Type 2 Diabetic Nephropathy in Uncontrolled Patients Treated with Daonil® and Glucophage®

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Abstract

Type 2 diabetes mellitus is similar to type 1 and other forms of diabetes in that it is defined by high levels of plasma glucose and is associated with many long-term complications caused or enhanced by hyperglycemia and related metabolic abnormalities.

Levels of glucose,glycohemoglobin(HbA1c),insulin, insulin resistance, ,creatinine , urea, eGFR, microalbominurea ,β2-microglobulin concentraions in sera ,blood, and urine of 298 patients diabetes mellitus treated with daonil(149) , glucophage (149) and 83 apparently healthy who served as control.

The results showed a highly significant increase $p<0.001$ in glucose, HbA1c, insulin, insulin resistance , albumin , creatinine ,microalbominurea, concentrations in patient with diabetes mellitus treatment with daonil and glucophage (less and more than 5y) when compared to control group.

Also a highly significant increase $p<0.001$ of urea, eGFR, β2-microglobulin concentrations(more than 5y).β2-microglobulin concentrations a significant increase $p<0.05$ in the urine(≤ 5y).

الخلاصة

يشبه داء السكر من النوع الثاني داء السكر من النوع الاول بارتفاع مستويات الكلوكوز وترابطها مع مضاعفات طويلة الامد.في هذة الدراسة تم قياس مستوبات الكلوكوز والHbA1c والانسولين ومقاومو الانسولين والكرياتنين واليوريا وسرعة ترشيح الكبيبات والالبومين المايكروي البولي في امصال وبول ٢٨٩ مريض مصاب بالسكر ويتعالج بالداؤنيل (١٤٩) والكلوكوفيج(١٤٩) و(٨٣) شخصا سليما كمجوعة سيطرة.

اظهرت النتائج ارتفاعا معنويا بالكلوكوز والـ HbA1c والانسولين ومقاومة الانسولين والالبومين والكرياتنين والالبومين المايكروي البولي عند مرضى السكر(المعالجين لمدة اقل واكثر من ٥ سنوات) عند المقارنة بجموعة السيطرة.كما اظهرت النتائج ارتفاعا معنويا في واليوريا وسرعة ترشيح الكبيبات والالبومين المايكروي البولي−β2 والذي ارتفع مستواه في البول ايضا.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by high levels of blood glucose resulting from defects in insulin secretion, insulin action, or both. It is by far the most commonly occurring disorder of the endocrine system in all populations and in all age groups. Insulin is a hormone produced in the pancreas that helps transport glucose (blood sugar) from the bloodstream into the cells so they can break it down and use it for fuel. People cannot live without insulin[1].

Diabetes results in abnormal levels of glucose in the blood stream. This can be cause severe short-term and long term consequences ranging from brain damage to amputations and heart disease [2].Classic signs and symptoms of diabetes include polyuria, polydipsia, polyphagia, weight loss, headache, tachycardia, palpitations, and blurred vision [2].The incidence of diabetes has soared worldwide in recent years and is expected to keep

growing, with the greatest increase seen in metabolic forms of diabetes, notably type 2.The estimated number of people with diabetes has jumped from 30 million in 1985 to 150 million in 2000 and then to 246 million in 2007, according to the International Diabetes Federation. It expects this number to hit 380 million by 2025[4]

In the year 2000 the worldwide prevalence of type 2 diabetes was estimated to be 150 million people and it is expected to increase to 220 million by 2010 [5].In the U.S. the prevalence is calculated to be approximately 16 million people with type 2 diabetes and an additional 30-40 million with impaired glucose tolerance [6]. Type 2 diabetes has a stronger genetic component than type 1, with a concordance rate of up to 90% in identical twins [7]. While over 250 genes have been tested for possible relationships with type 2 diabetes, none has shown consistent associations in multiple study populations [8]. In addition to genetic risk factors for type 2 diabetes, acquired or environmental factors play a major role; foremost among these is obesity.

Type 2 diabetes used to be called adult-onset diabetes and non-insulin-dependent diabetes mellitus (NIDDM). Those terms are not accurate because children can also develop this disease, and some patients require insulin therapy[9].In addition to high levels of glucose in plasma, type 2 diabetes is characterized by high levels of free fatty acids (FFAs) and abnormal lipoprotein patterns, as well as by changes in various hormonal and neural regulatory mechanisms affecting all tissues of the body . Excess body fat underlies 64% of cases of diabetes in men and 77% of cases in women[10].

Epidemiologic studies in persons without diabetes or with prediabetes show a correlation between plasma insulin levels and cardiovascular risk[11].

Complications of diabetes are due to pathologic changes that involve and large blood vessels, cranial and peripheral nerves, the skin, and the lens of the eye. Macrovascular complications involve damage to the large blood vessels of the brain, heart, and extremities[12]. Microvascular complications of diabetes include retinopathy, nephropathy and neuropathy are thought to be a result of an abnormal thickening of the basement membrane of the capillaries [13].

Diabetic nephropathy is a serious microvascular complication of diabetes. Diabetic nephropathy is the most common cause of end stage renal disease and constitutes approximately 40% of all patients needing renal replacement therapy [14]. Type 2 diabetes is the largest and fastest growing disease which is in need of renal replacement therapy. The risk factors for nephropathy are older age, male sex, non-Caucasian race, and poor blood pressure, glycemic, and lipid control [15].

The first sign of diabetic nephropathy is usually albuminuria and the first symptom peripheral oedema. The grade of decline in GFR in the natural history of diabetic nephropathy has been found to be highly variable (2-20 ml/min/year) with a mean of 12 ml/min/year [15].

Diabetic nephropathy is characterised by changes in the glomerulus filtration rate, expansion of extracellular matrix in the mesangial part, i.e. in the central part of the glomerulus, glomeruluar capillary crowding and overt renal occlusion leading to kidney failure [16].The clinical hallmark of diabetic nephropathy is persistent proteinuria greater than 500 mg/24 hours. This is equivalent to urinary albumin

excreation rate of 300 mg/day (or over 200 μg/min))in a person with diabetes, and without any other renal disease[17].

The current study will evaluate the role of duration in diabetes mellitus (type 2) patients in less and more than five years and investigate the complication of diabetes mellitus(nephropathy) in different treatment (daonil and glucophage) by determination the fasting glucose , Glycohemoglobin (HbA1c) the insulin and insulin resistance serum albumin ,creatinine in serum and urine, microalbumine,β2-Microglobuin,urea in serum and urine concentrations.

Materials and Methods

298 patients suffering from type 2 diabetes mellitus (150 male, and 148 female) aged between 25 to 67 years with a mean age of (45.11±12.03) were included in this study. The study was carried out from first of November 2009 to the day 31 of May 2010.The samples were obtained from Al-Kassim hospital and Merjan Teaching Hospital in Hilla city .The practical side of the study was performed at the laboratory of biochemistry department in College of Medicine /Babylon University.

The diabetic patients were diagnosed on the basis of WHO criteria. The general criteria for all subjects in this study include all patients not suffering from any disease(e.g. Hypertension, asthma ,smoker ,alcoholism, etc.) and not given any medication only treatment with daonil or glucophage, any subject that have not these criteria are excluded from this study.The study included three groups:

Group one: 149 patients (76 males, 73 females) with type 2 diabetes mellitus treatment with daonil .**Group two:** 149 patients (74males, 75 females) with type 2 diabetes mellitus treatment with glucophage .**Group three:** 83 (42 males, and 41females) apparently healthy subjects were chosen as healthy people, they were non smoker, alcohols, don't have any history of chronic diseases.

The study samples were serum, blood and urine , samples which should be absolutely free from haemolysis[18]were aspirated and divided into four parts, and stored at -20 ^{0}C until analysis. determined of glucose[19],HbA1c[21] ,insulin[20]resistance determined by HOMA SCORE [22], albumin[24], creatinine[20], β2-microglobulin[25]and urea by kits[26]microalbominurea by albumin/ creatinine ratio in urine[27]and eGFR by this equation eGFR = 186×[SCr]-1.54 × [age]-0.203× [0.742 if patients is female[28].Student's t-test was used to estimate differences between the groups .The differences were considered significant when the probability was ($p<0.05$) and highly significant at ($p<0.001$).

Results

1-Determination of fasting glucose concentration

The fasting glucose concentration(mmol/l) was determined in sera of control group and patients with DM (type 2).The figure 1- represents the results of glucose concentration in DM(type 2)patients treatment(≤5 y) with daonil 7.19 ± 0.72 and glucophage 7.16 ± 0.82 and compared with control groups 4.56 ± 0.62 mmol/l.

The test shows a highly significant increase $p<0.001$ in glucose concentration in DM patients compared with the healthy control group.Also a highly significant increase $p<0.001$ of glucose concentration in sera of DM patients treatment(6-15y) with daonil and glucophage compared with that of

healthy control group as shown in figure 2.No significant difference was found in the males and females of DM patients treatment with daonil and glucophage (≤5 y or 6-15 y) when compared with control group P>0.05 as shown in figure 1 and.2 respectively.

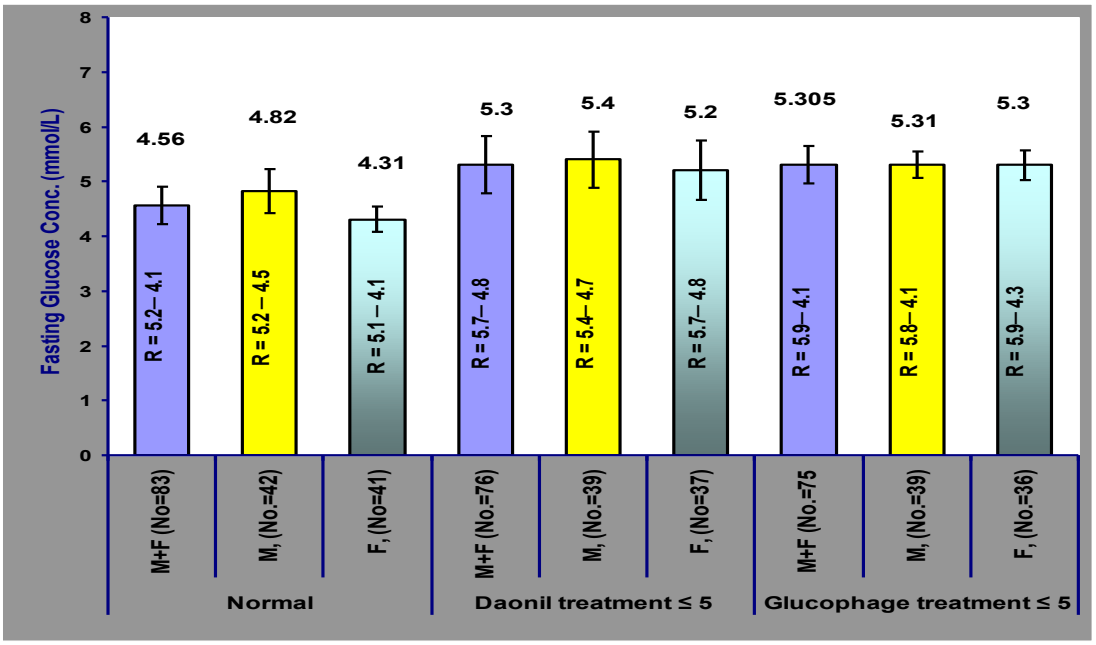

Figure1 Glucose conc.in sera of control and patients with DM(type 2) treated with daonil and glucophage ≤ 5 y.

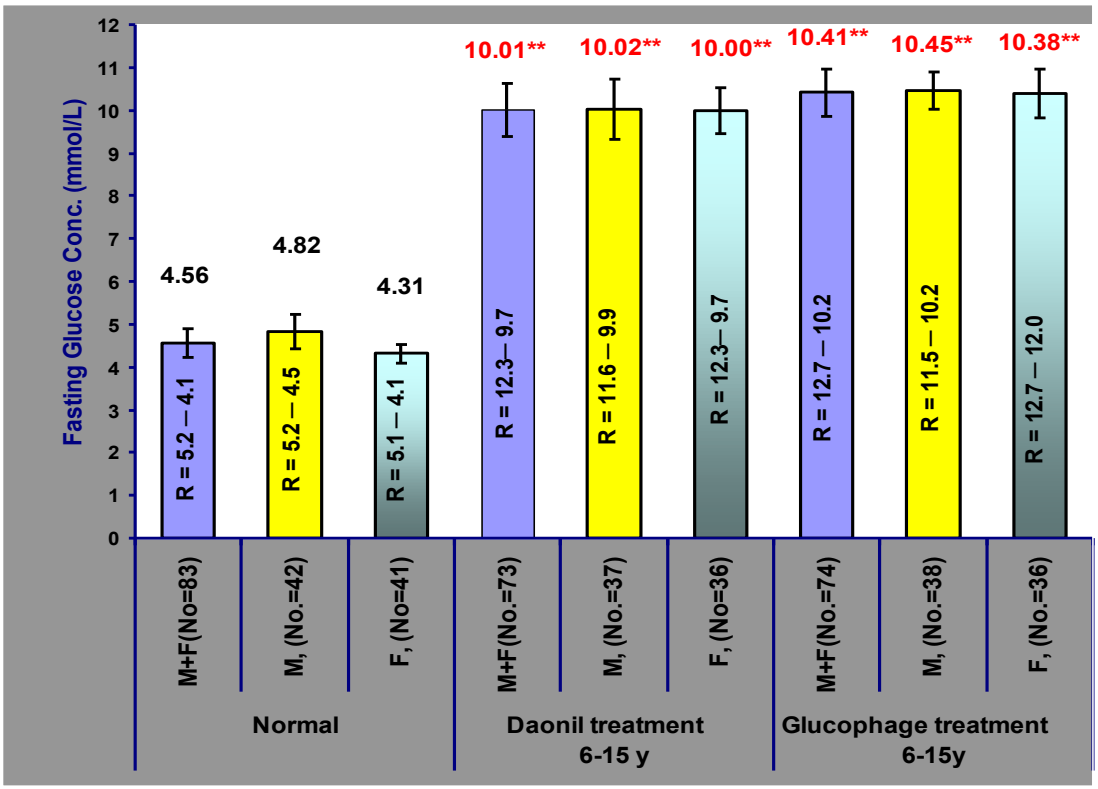

Figure 2 Glucose concentration in sera of control and patients with DM(type 2) treated with daonil and glucophage 6-15 y.

2-Determination of HbA1c

The results show a highly significant increase p<0.001 of HbA1c concentration in patients with DM(type 2) treated with daonil and glucophage (less than 5 y or 6-15y) when compared with that of control group as shown in figure 3 and 4.

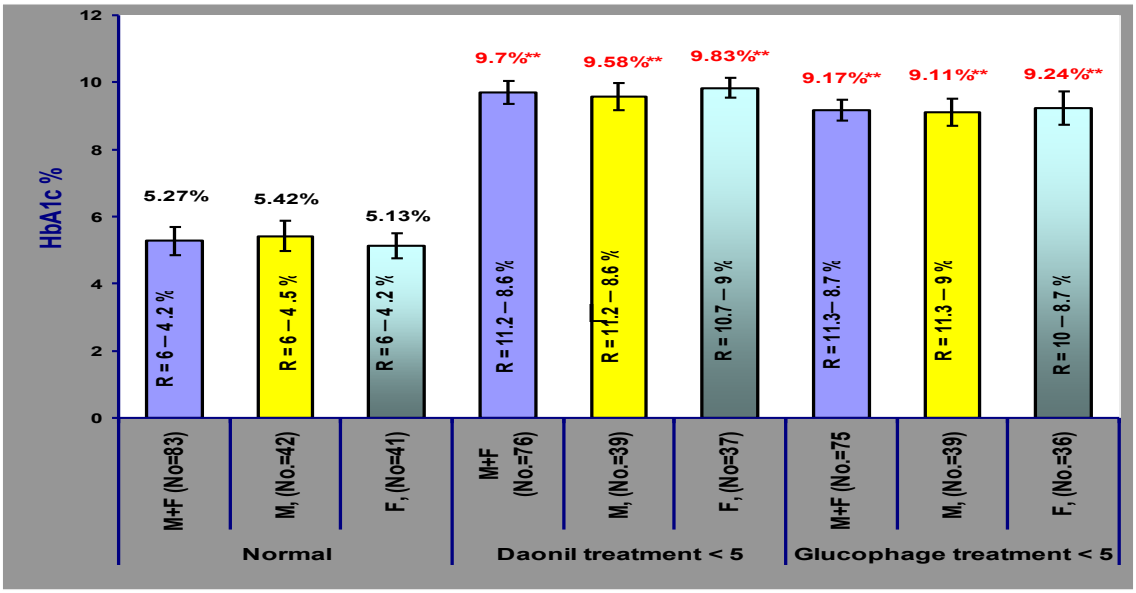

Figure 3 Glycohemoglobin (HbA1c) concentration in blood of control and patients with DM treated with daonil and glucophage≤ y

A highly significant increase p<0.001 was found in the HbA1c concentration in blood of patients with males and females of diabetes mellitus when compared with that of control group .

No significant difference was observed in HbA1c concentration in both male and females in patients with diabetes mellitus treatment with daonil and glucophage (≤5 y) when compared with that of more than five years as shown in figure 3.

Also a highly significant increase p<0.001 of HbA1c concentration in patients with diabetes mellitus(type 2) treatment with daonil and glucophage ≤5 y when compared with that of more than five years.

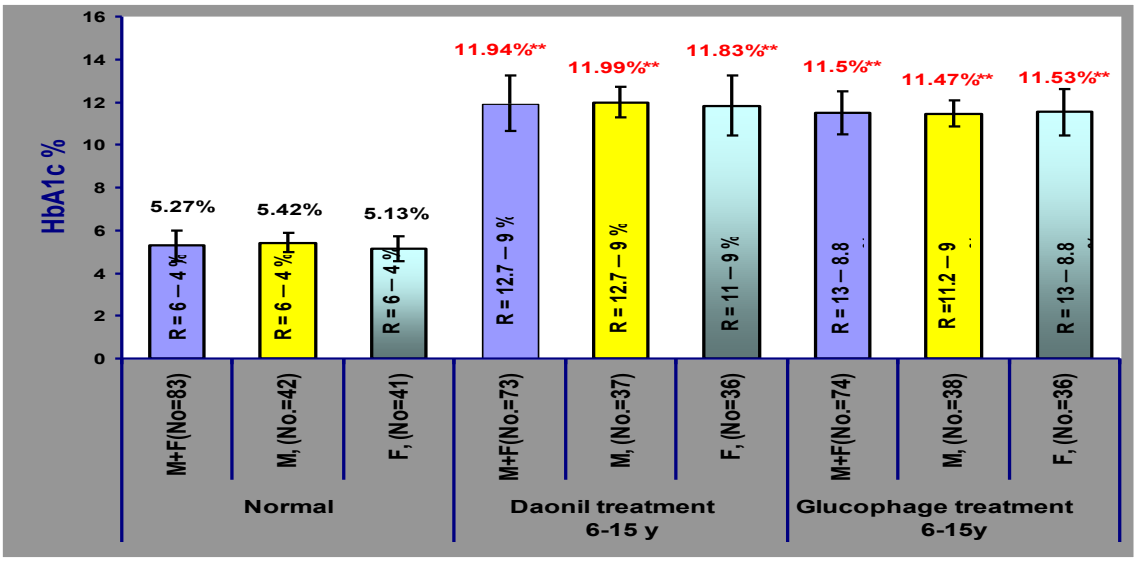

Figure 4 Glycohemoglobin (HbA1c) concentration in blood of control and patients with DM treated with daonil and glucophage 6-15 y

3-Determination of Fasting Insulin Concentration and Insulin Resistance

The results show a highly significant increase $p<0.001$ in insulin concentration in sera of DM groups compared to the control group. No significant difference $p>0.05$ was found in insulin concentration in both male and females in patients with DM treatment with daonil and glucophage (≤5y) when compared with that of more than five years or when compared with subgroups as shown in fig. 5 and 6.The results of our study have shown that there are a highly significant increase $p<0.001$ in patients with DM treatment with daonil and glucophage (≤5 y) when compared with that of more than five years as shown in figure 5 and 6.

The results of this study in table 1 shown increased in levels of IS_{HOMA} ,but not significant $p>0.05$ in patients with diabetes mellitus treatment with daonil and glucophage ≤5 y when compared with that of control and more than five years .Also not difference shows in males and females between the same groups.

The results in table 1 show a highly significant increase $p<0.001$ in levels of IS_{HOMA} in DM groups treatment more than 5 years when compared with those of the control group and ≤5years. Also a highly significant increase $p<0.001$ shows in males and females between the same groups.

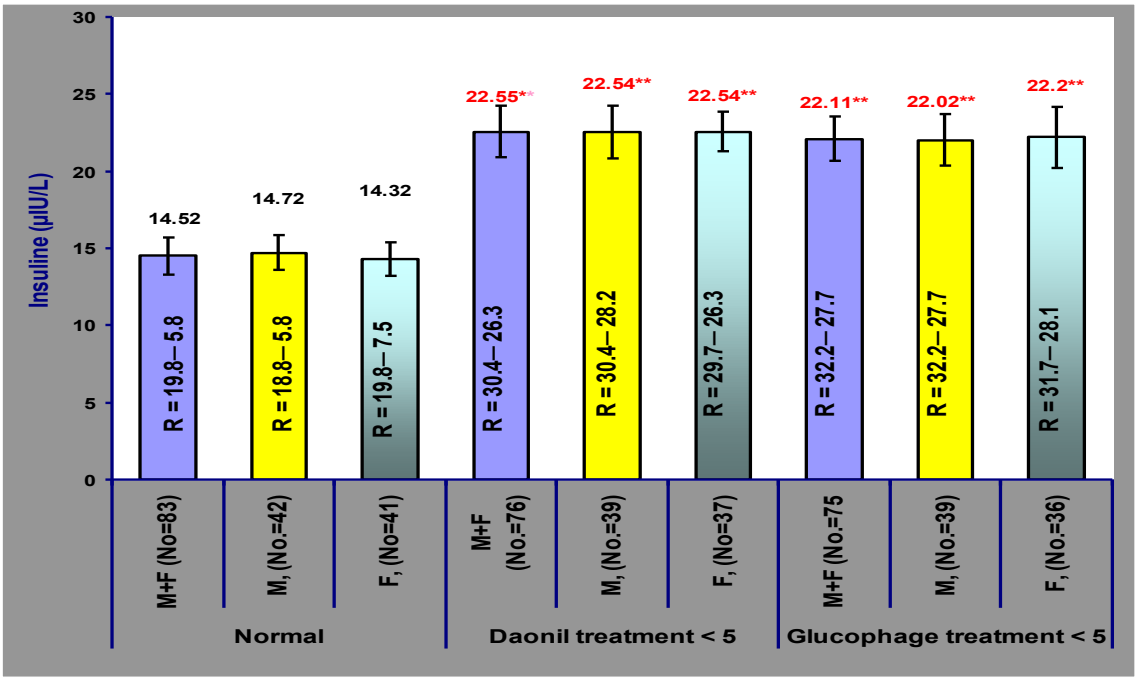

Figure 5 Fasting insulin concentration in blood of control and patients with diabetes mellitus treated with daonil and glucophage ≤ 5 y.

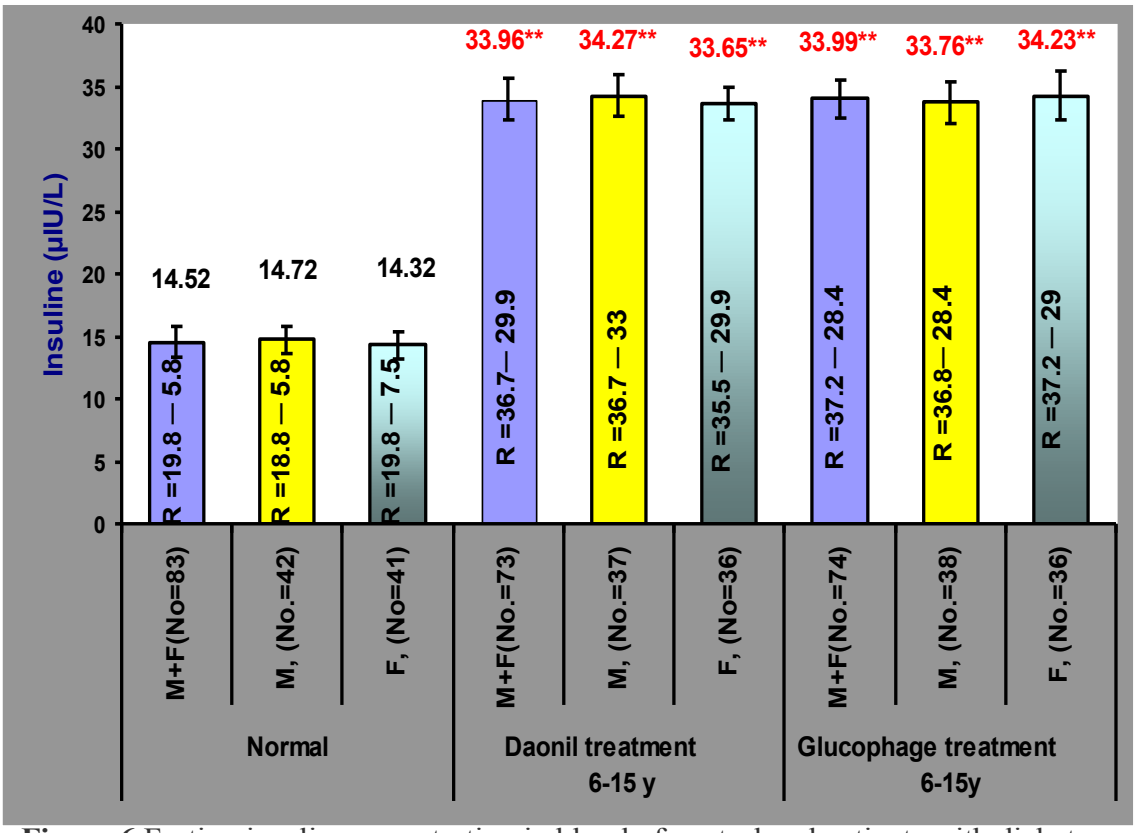

Figure 6 Fasting insulin concentration in blood of control and patients with diabetes mellitus treated with daonil and glucophage 6-15 y.

Table 1 Insulin resistance concentration in blood of control and patients with diabetes mellitus(type 2) treated with daonil and glucophage(A:≤5 treatment, B:6-15y treatment) (M=males, F=females, Mean Value± SD,**= p< 0.001

Parameter	Duration	Gender	No.	Mean Value ±SD	Range Value
Control					
insulin resistance		M+F	83	1920±14.24	2010-1867
DM Patients Treatments with Daonil					
insulin resistance	≤ 5 Y	M+F	76	2178**±12.43	2265-1847
	6 -15 Y	M+F	75	3258**±13.75	3295-2888
DM Patients Treatments with Glocophage					
insulin resistance	≤ 5 Y	M+F	76	2145**±11.33	2185-1955
	6 -15 Y	M+F	75	3301**±14.33	3392-3122

4-Determination of urea concentration:

It is clear from fig. 7 that sera urea concentrations in patient with DM treated with daonil and glucophage (≤ 5y) not apparently significant p>0.05 when compared with control group.

In our study we found that a highly significant increase p<0.001 in urea concentrations in DM patients more than five years when compared to control group,Also a highly significant increase p<0.001 of urea concentrations in sera of DM patients more than five years when compared to diabetes mellitus patients more than five years fig. 8

5-Determination of Estimation of Glomerular Filtration Rate (eGFR) (ml/min/1.73m2):

Figure 9 show that increased in estimation glomerular filtration rate (ml/min/1.73m2)of DM treated with daonil (92.3±6.7) and glucophage (91.4±10.2) ≤5 years when compared to control group(88.5±7.7) , but not significant p>0.05.

While the results show a highly significant decrease p<0.001 of estimation glomerular filtration rate in patients with DM treatment with daonil((59.15±9.5) and glucophage (60.75±10.2) more than 5 y when compared with that of control group (88.5±7.7)as shown in fig. 10 .

On the other hand no significant difference were found between males and females patients in subgroups,

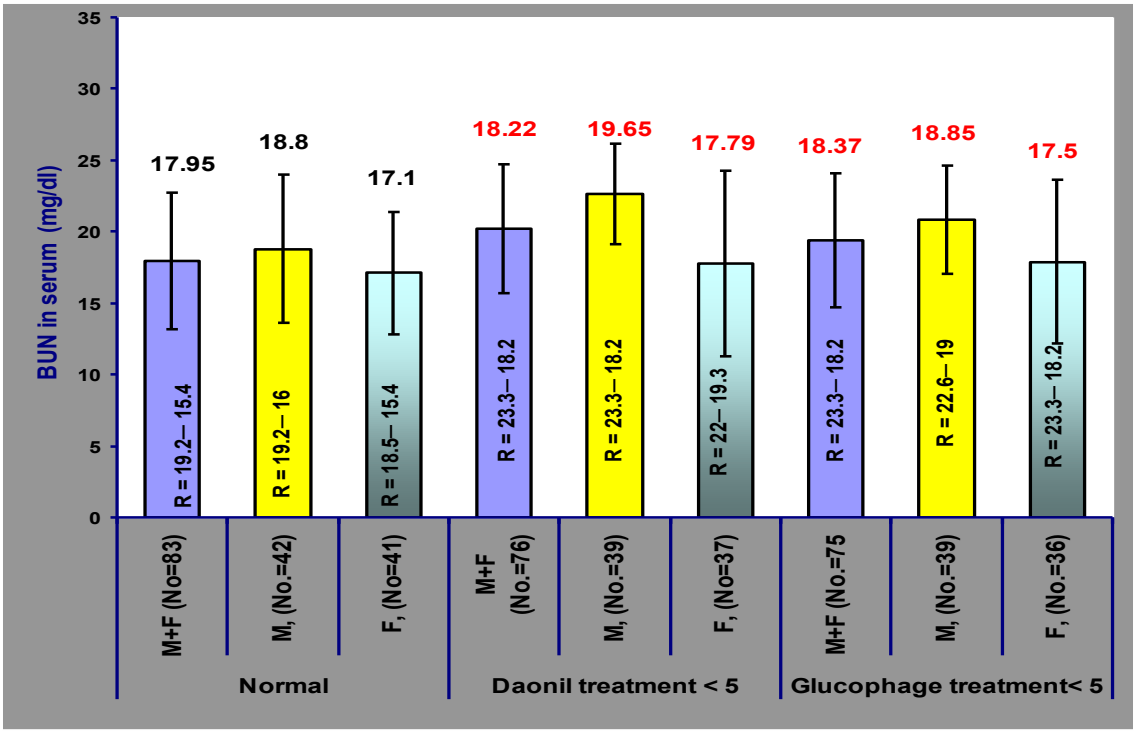

Figure 7 Urea concentration in sera of control and patients with DM(type 2) treated with daonil and glucophage ≤ 5y.

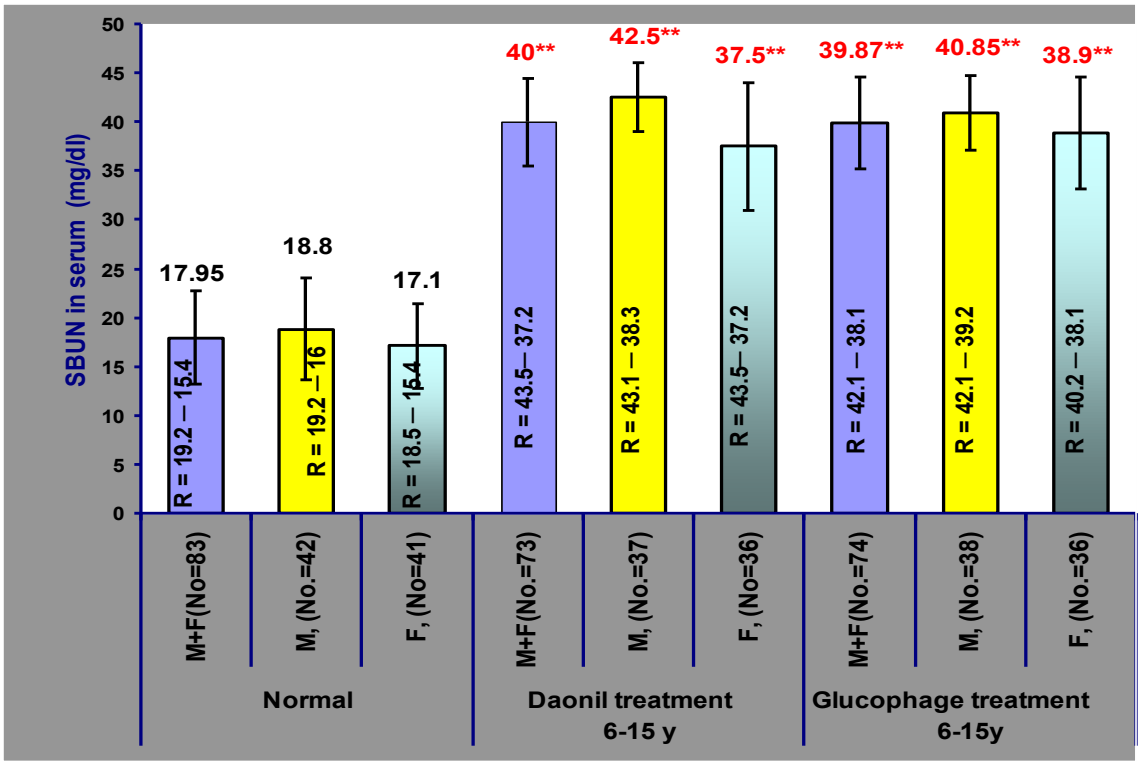

Figure 8 Urea concentration in sera of control and patients with DM(type 2) treated with daonil and glucophage 6-15y.

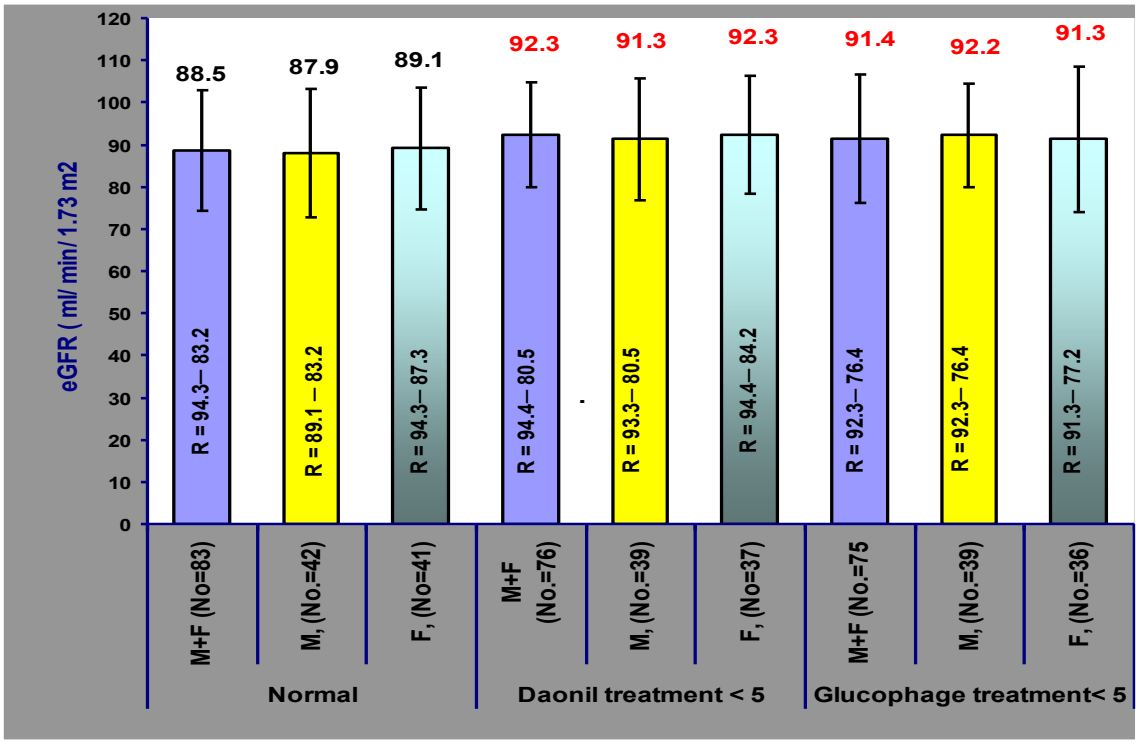

Figure 9 eGFR(ml/min/1.73m2) rate in sera of control and patients with DM(type 2) treated with daonil and glucophage ≤ 5y.

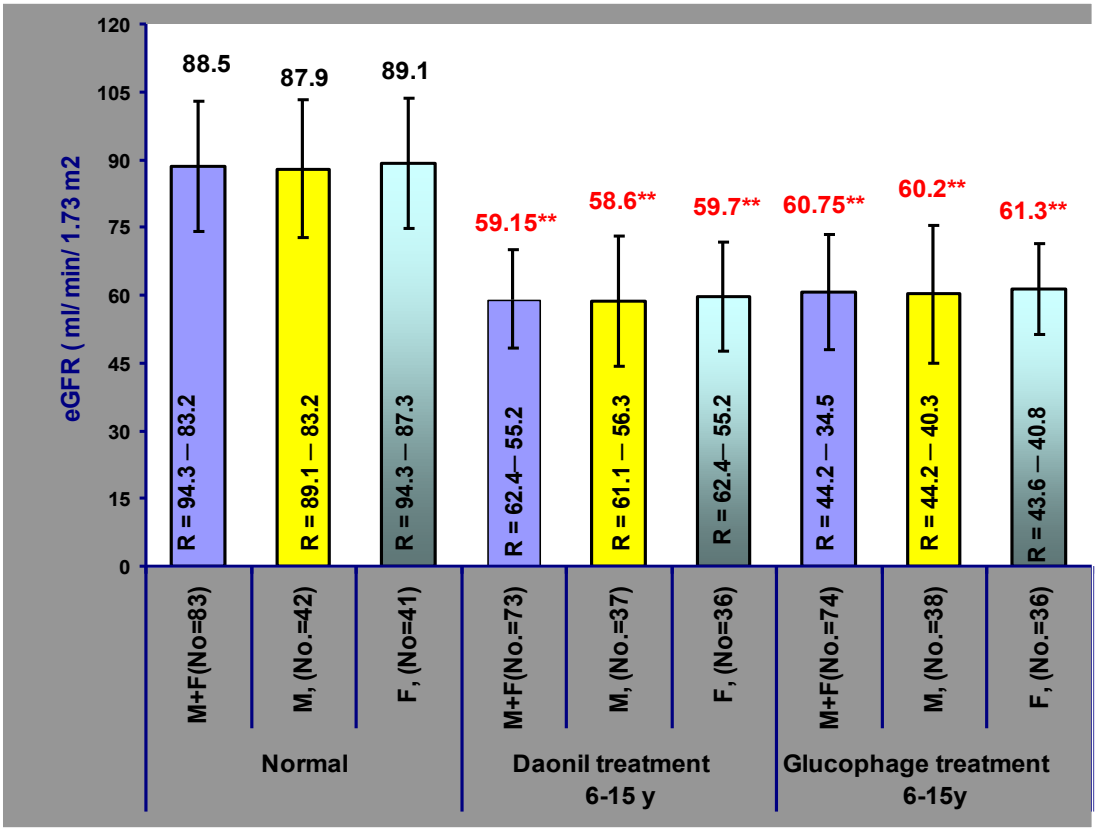

Figure 10 eGFR(ml/min/1.73m2) rate in sera of control and patients with DM(type 2) treated with daonil and glucophage < 6-15y.

7.Determination of albumin concentration in Urine:

In table 2 ,the results revealed a highly significant increased p< 0.001 in urine albumin concentration of DM patients groups(daonil and glucophage) more than five years treated when compared with those of the control group or less than five years treatment. No significant difference between males and females in same groups above.

Table 2 Albumin in urine of control and patients with diabetes mellitus(type2) treated with daonil and glucophage (M=males, F=females, Mean Value± SD,**= p< 0.001

Parameter	Duration	Gender	No.	Mean Value ±SD	Range Value
Control					
Albumine µg/dl		M+F	83	1920±14.24	2010-1867
DM Patients Treatments with Daonil					
Albumin µg/dl	≤ 5 Y	M+F	76	2178**±12.43	2265-1847
	6 -15 Y	M+F	75	3258**±13.75	3295-2888
DM Patients Treatments with Glocophage					
Albumin µg/dl	≤ 5 Y	M+F	76	2145**±11.33	2185-1955
	6 -15 Y	M+F	75	3301**±14.33	3392-3122

8.Determination of creatinine concentration in urine

The results in table 3 show a highly significant decreased p< 0.001 in creatinine concentration in urine of DM patients groups (daonil 64.42**±11.35 and glucophage 66.07**±14.43)more than five years when compared with those of the control group 70.06±7.54 or less than five years treatment (70.12±10.53 and71.035±12.43 respectively). Also a highly significant decreased P< 0.001 in creatinine concentration in urine of males(65.51**±13.47 and 67.12** ± 12.54 respectively) and females (63.34** ±12.34 and 65.02 ** ± 13.74 respectively) in same groups above when compared with those of the control group(70.11±8.32 and 70.01±8.46) and less than five years treatment (70.22±8.35, 70.01±8.46 and 71.72±12.34, 70.35 ± 13.36 respectively).

No significant difference p>0.05 between all groups in sera of diabetes mellitus patients less than five years (daonil 70.12±12.47 and glucophage 71.035±11.33 when compared to control group 70.06±14.24 as shown in table 3. Also no significant difference between males and females in same groups above.

Table 3 Creatinine (g/dl)concentration in urine of control and patients with diabetes mellitus(type2) treated with daonil and glucophage(M=males, F=females, Mean Value± SD,**= p< 0.001

Parameter	Duration	Gender	No.	Mean Value ±SD	Range Value
Control					
Creatinine g/dl		M+F	83	70.06±7.54	78.35-63.2
DM Patients Treatments with Daonil					
Creatinine g/dl	**≤ 5 Y**	M+F	76	70.12±10.53	76.6-65.2
	6 -15 Y	M+F	75	64.42**±11.35	68.5-58.2
DM Patients Treatments with Glocophage					
Creatinine g/dl	**≤ 5 Y**	M+F	76	71.035±12.43	75.8-62.4
	6 -15 Y	M+F	75	66.07**±14.43	70.0-57.8

8.Determination of Microalbuminurea concentration in urine

It was observed that microalbominurea concentration had highly increased p<0.001 in all groups of patients with diabetes mellitus treated with daonil and glucophage (less or more than 5y) when compared to its values in healthy individuals as control group. as shown in figure 11.

The mean values of microalbominurea concentration of

patients treatment with daonil and glocophage less than five years (30.28 ± 4.3, 29.93 ± 3.7 respectively),while highly increased p<0.001 in more than five years (70.76 ± 5.2, 67.75 ± 4.3 respectively) when compared with less than five years and control groups as shown Figure 12

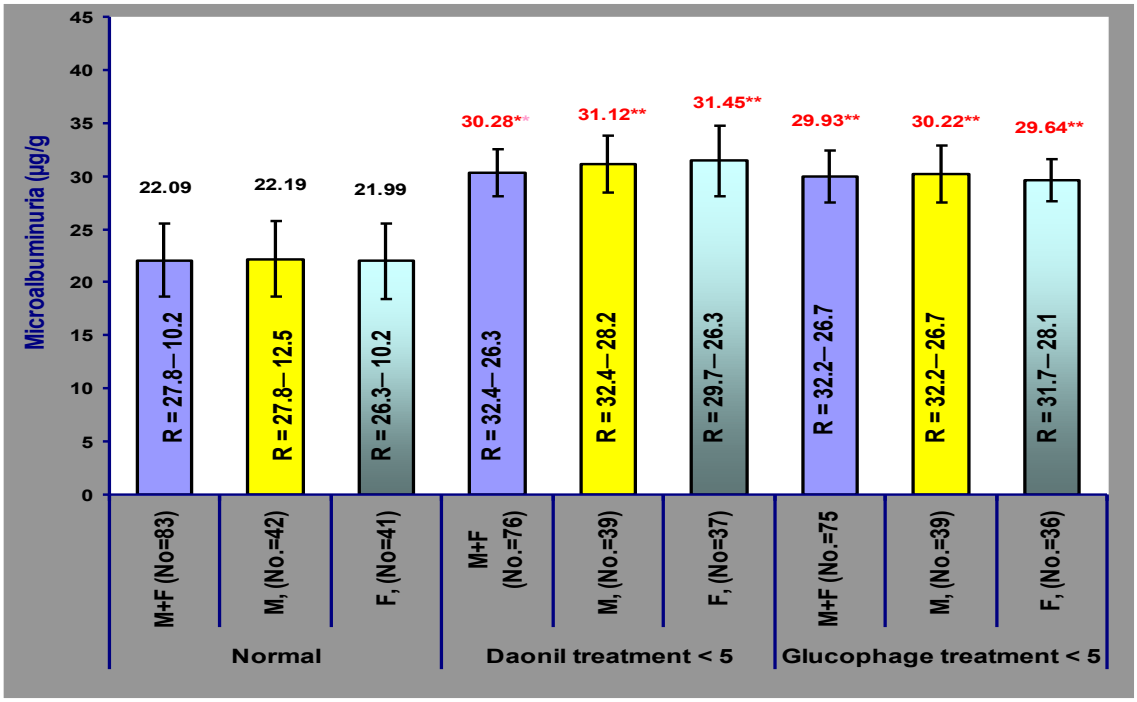

Figure 11 Microalbominurea conc(µg/g) in urine of control and patients with diabetes mellitus(type2) treated with daonil and glucophage < 5 y.

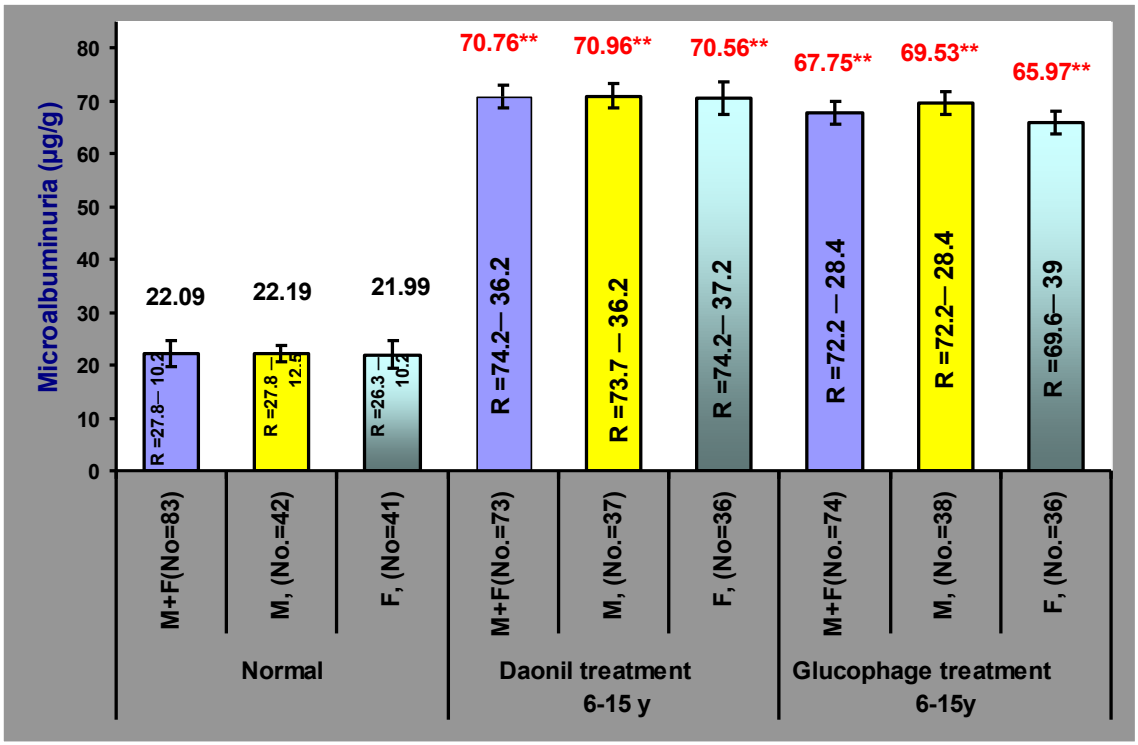

Figure 12 Microalbominurea conc(µg/g) in urine of control and patients with diabetes mellitus(type2) treated with daonil and glucophage 6-15 y.

9.Determination of β2-Microglobulin Concentration in Urine

Firstly: a significant increase $p<0.05$ in the urine β2-microglobulin concentration of DM treated with daonil and glucophage ≤5years when compared to control group.

Secondly, β2-microglobulin concentration shows a highly significant increase $p<0.001$ in the of DM patients treatment with daonil and glucophage more than 5 years when compared to control group. as shown in table 4.

Table 4 β2-Microglobulin conc(mg/L) in urine of control and patients with diabetes mellitus(type2)treatment with daonil and glucophage

Parameter	Duration	Gender	No.	Mean Value ±SD	Range Value
Control					
β2-Microglobulin mg/L		M+F	83	0.13 ± 0.02	0.19-0.11
		M	42	0.13 ± 0.04	0.19-0.15
		F	41	0.14 ± 0.08	0.17-0.11
DM Patients Treatments with Daonil					
β2-Microglobulin mg/L	≤ 5 Y	M+F	76	0.18*± 0.03	0.24-0.16
		M	39	0.19*± 0.07	0.21-0.14
		F	37	0.18*± 0.06	0.24-0.16
	6 -15 Y	M+F	75	0.25**± 0.11	0.31-0.21
		M	39	0.24**± 0.09	0.31-0.19
		F	36	0.26**± 006	0.28-0.21
DM Patients Treatments with Glocophage					
β2-Microglobulin mg/L	≤ 5 Y	M+F	76	0.19*± 0.04	0.24-0.13
		M	39	0.17*± 0.07	0.24-0.15
		F	37	0.19*± 0.06	0.24-0.13
	6 -15 Y	M+F	75	0.25**± 0.14	0.34-0.15
		M	39	0.27**± 0.12	0.34-0.17
		F	36	0.23**± 0.16	0.27-0.15

Thirdly, urine β2-microglobulin concentration shows a highly significant increase $p<0.001$ in the males and females of DM patients treated with daonil and glucophage less and more than 5 years when compared to control group and sub groups.

Finally, β2-microglobulin concentration has a non-significant difference $p>0.05$ between the males and females when the compared in the same group(control, daonil and glucophage) in all duration.

Discussion

Elevation of fasting glucose is used for the definition of fasting glucose (FG). Both the ADA and WHO recommend a fasting plasma glucose

concentration of 7.0 mmol/L for the diagnosis of diabetes, but according to the WHO criteria [29], diabetes can be also diagnosed if the 2-h glucose concentration is at least 11.1mmol/L. For the asymptomatic person, at least one additional glucose test result with a value in the diabetic range is essential, from a random (casual) sample, or from the oral glucose tolerance test[30].

HbA1c level was elevated in the blood of both DM patients treatment with daonil and glucophage subjects. The mean HbA1c of all treatment groups increased by about 2%, reflecting the overall loss of beta-cell function. However, this increase in HbA1c was seen in all treatment groups and was not a consequence of sulfonylurea treatment, but rather was a consequence of the disease itself. It has been reported that the diabetic patients possess elevated level of Hb1Ac[31]which is in good agreement with our present findings.The diabetic subjects with HbA1c may create various clinical situations for the development of diabetes complications[32].

The present findings suggest that there was a strong relationship between fasting, blood sugar and HbA1c level in diabetic patients. Black has similarly reported that the blood glucose and HbA1c levels considerably increased in diabetic patients[33]Further, it has been reported from another study that there is elevated levels of fasting sugar, post prandial sugar and Hb1Ac in diabetes associated hyperlipidemia[34]It has been suggested that there are certain clinical diagnoses and utmost care is required to diabetic patients in order to effectively suppress the severity of diabetes. Here, monitoring of the Hb1Ac was carried out in every three months to maintain proper data for the effective diabetes managements. Thus, HbA1c was observed to play a major role in diabetic complications and in the improvement of good glycemic control[34,35].

Our study revealed that overall glycemic control of our diabetic patients was poor with fasting blood sugar of more than 126 mg/dl in 99% of patients.Random blood sugar was more then 200 mg/dl in 95% and HbA1c of more then 7% was present in 97% patients. Our study clearly demonstrates an association between poor glycemic control and the frequency of complications

Insulin resistance is present when the biological effects of insulin are subnormal for both glucose disposal in skeletal muscle and suppression of endogenous glucose productionprimarily in liver [36].

The degree of insulin resistance varies between different ethnic groups. For example, in the insulin resistance atherosclerosis study including 1100 healthy subjects African-Americans and Mexican Americans had a lower insulin sensitivity than non-Hispanic whites. [37] The first study to demonstrate that a combination of insulin resistance and impaired insulin secretion predicts type 2 diabetes was published on Pima Indians. Lillioja *et al.* [38]showed that low insulin secretory response and increased insulin resistance were both predictors of type 2 diabetes. Furthermore, both impaired insulin secretion and insulin resistance acted as an independent risk factor. Quite similar results were published on Mexican Americans.

Elevations in urea occur as the number of functional nephrons decreases[39]Uremia is a serious condition in which nitrogen based toxins such as urea, the primary waste products of metabolism, accumulate in the blood because the kidneys are unable to filter them out and pass them from the body via the urine. Uremia

indicates renal failure. Urologists sometimes use the term azotemia to designate preclinical uremia-that is, rising levels of urea in the blood that have not yet reached a level at which they cause symptoms[40] .

Nobuko Harita *et al*[41], hypothesized that, lower serum creatinine is associated with an increased risk of type 2 diabetes, which might reflect a lower volume of skeletal muscle. Skeletal muscle is a major target tissue of insulin and a lower volume of skeletal muscle would mean fewer target sites for insulin which causes increase in insulin resistance. This leads to the development of type 2 diabetes[42]This may explain in part the pathogenesis of type 2 diabetes associated with lower serum creatinine.

GFR is the best measure of kidney function since it accounts for age, BMI and sex. GFR measures the rate at which the kidneys' two million glomeruli filter plasma in order to process it and remove waste products from it.Formula-derived eGFR results have become widely used in clinical practice. The National Service Framework for Renal Services in the U.K. recommends the adoption of formula-derived eGFR in the annual evaluation of all patients with diabetes[43]

Creatinine is also used in clinical practice and studies to estimate GFR and predict the grade of chronic kidney disease [44,45]Renal failure due to diabetes was e.g. in the UKPDS defined as creatinine above 250 or need of dialysis and that no other acute disease can have caused the renal impairment. Another reason why creatinine is measured is that studies have found decreased GFR in several adult diabetes patients although normoalbuminuria is present [44].Hence, in some patients measurements of albuminuria could miss detecting an impairment in renal function.

Microalbuminuria is defined as persistent urine albumin excretion in the range of 30-300 mg/24 hours or 20-200 μg/min [44,46]It is an important marker of increased risk for nephropathy, ESRD as well as cardiovascular morbidity . It has therefore been possible to identify patients with high risk at an early stage of disease. Patients progressing from microalbuminuria to macroalbuminuria have a large risk of progressing to ESRD [44].In general the albumin/creatinine ratio is measured in clinical practice to estimate micro and macroalbuminuria.

Our study showed that urinary excretion of β_2-microglobulin was significantly higher in diabetic pateints ($p<0.0001$) than controls with positive correlation with the duration of diabetes ($p<0.001$), which indicates an increase in glomerular permeability and/ or decrease re-absorption of proximal tubules.

Detection of renal tubular proteins and enzymes may precede glomerular involvement, as several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminurea Jung *et al*. [47]stated that, urinary excretion of renal tubular enzymes and low molecular weight proteins have been recommended as useful markers for detection of minor changes in proximal tubular function long before elevation in other markers as proteinuria and rise in serum crearinine.

Musialik *et al*[48]. stated that urinary B2-microglobulin is a sensitive marker of increased glomerular filtration and proximal renal t ubular function. Chiaramonte *et* al[49]stated t hat elevated urinary microprotein (β2microglobulin) might be a useful marker of renal injury in children.

On the other hand, Mojiminiyi and Abdella [50]showed that there is no significant difference between diabetic and controls as regard β_2-microglobulin, but Mortada *et al* [51]determined urinary excretion of β 2- microglobulin, as a marker of tubular damage. Aksun *et al*[52]., stated that increased B2-microglobulin in diabetes

This study suggests that, tubular dysfunction is an important component of diabetic renal disease. For early detect ion of diabetic nephropathy, it would be necessary to include some markers of tubular dysfunction. Of these, B2-microglobulin appear to be useful. These documented tubular dysfunctions appear to be correlated with duration of type 2 diabetes and glycemic control (HbAlc).

Conclusions

From the results of our study ,the following conclusions have been abstracted:

1-Type 2 diabetes mellitus patients is associated with elevation in both ,serum HbA1c and insulin concentrations after five years happened. These elevations due to the uncontrolled in glucose level in duration of diabetes especially in patients treatment more than five years by daonil and glucophage drugs.

2-Kidney function is significantly compromised in patients with DM and this is indicated by highly significantly increased in urea and SCr concentration and highly significantly decreased eGFR.

3-Kidney function of patients with type 2 diabetes mellitus (more than five years)is more compromised compared to those with patients less than five years.

4-Nephropathy (complication of DM)is associated with type 2 diabetes mellitus especially in patients treatment more than five years by daonil and glucophage drugs by elevated microalbumineurea and β2-microglobuline in urine samples.

5-The significantly increase in total protein may be due to the change in the cell membrane permeability cause an increase of the release of different protein from tissue to the body fluids.

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