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A Study Of Association Between Neutrophil – Lymphocyte Ratio And Proteinuria Among Chronic Kidney Disease Patients

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Abstract

Background: Chronic kidney disease is a chronic inflammatory disorder, which usually associated with changes in the renal tissue architecture and further causes glomerular dysfunctions in the nephron. So this glomerular dysfunction leads to the abnormal excretion of protein and albumin in the urine. Since neutrophils and lymphocytes are usually elevated in inflammatory conditions, neutrophil-lymphocyte ratio is considered as a biomarker of inflammation. The main objective of this study is to reveal the association between neutrophil-lymphocyte ratio and proteinuria among chronic kidney disease patients.

Materials and Methods: This was a case-control study, which involves 100 cases and 100 controls. Neutrophil-lymphocyte ratio was calculated from complete blood count report. Estimation of proteinuria was done in the spot urine sample. The statistical analysis was done using the SPSS software version 24.

Results: In this study, there were significant increase (p value < 0.0001) in neutrophil-lymphocyte ratio, spot urine protein excretion and spot urine protein-creatinine ratio among chronic kidney disease patients when compared to the control group. Chronic kidney disease patients with hemodialysis were associated with very high neutrophil-lymphocyte ratio values, which indicates that these patients were in severe inflammatory stress.

Conclusions: Neutrophil-lymphocyte ratio plays a greater role in revealing the underlying degree of inflammation and severity of proteinuria among chronic kidney disease.

Keywords: Chronic kidney disease, neutrophil-lymphocyte ratio, proteinuria, inflammatory markers

Introduction

Chronic Kidney Disease (CKD) patients is defined based on the two clinical criteria, first criteria includes glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2 for ≥ 3 months with or without evidence of kidney damage and the second criteria includes the evidence of kidney damage with or without decreased GFR for ≥ 3 months [1]. Global prevalence of CKD is about 11 – 13 % and worldwide incidence is 10%. Prevalence of CKD in India is about 17.2%. The stage wise prevalence of CKD includes, 1 – 7 % patients with stage – 1, 4.3 % with stage 2 & 3 and 0.8% with stage 4 & 5 respectively [2,3].

Nephron is the basic structural unit of the kidney. And the glomerulus part of the nephron has a vital role in filtrating all the solutes and proteins from the human body. Any changes in the normal physiological structure of glomerulus, causes urinary excretion of protein in larger amount [4].

CKD causes some changes and alterations in the architechture of the renal tissues, which causes

glomerular damage and further leads to the decreased GFR and increased protein and albumin excretion in the urine [5].

Normal urinary protein excretion is < 150 mg/day and albumin excretion is < 30 mg/day respectively. Most of the protein that are normally excreted in the urine includes, Tamn-Horsfall glycoprotein, which is otherwise known as uromodulin. This uromodulin is secreted mainly by the thick part of ascending loop of Henle. And this uromodulin serves as a protective factor for urinary tract infection (UTI) and renal stones.

Normal spot urinary protein is about < 15 mg/dl and creatinine excretion is about 20 – 300 mg/dl respectively. And normal spot urinary protein-creatinine ratio is < 0.2 and CKD patients usually presents with the features of high urinary protein excretion and low urinary creatinine excretion with very high urine protein-creatinine ratio [6,7].

Total leucocyte count (TLC) is considered as an ideal marker for inflammation and infection. TLC includes neutrophils, lymphocytes, monocytes, basophils and eosinophils. Among these parameters, neutrophils and lymphocytes plays a vital role in the process of inflammation. And hence, neutrophil and lymphocyte ratio (NLR) can be used as a marker for subclinical inflammation. NLR is calculated by dividing the number of neutrophils by the number of lymphocytes. These number of neutrophils and lymphocytes are usually taken from the complete blood count (CBC) report [8,9].

And moreover, CKD is an inflammatory state with elevated levels of pro-inflammatory cytokines and acute phase proteins. So the high NLR value is usually associated with high degree inflammation in the CKD patients. This type of chronic inflammation causes progressive damage of glomerulus and causes proteinuria.

So the purpose of the study was to reveal the association between NLR and proteinuria among CKD patients in Tiruvannamalai.

Methods

This was a case-control study done at Arunai Medical College and Hospital, Tiruvannamalai from September 2021 to March 2022, after obtaining permission from the Institutional Ethics Committee (Ethical Code: 07/2021). This study includes 100 CKD patients and 100 controls.

CKD patients were selected from Nephrology department and controls were selected from General Medicine out-patient department (OPD).

Among the 100 CKD patients, 50 patients were undergoing hemodialysis and 50 CKD patients were without hemodialysis.

Inclusion criteria for cases:

1. CKD patients with age group 45 – 70 years were included in this study.
2. CKD patients with serum urea value > 40mg/dl
3. CKD patients with serum creatinine > 1.2mg/dl
4. CKD patients with spot urine protein excretion > 20mg/dl
5. CKD patients with spot urine creatinine excretion < 20mg/dl
6. CKD patients with spot urine protein-creatinine ratio > 0.2
7. CKD patients with neutrophil-lymphocyte ratio < 6
8. CKD patients with GFR < 60ml/min/1.73m^2

Where GFR was calculated using the modification of diet in rural disease formula (MDRD) [10].

9. CKD patients undergoing hemodialysis were also included in this study.
10. CKD study participants were staged based on the KDIGO (Kidney Disease Improving Global Outcomes) CKD staging by using the GFR [11].

Inclusion criteria for controls:

1. Study participants with age group 45 – 70 years.
2. Subjects with normal serum urea, creatinine , GFR, spot urine protein, spot urine creatinine and spot urine protein-creatinine ratio were included in this study.

Exclusion criteria:

1. Patients with acute renal failure.

Volume 5, Issue 3; May-June 2022; Page No 602-608

2. Patients with renal cell carcinoma.

Sample collection: After getting proper informed consent, blood samples and random or spot urinary samples were collected from all the study participants.

Biochemical investigations

Biochemical analysis: Estimation of serum creatinine and urea for all the study participants were done by EM 200 auto-analyzer.

Serum creatinine estimation was done by Jaffe's method (Erba Kit) and blood urea estimation was done by Urease-Glutamate Dehydrogenase method (Erba Kit) respectively.

Hematological investigations

2ml of Ethylene Diamine Tetra Acetic acid (EDTA) sample was collected from all the study participants. And total leucocyte count was done by Erba 5-part haematological H560 auto-analyzer. NLR can be calculated by dividing the number of neutrophils by the number of lymphocytes and this calculation was done with the help of complete blood count (CBC) report.

Urine analysis

Estimation of urinary excretion of protein, creatinine and protein-creatinine ratio were done in the spot or random urine sample.

Spot urinary protein estimation was done by pyrogallol method (Erba Kit) and urinary spot creatinine estimation was done by Jaffe's method (Erba Kit). Spot urine protein-creatinine ratio was done by dividing the urine protein value by the urine creatinine value respectively.

Statistical analysis:

Statistical analysis was done using SPSS software version 24.0. Comparison of the study variables among CKD patients and controls were expressed as mean ± SD and were done by student t test. The association between NLR and end stage renal disease (ESRD) among CKD study participants was calculated using Odds ratio (OR) and 95 % confidence intervals (CI). p values < 0.05 were considered statistically significant.

Results

Demographic characteristics of the study participants

Table 1 shows the comparison of demographic characteristics of study variables among CKD patients and controls. Among 100 CKD patients, 50 patients were undergoing hemodialysis. There were significant increase (p value < 0.0001) in serum creatinine, urea, NLR, spot urine protein excretion and protein-creatinine ratio in CKD patients when compared to the control group. And further there were significant decrease (p value < 0.0001) in GFR and spot urine creatinine excretion among CKD patients than compared to the control group.

Distribution of neutrophil-lymphocyte ratio

Table 2 shows the distribution of NLR among the CKD patients. Out of the 50 CKD patients with hemodialysis, 3 patients were having NLR values between 6 – 8 (mild inflammatory stress), 11 patients were having NLR values between 9 – 18 (moderate inflammatory stress) and 36 patients were having NLR values of > 18 (severe inflammatory stress).

Out of the 50 CKD patients without hemodialysis, 38 patients having mild inflammatory stress, 9 patients were having moderate inflammatory stress and 3 patients were having severe inflammatory stress.

Thus majority of the CKD patients with hemodialysis were with NLR values of more than 18, which indicates that these patients were in severe inflammatory stress. And further majority of CKD patients without hemodialysis were with NLR values between 6 – 9, which indicates that these patients were in less inflammatory stress.

Association of NLR and ESRD

Table 3 shows the association of NLR and ESRD among CKD study participants. CKD patients with GFR < 15ml/min/1.73m^2 & undergoing hemodialysis were considered as ESRD. In this study, the odds of developing ESRD in CKD patients were 36 times higher (OR = 36 ; 95% CI = 11.34 to 114.25, p = < 0.0001) in patients with NLR value > 18 (severe inflammatory stress).

Volume 5, Issue 3; May-June 2022; Page No 602-608

Discussion

The primary objective of this study was to find the association between NLR and proteinuria among CKD patients in Puducherry. In this study we demonstrated that the CKD patients on hemodialysis showed high NLR values and massive proteinuria when compared to the CKD patients without hemodialysis.

Although 24 hours urine sample is gold standard method for measuring urine protein and creatinine excretion [12,13], for this study we found difficulty in accurate collection of 24 hours urine sample from patients and controls. So we focused on the concept of spot urine sample collection from CKD patients and controls. In current scenario, many studies prefer taking spot urinary samples for the quantitative as well as qualitative analysis of urinary protein excretion [14].

A study conducted in Turkey, found a positive correlation between NLR and proteinuria in CKD patients. And further two more Turkish studies found that NLR was one of the important marker in predicting proteinuria in CKD patients and NLR values were significantly increased and were also correlated with proteinuria in CKD patients [15-17].

The result of a Chinese cohort study suggest us that NLR was associated with the risk of ESRD and stage 4 CKD in Chinese population [18].

Another Chinese study found that the NLR were very cheap and reliable biomarker among CKD patients. And they further demonstrated that high NLR values were associated with poor survival rates in patients with hemodialysis. And further another Chinese study concluded that NLR was considered as a non-expensive inflammatory marker in hemodialysis patients [19].

A study conducted by the University of South China reported that NLR was positively correlated in ESRD patients [20].

An Indian study demonstrated that NLR was a simple and reliable indicator of worsening renal function in CKD patients [21].

In our study, a statistically significant relationship was observed between NLR and degree of proteinuria among CKD patients in Tiruvannalai.

Limitations of this study are the limited sample size, the study excluded the CKD patients on peritoneal dialysis and there is no long-term follow up of CKD study participants.

Conclusions

Our study revealed that NLR was significantly higher in CKD patients and it was also associated with proteinuria among CKD patients. NLR is considered as the most reliable and cost-effective biomarker of inflammation and it is also further added to the list of potential prognostic markers for physicians in determining the CKD risk profile. And NLR further highlights the concepts of anti-inflammatory therapy and interventions in the CKD patient treatment protocol.

This study needs to be extended in a larger sample size and in a different populations to establish the association between NLR & proteinuria more accurately among CKD patients.

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Volume 5, Issue 3; May-June 2022; Page No 602-608

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Tables

Volume 5, Issue 3; May-June 2022; Page No 602-608

Table 1 : Comparison of demographic characteristics of the study variables among CKD patients and controls

Sl.No	Study Variables	CKD patients (n = 100)	Controls (n = 100)	p value
1.	Age (years)	57.66 ± 6.57	57.57 ± 7.51	0.928
2.	Gender (%) Male Female	 53 (53%) 47 (47%)	 51 (51%) 49 (49%)	
3.	Blood urea (mg/dl)	94.82 ± 15.11	25.12 ± 10.08	< 0.0001*
4.	Serum creatinine (mg/dl)	5.93 ± 1.26	0.79 ± 0.21	< 0.0001*
5.	GFR (ml/min/1.73m^2)	35.88 ± 15.24	110.49 ± 10.01	< 0.0001*
6.	NLR	13.24 ± 6.89	2.12 ± 1.80	< 0.0001*
7.	Spot urine protein (mg/dl)	1208.12 ± 600.29	10.99 ± 2.01	< 0.0001*
8.	Spot urine creatinine (mg/dl)	12.17 ± 6.89	98.89 ± 56.12	< 0.0001*
9.	Spot urine protein-creatinine ratio	2.89 ± 0.99	0.12 ± 0.01	< 0.0001*

Data are represented in mean ± SD. n: Number of subjects, % : Percentage,

CKD: Chronic Kidney Disease, GFR: Glomerular Filtration Rate, NLR: Neutrophil-Lymphocyte Ratio.

Statistical Analysis done by Student t test, * considered as significant

Table 2 : Distribution of NLR among the CKD patients

Sl.No	NLR	CKD patients with Hemodialysis (n = 50)	CKD patients without Hemodialysis (n = 50)

Volume 5, Issue 3; May-June 2022; Page No 602-608

1.	**6 – 8** Mild inflammatory stress	3	38
2.	**9 – 18** Moderate inflammatory stress	11	9
3.	**>18** Severe inflammatory stress	36	3

CKD: Chronic Kidney Disease, NLR: Neutrophil-Lymphocyte Ratio, n: Number of subjects

Table 3: Association of NLR and ESRD among CKD study participants

Sl. No	**NLR**	**CKD patients with Hemodialysis** **(n = 50)**	**CKD patients without Hemodialysis** **(n = 50)**	**OR** **(95% CI)**	**p value**
1.	> 18 Severe stress	40	5	36 (11.34 - 114.25)	< 0.0001*
2.	6 – 18 Mild to moderate stress	10	45		

NLR: Neutrophil-Lymphocyte Ratio, CKD: Chronic Kidney Disease, OR: Odds ratio,
CI: Confidence interval, n: Number of patients
Statistical Analysis done by Odds Ratio, *Considered as significant

Volume 5, Issue 3; May-June 2022; Page No 602-608