointerstitial lesions.

Giannico and Fogo, [75] reported that the presence of active lesions (i.e., endocapillary proliferation, necrosis, and cellular crescents) versus chronic lesions (i.e., sclerosis, fibrous crescents, and interstitial fibrosis), in addition to class of LN, influence the response to therapy in patients with LN.

Different studies showed that the presence of autoantibodies; with high-avidity anti-dsDNA antibodies, anti-nucleosome and anti-C1q antibodies with decrease in complement were important for renal involvement. [250, 251, 267, 268] Also, Manson et al, [256] reported that levels of anti-nucleosome and high-avidity anti-dsDNA antibodies were positively correlated with each other and over follow-up period associated positively with renal disease activity in patients with lupus nephritis.

From these different studies, it can not be suggested that any biological or immunological measurement is able to replace renal biopsy and that the renal biopsy represents the gold standard in management of LN. The role of the renal biopsy in diagnosis, assessment of disease activity, response to treatment, management, and follow-up of LN is critical. Routine performance of a renal biopsy has been advocated by some nephrologists in SLE. [75]

When comparing the three groups as regard hematological data, both groups of patients showed a significant decrease in hemoglobin concentration, total white blood count and monocyte count than the control group with insignificant difference between both groups of patients except for hemoglobin that showed a significant decrease in patients with LN than Patients with SLE. Platelets count showed a significant decrease in patients with LN than both patients with SLE and controls. So, patients with SLE especially patients with LN had more severe anemia, leucopenia, monocytopenia and thrombocytopenia.

Anemia is a common clinical finding in SLE patients. Multiple factors are implicated such as iron deficiency anemia (IDA), drug-induced myelotoxicity, anemia of chronic disease (ACD) and autoimmune hemolytic anemia which accounts for only 5-10% of patients with SLE. Inadequate erythropoetin (EPO) response which was documented in 42.4% of SLE patients with ACD can affect erythroid precursor cell proliferation, differentiation and maturation, as well as reduction of haemoglobin synthesis. [269] Decrease in EPO production, resistance to EPO action, and the presence of anti-EPO receptor antibodies were suggested to the presence of anemia and deceased response to EPO. [269-271] The presence of hemolytic anemia and even HUS were reported in patients with SLE. [83, 272]

It was found that the use of IFNα or IFNλ could be associated with different degrees of anemia in the treatment of patients with HCV infection, but IFNλ showed less degree of anemia and thrombocytopenia than IFNα. [273]

The thrombocytopenia in patients with SLE and LN in this study was negatively correlated with SLEDAI and with TLR7, ANA, anti-dsDNA and CRP in patients with

SLE. It was negatively correlated with IL-29 in patients with LN. These results suggest the role TLR7 and IL-29 and in the pathogenesis of thrombocytopenia in patients with SLE and LN respectively and it could be a marker of disease activity in these patients.

It was found that although thrombocytopenia is also common in patients with active SLE, they rarely die of bleeding complications. It can be attributed to either peripheral autoimmune platelet destruction which accounts for 20-40% of cases or aggressive immunosuppressive treatment. [274] Zhao et al, [274] showed that SLE patients with thrombocytopenia are more predisposed to a higher disease activity, more possibility of end organ damage, especially lupus nephritis even without skin rash, and a higher mortality than those SLE patients without thrombocytopenia.

Monocyte subsets have been examined in SLE, but the findings vary among several studies. Cairns et al, [275] reported that the percentages of CD14+CD16- monocytes in SLE versus healthy subjects were not significantly different. In addition, while Sumegi et al [276] reported a non significant expansion of CD14+CD16- monocytes, the number of these monocytes decreased after steroid treatment in a dose-related manner. Furthermore, Li et al [277, 278] demonstrated that although the absolute total monocyte count is decreased in SLE patients, there is no relative difference in proportion of the different monocyte subsets between the groups and neither the percentage nor the absolute number of monocytes in the different subsets correlates with disease activity or manifestations. This reduction in total monocytes count in SLE can be attributed to either a disease activity related defect in the monocyte development, immunomodulatory effect by glucocorticoids [276] or infiltration of these cells to sites of inflammation, such as the kidneys as evidenced by the increased expression of CX3CL1 and accumulation of CD16+monocytes in glomeruli of active lupus nephritis, which had been correlated with impaired renal function and the presence of anti-ds.DNA autoantibodies. [279]

SUMMARY

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease of a multifactorial etiology characterized mainly by deregulation of the immune system. It affects mainly females in the childbearing age and involves almost all organs in the body in varying degrees of activity leading to multi-organ damage.

Lupus nephritis (LN) is the main cause of morbidity and mortality in patients with SLE worldwide. Variable clinical presentations have been reported; asymptomatic and accidentally discovered during the routine work up, puffiness and oedema, and urinary changes. Heterogeneity of the pathogenic mechanisms of LN results in heterogeneity of the pathological features of the disease. The main pathologic feature of injury in LN is immune complex (IC) deposition with complement activation in the different tissues of kidneys. These deposited ICs activate local and systemic inflammatory cells through binding of antinuclear antibodies to their specific TLRs with release of inflammatory cytokines leading to podocyte injury and proteinuria resulting in further tissue damage.

Toll-like receptors (TLRs) are evolutionarily conserved transmembane proteins that are expressed by many cell types and play crucial roles in activation of innate and adaptive immunity. TLR7 is expressed intracellularly in plasmacytoid DCs (pDCs), macrophages and B lymphocytes and can bind to single-stranded RNA (ssRNA) containing viruses or self ssRNA. The activation of TLR can lead to the production of many cytokines as IFN-α, and IL-12. The released IFNs produce powerful modulation of cell physiology including cell proliferation, survival, differentiation and protein translation.

IFN-λ1 (IL-29) is one of Type III interferons (IFNs-III/ IFN lambdas) that represent a newly identified group of the class II cytokine family and are produced from a limited group of cells and signal through a different receptor IFN-λ receptor (IFNLR). Binding of TLRs to their respective ligands result in a downstream signalling cascade that leads to induction of IFN-λ genes. The released IFN-λ binds to IFNLR and activates various IFN-stimulated genes (ISG) which encode synthesis of proteins that mediate antiviral, immunomodulatory and antitumor activities.

So, the aim of the present work was to determine TLR7 in peripheral blood monocytes and serum IL-29 in patients with SLE, and their correlation to disease activity and LN.

This study was conducted on 45 subjects; they were classified into three groups:

Group I: Fifteen patients with SLE without laboratory evidence of lupus nephritis.

Group II: Fifteen patients with SLE with laboratory evidence of lupus nephritis.

Group III: Fifteen normal subjects of matched age and sex as controls.

All patients and controls were subjected to the following:

I- Thorough history taking

II- Clinical examination: with assessment of Systemic Lupus Erythematosus Disease Activity Index in SLE patients (SLEDAI) using SLEDAI 2000 (SLEDAI-2K).

III- Laboratory Investigations:

1. Complete blood picture.
2. Renal function tests; Blood urea, serum creatinine (S.Cr) and estimated glomerular filtration rate (e-GFR) using the Modification of Diet in Renal Disease (MDRD).
3. Complete urine analysis and estimation of urinary albumin/urinary creatinine ratio.
4. Estimation of erythrocyte sedimentation rate (ESR) 1st hour.
5. Determination of C-reactive protein (CRP).
6. Estimation of serum complement 3 and 4 (C_3 and C_4 respectively).
7. Antinuclear antibody titer (ANA) and anti-double stranded DNA antibodies (anti-ds.DNA).
8. Measurement of Serum Levels of Interleukin-29 by using Enzyme Linked Immunosorbent Assay Kit (ELISA)
9. Enumeration of toll like receptor 7 in peripheral blood monocytes by color flow cytometric assay

IV- Renal biopsy: was done for patients with LN who have no contraindications.

Statistical analysis of data obtained from the present study showed the following results:

- In patients with group I, one patient presented by fever (6.67%), 13 with photosensitivity (86%), 5 with skin lesions (33.33%); five patients with malar rash, 12 with hair fall (80%), 2 with alopecia areata (13.33%); 15 with anorexia (100%), 9 patients alleged weight loss (60%), one with deep cervical lymphadenopathy (6.67%). None of the patients had serositis.
- In patients with group II, 6 patient presented by fever (40%), 14 with photosensitivity (93%), 5 with skin lesions (33.33%); five patients had malar rash, and only one of them also had discoid lupus, 12 with hair fall (80%), 6 with alopecia areata (40%), 14 with anorexia (93%), 13 weight loss (86%), 2 with serositis (13.33%); one patient suffered pericardial effusion, pleural effusion and ascites the other one had aseptic cardiac tamponade. None of the patients had lymphadenopathy (0%).
- Hemoglobin concentration was statistically significant lower in both group I and group II than group III. It was statistically significant lower in group II than group I.
- Total white blood cell count and monocytes count were statistically significant lower in both group I and group II than group III, however there was no statistically significant difference between group I and group II.
- Platelets count was statistically significant lower in group II than both group I and group III, with statistically insignificant difference between group I and group III.
- There was a statistically significant increase in blood urea, serum creatinine and urinary albumin/urinary creatinine ratio and a significant decrease in e-GFR in group II than both group I and group III, with statistically insignificant difference between group I and group III.

- There was a statistically significant increase in ESR, CRP in both groups of patients than the controls with statistically insignificant difference between group I and group II.
- The immunological parameters; C3 and C4 were statistically significant lower, while the ANA titer and anti-dsDNA were statistically significant higher in both group I and group II than group III, with statistically insignificant difference between group I and group II.
- The clinical activity index in patients with SLE (SLEDAI) was statistically significantly higher in group II than group I.
- The number of TLR7 in peripheral blood monocytes and serum IL-29 were statistically significant higher in both groups of patients and were higher in patients with LN than patients with SLE without nephritis. By plotting a ROC curve, the sensitivity and specificity of TLR7 and IL29 in discriminating patients with LN from patients with SLE were found in TLR7 to be 100% and 93.33% respectively, at a cut-off value of 28 cell/μl. The area under curve = 0.996. Also, it was found in IL29 to be 93% and 40% respectively, at a cut-off value of 29.8 pg/ml. The area under curve = 0.687.
- According to ISN/RPS classification, consensus 2003 renal biopsy of 14 patients with LN showed that 4 were class II LN; one of each was (IIC), (IIA/C), (IIA) and (II) LN, 3 were class III LN; one of each was class (III A), (III A/C) and one (III) LN, 7 were class IV LN; one of them class (IV A), 8 were class V LN and 8 had combined classes; one class III + IV (A), one class III (A/C) + V and six class IV + V (A) LN. The activity index was in one of each biopsy with 0/24, 4/24 and 7/24, in two biopsies of each with 1/24, 2/24, 6/24, 8/24, and in three with 5/24. The chronicity index was 0/12 and, 1/12 in five biopsies each, in three biopsies 2/12 and one 4/12. The walls of blood vessels; 7 biopsies were within normal range of thickness, 3 were mildly thickened and 4 were thickened and hyalinized.
- The number of TLR7 in peripheral blood monocytes was positively correlated with IL-29, CRP, ANA, anti-ds.DNA antibody and SLEDAI and was negatively correlated with C3 and C4 in patients with SLE with and without nephropathy. It was positively correlated with S.Cr and urinary albumin/urinary creatinine ratio in patients with LN.
- The IL-29 was positively correlated with S.Cr, urinary albumin/urinary creatinine ratio, ESR, CRP, ANA antibody and anti-ds.DNA antibody SLEDAI, and was negatively correlated with e-GFR, C3 and C4 in patients with SLE with and without nephropathy.
- The SLEDAI was positively correlated with S.Cr, ESR, CRP, ANA antibody and anti-ds.DNA antibody and was negatively correlated with platelets count, e-GFR, C3 and C4 in patients with SLE with and without nephropathy. It was positively correlated with urinary albumin/urinary creatinine ratio in patients with LN.
- The CRP was positively correlated with S.Cr, ESR, ANA antibody and anti-ds.DNA antibody and was negatively correlated with C3 and C4 in patients with SLE with and without nephropathy. It was negatively correlated with platelets count, e-GFR in patients with SLE without nephropathy.
- The serum C3 and C4 were positively correlated with each other and with e-GFR and was negatively correlated with S.Cr, urinary albumin/urinary creatinine ratio,

ANA antibody and anti-ds.DNA antibody in patients with SLE with and without nephropathy

- The ANA antibody and anti-ds.DNA antibody were positively correlated with each other and with S.Cr, ESR in patients with SLE with and without nephropathy and was negatively correlated with platelets count in patients with SLE without nephropathy.
- Serum creatinine was positively correlated with urinary albumin/urinary creatinine ratio, and was negatively correlated with e-GFR in patients with SLE with and without nephropathy and it was negatively correlated with platelets count in patients with SLE without nephropathy.
- The activity index of renal biopsy in patients with LN was positively correlated with the number of TLR7 in peripheral blood monocytes, IL29, S.Cr, urinary albumin/urinary creatinine ratio, CRP, ANA, and was negatively correlated with C3 and C4. The chronicity index of renal biopsy was negatively correlated with IL-29.

CONCLUSION

Based on the results of the present study, it could be concluded that:

- The number TLR7 in PBMCs and serum IL-29 were significantly high in patients with SLE especially in patients with LN. So, there was an up-regulation of TLR7 that increases with renal dysfunction and LN. TLR7 has a potential influence on the disease and could play a role in the pathogenesis of LN and could be a marker for diagnosis and follow up of patients with SLE and LN.

- Serum IL-29 was high in patients with SLE, especially in patients with organ affection and LN. This indicating an enhanced stimulation and activity of IFN-λ1that increases with deterioration of renal function and LN and that IL-29 may also have a role in the pathogenesis of SLE.

- The stimulation of both the TLR7 and IL-29 could be good laboratory markers in assessment of disease activity, the inflammatory process and the renal involvement in SLE as they were positively correlated with the inflammatory, disease activity markers and with renal function and negatively correlated with C3 and C4.

- IL-29 could trigger TLRs expression and TLRs stimulation leads to induction of IFN-λ in patients with SLE as there was a positive correlation between IL-29 and TLR7.

- Both measurement of serum IL-29 and enumeration of PBMS that express TLR7 could be good prognostic markers during the follow up of patients with SLE and for early detection of lupus nephritis as they were positively correlated with renal function. However enumeration of PBMS that express TLR7 is more reliable and accurate than serum IL-29 in discriminating patients with LN from patients without renal affection, early prediction of LN and for the follow up the progression of kidney disease, as the sensitivity and specificity were higher for TLR 7 than IL-29. (100% and 93.33%), (93% and 40%) respectively.

- The disease activity index (SLEDAI) is a reliable index for assessment of disease activity and follow up of patients, as it was correlated with the other markers of disease activity, TLR7 and IL-29. Also, the use of combination of activity markers may provide the most useful clinical information on SLE disease activity, in particular patients with lupus nephritis.

- The measurement of IL-29 and TLR7 could be an indirect assessment of activity index in renal tissues as they were positively correlated with the activity index of renal biopsy. Also, the increase in auto-antibodies with the secondary decrease of complement were associated with the increase of activity index of the renal tissues with increase in protein excretion and renal dysfunction, as there was there was a positive correlation with activity and inflammatory markers associated with hypo-complementeamia and a positive correlation with renal function and the degree of protein excretion. IL-29 can be implicated as anti-fibrotic substance as it has a negative correlation with the chronicity index but enhances the activity reaction in patients with LN.

- Patients with SLE especially patients with LN had more severe anaemia, leucopenia, monocytopenia and thrombocytopenia.
- Thrombocytopenia was negatively correlated with disease activity, inflammatory markers, TLR7 and IL-29. These suggested the role TLR7 and IL-29 in the pathogenesis of thrombocytopenia and that thrombocytopenia could be a marker of disease activity.