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Speculation of diabetic nephropathy by pro-inflammatory cytokines (IL-6, IL-10) & renal biomolecules

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Abstract

Introduction: Type 2 diabetic nephropathy (T2DN) develops inflammation in kidney which leads to failure. Study designed to evaluate risk of diabetic nephropathy (DN) by inflammatory gene expressions (IL6, IL10) and renal parameters at the early stage in type II diabetes mellitus (T2DM) in Indian population.

Methods: This study includes 241 subjects (118 men, 123 women, and age ranges 30-70 years, distributed in three groups on the basis of age. Subjects recruited after screening for T2DM by measurement of blood glucose in fasting, post-prandial and glycosylated hemoglobin, microalbumin was measured from spot urine for screening of DN.

Results: Analysis of data of routinely used renal parameters found within reference interval except e-GFR marginally deviated from control group ($p < .00$). Other parameters showed marginal significance within and between the groups. Pro-inflammatory cytokines IL-6 and IL-10 evaluated by rt-PCR, showed high degree of significance ($p < .00$) in both study groups. Results of this study suggest that measurements of renal parameters are not enough to diagnose early stage of diabetic nephropathy (DN) and eventually to prevent progression of the disease.

Conclusion: It was concluded that early measurement of IL6, IL10 along with direct and indirect renal marker was suggested to prevent morbidity & mortality in DN.

Keywords: Diabetes mellitus, diabetes nephropathy, inflammatory gene expressions (IL6, IL10, risk prediction.

Introduction

Recent estimates by the National Institutes of Health indicate that diabetes represents the single largest cause of end-stage renal disease (ESRD). However, early renal involvement in T2DM was considered as a benign kidney condition in uncontrolled T2DM, though loss of renal function similar to that is also seen in the normal aging process at later stage of life. On a global level, the burden of chronic kidney disease (CKD) continues to increase & more than 1 million people die annually due to ESRD.[1,2] Routinely used biomarkers to diagnose kidney injury have several disadvantages. Serum creatinine and blood urea nitrogen is a routinely used parameter in kidney function assessment. But raised serum creatinine usually reported after 50-60% loss of the kidney function. Inadequate tools have failed to recognize DN at an early stage which leads to ESRD. So it is necessary to evaluate highly sensitive biomarkers for early detection of DN. The aim of the present study is to establish correlation between inflammatory gene expressions (IL-6, IL-10) and conventional renal parameters.

Material and Methods

Present research is prospective study was conducted at the Department of Biochemistry, D. Y. Patil University, School of Medicine, Navi Mumbai between December 2014 to February 2016. This study is conducted in albuminuric subjects and control group was normoalbuminuria. Total number of patients is 241 out of which 118 Men and 123 Women. The patient attended medicine OPD & diabetic clinic were selected after screening for T2DM & DN. Further they were distributed into three different groups; subjects of T2DM in the range of age of 30-45 years; 46-70 years and healthy volunteers (Non-diabetic) between 30-70 years.

Inclusion -Exclusion Criteria and biochemical parameters: T2DM of duration 3-5 years, HbA1c $\geq$ 7.0%, pre-prandial blood glucose (FBS) $\geq$ 6.0 mmol/L (126 mg/dl), post-prandial glucose (PPBS) $\geq$ 8.0 mmol/L (200 mg/dl) and micro albuminuria (microalbumin less than 260 mg5/dl, it is pre-DN condition) were measured for screening of DN. Since micro albumin is screening parameter single spot urine sample is taken for detection of microalbuminuria. Subjects satisfying above criteria but suffering with chronic conditions like uncontrolled diabetes with macro & micro-vascular complications, renal failure, pregnancy, nursing mothers, subject taking treatment for chronic illness were excluded from the study. The primary goal of this study was to prevent DN by risk prediction without surgical intervention by quantifying expressions of cytokines genes. Renal biopsy is gold standard method but subjects studied in this research projects are newly diagnosed T2DM therefore renal biopsy was not advisable. Research design was approved by institutional ethic committee & every participant signed consent voluntarily as per the GCP guidelines.

Other renal parameters such as blood urea, serum creatinine, urine creatinine calcium and uric acid was measured and e-glomerular filtration rate (e-GFR, albumin-creatinine ratio (ACR) were calculated. Three milliliter of whole blood was collected for gene polymorphism separately. All the biochemical renal parameters were measured by Dade Dimension dry chemistry auto-analyzer (Roche Diagnostics) and IL6, IL10 polymorphisms by rt-PCR (Qiagen & Takara Bio, Inc).

Statistical Analysis: The R test was used to assess the standard error of distribution of investigated parameters. All parameters in our study were distributed normally. Data were expressed as mean ± standard deviation. Differences were tested by two-tailed t-test. The values $p<0.05$ were considered statically significant. Stastical analysis was done using R Statistical software (Bell laboratories Lucent technologies by john chamber).

Results and Discussion

Glycosylated hemoglobin (HbA1C) is an average blood glucose (ABG) level for the past 2 to 3 months. It gives an idea about diabetes prognosis of the individual. In this study there was a significant difference between control and study groups (p>.00). There is no significant difference within study groups of T2DM. Measurement of glycosylated hemoglobin is useful parameter in diabetic nephropathy for monitoring T2DM treatment and its prognosis.

In this study, on applying post hoc test within study groups significant difference between control and study groups in their FBS and PBS was found. (<45 years and >45 years; p>.00 and .00).

Study done by Nobuko Harita et al. (2009), showed that lower serum creatinine increased the risk of T2DM.[3] Skeletal muscle is major target tissue of insulin and its resistance leads to the development of T2DM.[4] Creatinine is commonly used to determine GFR. In our study average serum creatinine reported within the normal range in control and study groups, similar findings were reported by Harita, et al.,[3] urinary excretion of creatinine was almost two fold higher in both the study groups against the control group. Further post hoc analysis within study groups (<45 years and >45 years) irrespective of gender showed significant P-value (p>.00 and .00). In this study it was found that no significant difference in urine creatinine was observed between control and study groups.

Micro-albuminuria is a gold standard parameter in diagnosis of renal diseases. Albumin/ creatinine ratio (ACR) is greater than or equal to 2.5 (men) or 3.5 (women), or albumin concentration greater than or equal to 20 mg/L is significant observation in diagnosis of renal diseases. Literature survey reveals that early stage of kidney disease demonstrates an abnormal ACR. This study reported marginally significant difference of ACR between control and study groups. Further post hoc analysis also showed similar observations between control, less than 45age and above 45age. This observation does not indicate any confirmatory outcome. So it was recommended to undertake study on a larger population to achieve final conclusion. After literature survey it was fond that ACR is an important marker in diagnosis of DN but values reported in this study does not support, reason of it could be the clinical stage of T2DM & renal physiology.

Serum calcium concentrations are maintained in the normal range in healthy individuals. Very few studies have been done to understand the role of calcium in DN. Hypercalcimia may cause renal dysfunction. Deposition of calcium oxalate crystal may derange kidney function. In this study the level of calcium in control and study groups was practically the same and within the normal range and its role in prognosis of disease was not clear.

It was recommend by the American Diabetes Association and the National Institutes of Health that in all the people with diabetes for detection of kidney dysfunction, e-GFR must be calculated from serum Creatinine at least once a year.[5] In this study e-GFR was calculated by MDRD (Modification of diet in renal disease) study group equation.[6] There is significant difference between control and study groups (p>.00). Further analysis by post hoc test within the study groups (<45 years and >45 years) and control showed the significant difference (p>.00). Therefore measurement of e-GFR is useful indicator in monitoring diabetic nephropathy associated with T2DM. All above statements are tabulated in Table 1 & 2.

Table 1: Descriptive analysis of renal parameters, Microalbumin (MALB), Glycosylated Hemoglobin (HbA_1C) and Albumin-Creatinine ratio (ACR) within groups

Groups / Parameters	Control		45 years and less		More than 45 years	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**
Glycosylated Hemoglobin	5.6	0.052	8.0	0.157	8.0	0.129
Blood glucose (F) (70-99 mg/dL)	96	0.806	147	4.638	156	5.941
Blood glucose (PP) (less than 140 mg/dL)	108	0.921	175	4.242	197	7.696
Microalbumin (30-300 mg/dl)	14.13	0.401	235.28	5.970	263.37	9.462
Urine Creatinine (20-370 mg/dl)	60.99	4.335	121.06	9.231	134..65	13.960
Albumin/ creatinine ratio (30-299 mg/gm of creatinine)	0.44	2.113	3.35	3.556	3.35	2.623
Calcium (8.8-10.6 mg/dL)	0.94	0.064	9.6	0.069	9.6	0.057
Blood urea nitrogen (8-20 mg/dL)	10	0.284	10	0.237	11	0.335
Uric Acid (2.6-6.0 mg/dL)	4.8	0.112	5.0	0.203	5.3	0.139
Serum Creatinine (0.51-0.95 mg/dL)	0.79	0.02	0.716	0.019	0.854.	0.023
e-GFR (>/=90 ml/min/ 1.73m^2)	100	2.46	94	114	90	2.077

Abbreviations**:** SE-standard error; e-GFR; estimated Glomerular filtration rate

Table 2: P Value of Post Hoc Tests of renal parameters within groups and between the groups (Tukey HSD)

Dependent Variable	Control group		<45 years group		>45 years group	
	<45 yrs	**>45 yrs**	**Control**	**>45 yrs**	**Control**	**<45 yrs**
HbA_1C	.00	.00	.00	.948	.00	.948
Urine creatinine	.00	.00	.00	.822	.00	.822
Serum Creatinine	.034	.074	.034	.00	.074	.00
ACR	.008	.420	.008	.186	.420	.186
e-GFR	.00	.012	.00	.00	.012	.00
Calcium	.048	.146	.048	.871	.146	.871
BUN	.751	.040	.751	.197	.040	.197

Table 3: Post hoc test for threshold cycle (CT) between study groups and inflammatory markers (Tukey HSD)

Dependent Variable	Control		45 years and less		More than 45 years	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**
CT OF IL-6	25.38	0.060	22.46	0.098	20.48	0.124
CT OF IL-10	26.09	0.121	23.64	0.077	23.20	0.086

IL-6 is one of the most extensively studied pro-inflammatory cytokines.[7,8] Cortical mRNA expression of IL-6 is increased in diabetic kidney in comparison with normal rodents and is positively associated with elevated urinary concentrations of albumin. Similarly, other researcher published work on interstitial expression of IL-6 mRNA in human renal tissue from individuals with diabetes, correlates with histological features of interstitial injury.[7] Gene expressions were measured by rt-PCR and value of CT calculated using

Taq Man Gene Expression Assays, calculated fold change values correlate better to expected fold change values when the comparative C_T method is used. There are other methods like standard curve method but the method used in this study is more compatible for larger number of samples.

IL-10, an important Th2 cytokine, exerts predominantly anti-inflammatory and immunosuppressive effects.[9] In present study data analyzed by R-software showed positive expression of IL-6 and IL-10 in both the groups irrespective of gender. It indicates that there is inflammation and its degree of severity is correlated with renal injury in T2DM. In this study, High degree of significance ($p>.00$) was found in pro-inflammatory cytokines (IL6 & IL10) in both study groups which is tabulated in Table 3. Researcher reported found some other gene biomarker expressed significantly in T2DM.[10,11]

Researchers found that plasma concentrations of inflammatory molecules including proinflammatory cytokines IL-6, IL-10 are elevated in diabetic patients.[12] Recent studies have shown that concentrations of these substances increase with the progression of nephropathy.[13,14] In this study the similar observations were observed. Shikano M, et al., and Myśliwska J[15] et al., studied usefulness of IL-6 and IL10 diabetic nephropathy showed that increased adipose tissue IL-6 mRNA may be the cause of insulin resistant humans and the elevated mRNA levels correlated with reduced rates of insulin stimulated glucose disposal[14,16] which further might progress into insulin resistance diabetes nephropathy.

In 1991, Suzuki et al.,[7] reported that serum levels of IL-6 were significantly higher in patients with Type 2 DN than the levels observed in diabetic patients without nephropathy, which suggests that IL-6 may have a role in the pathogenesis of DN. Later Suzuki et al.,[7] performed situ hybridization of IL-6 in diabetic nephropathy, analyzed kidney biopsies in patients with DN by high-resolution in situ hybridization. Outcome of further experiment showed that cells infiltrating the mesangium, interstitium, and tubules were positive for mRNA encoding IL-6. Also they found a relationship between the severity of diabetic glomerulopathy (mesangial expansion) and expression of IL-6 mRNA in glomerular cells (mesangial cells and podocyte), which indicated that IL-6 may affect the dynamics of extracellular matrix surrounding those cells. Many in-vitro studies have confirmed that IL-6 affects extracellular matrix dynamics at mesangial and podocyte levels, stimulates mesangial cell proliferation, increases fibronectin expression, and enhances endothelial permeability. This mechanism has been proved in the development of kidney injury in patients with T2DM. Moreover, high serum and urinary concentrations of IL-6 are associated with greater albuminuria in patients with DN; however, serum and urinary levels do not correlate with each other. [16]

IL-10 plays an essential role in the pathogenesis of DN. Mesangial expansion has also been found to be attributable to hyperglycemia related inflammation.[17] Mesangial cells are the major local source of IL-10 in the normal adult kidney. Several studies have demonstrated the association between up regulation of IL-10 and the pathophysiology of various kidney diseases such as mesangio-proliferative glomerulonephritis.[18-20] Increased serum levels of circulating IL-10 have been found and correlate with albuminuria and diabetic nephropathy IL-10 plays an essential role in the pathogenesis of DN. These factors may slow down the course of diabetic nephropathy through a reduction of the inflammatory processes. The study done by Inna Sinuai et al., suggested that IL-10 gene expression and IL-10 induced signaling pathways have an important role in the regulation and maintenance of normal renal function. In this study IL6 & IL10 expressions are observed which is relates to the conclusion of several research papers referred in literature.

Conventional renal markers such as microalbuminuria, e-GFR, blood urea & serum creatinine can be used to detect renal injury in diabetic patients, but these are the less sensitive & delayed markers. Similar observations were reported in this study.

The earliest stage of DN is hyper-filtration, where the GFR is found significantly higher than normal. Detection of hyper- filtration is not clinically useful, as it is difficult to determine from routine testing. Constant albuminuria is considered the earliest clinical sign of DN. At the beginning, small amounts of albumin are leaked which may be within normal range but above target value and below the detection level of a urine dipstick. This stage is referred to as microalbuminuria (30-300 mcg/mg of creatinine). In this stage the urinary albumin excretion becomes sufficiently high detectable by a urine dipstick, known as overt nephropathy. The rate of progression from normoalbuminuria, ~~to~~ microalbuminuria to overt nephropathy usually is gradual, typically taking few years (more than 5 years of diabetic duration) or more to progress through each stage.[21,22] In this study ESRD is exclusion criteria & subject with microalbuminuria value less than 300 mg/dl were included. Statistical analysis of p-value showed that significance between study group and control.

The measurement of GFR requires a substance that has a stable plasma concentration, is freely filtered and is not metabolized or actively transported by the tubules. The plasma concentration of creatinine changes, until the amount excreted again equals the production. The plasma levels continue to increase as the GFR decreases, because no significant tubular adjustment occurs for creatinine. This relationship between blood concentration and renal excretion of

creatinine allows plasma creatinine concentration to serve as an estimate of changing glomerular function.

During the early stages of DN, the rate of loss of renal function is relatively slow GFR (1 to 2 mL/min/1.73 m^2 per year) and not impressively higher compared to the general population (0.5 to 1 mL/min/1.73 m^2 per year). However, late in the overt nephropathy phase, the failure rate of renal function can accelerate (5 to 10 mL/min/1.73 m^2 per year). Thus, significant renal dysfunction is not usually seen until late in the course of DN[23] which is hindered risk in T2DM patients. To identify risk of DN in borderline microalbuminuria & reduced GFR is motive of this study.

Plasma creatinine levels in blood may not alter unless and until 50-60% of kidney function has been lost. This makes plasma creatinine levels a suitable biomarker for chronic kidney injury, but not for acute kidney injury.[24,25] Serum creatinine is the most common measurement of kidney function; however, it cannot reflect accurate renal function in many scenarios, particularly in extremes of patient age or body mass index. Indeed, in T2DM, the GFR usually will be less than half of normal before the serum creatinine exceeds the lab normal range.[26-28]

However, the link between glomerular hyper-filtration and subsequent albuminuria or e-GFR loss in humans has not been consistently proven. A meta-analysis suggested that there was a 2.7-fold increased risk for the development of microalbuminuria (30-300 mg/24 hrs, moderately increased) in those with prior hyper-filtration.[29]

Screening with albumin excretion rate alone would miss >20% of progressive disease. Serum creatinine with e-GFR should therefore be assessed at least annually in all adults with diabetes, regardless of the degree of urine albumin excretion.

The epidemiology of micro-albuminuria reveals a close association with systemic endothelial, dysfunction and with vascular disease, also implicating glomerular endothelial dysfunction in micro-albuminuria. Micro-albuminuria predicts the development of overt diabetic nephropathy in T1DM and T2DM; however, the relationship in T2DM is less clear because of the greater heterogeneity of this condition and the presence of other risk factors for micro albuminuria, usually in elderly patients.[28] Micro albuminuria invariably precedes overt diabetic nephropathy, and although micro albuminuria may regress spontaneously in a proportion of cases, it remains the best documented predictor for high risk of development of diabetic nephropathy in both T1DM and T2DM.[30]

In conclusion, our results suggest that for early detection of renal injury routine renal markers (blood urea nitrogen and serum/ urine creatinine) are not enough, these routine markers can be used for monitoring the treatment & progression of the disease rather than for diagnosis. Other parameters (GFR, micro albumin& urine albumin) have important role in screening of renal dysfunction. Though they play important role in diagnosis, reversion of renal dysfunction even at early stage is impossible. FBS, PPBS & HbA_1C these three are very important parameters as far as progression of renal disease is concerned. Etiology of calcium & uric acid crystal deposition in kidney has many other reasons other than T2DM. But monitoring their levels in T2DM may reduce chances of renal failure. Inflammation of kidney may progress to renal failure; Pro-inflammatory cytokines (Il-6 and IL-10) are secreted into circulation and upregulate their expressions shown in rt-PCR amplification plots (Fig. 1 & 2). Threshold cycle (CT) quantified from amplification plot and their significance was analyzed. We found both cytokines p-value ($p<.000$) in T2DM (Table 3). More than 60-70% uncontrolled T2DM subjects demonstrate some degree of renal dysfunction. The renal bio-molecules studied showed significant abnormal level indicating damage to kidney. Relationship between T2DM & MABL is unclear, which can be superseded by gene polymorphisms of IL6- and IL-10. Early measurement of IL6, IL10 may prevent morbidity & mortality. The present study was carried out in limited number of T2DM subjects. Further extensive research on large number of subjects with population diversity has been recommended.

Consent

All authors declare that 'written informed consent was obtained from patients for publication of outcome of this study' copy of written consent may retrieve from us, if required.

Ethical Approval

All authors are here by declared that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

References

1. Lozano R, Naghavi M. Correa, Foreman K, et al. 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2095–128.
2. Hamer RA, El Nahas AM. 2006. The burden of chronic kidney disease. (2006); Br Med J 332:563–4.
3. Harita N, Hayashi T, Sato KK, Yoneda T, Endo G, Kambe H. 2009. Lower serum creatinine is a new risk factor of T2DM: the Kansai healthcare study. Diabetes care 32; 424-426.
4. Zierath JR, KrookA, Wallberg-Henriksson H. 2000. Insulin action and insulin resistance in human skeletal muscle. Diabetologica 43:821-835.
5. Dabla PK. Renal function in diabetic nephropathy. 2010. World J Diabetes 1(2):48-56.

6. M. H. Stanley Chan, Andrew L. Carey, Matthew J. Watt, Mark A. Febbraio. 2004. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 287 (2), R322-R327.
7. Suzuki D, Miyazaki M, Naka R, Koji T, Yagame M, Jinde K, Endoh M, Nomoto Y, Sakai H. 1995. In situ hybridization of interleukin 6 in diabetic nephropathy. Diabetes 44:1233–1238.
8. Van Snick J. Interleukin-6: An overview. 1990. Annu Rev Immunol. 8:253–278.
9. Zimmet PZ. 1999. Diabetologia. Diabetes epidemiology as a tool to trigger diabetes research and care 42(5):499-518.
10. Khot VV, Yadav KS. Correlation between LDLr and CD-36 with Lipids in Pre-phase of Diabetic Nephropathy. 2016 Journal of Applied Life Sciences. 8(1):1-6.
11. Yadav KS, Khot VV. Prediction of Renal Injury Risk by Expressions of KIM-1 and NGAL in Type 2 Diabetic Nephropathy.2016. British Journal of Medicine and Medical Research. 17(9):1-8.
12. Pickup J. C., Chusney G. D., Thomas S. M., Burt D., Bargero G, Ferrero S, Pagano G., et al. 2000. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. Life Sciences 67(3):291–300.
13. Bruno G., Merletti F., Biggeri A. 2003. Diabetes Care. Progression to overt nephropathy in type 2 diabetes: the Casale Monferrato Study 26(7):2150–2155.
14. Festa A., D'Agostino R., Howard G., Mykkänen L., Tracy R. P., Haffner S. M. 2000. Inflammation and microalbuminuria in non-diabetic and type 2 diabetic subjects: The Insulin Resistance Atherosclerosis Study. Kidney Int. 58(4):1703-10.
15. Shikano M, Sobajima H, Yoshikawa H, Toba T, Kushimoto H, Katsumata H, Tomita M, Kawashima S. 2000. Usefulness of a highly sensitive urinary and serum IL-6 assay in patients with diabetic nephropathy. Nephron 85:81–85.
16. Bastard JP, Maachi M, Van Nhieu JT, Jardel C, Bruckert E, et al. 2002. Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. J Clin Endocrinol Metab 87:2084–2089.
17. Morcos M, Sayed AA, Bierhaus A, Yard B, Waldherr R, et al. 2002.Activation of tubular epithelial cells in diabetic nephropathy. Diabetes. 51:3532-3544.
18. Yano N, Endoh M, Nomoto Y, Sakai H, Fadden K, Rifai A. 1997. Phenotypic characterization of cytokine expression in patients with IgA nephropathy. J Clin Immunol. 17:396-403.
19. Niemir ZI, Ondracek M, Dworacki G, Stein H, Waldherr R, Ritz E, Otto HF. 1998. In situ up regulation of IL-10 reflects the activity of human glomerulonephritides. Am J Kidney Dis. 32:80-92.
20. Hirschberg R and Adler S. Review. 1998. Am J Kidney Diseases. 31(6):901-919.
21. Lemley KV, Abdullah I, Myers BD. 2000. Evolution of incipient nephropathy in Type 2 diabetic mellitus. Kidney Int. 58(3):1228-37.
22. Gall MA, Nielsen FS, Smidt UM, Parving HH.1993. The course of kidney function in type 2 (non-insulin-dependent) diabetic patients with diabetic nephropathy. Diabetologia. 36(10):1071-8.
23. Jacobsen P, Rossing K, Tarnow L. 1999. Progression of DN in normotensive type 1 diabetic patients. Kidney Int Suppl. 71:S101-5.
24. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. 1999. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 130(6):461-70.
25. Gault MH, Longerich LL, Harnett JD, Wesolowski C. 1992. Predicting glomerular function from adjusted serum creatinine. Nephron. 62(3):249-56.
26. Bending JJ, Keen H, Viberti. GC. 1985. Creatinine is a poor marker of renal failure. Diabet Med. 2(1):65-6.
27. Shemesh O, Golbetz H, Kriss JP, Myers BD. 1985. Limitations of creatinine as a filtration marker in glomerulopathic patients. Kidney Int.1985;28(5):830-8.
28. Adler AI, Stevens RJ, Nanley SE, Bilous RD, Cull CA, Holman RR. 2003. The United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int. 63:225–232.
29. Magee GM, Bilous RW, Cardwell CR, Hunter SJ, Kee F, Fogarty DG.2009. Is hyperfiltration associated with the future risk of developing DN? A meta-analysis. Diabetologia. 52(4):691-7.
30. Nahar Avi, Yadav K.S., P. Suresh Kumar. 2014. Novel biomarkers in Diabetic nephropathy. DYPJHS. 2(3):35-39.