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Role of IL-6 and IL-10 in IgA Nephropathy and Lupus Nephritis

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Abstract:

Background: IgA nephropathy (Berger's disease or mesangial IgA deposition) is characterized by deposition of the IgA auto-antibody in the glomerulus causes a variety of lesions and inflammation in renal tissue. The clinical course of IgA nephropathy is usually mild, although in 40% of the cases it may lead to end-stage renal disease and it is considered is the most common type of primary GN in the world.One of the mostfrequent organ manifestations of SLE is LN, The disease most affected patients are women of childbearing age, and it is characterized by overproduction of antibodies to self-antigens, which are mostly extracted from cell components including the nucleus, cell membranes, cytoplasm, and ribosomes.which in many cases leads to end-stage renal disease.

Objective:biopsy is the best way to diagnose patients with IgA Nephropathy and Lupus Nephritis But it is considered an invasive method for diagnosis, therefore present study suggests to evaluate the significant importance of IL-6 and IL-10 as biomarkers for diagnosis of IgA Nephropathy and Lupus Nephritis

Materials and Methods :A case control study based on three groups, first group consist from 30 patients previously diagnosed as IgA Nephropathy(IgAN) which include (19 male and 11 female), also Second group was 30 patients who have Lupus Nephritis(LN) which include (10 male and 20 female)who were observation in Al Hussein Teaching Hospital in Holy karbala province, the period from December2019 toDecember 2020 under the supervision of nephrology specialists**.** Third group was include 30 healthy volunteers (non IgA Nephropathy and non Lupus Nephritis). The blood sample were collected by venipuncture from three groups were drawing approximately five millimeter of venous blood by disposable syringe under aseptic technique ,Each blood sample of these groups were collected in plane tube then serum was

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separated by centrifugation 13000 for 5 minute,IL-6 and IL-10 ELISA assays were performed using serum collected in Eppendrof tubes and stored at -20°C.

Result : The demographic characteristics, age and gender, of patients with IgA nephropathy, lupus nephritis and control subjects. The group of IgA nephropathy included significantly more males than control subjects; whereas, the group of lupus nephritis included more females than control group (p = 0.008). There was also highly significant difference in mean age among patients and control groups ($p < 0.001$), in such a way that highest age was observed in lupus nephritis group followed by IgA nephropathy and lastly by control group, 46.03 ±12.43 years versus 39.17 ±10.40 years versus 25.87 ±6.67 years, respectively.

There was highly significant difference in serum IL-6 level among study groups ($p< 0.001$); the level being highest in lupus nephritis and IgA nephropathy groups and then followed by control group.There was highly significant difference in serum IL -10 level among study groups ($p< 0.001$); the level being highest in lupus nephritis and IgA nephropathy groups and then followed by control group.Regarding IgA nephropathy ,the results showed that the cutoff values for IL-6 and IL-10 were >8.62 and >136.04, respectively; the most accurate one was IL-6 followed by IL-10 and accuracy levels of 96.2 and 95.1, respectively.while Regarding lupus nephritis ,the results also showed that the cutoff values for IL-6 and IL-10 were >7.99 and >136.04, respectively; the most accurate one was IL-6 followed by IL-10 and accuracy levels of 99.2 and 94.8, respectively.

Conclusion: Present study concluded that, IL-6 consider a good biomarker with high sensitivity and specificity for IgA nephropathy and Lupus nephritis, while IL-10 consider an inflammatory biomarker for nephritis in general. More studies are needed to solidify the result of present study.

Introduction

IgA nephropathy (Berger's disease or mesangial IgA deposition) is characterized by deposition of the IgA auto-antibody in the glomerulus causes a variety of lesions and inflammation in renal tissue(1). The clinical course of IgA nephropathy is usually mild, although in 40% of the cases it may lead to end-stage renal disease and it is considered is the most common type of primary GN in the world(2)

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One of the mostfrequent organ manifestations of SLE is LN, The disease most affected patients are women of childbearing age, and it is characterized by overproduction of antibodies to self-antigens, which are mostly extracted from cell components including the nucleus, cell membranes, cytoplasm, and ribosomes.which in many cases leads to end-stage renal disease(3).It has been hypothesized that Multiple factors are associated with the development of glomerulonephritis, including: genetic, racial, hormonal, and environmental factors(4).

IL-6 is a pleiotropic cytokine that influences metabolism, hematopoiesis, and organ growth in addition to the immune and inflammatory responses. IL-6 can induce distinct or even conflicting physio-pathological processes at the same time, which is possibly differentiated by signaling cascades known as classic and trans-signaling (5).IL-6 dysregulation has been linked to a variety of inflammatory diseases, metabolic disorders, cancers, and autoimmune diseases, including Glomerulonephritis, IgAN, and LN (6). Mesangial cells, endothelial cells, podocytes, and tubular epithelial cells all secrete IL-6.The roles of IL-6 development and progression several renal diseases, such as IgA nephropathy, lupus nephritis, diabetic nephropathy, acute kidney injury, and chronic kidney disease(7).

IL-10 is primarily expressed in endothelial and mesangial cells, where it acts as an autocrine cell growth factor. IL-10 promotes mesangial cell proliferation by increasing the synthesis of cytokines, chemokines, and growth factors.

This causes structural intraglomerular and tubulointerstitial changes, such as glomerular basement membrane thickening, cell hypertrophy, mesangial matrix aggregation, glomerulosclerosis developmentand overt proteinuria(8). One of the supportive findings that enforces the inflammatory role in LN are the evidences which have shown that anti-lupus nephritis drugs can modulate components of the inflammatory-related pathways such as decreasing in serum levels of pro-inflammatory cytokines and raising the anti-inflamatory cytokines **,**In recent years, there has been search for blood-based biomarkers for LN as a valid alternative(9)

Material and methods

Patients and Sample Collection

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A case control study based on three groups, first group consist from 30 patients previously diagnosed as IgA Nephropathy(IgAN) which include (19 male and 11 female), also Second group was 30 patients who have Lupus Nephritis(LN) which include (10 male and 20 female)who were observation in Al Hussein Teaching Hospital in Holy karbala province, the period from December2019 toDecember 2020 under the supervision of nephrology specialists**.**

Third group was include 30 healthy volunteers (non IgA Nephropathy and non Lupus Nephritis). The blood sample were collected by venipuncture from three groups were drawing approximately five millimeter of venous blood by disposable syringe under aseptic technique ,Each blood sample of these groups were collected in plane tube then serum was separated by centrifugation 13000 for 5 minute,IL-6 and IL-10 ELISA assays were performed using serum collected in Eppendrof tubes and stored at -20°C.

Serum IL-6 and IL-10 levels were value in IgA Nephropathy , Lupus Nephritis and healthy controls using Enzyme linked immuno-sorbent assay (ELISA) according to the manufacturer's instructions (Elab science -UK).Such kit of ELISA utilizes principle of Sandwich ELISA. In this kit, provided microtiter was precoated by specific antibody to IL-6 and IL-10 of human. Standards or samples to microtiter wells of ELISA were added as well as combined along antibody specific. then, Human IL-6 and IL-10 as biotinylated detection specific antibody as well as conjugate of Avidin –Horseradish peroxidase (HRP) were added to every well respectively . Wells containing biotinylated detection antibody, Avidin-HRP conjugated, and Human IL-6 and IL-10 aappear blue color. Reaction of enzyme-substrate is terminated by adding stop solution and color changes to yellow. OD is spectrophotometrically measured at 450nm ± 2nm wavelength. Value of OD is proportional to Human IL-6 and IL-10 concentration,we can measure Human IL-6 and IL-10 sample concentration via comparing standard curve to sample OD.

RESULT:

Distribution of patients and control subjects according to age and gender

The demographic characteristics, age and gender, of patients with IgA nephropathy, lupus nephritis and control subjects are shown in table 1. The group of IgA nephropathy included significantly more males than control subjects; whereas, the group of lupus nephritis included

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more females than control group (p = 0.008). There was also highly significant difference in mean age among patients and control groups (p < 0.001), in such a way that highest age was observed in lupus nephritis group followed by IgA nephropathy and lastly by control group, 46.03 ±12.43 years versus 39.17 ±10.40 years versus 25.87 ±6.67 years, respectively.

Table 1: Distribution of patients and control subjects according to age and gender

Characteristic	**Control** ***n*** **= 30**	**IgA nephropathy** ***n*** **= 30**	**Lupus nephritis** ***n*** **= 30**	***p***
Gender				
Male, *n* (%)	15 (50.0 %) B	20 (66.7 %) A	8 (26.7 %) C	0.008 C HS
Female, *n* (%)	15 (50.0 %) B	10 (33.3 %) C	22 (73.3 %) A	
Age (years)				
Mean ±SD	25.87±6.67 C	39.17±10.40 B	46.03±12.43 A	< 0.001 O HS
Range	16 -40	18 -64	24 -73	

n: number of cases; **SD**: standard deviation; **C**: Chi-square test; **O**: one way **ANOVA**; Capital letters (A, B and C) were used to indicate the level of significance following 2-groups Chi-square of post hoc **LSD** test so that A indicate the highest value followed by B and then C and that different letters indicate significant difference at $p \leq 0.05$

Comparison of interleukin (IL-6 and IL-10) levelsamong patients and control groups

Comparison of serum IL-6 is shown in table 2 and figure 1. There was highly significant difference in serum IL level among study groups (p< 0.001); the level being highest in lupus nephritis and IgA nephropathy groups and then followed by control group.

Comparison of serum IL-10 is shown in table 2 and figure 2. There was highly significant difference in serum IL level among study groups (p< 0.001); the level being highest in lupus nephritis and IgA nephropathy groups and then followed by control group.

Table2: Comparison of serum interleukin levels (IL-6 and IL-10) among patients and control groups

Characteristic	**Control** ***n*** **= 30**	**IgA nephropathy** ***n*** **= 30**	**Lupus nephritis** ***n*** **= 30**	***p***

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IL-6				
Median (IQR)	6.36 (2.30) B	20.48 (18.49) A	20.61 (10.63) A	<0.001 K HS
Range	3.36 -12.97 C	6.35 -36.86 B	8.40 -29.91 A	
IL-10				
Median (IQR)	60.83 (42.54) B	202.97 (153.52) A	211.56 (136.28) A	<0.001 K HS
Range	41.96 -136.04	57.20 -395.68	53.23 -395.86	

n: number of cases; **IQR**: Inter-quartile range; **K**: Kruskal Wallis test; Capital letters (A, B and C) were used to indicate the level of significance following **Mann Whitney U** test so that A indicate the highest value followed by B and then C and that different letters indicate significant difference at $p \leq 0.05$; whereas, same letters indicate no significant difference at $p > 0.05$; **HS**: highly significant at $p < 0.001$

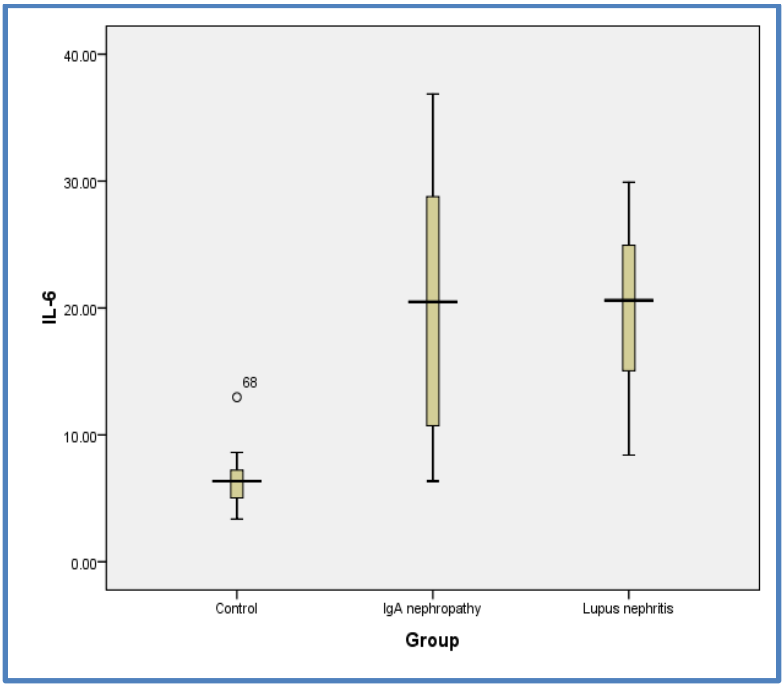

Figure 1: Box plot showingcomparison of IL-6 serum level in patients and control subjects

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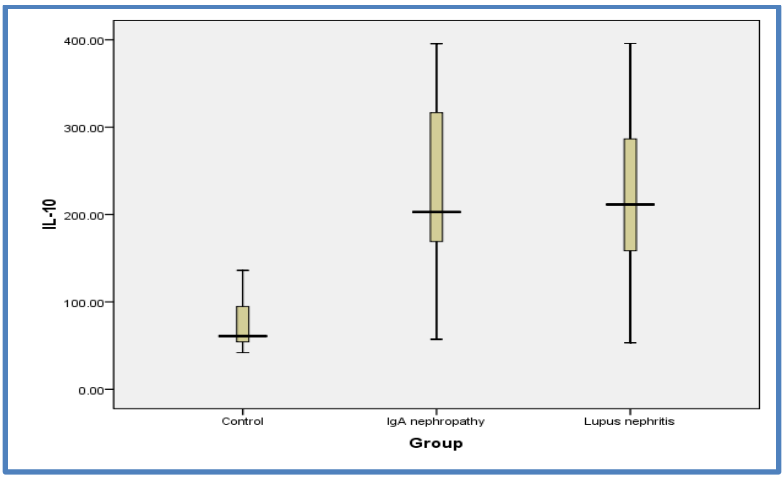

Figure 2: Box plot showingcomparison of IL-10 serum level in patients and control subjects

The diagnostic role serum IL levels in IgA nephropathy and lupus nephritis

In order to figure out the diagnostic role of serum IL levels in IgA nephropathy and lupus nephritis receiver operator characteristic curve (ROC) analysis was carried out and the results are shown in tables 3 through 4 .

Regarding IgA nephropathy ,the results showed that the cutoff values for IL-6 and IL-10 were >8.62 and >136.04, respectively; the most accurate one was IL-6 followed by IL-10 and accuracy levels of 96.2 and 95.1, respectively, table 3 and figures 3 and 4.

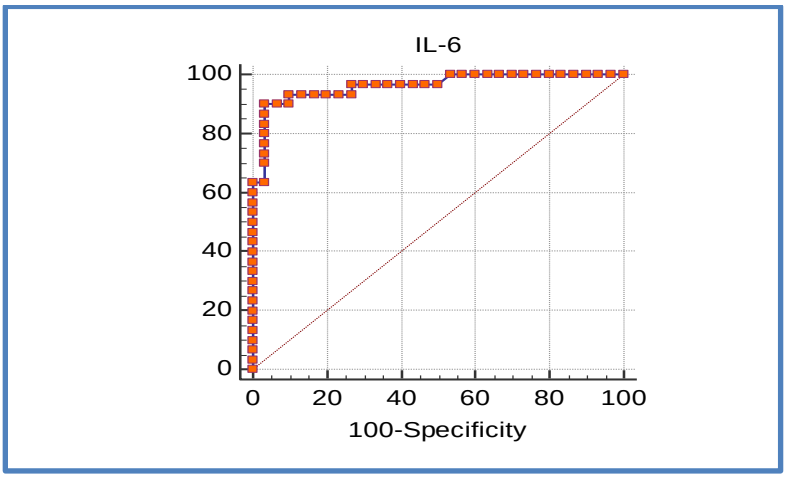

Figure 3: Receiver operator characteristic (ROC) curve analysis to find the cutoff value of serum IL-6 that predict a diagnosis of IgAnephropathy with best accuracy

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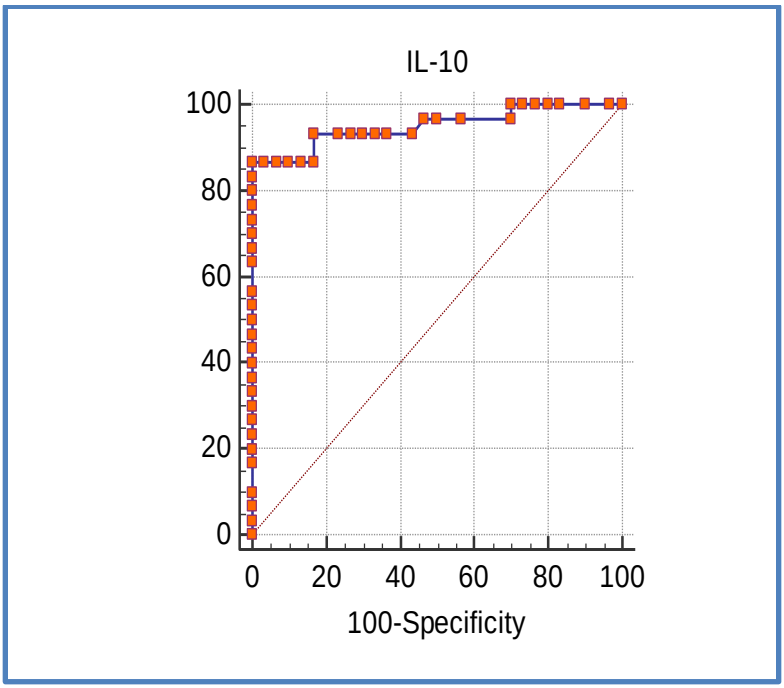

Figure 4: Receiver operator characteristic (ROC) curve analysis to find the cutoff value of serum IL-10 that predict a diagnosis of IgAnephropathy with best accuracy

Table 3: The characteristics of ROC curve when IgA nephropathy group where compared to control group

Characteristic	**IL-6**	**IL-10**
Cutoff	>8.62	>136.04
AUC	0.962	0.951
95 % CI	0.877 to 0.994	0.862 to 0.990
Accuracy %	96.2	95.1
p-value	< 0.001	< 0.001
Sensitivity %	90.0	86.67
Specificity %	96.67	100.0

AUC: area under curve; **CI**: confidence interval; **HS**: highly significant

Regarding lupus nephritis ,the results also showed that the cutoff values for IL-6 and IL-10 were >7.99 and >136.04, respectively; the most accurate one was IL-6 followed by IL-10 and accuracy levels of 99.2 and 94.8, respectively, table 4,figures 4 and 5.

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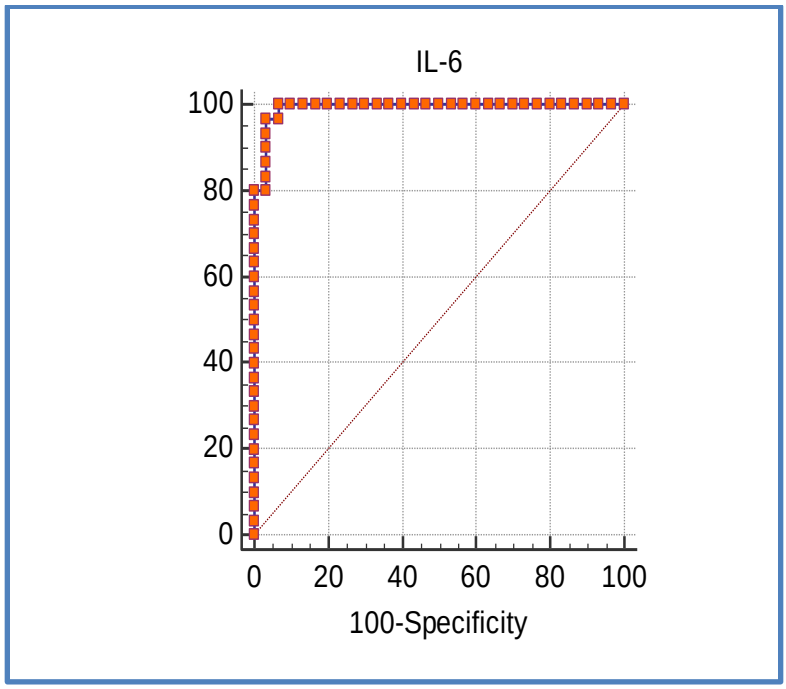

Figure 5: Receiver operator characteristic (ROC) curve analysis to find the cutoff value of serum IL-6 that predict a diagnosis of lupusnephritis with best accuracy

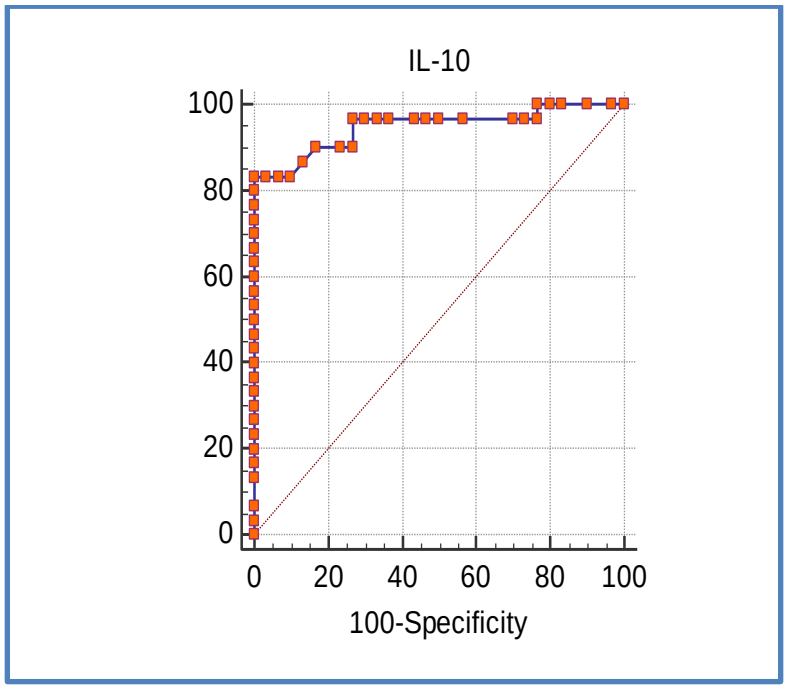

Figure 6: Receiver operator characteristic (ROC) curve analysis to find the cutoff value of serum IL-10 that predict a diagnosis of lupusnephritis with best accuracy

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Table 4: The characteristics of ROC curve when lupus nephritis group where compared to control group

Characteristic	IL-6	IL-10
Cutoff	>7.99	>136.04
AUC	0.992	0.948
95 % CI	0.926 to 1.000	0.858 to 0.989
Accuracy %	99.2	94.8
p-value	<0.001	<0.001
Sensitivity %	100.0	83.33
Specificity %	93.3	100.0

AUC: area under curve; **CI**: confidence interval; **HS**: highly significant

DISCUSSION

This result came in agreement with most studies in the world showed wide range of 84%female: 16%male in LN where39 %female: 61%male in IgA nephropathy , suggesting that hormones may play a significant role in determining susceptibility to GN(10).

The age group (15-45) years whether females or males was more affected statistically than other age group(11). This result were in agreement with studies in Southern Asia And other studies in Eastern Asia shows that, SLE disease can affect all ages but most commonly the age group (15-45) years (12).

Many studies have shown that IgA nephropathy is accompanied by activation of the immune/inflammatory system.Numerous studies have discovered that people with IgA nephropathy have higher levels of inflammatory cytokines in their blood (13). These studies reveal two critical themes. First, inflammatory defects are present in prednisolone-treated subjects as compared to controls, prednisolone therapy, a humanized anti-human IL-6 receptor antibody, IL-6 now becomes a realistic new option for lupus nephritis treatment. Selective blockade of IL-6 transsignaling may be another therapeutic choice. (14). Second, certain inflammatory molecules' concentrations can differ depending on a patient's clinical status: Interleukin IL-6 is one of the state-related markers.During an exacerbation of symptoms, people

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with IgA nephropathy have higher concentrations of these cytokines than controls, yet there is no difference during times of clinical stability (15).

IL-10 upregulation has been linked to the pathophysiology of several kidney diseases, including mesangioproliferative glomerulonephritis, IgA nephropathy, and the acute phase of microscopic polyangiitis, all of which are associated with mesangial cell proliferation (16).The human IL-10 gene (5.1 kb pairs) is found on chromosome 1 and consists of five exons. Several single nucleotide polymorphisms (SNPs) in the IL-10 promoter region affect IL-10 expression and function (17).

Elevated serum IL-10 levels were found to be higher in the LN group than in controls, but not in the SLE group, according to Lit et al (18). Anti-IL-10 therapy of mice with systemic lupus erythematosus (SLE) or mice injected with human SLE patients' peripheral blood mononuclear cells prevents the onset of autoimmune manifestations.Immune complex accumulation in the glomeruli is reduced, and glomerular hypercellularity and mesangial expansion are prevented, as well as proteinuria is reduced (19).

Moreover, a study done by (Ishibashi et al. 2018) they found that according to ROC analysis IL-6 has high sensitivity, specifity and accuracy in IgAN patient indicated that Anti-IL-6 antibodies are part of a new generation of IL-6 inhibitorsIt is also used as a prognostic marker to predict the efficacy of ocilizumab (Actemra), which has already been approved for use in patients with autoimmune disorders including rheumatoid arthritis in over 100 countries, including the European Union, the United States, Brazil, and India. And in Japan.Also anthor study mentioned that Receiver operating characteristic curve analysis revealed that IL- 6producing CD4+CD161+Tcell are apotential biomarker of IgAN disease Activity with good sensitivity and specifity(20).

The present study come in agreement with previous study done by (Silosi, Boldeanu et al. 2016) they were demonstrated that IL-10 serum level in IgAN patients when compared with those in control have high sensitivity for IL-10and were 100% and specifity for these marker were100% and 86% respectively might be involved in pathogenesis of IgAN.In the normal adult kidney, mesangial cells are the major local source of IL-10, and they are also the main regulators of kidney function (21).

Recent work suggests numbers of proinflammatory cytokine was estimated that there are more than 300 IL-6 in LN. Differences in IL-6 levels between GN kidney diseases may be

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diluted within the systemic circulation. Clinically, the most interesting comparisons are between GN cases either on active lesion or remission and the controls. Subtypes of each condition should ideally be possible to differentiate using a biomarker. Here present study identified a set of IL-6 and IL-10 which were differentially expressed in lupus nephritis cases compared to controls. ROC analysis supported good diagnostic accuracy for IL6(22).

In a study done by(Cui et al. 2011) they found that according to ROC analysis IL-10 has high sensitivity, specifity and accuracy in LN patient indicated that IL-10considered as effective a prognositic marker in LN patient associated with SLE(23).Also anthor study mentioned that Receiver operating characteristic curve analysis revealed that IL-10R1 expression and signaling were found to be down-regulated in CD4+ cells from patients with lupus nephritis (LN)and apotential biomarker of LN disease Activity with good sensitivity and specifity(24).

Conclusion:

Present study concluded that, IL-6 consider a good biomarker with high sensitivity and specificity for IgA nephropathy and Lupus nephritis, while IL-10 consider an inflammatory biomarker for nephritis in general. More studies are needed to solidify the result of present study.

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