RESEARCH ARTICLE

SERUM IMMUNOGLOBULIN G (IgG) AS A PREDICTIVE MARKER OF EARLY RENAL AFFECTION IN TYPE 2 DIABETIC PATIENTS.

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Manuscript Info	*Abstract*

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Background:- Diabetic nephropathy (DN) is one of the most common diabetic microvascular complications. Albuminuria is considered the gold standard of onset or progression of DN. However albuminuria has certain limitations, clinical practice suggested detectable albuminuria is later than the onset of DN. Thus, the request for more reliable highly sensitive and specific biomarkers is needed for early prediction the onset and progression of DN, which is important to slow down or prevent the renal function decline in diabetic patients. Immunoglobulin G (IgG) is a high molecular weight protein that has been proposed to be a marker for DN and is freely filtered by the glomeruli and completely catabolized in the proximal tubules. **In this study we aimed to** evaluate serum IgG as a marker of early renal affection in type 2 diabetic patients.

Subjects and methods:- This study was performed on 52 type 2 diabetic patients and were compared with 26 healthy subjects as a control group, they were classified as the following; **Group** I: Including 26 healthy subjects ,**Group** II: Including 26 type 2 diabetic patients without diabetic nephropathy and **Group** III: Including 26 type 2 diabetic patients with diabetic nephropathy. All studied patients were subjected to complete medical history and detailed clinical examination, routine laboratory tests including fasting blood glucose(FBS),2-h postprandial blood glucose (PPS) and glycosylated hemoglobin (HbA1c %), serum creatinine, urinary creatinine, urinary albumin , urine albumin/creatinine ratio, measurement of creatinine clearance ,blood urea and serum IgG.

Results:- Serum IgG increased significantly in diabetic patients without nephropathy compared to control group and diabetic patients with nephropathy and significantly increased in diabetic patients with nephropathy compared to control group (P <0.001).Also there was significant correlation between serum IgG and duration of treatment ,FBS,PPS,HBA1C, serum creatinine, urine albumin , urine creatinine , urine albumin / creatinine ratio and creatinine clearance (P<.001).

Conclusion:- Serum IgG may be useful in detecting early renal impairment in type 2 diabetes mellitus (DM).

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Introduction:-

Diabetes mellitus is a chronic disease that affects 366 million people worldwide (6.4% of the adult population) and is expected to rise to 552 million by 2030 **(1).** Diabetic nephropathy occurs in approximately one-third of all people with diabetes and is the leading cause of renal failure in developed and developing countries (**2).**Clinically, the first sign of diabetic nephropathy is considered to be microalbuminuria. As the disease progresses, patients develop macroalbuminuria, and the kidney function declines until patients end up requiring renal replacement therapy **(3).**Although microalbuminuria in diabetic patients is considered to be the best predictor of progression to end-stage renal disease and cardiovascular events ; earlier, more sensitive and specific markers of kidney damage might help diagnose and treat diabetic nephropathy at an earlier stage to prevent the progression to renal failure**(4-6).** ImmunoglobulinG (IgG) is a protein synthesized and secreted by plasma cells that is mainly involved in the secondary immune response, with a molecular weight of 150 kDa **(7,8).** Total urinary IgG excretion is higher in diabetic patients compared to controls, even before they develop microalbuminuria(**6**). Serum IgG and urinary IgG show a significant increase in diabetic patients without complications and increase in diabetic nephropathy patients in comparison to control. Serum IgG may play an important role in host defence mechanism in diabetic patients and may be a good marker of renal injury because it combines information on level of protinuria and loss of size selectivity (**9). In this study we aimed to** evaluate serum IgG as a marker of early renal affection in type 2 diabetic patients.

Subjects and Methods:-

Before selection of the subjects, the individuals were subjected to full clinical and laboratory examination to exclude the factors that affect the parameters of our study. *Exclusion criteria* included: Ages <30 or >70, Patients with type 1 diabetes millets, Pregnant and lactating females, Patients with any congenital diseases ,Infection ,Smokers ,alcoholics and drug abusers.The studied subjects were categorized into 3 groups as follow: *Group I*: Including 26 healthy subjects matched with age and sex of patients in our study (*control group*), *Group II*: Including of 26 type 2 diabetic patients without diabetic nephropathy and *Group III*: Including 26 type 2 diabetic patients with diabetic nephropathy . Informed consent was obtained from all participitants in our studied groups and subjected to full history and clinical examination, Body mass index (BMI) which was calculated as weight (kg) / Height (m2) , laboratory investigations including renal function tests, liver function tests, complete blood picture ,urine analysis ,serum IgG, fasting and 2-hours postprandial blood glucose, glycohemoglobin (HbA1c) in the blood, serum and urinary creatinine, Blood urea , urinary albumin , urine albumin/creatinine ratio and glomerular filtration rate using creatinine clearance . 5 ml of peripheral venous blood were taken from each subject under complete aseptic conditions and were divided into 3 portions.1 ml collected on fluoride oxalate(2:1) 2mg/ml for estimation of plasma glucose (fasting & 2 hr postprandial).1 ml collected with potassium EDTA 1mg/ml for measurement for glycosylated hemoglobin ((HbA1c).3 ml were left for 30-60 minutes for spontaneous clotting then centrifuged at 3000 rpm for 10 minutes, serum samples were separated into another set of tubes and kept frozen at -80°C till use. Morning urine sample (60 ml) was collected in a relabeled sterilized bottle for estimation of creatinine and albumin. 24 hours urine were collected for detection of albumin. The IgG test is based upon the reactions between IgG and latex-covalently bound antibodies against human. IgG values are determined turbidmetrically using fixed-time measurement with sample blank correction. The relationship between absorbance and concentration permits a multipoint calibration with a measuring range between 0-10 mg/l. The measuring temperature is 37°C. **STATISTICAL ANALYSIS** was performed using a computer based program (SPSS, version 11) the data are presented as mean ± standard deviation. Continuous data were compared with a two-tailed unpaired *t* test. The strength of the link between the Continuous variables was tested by Pearson correlation coefficient *r*. *P* value less than 0.05 indicated statistical significance.

Results:-

Table 1:- Comparison of the mean values of demographic parameters among different groups.

	Group I(n=26)			Group II(n=26)			Group III(n=26)			F	P
Age(years)											
Mean ± SD	51.26	±	9.79	53.46	±	10.11	55.07	±	8.21	0.98	0.53
											(NS)
Range	45	-	63	45	-	62	48	-	63		
Gender											
Male	16	55%		17	55%		20	60%		X=3.6	0.57
Female	10	45%		9	44%		6	40%			(NS)
Duration of ttt(years)											
Mean ± SD	0	±	0	9.57	±	2.402	16.11	±	3.26	311.7	<0.001
											(HS)
Range	0	-	0	6	-	15	12	-	20		
BMI											<0.001
Mean ± SD	24.26	±	2.8	25.57	±	2.5	28.25	±	2.1	17.28	(HS)
Range	20	-	28	21	-	28	21	-	28		
SBP(mmHg)											<0.001
Mean ± SD	113.1	±	67.86	125.96	±	8.07	140.92	±	31.73	4.76	(HS)
Range	115	-	125	115	-	135	115	-	140		
DBP(mmHg)											<0.001
Mean ± SD	77.3	±	4.74	80.18	±	64	93.22	±	25	7.2	(HS)
Range	70	-	85	64	-	85	70	-	85		

Duration of treatment (ttt),body mass index (BMI) , systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Table 2:- Comparison of the mean values ± SD of Fasting blood sugar (FBS) (mg/dl) and Post prandial blood sugar (PPBS) (mg/dl) ,glycosylated hemoglobin (HbA1c%)and serum ImmunoglobulinG (IgG) among different groups.

	Group I(n=26)			Group II(n=26)			Group III(n=26)			F	P
FBS(mg/dl)											
Mean ± SD	89.69	±	10.54	137.88	±	37.6	222.8	±	26.8	157.9	<0.001
											(HS)
Range	75	-	100	100	-	250	150	-	240		
PPBS(mg/dl)										125.1	<0.001
Mean ± SD	120.07	±	12.9	227.7	±	70	348.3	±	55.3		
Range	100	-	135	135	-	300	165	-	395		(HS)
HbA1c%											<0.001
Mean ± SD	4.32	±	1.12	7.21	±	0.65	9.6	±	2.57	65.45	(HS)
Range	0	-	5.5	6	-	8	9	-	11		
Serum IgG(mg/dl)											<0.001
Mean ± SD	841.76	±	129.23	1495.42	±	241.04	1222.73	±	248.88	61.48	(HS)
Range	710	-	1119	1005	-	1844	700	-	1600		

Table 3:- Comparison of the mean values ± SD of serum creatinine (mg/dl),creatinine clearance (ml / min / 1.73 m2) and urine albumin (mg/24H), urine creatinine (g/l) ,Urine albumin (alb.) / creatinine Ratio (mg/g) and creatinine clearance among different groups .

	Group I(n=26)			Group II(n=26)			Group III(n=26)			F	P
Serum creatinine(mg/dl)											
Mean ± SD	0.88	±	0.17	0.98	±	0.21	2.77	±	1.26	52	**<0.001**
Range	0.6	-	1.1	0.6	-	1.2	1.5	-	4		**(HS)**
Urine albumin(mg/24h)											**<0.001**
Mean ± SD	12.75	±	4.92	14.57	±	3.51	1732.11	±	908.2	93	
Range	1	-	17	8	-	19	500	-	2700		**(HS)**
Urine creatinine(g/l)											
Mean ± SD	1.56	±	2.2	1.6 3	±	0.16	2.47	±	2.97	3	**<0.001**
Range	0	-	9	0.7	-	1.2	0.6	-	0.8		**(HS)**
Urine Alb/creatinine ratio(mg/g)											**<0.001**
Mean ± SD	12.96	±	4.54	14.6	±	0.9	2923.43	±	344.85	71	
Range	1	-	18	7.5	-	19	625	-	4500		**(HS)**
Creatinine clearance(ml/min)											
Mean ± SD	111.25	±	28.07	116.38	±	16.35	73.53	±	36.69	18	**<0.001**
Range	0.5	-	135	94	-	135	25	-	85		**(HS)**

Figure 1:- Comparison of mean value for serum IgG (mg/dl) in all studied groups.
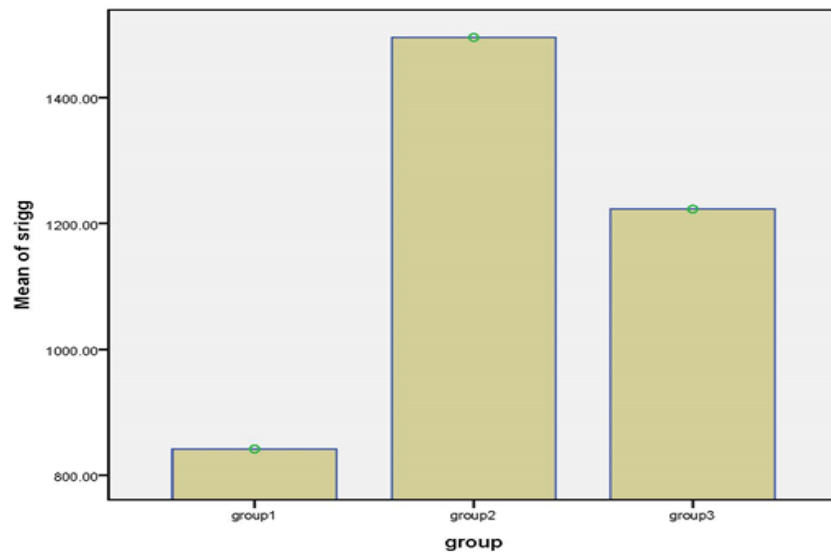
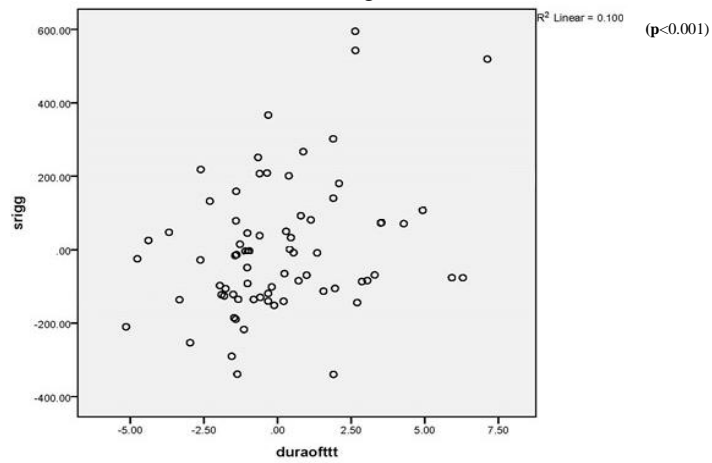**Figure 2:-** Correlation between serum IgG and duration of treatment.

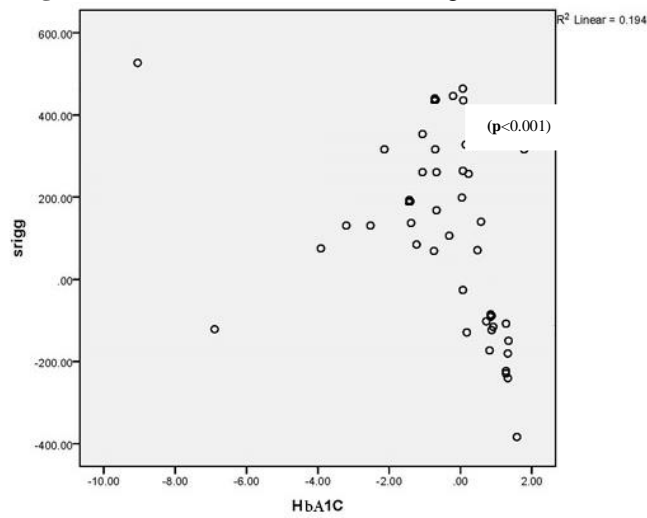**Figure 3:-** Correlation between serum IgG and HbA1C.

Figure 4:- Correlation between serum IgG and serum creatinine.
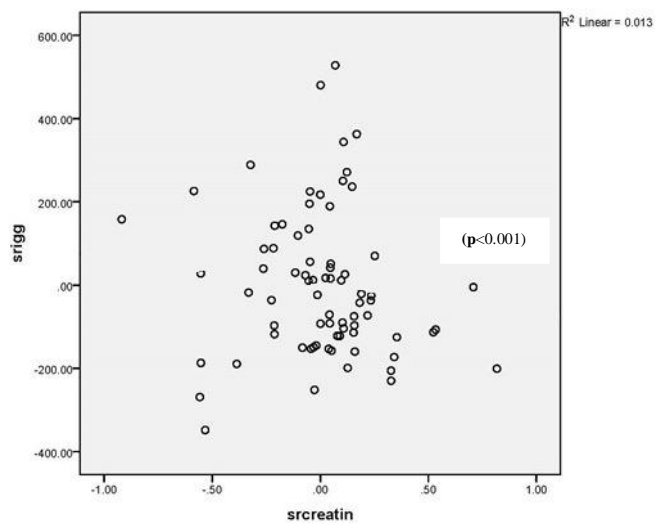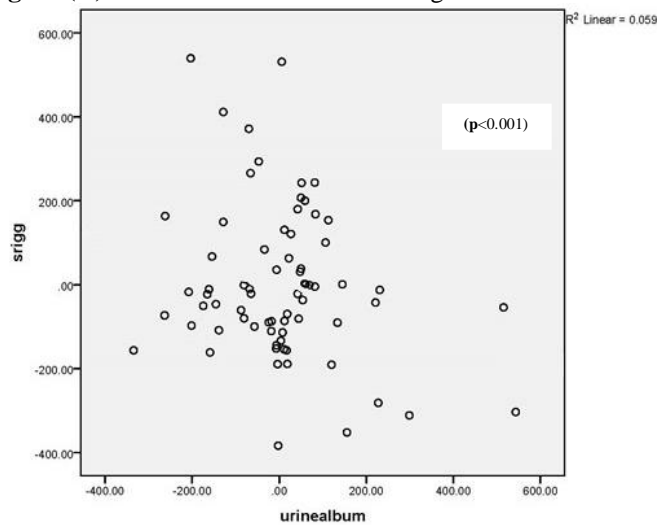
Figure (5) : Correlation between serum IgG and urine albumin

In our study there was no significant differences among the studied groups as regard age , gender (P>0.05) ,while there was significant differences in duration of treatment, BMI, systolic blood pressure (SBP) and, diastolic blood pressure (DBP) (P<0.001) as   illustrated in *(Table I).*

The comparison of the mean values of fasting blood sugar (FBS) (mg / dl) and post prandial blood sugar (PPBS) (mg / dl) , glycosylated hemoglobin (HbA1c%) and serum immunoglobulin G (IgG) among different groups of our study showed there was high significant differences among studied groups as regard all these parameters (P<0.001) (*Table 2).*

In **Table 3**, comparison of the(mean values ± SD) of serum creatinine (mg/dl) , creatinine clearance (ml / min / 1.73 m2) and urine albumin (mg/24H), urine creatinine (g/l) and urine albumin / creatinine Ratio (mg/g) among different groups of the study showed that there was high significant differences among studied groups as regard all these parameters.

(Figure 1) showed high significant increase in serum IgG in group II more than (group I and group III) and significant increase in group III more than group I (p<0.001).

The figures from **2 to 5** show significant correlations between serum IgG and {duration of treatment , glycosylated hemoglobin (HbA1C), serum creatinine and urine albumin } (P<.001).

Discussion:-
Diabetic nephropathy is a severe complication occurring in diabetic patients and it is associated with an increased risk of all-cause mortality, cardiovascular disease and progression to end stage renal disease (ESRD), requiring costly renal replacement therapy in the form of dialysis or transplantation. Diagnostic marker to detect DN at early stage is important as early intervention can slow the loss of kidney function and reduce adverse outcomes(*10-12*)*.* Microalbuminuria has been accepted as the earliest marker for development of DN, However albuminuria has several confounding issues associated with it such as exercise, urinary tract infection, acute illness and cardiac failure, Furthermore, it has been reported to occur in the urine of non-diabetic(*13*)*.*The interest for the use of biomarkers for early DN derives from the observation that patients with type 2 diabetes pass through a period of pre-diabetes and may experience renal impairment at the time of diagnosis , Thus it is necessary to implement different strategies for   detecting early DN in patients with type 2 diabetes aiming to delay its progression and improve outcomes(*14*).Serum creatinine is considered specific but not very sensitive as its level does not significantly increase until the glomerular filtration rate (GFR) is reduced to less than 50% of normal , In addition, serum creatinine concentration is significantly affected by many factors: age, sex, muscle mass, dietary intake, changes in tubular secretion and various drugs as well as endogenous substances that interfere with its assay(*15*).Ig G is a high molecular weight protein produced by plasma cells, with a stable production rate, It is filtered by the glomerulus and catabolized primarily by proximal tubular cells (**9**)**.**

In our study we found a significant difference regarding the duration of DM between studied groups, where the patients with diabetic nephropathy (**group III**) showed longer duration of DM (16.11 ± 3.2)years than patients with type 2 diabetes without complications (**group II**) (9.57 ± 2.4)years.The assessment of glycemic state was accomplished during this study by measuring fasting blood sugar (FBS), 2h-Post prandial blood sugar (PPS) and glycosylated hemoglobin(HbA1c%) ,Diabetic patients in **group III** as regard FBS , 2h-PP and HbA1c,had significantly higher levels (222.80 ± 26.80, 348 ± 55.3, 9.60 ± 2.5) in respectively more than **group II** (137.88 ± 37.6, 227.6 ± 70, 7.21 ± 0.65) and more than control group (89.69 ± 10.5,120.07 ± 12.9, 4.32 ± 1.12) reflecting a poor glycemic control. These results are consistent with the general consensus that microvascular and macrovascular complications of DM are more evident as the disease progress. The aforementioned findings go in agreement with those of *Girach et al.,* who concluded that the strongest risk factors for diabetic microvascular complications are glycemic control and diabetes duration; however, other modifiable risk factors such as hypertension, hyperlipidemia, smoking, and unmodifiable risk factors including age at onset of diabetes and genetic factors may play a part (**16**). In the same context *Vaag* reported that improving glycemic control in patient with type 2 DM may be as important as or even more important than treating hypertension and dyslipidemia for prevention of both microvascular and macrovascular complications, particularly when aggressive treatment is initiated at an early stage of the disease(**17**). Moreover, *DeVriese et al.,* established that hyperglycemia is a major risk factor for the development and progression of diabetic nephropathy as it induces multiple cellular and molecular alteration that presage the development of renal vascular dysfunction(**18**).

In the current study the serum IgG attained the highest level(1495.42 ± 241.04) in **group II** (patients with type 2 diabetis without complications) compared to any of the studied groups { the patients with diabetic nephropathy (**group III**) (1222.73 ± 248.8) and control group (**group I**) (841.76 ± 129.23)}. Going with our results Mistry and Kalia revealed that diabetic patients without nephropathy had the higher level of serum IgG (1168.4 ± 29.7) compared to diabetic patients with nephropathy(1041.2 ± 32.97) and healthy control group (772.10 ± 8.0) (**9**).In the same context *Yashima et al.,* founded that serum IgG was significantly increased in diabetic patients without any complications as compared with the healthy controls(**19**) . It is generally assumed that serum IgG may play an important role in the host defense mechanism in diabetic patients and also there was 52 % increase in IgG levels in diabetics compared to normal healthy individuals *(20).* Abnormal levels of Immunoglobulin are very common in diabetic patients and the probability that changes in Immunoglobulin levels are implicated in the pathogenesis of infection requires further exploration. Compared to diabetic patients without any complications, patients with diabetic nephropathy showed a marked depletion of serum IgG level, which could be attributed to their increased vascular clearance and to the extent of nephropathy. There was no significant increase in serum IgG level observed in non-diabetic nephropathy. Thus the increase in IgG level might be playing a role in diabetics as well as in pathogenesis of diabetic nephropathy (**21**).

In our study there is significant correlations between serum Immunoglobulin G (IgG) and urine albumin, these findings were going hand in hand with those reported by Yang *et al., (22).* Also there is significant correlations between serum IgG and HbA1C, FBS, PPBS, serum creatinin and duration of treatment ($p<.001$)

The results of the present study thus suggest that the serum Immunoglobulin G (IgG) might be more useful in detection of the onset and/or early stage of diabetic nephropathy, however, requires further detailed studies.

Conclusion:-
- Serum IgG levels are higher in diabetic patients with or without nephropathy than control healthy subjects.
- Serum IgG levels are correlated significantly to FBS, PPS and HbA1C.
- Serum IgG might have a role in pathogenesis of diabetic nephropathy so that may be used as an early marker for kidney affection.
- Further studies are recommended using large number of patients to confirm our results.

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