Correlation of Urinary Markers and Urine Creatinine in Glomerulopathy at the Onset of Type –II Diabetic Subjects

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ABSTRACT

Background: *Diabetic glomerulopathy in hyper glycemic state impaired kidney filtrations. Micro albuminuriais primary urinary marker simply indicating kidney dysfunction. IgG and transferrins are authenticating urinary markers of kidney dysfunction. High urine creatinine (Crt)) is waste product of unusual high protein metabolism.*

Materials and Methods: *The present study was conducted over 400 diabetes mellitus type –II (DMT-II) complicated patients; they were randomly selected on micro albuminuria using dipstic method. Urinary markers: Albumin (Alb), Immunoglobulin G (IgG), Transferrin (Trf), and Urine Crt were analyzed using enzyme linked immunosorvant assay (ELISA) kit method and Jaffe's reaction respectively. Statistical analysis was performed using IBM, SPSS version 20.0 (Illinois). Data was expressed as mean ± S.D., Correlation between parameters was assessed using Pearson's correlation coefficients (r) by linear regression analysis.*

Result: *276 patients were reported as hyperalbuminic (More than normoalbuminuria 30mg/day) cases among 400 DM type-II complications. 55.15%hyperimmunoglobulinic-G(more than normal level of IgG) and 67.88 % hyper transferrinic cases were reported and found positive correlation between Crt & Alb (r^2 = 0.561, P-0.01), Crt & Trf (r^2 = 0.142, P-0.01), Crt & IgG (r^2 = 0.205, P-0.01).*

Key worlds: *Urinary markers, transferrin, IgG, creatinine*

INTRODUCTION

Diabetes mellitus is metabolic disorders, simply characterized by high glucose levels in blood (hyperglycemia) and excretion of glucose in urine (glucosuria). Diabetes affected more than 387 million people worldwide and the number will rise to 592 million people by 2035. The number of people with DM type-2 is increasing in every country. 77% of people with diabetes live in low- and middle-income countries. Maximum number of people reported as diabetics under the age group of 40 to 59 years. About 179 million people with diabetes are undiagnosed. Diabetes caused 4.9 million deaths in 2014. The world diabetes report says that every seven seconds a person dies from diabetes. In South-East Asia, almost half of people with diabetes are undiagnosed[1]. Aboutone third population of those affected will eventually have progressive deterioration of renal function[2]. Renal malfunction can predict on the presence of a number of detectable proteins and factors, these factors are known as urinary markers.

Urinary markers: Diabetic glomerulopathy (DG) is the leading cause of renal failure worldwide, affects approximately one-third of all people with diabetes. Leakage of albumin abnormally in small amount termed as Micro albuminuria which is considered the first sign and the best predictor of progression to renal failure. As the disease progresses, patients develop advance macro albuminuria, and the kidney function declines until patients end up requiring renal replacement therapy[3]. Although micro albuminuria in diabetic patients is considered to be the best predictor of progression to end-stage renal disease (ESRD) and specific markers of kidney damage which might help in diagnosis and treat DG at an earlier stage to prevent the progression to renal failure. DG affects all the kidney cellular elements, that is, glomerular endothelia, mesangial cells, and podocytes. Baseline albuminuria is the strongest predictor of (ESRD) for DMT-2 patients. Approximately 20 to 40% of diabetic patients develop micro albuminuria within 10–15 years of diagnosis, whereas macro albuminuria occurs within 15–20 years in 20–40% of patients[4]. It is characterized by excessive accumulation of extracellular matrix with thickening of glomerular basement membranes and increased amount of mesangial matrix, which ultimately progresses to glomerulosclerosis, due to elevated urine Alb excretion is considered a well-established urinary marker of glomerular damage[5]. Multiple biomarkers in urine have been studied that represent different mechanisms or structural damage, based on which they have been classified as markers of glomerular injury, tubular injury, oxidative stress, inflammation, and endothelial damage. Urinary markers of glomerular damage represent either, increased

permeability to plasma proteins: IgG, albumin and transferrin.[6]

Albumin: Albumin is a 65-kDa protein produced in the liver it is the most abundant plasma protein in the body. The main function of albumin is to regulate the oncotic pressure which is to act as an acid/base buffer and to mediate the transportation of metabolites, hormones, vitamins, and drugs. In normal subjects, a small amount of albumin is filtered in the glomerulus, but almost all of it is reabsorbed by the tubules. In addition, it is known that tubular dysfunction by itself may cause albuminuria owing to decreased re-absorption of filtered albumin[7]. The urine albumin excretion is considered normal when it is less than 30mg/day or 20microg/min (normoalbuminuria). This threshold was determined because the urine albumin excretion of 95% of "normal" patients falls below this value[8]. Based upon the ability of dipstick to measure urine albumin, the urine albumin excretion has been classified as micro albuminuria, when the urine albumin excretion is 30 to 300mg/day or 20 to 200microg/min; macro albuminuria, when the urine albumin excretion is above 300mg/day or 200microg/min[9].

Transferrin: Transferrin is a plasma protein having molecular weight 76.5k Da, it is similar in weight to albumin, but slightly larger. It is less anionic than albumin with an isoelectric point one unit higher, therefore, expected to be filtered more readily through the glomerular barrier. Transferrin is the major iron-binding protein in the serum, and it transports ferric ions to all proliferative cells in the body. Among DMT-II patients, urinary transferrin significantly increase with respect to the progress of biopsy proven glomerular diffuse lesions and has been shown that some DMT-II patients with diffuse glomerular lesions without micro albuminuria had microtransferrinuria[10]. Transferrin excretion is higher in diabetic patients, even before they develop micro albuminuria. Because diabetic patients are more likely to have transferrinuria than albuminuria because the albumin/transferrin ratio was significantly smaller in normoalbuminuric and micro albuminuric compared to macro albuminuric patients, urinary transferrin is considered to be a more sensitive marker of glomerular damage in diabetic patients. Furthermore, increased urinary transferrin excretion predicts the development of micro albuminuria in DMT-II patients with normoalbuminuria in patients that already developed albuminuria; the urinary transferrin excretion has a linear relationship with urinary albumin excretion. Urinary transferrin excretion is elevated in primary glomerulonephritis and other diseases that affect the glomerulus and is not specific to diabetic nephropathy. Urinary transferring excretion is not correlated with glycemic control; supporting the hypothesis that transferrinuria is caused by intrinsic renal damage[11].

IgG: Diabetic hypertensive renal dissociation is one of the developed high blood pressure renal abnormalities causes diffusion of Immunoglobulin G (IgG). Furthermore, a diurnal change in systolic blood pressure significantly correlates with urinary IgG excretion, but not with urinary albumin excreation[12]. IgG is a protein synthesized and secreted by plasma cells than is mainly involved in the secondary immune response. It is larger than albumin, with a molecular weight of 150kDa and molecular radii of 62Å, compared to albumin 65kDa and 36Å, respectively[13]. Total urinary IgG excretion is higher in diabetic patients compared to controls. Urinary IgG excretion in normoalbuminuric diabetic patients predicts the development of micro albuminuria; it correlates with the progression of glomerular diffuse lesions[14]. Urinary IgG excretion correlates well with urinary excretion of transferrin, but it has a weak and nonlinear relationship with urinary albumin excretion, indicating that the urinary excretion of IgG rises later and moves slower than that of albumin[15-16].

MATERIAL AND METHOD

The present study was conducted over 400 diabetics (attending diabetic clinic, OPD and indoor patients admitted in medical wards of Cancer Hospital and research Institute, Gwalior M.P.). 100 age and sex matched healthy volunteers were selected as control. Age, sex, blood pressure and anthropometric parameters such as height, weight were recorded and parameters regarding kidney mall function as secondary complication was also analyzed and recorded. Past history and family history of diabetes, hypertension, chest pain or myocardial infection were enquired particularly. Detailed clinical examination has been done with more emphasis on blood pressure, height and weight, blood and urine analysis. Fasting and PP blood sugar were also analyzed.

Method for urine creatinine (Jaffe's reaction): Urine creatinine was measured colorimetrically by the reaction of creatinine in urine with picric acid in alkaline condition to form a yellow – orange color complex (creatinine picrate). The intensity of the orange color is read using green filter (540 nm). The normal creatinine level for men is 0.5-1.5 mg/dl. The normal level for women is 0.6-1.2 mg/dl. Very high muscular have slightly higher creatinine levels. A high creatinine level indicates kidney dysfunction and damage. One instance of elevated creatinine is not enough to diagnose kidney disease.

Estimation of Albumin: (Comper and Colleagues method) The human Urine sample reacts upon a specific antibody for human albumin and the turbidity induced by the formation of immune complexes is recorded at 340 nm. The turbidity measured is directly proportional to the albumin sample concentration.

Estimation of IgG: (R&D Bio systems ELISA kit method) Heavy loss of urine IgG, over 8.8mg/lit, is a sign that glomerular filtration membrane is severely impaired. Urine from the same spot sample was analyzed by ELISA for IgG.

Estimation of transferrin: (Abeam's transferrin Human in vitro ELISA kit method) Urine transferrin test can detect early damages of the change barrier functions on glomerular filtration membrane. Urine transferrin over 1.9mg/lit. will be an abnormal test result to diagnose glomerular damage. Spectrophotometric based transferrin quantitative sandwich ELISA Kit with 1x96 well plate, type of sample -urine, sensitivity range 1.563 ng/ml to 100ng/ml, assay time is 4 hrs was used.

Statistical analysis: (IBM, SPSS, Version 20.0 Illinois) Statistical analyses were performed using SPSS version 20.0 (Illinois). Data was expressed as mean ± SD. The significance of difference was assessed by t-test. Correlation between parameters were assessed using Pearson correlation coefficients (r) and coefficient of determination (r^2) performed by linear regression analysis.

RESULT

The present study was conducted over 500 (Test:T-400+ Control:C-100) diabetics, who were reported in the OPD and general ward in cancer hospital and research institute (CHRI), Gwalior. As per the age and sex distribution of diabetics 400 cases were analyzed, among 400 cases 74.00 % were male and 26.00 % were female constituting a ratio 2.85:1. Majority of male were in 46-55 years and female patients were in age groups of 56-65 years (Table1). Allied pictograph is showing age and sex distribution of diabetics in fig. 1.

Table 1: Age and Sex distribution of diabetics (T-400)

Age	Male		Female		Total	
	No. of Patients	Percentage	No. of Patients	Percentage	No. of Patients	Percentage
15-25 years	00	00.00	02	01.88	02	00.48
26-35 years	07	02.365	03	02.83	10	02.38
36-45 years	44	14.865	26	26.41	75	17.82
46-55 years	113	38.176	31	29.24	152	36.10
56-65 years	72	24.324	34	32.07	113	26.84
66-75 years	59	19.932	08	07.54	68	16.15
76-85 years	01	00.338	00	00.00	01	00.23
Total	**296**	**74.00%**	**104**	**26.00%**	**400**	**100%**

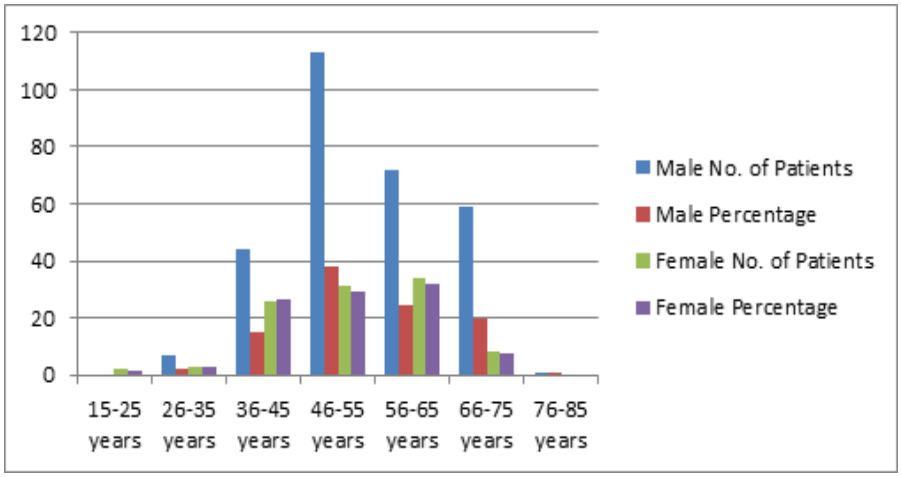
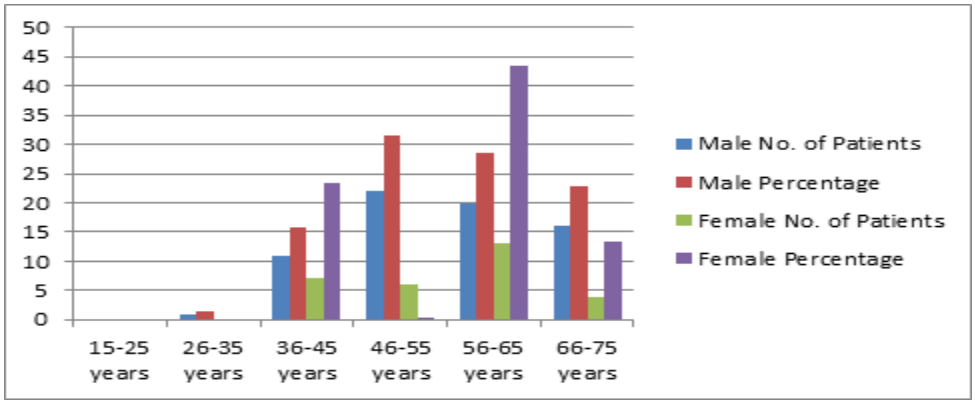


Fig. 1: Age and sex distribution of diabetics (T-400)

Considering the data in control subjects age and sex distribution were analyzed over 1**00** case, among them **70.00 %** male and **30.00 %** female were found constituting a ratio **2.33 : 1**. Majority of male fall into **46-55** years group and female were fall into in the age groups of **56-65 years**. (Table 2), Allied pictograph is showing age and sex distribution of controls in fig. 2.

Table 2: Age and sex distribution of controls (C-100)

Age	Male		Female		Total	
	No. of Patients	Percentage	No. of Patients	Percentage	No. of Patients	Percentage
15-25 years	00	00.00	00	00.00	00	00.00
26-35 years	01	01.43	00	00.00	01	01.00
36-45 years	11	15.72	07	23.33	18	18.00
46-55 years	22	31.43	06	00.20	28	28.00
56-65 years	20	28.57	13	43.33	33	33.00
66-75 years	16	22.86	04	13.33	20	20.00
Total	**70**	**70%**	**30**	**30%**	**100**	**100%**



Age→

Fig. 2: Age and sex distribution of diabetics (C-100).

Albumin: Among 400 cases 276 patients were reported as hyperalbuminic (albumin excreted in urine more than reference value 0.5-5.4gm/dl). Mean value of excreted albumin in urine is 8.601gm/dl±0.23751 (Table and Fig. 3). Albumin found in urine is defined, by various authors as early diagnostic and prognostic markers in diabetic complications.

Table 3: Case distribution of kidney mall function with reference to albumin in diabetics N=400 (Mean-8.6018gm/dl±0.23751)

Albumin level(gm/dl)	Normal range (5.0-5.4)	Hyper value	Total
Case	124	276	400
%	31	69	100

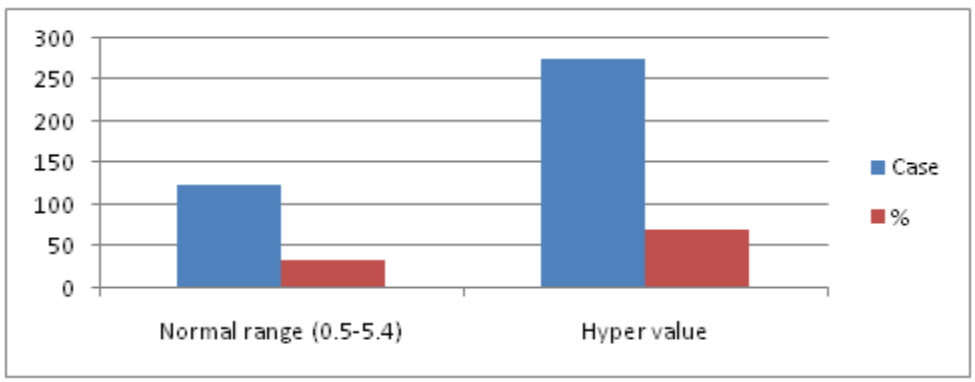


Fig. 3

IgG: 330 cases was reported as positive IgG value in urine, the mean value of IgGis5.59mg/dl ± 4.96, (70 cases are notexcreting IgG in urine beside their micro albuminuria) 55.15% hyper immunoglobulin-G (IgG excreted in urine more than normal value), hence 55.15%cases were reported positive IgG in Urine (Table 4 & Fig. 4)

Table 4: Case distribution of kidney mall function with reference to IgG in diabetics

IgG level(mg/lit)	Normal range(<8.8)	Hyper value(>8.8)	Total
Case	148	182	330
%	44.85	55.15	100

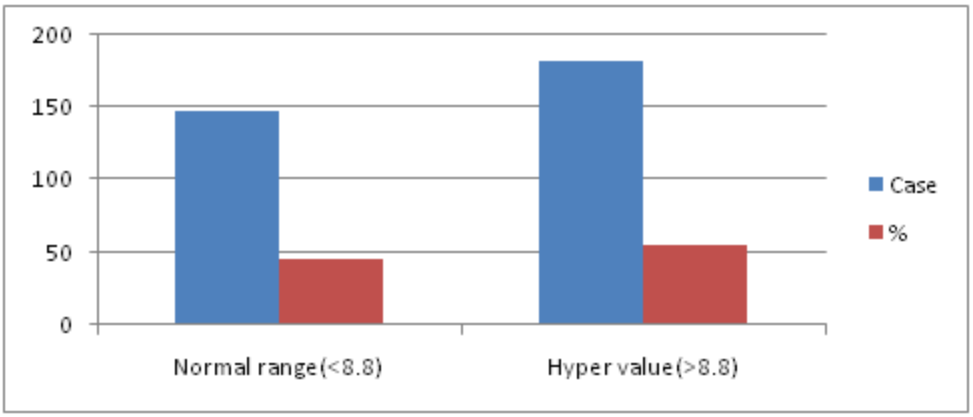


Fig. 4

Transferrin: 330 cases were reported as positive transferrin in urine, the mean value of transferrin is 3.7635 mg/lit±2.59903 (70 cases are non-transferrin diabetics) and 67.88 % was reported hypertransferrin (transferring excreted more than normal value) cases (Table5 and Fig. 5), they were significantly correlated with 69% micro albuminic subjects.

Table 5: Case distribution of kidney mall function with reference to transferrin in diabetics

Transferrin level mg/lit	Normal range(<1.9)	Hyper value(>1.9)	Total
Case	106	224	330
%	32.12	67.88	100

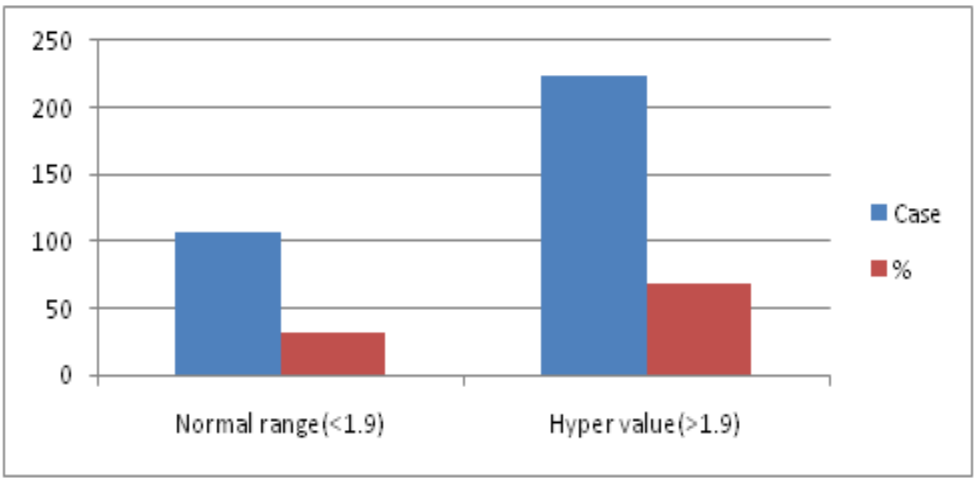
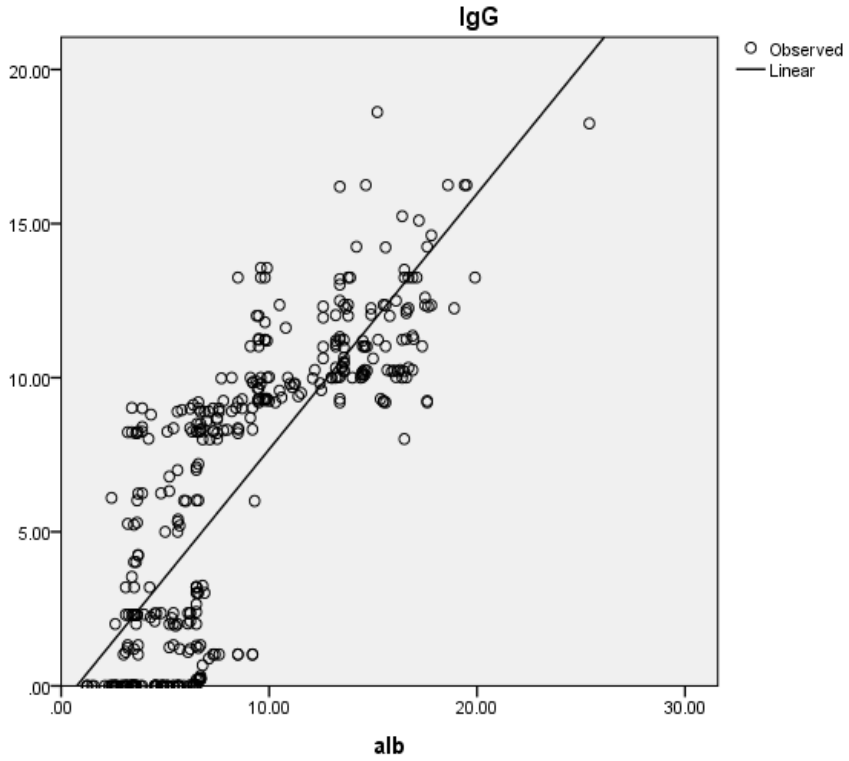


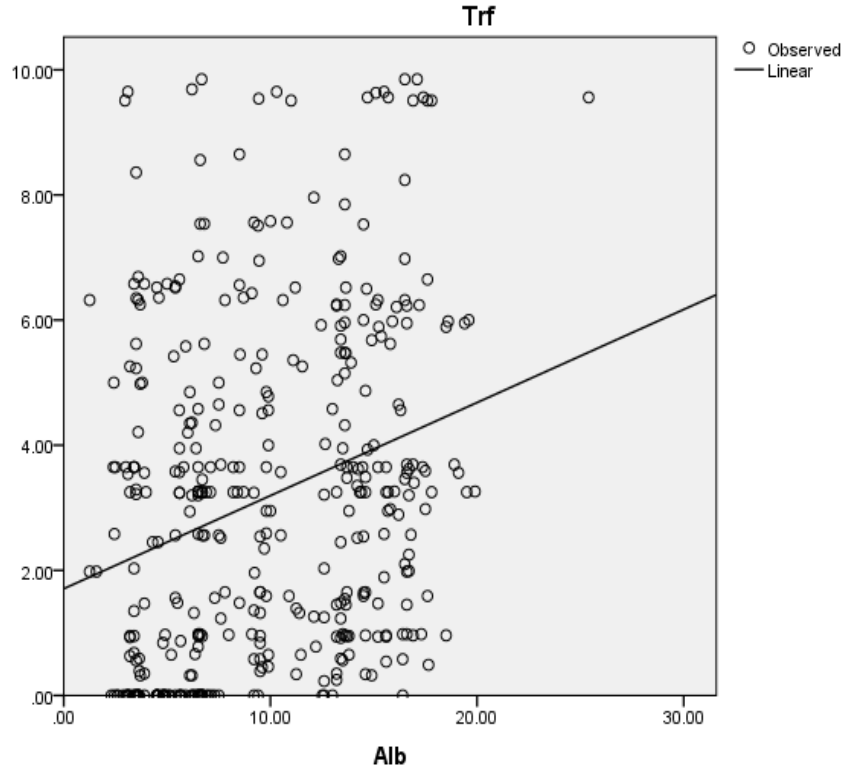
Fig. 5

Correlations: **(Significance = P, Pearson correlation coefficient = r, Coefficient of Determination= r^2)** Urinary albumin (Alb) as well as urinary transferrin (Trf) and Immunoglobulin G (IgG) excretion in urine is often observed in impaired kidney filtration due to multiple complications. In the present study, a positive correlation was found between albuminuria and IgG ($r^2 = 0.651$, $r = 0.807$ $P<0.01$) & albuminuria and transferrinuria ($r^2 = 0.073$, $r = 0.305$, $P < 0.01$), regression lines of correlations are showing in the fig no. 6 & 7.



(r^2 = 0.651, r =0.807 P<0.01)

Fig. 6: Relationship between urinary albumin and IgG in diabetic glomerulopathy



(r^2 = 0.073, r = 0.305, P < 0.01)

Fig. 7: Relationship between urinary albumin and IgG in diabetic glomerulopathy

Correlation of Urinary Markers: Using Pearson correlation methods by the help of SPSS version 20 software were found the correlation among all three urinary markers- urinary albumin, IgG and transfferin, correlation is significant at the 0.01 level (2tailed, Table6). Alb is independent variable and IgG and Trf using as dependent variables (Table7 & Table8).

Table 6: Correlations of urinary markers (Albumin, IgG, and Transferrin)

		alb	IgG	Trf
alb	Pearson Correlation	1	.807**	.305**
	Sig. (2-tailed)		.000	.000
	N	400	400	400
IgG	Pearson Correlation	.807**	1	.396**
	Sig. (2-tailed)	.000		.000
	N	400	400	400
Trf	Pearson Correlation	.305**	.396**	1
	Sig. (2-tailed)	.000	.000	
	N	400	400	400

**. Correlation is significant at the 0.01 level (2-tailed).

Table 7: Model Summary and Parameter Estimates

Dependent Variable: IgG

Equation	Model Summary					Parameter Estimates	
	r^2	F	df1	df2	Sig.	Constant	b1
Linear	.651	741.628	1	398	.000	-.623	.830

The independent variable is alb.

Table 8: Model Summary and Parameter Estimates

Dependent Variable: Trf

Equation	Model Summary					Parameter Estimates	
	r^2	F	df1	df2	Sig.	Constant	b1
Linear	.073	31.514	1	398	.000	1.707	.149

The independent variable is Alb.

Creatinine excretion in urine was reported positive correlation with albumin, IgG and Transferrin: Crt & Alb (r^2 = 0.561), Crt & IgG (r^2 = 0.205), Crt & Trf (r^2 = 0.142) respectively. All correlations were found positively significant at the 0.01 level in all respect (P<0.01) (Table: 9,10,11,12 & Fig. 8,9,10).

Table 9: Correlation of Crt to U. Markers Alb, IgG, and Trf:

		Alb	IgG	Trf	Crt
Alb	Pearson Correlation	1	.243**	.244**	.749**
	Sig. (2-tailed)		.000	.000	.000
	N	400	400	400	400
IgG	Pearson Correlation	.243**	1	.411**	.271**
	Sig. (2-tailed)	.000		.000	.000
	N	400	400	400	400
Trf	Pearson Correlation	.244**	.411**	1	.237**
	Sig. (2-tailed)	.000	.000		.000
	N	400	400	400	400
Crt	Pearson Correlation	.749**	.271**	.237**	1
	Sig. (2-tailed)	.000	.000	.000	
	N	400	400	400	400

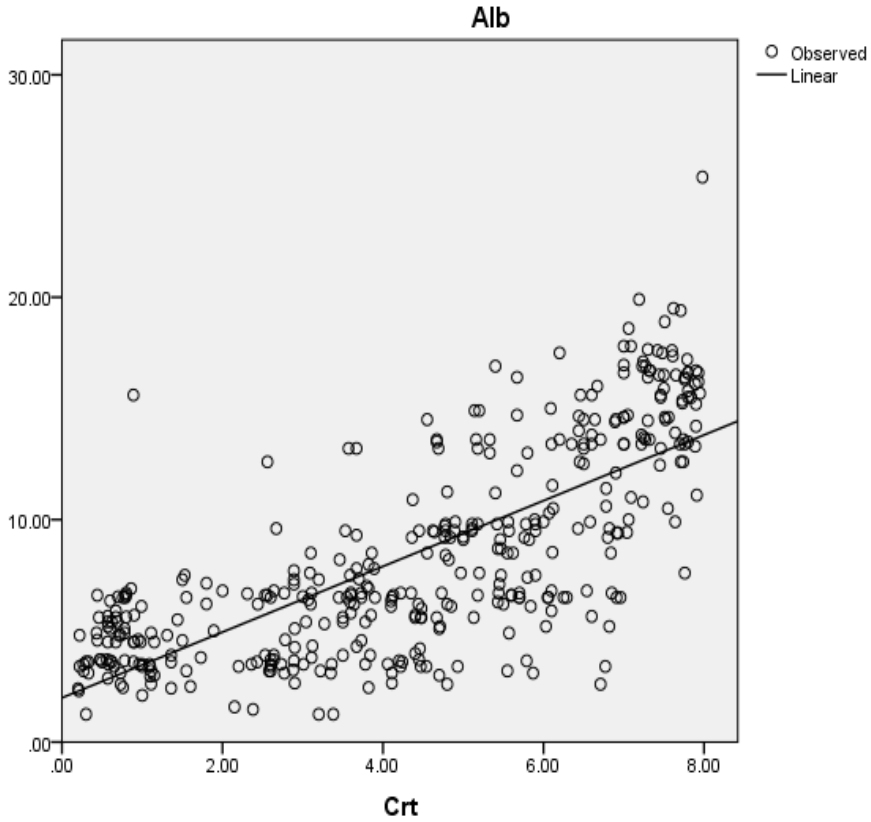
Correlation is significant at the 0.01 level (2-tailed)

Table 10: Model Summary and Parameter Estimates

Dependent Variable: Alb

Equation	Model Summary					Parameter Estimates	
	r^2	F	df1	df2	Sig.	Constant	b1
Linear	.561	509.056	1	398	.000	1.990	1.477

The Independent variable is Crt.



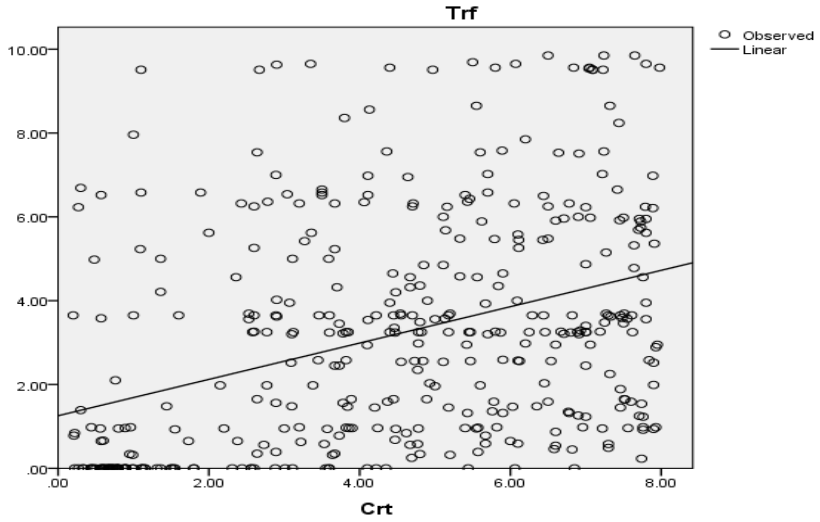
(r^2 = 0.561, r = 0.749, P<0.01)

Fig. 8: Relationship between urinary albumin and U.Creatinine in diabetic glomerulopathy

Table 11: Model Summary and Parameter Estimates

Dependent Variable: Trf

Equation	Model Summary					Parameter Estimates	
	r^2	F	df1	df2	Sig.	Constant	b1
Linear	.142	65.743	1	398	.000	1.255	.434



(r^2 = 0.142, r = 0.237, P<0.01)

Fig. 9: Relationship between urinary Crt. and urinary Trf. in diabetic glomerulopathy

Table 12: Model Summary and Parameter Estimates

Dependent Variable: IgG

Equation	Model Summary					Parameter Estimates	
	r^2	F	df1	df2	Sig.	Constant	b1
Linear	.205	102.848	1	398	.000	2.346	.930

The independent variable is Crt.

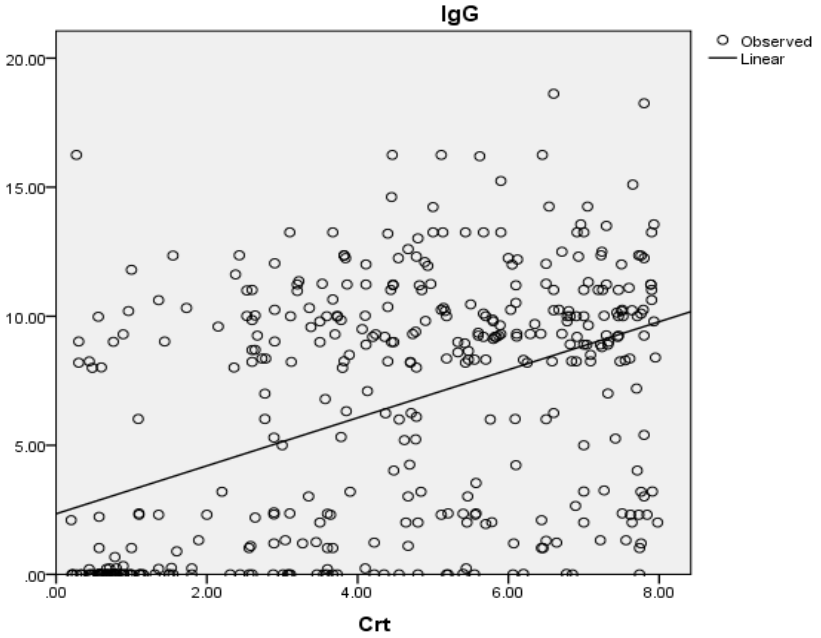


Fig. 10: Relationship between urinary Crt. and urinary IgG in diabetic glomerulopathy

DISCUSSION

400 micro albuminicpatients were selected randomly for test group, belonging to various age, and sex, micro albuminuria is the presence of albumin in urine above the normal level but below the detectable range of conventional urine dipstick methods. According to Moresco et. al. diabetic glomerulopathy is defined as a rise in the urinary albumin excretion (UAE) rate and abnormal renal function agreed with our findings.[17] Recently, changes in albuminuria are considered a hallmark of onset of progression of diabetic glomerupathy. The higher number of males and females were suffering from last five years and last ten years respectively numerous author agree with us.

Distribution of diabetics with kidney mall function with reference to urine creatinine levels was found 77.75 % in hyper group having value more than 6.3-23 mmol/24hrs.

Diabetes is a metabolic disorder of multiple chronic hyperglycemias with disturbances in carbohydrate, fat and protein metabolism that results in abnormality in insulin secretion, insulin action or both. It is a life style disorder and usually occurs after the age of 40 years which is also being shown during our investigation.

Current clinical markers of renal function include urine creatinine and urinary albumin excretion. Serum cystatin C is proposed as a superior biomarkers than serum creatinine of renal function identified by various others. During our investigation we found that 77.75 % diabetic subjects were suffering from high urine creatinine levels.

Creatnine and albumin were found positively correlated ($P<0.01$) in diabetic complicated subjects. Anoop et al agreed with our estimations they found that higher amounts of protein were excreted in the urine of diabetic patients, which was found to be statistically highly significant ($p< 0.001$). The elevated values of protein: Creatinine index (PCI) in diabetic patients were found to be statistically highly significant ($p< 0.001$)[18].

CONCLUSIONS

Uncontrolled hyperglycemic condition in DMT–II subjects may cause to develop complications associated with diabetes, other complications were reported under the age group of 5 to 10 years of duration of disease history. Most of the subjects were found under 7 to 10 years duration of disease case history, further, under such groups, urinary markers and creatin in eare positively correlated significantly at the 0.01 level. Excretion of

urinary markers in different stage and different part of duration of disease history gives valuable information about prognostic state and severity of disease. These urinary markers may use to diagnose the disease at earliest stage for better and effective treatment.

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