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RESEARCH ARTICLE

STUDY OF NEUTROPHIL LYMPHOCYTE RATIO AS INDICATOR OF NEPHROPATHY IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: Diabetic nephropathy is the leading cause of chronic kidney disease (CKD). However, the degree of albuminuria is not necessarily linked to disease progression in patients with DN. Several studies that have explored the relationship between systemic inflammation and vascular disease. Neutrophil-Lymphocyte ratio (NLR) may be considered as a marker of chronic inflammation. **Methods:** This was a cross sectional study which was conducted in Sardar patel medical college and associated group of hospital, Bikaner from November 2016 to October 2018 over 303 type 2 diabetes mellitus patient who fulfill inclusion and exclusion criteria. NLR was calculated by analyzing differential leukocyte count in complete blood picture. Albuminuria was detected by CHEMSTRIX-AG TEST STRIP by dipstick method. **Results:** Out of 303 patient 168 cases had their urine albumin positive while 135 cases had urine albumin Nil. when both group compare for complete blood count parameter haemoglobin had a non significant ($p>0.05$) relation with urine albumin while TLC, Neutrophil, ANC, Lymhocyte, ALC and NLR had a highly significant ($p<0.001$). In present study, mean NLR in normoalbuminuria was 1.72±0.49 and for microalbuminuria it was 2.43±0.57 ($p<0.001$). **Conclusion:** Microalbuminuria was found to be one of the earliest marker for DN. However, recent studies have shown that albuminuria is a less precise predictor of overt nephropathy risk than originally thought. . The results of our study have shown that there was a significant relation between NLR and DN. NLR is a simple, inexpensive test. Therefore, NLR may be considered as a predictor and a prognostic risk marker of DN.

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INTRODUCTION

Diabetes Mellitus has evolved into a global epidemic and India has the second largest population with diabetes. Diabetic nephropathy is the leading cause of chronic kidney disease (CKD) and account for 30-40 % of all end satge renal disease. However, the degree of albuminuria is not necessarily linked to disease progression in patients with DN associated with either type 1 or type 2 diabetes mellitus (T2DM) (Parving, 1983). Due to this, there is a need of early predictors of DN by which we can predict the disease and can halt the progression of the disease. Several studies that have explored the relationship between systemic inflammation and vascular disease indicated that chronic inflammation promotes the development and acceleration of micro and macro angiopathic complications in patients with diabetes (Rudiger *et al*., 2006; Choudhary, 2008). Systemic inflammation can be measured by using a variety of biochemical and haematological markers.

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Although novel disease specific biomarkers have been identified, most of which are time consuming and expensive. Neutrophil-lymphocyte ratio (NLR) may be considered a novel marker of chronic inflammation. It represents a combination of two markers; neutrophils, which represent the active nonspecific mediator initiating the first line of defense and lymphocytes, representing the regulatory or protective component of inflammation (Matthews, 1985). What might make NLR superior to other leukocyte parameters (e.g. neutrophil, lymphocyte and total leucocyte count) is its stability with less influence by physiological, pathological and physical factors (Gibson *et al*., 2007). Therefore, NLR has recently been defined as an essential and potential inflammatory marker in cardiac and non-cardiac disorders[6]. NLR that can be easily calculated from a simple peripheral blood count, is simpler and cheaper than measuring other inflammatory cytokines, such as IL-6, IL-1β and TNF-α (Ramachandran *et al*., 2012). Therefore, the current study was conducted to investigate the neutrophil lymphocyte ratio as a measure of systemic inflammation and its relationship, with microvascular complications of diabetes mellitus.

MATERIALS AND METHODS

This was a cross sectional study which was conducted in Sardar patel medical college and associated group of hospital, Bikaner from November 2016 to October 2018 over 303 type 2 diabetes mellitus patient who fulfill inclusion and exclusion criteria.Inclusion criteria was all cases of Type 2 diabetes mellitus patients attending medical outdoor, Pana devi binani government Geriatric research center and hospital,diabetic care and research centre and those admitted in medicine wards. Exclusion criteria were patients with type 1 DM , patients with infections, AIDS; patients with systemic disorder such as CVD, chronic kidney disease (CKD), chronic liver disease, blood disorders, autoimmune disorders, malignancy, poisoning; patients on anti-inflammatory drugs, systemic or topical steroids, any immunosuppressive therapy, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, alcohol; patients with uncontrolled blood pressure (BP); patients having diseases affecting urinary protein excretion as nephritic syndrome, urolithiasis, acute kidney injury, and UTI. All the subjects included in the study were interviewed regarding age, gender, residence, duration of diabetes, history of smoking, history of hypertension, or any other chronic disease, history of presenting illness, using a predesigned proforma. Medication history regarding use of anti-diabetes, antihypertensive were recorded through questionnaires and pill bottle review.

For each patient the following data were collected: Biochemical parameters (complete blood count including haemoglobin, total and differential leucocyte count, total platelet count, HbA1c, blood urea, serum creatinine, aspartate aminotransferase, alanine aminotransferase, total, direct and indirect bilirubin, serum triglycerides, serum HDL cholesterol, serum LDL cholesterol, serum VLDL, serum total cholesterol, urine albumin**.** Albuminuria was detected by CHEMSTRIX-AG TEST STRIP by dipstick method. Urine dipstick strips are clear plastic strips. Different reagent areas are affixed on the strip. These different cellulose areas are impregnated with specific testing chemicals according to the test which reacts with specific substances present in urine by changing the color. Color change chart is observed and compared to the color chart for the presence of abnormal levels of substances.

- Principle-This test is based on the protein-error-of-indicators principle. At a constant pH, the development of any green color is due to the presence of protein. Colors range from yellow for "Negative" through yellow-green and green to green-blue for "Positive" reactions.
- Urine dipsticks primarily measures albumin Indicator – Tetrabromophenol blue

Statistical Analysis: Data thus collected were analysed and chi square test, student 't' test, ANOVA test, regression analysis, multiple linear regression analysis were used by using SPSS 17.0, considering p value <0.05 as statistically significant.

RESULTS

In this study, among total 303 cases majority from urban population, male, non smoker and on oral hypoglycemic agent. Out of 303 patient 168 cases had their urine albumin positive while 135 cases had urine albumin Nil.Mean age in urine albumin positive cases was 61.57±10.53 years while in urine albumin negative cases it was 61.38±11.07 and this difference was found statistically insignificant (p>0.05). When both group compare for complete blood count parameter haemoglobin had a non significant (p>0.05) relation with urine albumin while TLC, Neutrophil, ANC, Lymhocyte, ALC and NLR had a highly significant (p<0.001). In present study, mean NLR in normoalbuminuria was 1.72±0.49 and for microalbuminuria it was 2.43±0.57 (p<0.001). as shown in Table 1. When we compare other biochemical parameters, Among aminotransferase Mean SGPT in our study was 26.76±5.86 in normoalbuminuria while in micrialbuminuria mean SGPT was 37.13±3.20 and this difference was found statistically highly significant (p<0.001).Mean HbA1c in urine albumin negative cases was 7.77±1.54% while in urine albumin positive cases it was 10.32±2.43% and this difference was found statistically highly significant (p<0.001).Mean duration of diabetes in urine albumin positive cases was 11.93±5.70 years while in urine albumin negative cases it was 4.92±3.52 years and this difference was found statistically highly significant (p<0.001).No relation was found for lipid profile ,blood urea ,creatinine between normoalbuminuria and microalbuminuria group (p>0.05 in all) shown in Table 2. We divided neutrophil lymphocyte (NLR) ratio into 3 groups i.e. <1.6, 1.6-2.40 and >2.40. When we studied different parameters with NLR, WBC, Neutrophils, ANC, ALC, Lymphocyte, SGPT, HbA1c had a highly significant difference (p<0.001) between 3 groups of NLR while haemoglobin, Monocyte, Platelet Count, Serum bilirubin total, serum bilirubin direct, SGOT, alkaline phosphatise, blood urea, serum creatinine and lipid profile had insignificant (p>0.05 in all) relation with NLR shown below in Table 3.

DISCUSSION

The key finding of this study was that NLR levels were found to be significantly associated (P=0.001) with patients who were diagnosed with early-stage DN as compared to those with normal albumin levels.NLR for without albuminuria was 1.72 and for with albuminuria it was 2.43. finding of our study was consistent with study conducted previously by , Huang *et al*. (2015). Many epidemiological studies have reported that DM is associated with chronic inflammation (Pitsavos, 2007), which may promote the acceleration of diabetic microangiopathy in addition to the development of macroangiopathy in diabetic patients (Fujita, 2013). The exact pathogenesis of DN is unknown. However, a cascade of pathological events (with glomerular damage being an early sign, which gives rise to proteinuria, followed by progressive renal damage, fibrosis, inflammation, and finally loss of functional nephrons) is known to play an important role in the development and progression of DN (Retnakaran, 2006). Counts of white blood cells and their subtypes are known as classic inflammatory markers, with low cost and wide availability, especially in cardiovascular disease. Numerous epidemiological and clinical studies have shown leukocytosis to be an independent predictor of insulin resistance, type 2 diabetes, microvascular and macrovascular complications of diabetes, and future cardiovascular events in patients with stable angina, unstable angina, or a history of myocardial infarction (Vozarova, 2002). Neutrophil-lymphocyte ratio (NLR) may be considered a novel marker of chronic inflammation.

Table 1. Relation between complete blood count parameters and urine albumin

Investigations	Urine Albumin				T	P
	Absent		Present			
	Mean	SD	Mean	SD		
Hb (gm/dl)	12.59	1.67	12.43	1.81	0.812	0.418
TLC (cells/cumm)	7077.11	1700.82	7830.89	1482.25	4.119	<0.001
Neutrophil %	55.50	2.92	63.56	5.23	16.013	<0.001
ANC cell/cumm	3913.32	932.66	4988.79	1161.94	8.729	<0.001
Lymphocyte (%)	34.03	2.95	26.02	3.10	22.838	<0.001
ALC (cells/cumm)	2419.29	650.17	2087.93	363.15	5.608	<0.001
NLR	1.72	0.49	2.43	0.57	11.467	<0.001
Monocyte (%)	2.26	0.88	2.34	1.11	0.709	0.479
Platelet Count (lacs/ml)	2.54	0.71	2.70	0.73	1.965	0.050

Table 2. Relation between different biochemical parameters with Urine albuminuria

Biochemical parameters	Urine albuine absent (n=135)	Urine albumine present (n=168)	P value
SGPT	26.76+_5.86	37.13+_3.20	<0.001
S.CREATININE	0.84±0.20	85±0.19	0.934
Hb1Ac	7.77+-1.54	10.32+-2.43	<0.001
Duration of Diabetes	4.92+-3.52	11.93+-5.70	<0.001
Total cholesterol	188.78±31.01	188.61±48.18	>0.05

Table 3. Relation of different parameters with Neutrophil Lymphocyte Ratio

Parameters	NLR						P
	<1.6 (n=57)		1.6-2.40 (n=157)		>2.40 (n=89)		
	Mean	SD	Mean	SD	Mean	SD	
Hb (gm/dl)	12.56	2.04	14.48	1.67	12.41	1.70	0.721
WBC (Th/mm^3)	7.60	1.27	7.11	1.74	8.11	1.40	<0.001
Neutrophils (%)	54.24	5.14	58.43	3.44	66.18	4.30	<0.001
ANC (Th/mm^3)	4.11	0.74	4.16	1.09	5.39	1.14	<0.001
ALC (Th/mm^3)	2.70	0.59	2.16	0.53	2.07	0.31	<0.001
Lymphocyte (%)	35.58	4.27	29.71	3.66	25.56	3.30	<0.001
NLR	1.41	0.11	1.92	0.23	2.89	0.57	<0.001
Monocyte (%)	2.20	0.87	2.23	1.05	2.50	1.02	0.104
Platelet (lacs/ml)	2.80	0.60	2.57	0.78	2.61	0.70	0.148
SBT (mg/dl)	0.88	0.14	0.91	0.29	0.84	0.19	0.099
SBD (mg/dl)	0.30	0.08	0.30	0.13	0.28	0.09	0.420
SGOT (u/L)	27.16	7.57	29.15	5.93	28.00	3.60	0.055
SGPT (u/L)	26.60	6.37	31.75	6.36	37.64	3.92	<0.001
ALP (u/L)	107.47	11.79	113.83	35.65	118.75	57.62	0.264
Blood Urea (mg/dl)	34.11	8.55	34.50	6.71	36.04	9.95	0.266
Serum Cr. (mg/dl)	0.82	0.16	0.83	0.22	0.88	0.15	0.146
HbA1c (%)	7.89	1.21	8.61	2.34	11.03	2.14	<0.001
TC (mg/dl)	190.63	34.82	186.69	46.10	190.94	36.27	0.687
TG (mg/dl)	139.95	19.26	140.68	37.38	131.56	22.41	0.071
HDL (mg/dl)	117.05	37.49	110.10	38.89	102.22	36.28	0.064
LDL (mg/dl)	44.18	16.86	47.61	19.56	52.26	27.32	0.077
VLDL (mg/dl)	28.00	3.89	28.43	7.40	26.89	4.03	0.153

It represents a combination of two markers; neutrophils, which represent the active nonspecific mediator initiating the first line of defense and lymphocytes, representing the regulatory or protective component of inflammation (Gibson, 2007). What might make NLR superior to other leukocyte parameters (e.g. neutrophil, lymphocyte and total leucocyte count) is its stability with less influence by physiological, pathological and physical factors (Nunez *et al*., 2008). Therefore, NLR has recently been defined as an essential and potential inflammatory marker in cardiac and non-cardiac disorders (Ramachandran, 2012). NLR that can be easily calculated from a simple peripheral blood count, is simpler and cheaper than measuring other inflammatory cytokines, such as IL-6, IL-1β and TNF-α (Huang *et al*., 2015). In present study, when we compared complete blood count with urine albumin**,** haemoglobin had a non significant (p>0.05) relation with urine albumin while TLC, Neutrophil, ANC, Lymhocyte, ALC and NLR had a highly significant (p<0.001) difference. Similar results also seen In the year 2005, by Chung *et al* in Taiwan reported that the total WBC, monocytes, and neutrophil counts increased in parallel with the advancement of diabetic nephropathy and in contrast, the lymphocyte count decreased (Lewis, 2008). These results also highlighted the role of inflammation in pathogenesies of diabetic nephropathy. Szydełko *et al* recent studies suggested the role of white blood cells in the pathogenesis of diabetic nephropathy (Arataeus, 1987). Several studies have reported an association between the presence of diabetic nephropathy and states of chronic inflammation. Few studies examined the relationship between white blood cells (WBC) count and urinary albumin excretion in diabetic patients and demonstrated that higher WBC counts were related to increased urinary albumin excretion rates (Ogawa, 2009; Goicoechea, 2010). In present study, in HbA1c group 6.50-8.50 mean NLR was 1.77±0.32, in HbA1c group 8.51-10.50 mean NLR was 2.44±0.83, in HbA1c group 10.51-12.50 it was 2.50±0.46 and in HbA1c group >12.50 it mean NLR was 2.55±0.67. Sefil *et al*. (2003) and Emanmoursay *et al*. (2012) reported a significant positive relation between Hb1Ac and NLR which favours results of our study.HbA1c levels are an indicator of blood glucose regulation, and increased HbA1c levels may be associated with increased risk of complications in patients with type 2 diabetes mellitus. Mean duration of diabetes in urine albumin positive cases was

11.93±5.70 years while in urine albumin negative cases it was 4.92±3.52 years and this difference was found statistically highly significant (p<0.001). Huraib *et al*. in Saudi Arabia, Varghese *et al*, and Mather *et al*.reported a significant relation between microalbuminuria and the duration of diabetes (Navarro-Gonzalez, 2009) on comparing other parameter like lipid profile, bloo urea, creatinine, haemoglobin, platelet, monocyte, billirubin no statically signigicant difference observed between patient with and without albuminuria.

Conclusion

Microalbuminuria was found to be one of the earliest marker for DN. However, recent studies have shown that albuminuria is a less precise predictor of overt nephropathy risk than originally thought .Thus there is an increasing question to find novel clinical biomarkers to identify individuals at risk of DN both onset and progression. The results of our study have shown that there was a significant relation between NLR and DN, implying that inflammation and endothelial dysfunction could be an integral part of DN. NLR is a simple, inexpensive test which can be an alternative for other costlier inflammatory markers such as ILs, TNF, cytokines, and high-sensitivity C-reactive protein. Therefore, NLR may be considered as a predictor and a prognostic risk marker of DN .Further research with a prospective design and multiple NLR measurements will shed more light on the role of NLR as a marker of inflammation and a probable risk factor for DN

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