

Study of the relationship between the level of angiotensinogen hormone and other vital variables with stage of nephropathy in type II diabetic patients

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Abstract:

Diabetes mellitus is the most common worldwide disease and one of its important complications is diabetic nephropathy. The activation of renin- angiotensin - aldosterone system (RAAS) is an important mediator of diabetic nephropathy. Urinary angiotensinogen, a novel biomarker of the intrarenal RAAS, is associated with the development of kidney damage. Therefore, the current study investigated the determinants of urinary angiotensinogen and its associations with staging of diabetic nephropathy and the early detection on some changes in kidney functions as result of diabetic disorder.

This study has performed in endocrine and diabetic center in Al-Sadder medical city /Al-Najaf governorate, and it is involved (60) type II diabetic patients for both gender, and (30) of healthy individuals from males and females as a control sample. The patients were divided according to the ratio of albumin to creatinine in urine (ACR) into three groups , the first group composed of (20) type II diabetic patients with normoalbuminuria stage , (20) type II diabetic patients with microalbuminuria and (20) type II diabetic patients with macroalbuminuria stage , their ages range between 30 -66 years old, the study continued from march 2017 until march 2018 .

The study involve assessment of albumin / creatinine ratio , angiotensinogen levels in urine , fasting blood sugar levels ,percentage of glycosylated hemoglobin, as well as urea, albumin ,and creatinine levels in serum .

The results of the present study have been revealed shown significant effects ($P < 0.05$) for age factor, duration of disease, diabetic nephropathy stage, body mass and gender in the levels of angiotensinogen in diabetic patients.

As well as the results revealed notable differences ($P < 0.05$) in angiotensinogen levels and some other biological parameters involved in the current study through comparison between males and females who have type II diabetes mellitus.

The conclusion of this study is diabetes mellitus has an effect on some physiological functions of kidneys which in turn lead to excretion of angiotensinogen with urine , angiotensinogen in urine could be one important vital criteria for the diagnosis of stages of diabetic nephropathy and it might be present before albuminuria and this is useful as an early biological marker to detect the activation of renin-angiotensin system in renal disorders resulted from diabetes mellitus .

Keywords: Diabetic mellitus, nephropathy, angiotensinogen ,Biomarkers

Introduction:

Diabetes mellitus is a metabolic disorder characterized by chronic increasing in blood sugar level (hyperglycemia) accompanied by disturbances in the storage of carbohydrates, proteins and lipids due to low insulin level or insulin dysfunction or both [1].

The pathogenesis of diabetes mellitus based on the histological structure of beta pancreatic cells and its secretions, but some common types of diabetes which increased hyperglycemia resulted from complex genetic reactions or environmental factors, as well as differences in life style [2].

Diabetes is a chronic complex disorder which represent the main source of a lot of diseases in the whole world , it is a group of metabolic disorders , and there are many common symptoms for diabetes including frequent urination , thirst , weight loss , and sometimes frequent drinking of water , difficulties in vision , weakness in the immune system which in turn causes many diseases[3,4].

Increasing the propagation of diabetes mellitus in the whole world as continuous and its about 24 millions of people in united states of America , which forms (8%) of total populations , it is should be noted that increasing infection rate with diabetes is due to microvascular complications such as diabetic retinopathy , diabetic nephropathy and diabetic neuropathy , as well as macrovascular complications such as cardiovascular diseases that lead to increasing death rate in diabetic patients[5].

The increment of blood glucose levels above the normal value after a meal during two hours is considered as a marker for type II diabetes mellitus, in which glucose value reach to 200mg/dl (in fasting state) , disorder occur due to lack in insulin production by beta cells in the pancreas insufficiently or when the pancreatic cells is not responding properly to make blood sugar level within the normal range , insulin help the body cells to uptake the glucose to use it in the metabolic reactions that lead to decrease blood sugar level , after having a meal . This regulating blood sugar level to 1 g/l, therefore any deficiency in the production of insulin hormone can cause hyperglycemia and this rise causes the emergence of diabetic complications [6].

Ratio of type I diabetes mellitus has been estimated to about 5-10% of total people infected with diabetes[7], this type is diagnosed in the early stages (childhood , adolescence)[8], while the propagation of type II diabetes in all ages in different parts of the world is about 171 million, which equal to 2-8% in 2000 , and it is expected to reach to 4.4% in 2030 [9].

The vascular complications resulted from diabetes mellitus including microvascular complications and macrovascular complications , these complications might be in relation with the changes in the lipid metabolism and hypertension , it has been found that the risks of cardiovascular complications has increased in patients with type II diabetes mellitus , and insulin resistance is the largest proof for this danger [10].

The pathological changes combined to microvascular complications caused by diabetes may cause organ osmosis, especially organs that depend completely on blood supply, such as retina, kidneys and peripheral nervous system [11].

Diabetic nephropathy is one of the most common complications associated with diabetes [12] , and occurs in both types of diabetes, is, type I and II [13] , characterized by the occurrence of several satisfactory changes in some serum criteria which lead to increase in mortality [14]. The value of patients arriving at this stage is estimated at about 40% compared with other causes of renal failure [15] .

It has found that about 7% of patients with type 2 diabetes had previously been diagnosed with microalbuminuria when they were diagnosed with diabetes [13] .

Diabetic nephropathy is the leading cause of chronic renal failure in Iraq. It is determined through proteinuria (more than 500 mg within 24 hours) in diabetics, and the microalbuminuria is defined that the excretion of albumin (30-299 mg) Within 24 hours, diabetic patients with microalbuminuria usually develop their disease into proteinuria (macroalbuminuria) , which in turn leads to diabetic nephropathy [13]. It is worth mentioning that diabetic nephropathy is characterized by the excretion of albumin molecules in urine and high blood pressure, as well as a decrease in the filtration activity of the renal glomeruli [12] .

Angiotensinogen (AGT) is the unique substrate of renin and precursors of angiotensin II in the renin-angiotensin system, which plays a major role in regulating blood pressure and the balance of extracellular fluid as well as sodium ion in the body [16]. The main source of angiotensinogen is the liver followed by fatty tissue [17].

Angiotensinogen is produced primarily in proximal tubular cells and secreted from the apical surface into the kidney cavity, first converted to angiotensin I (Ang I) and then to angiotensin II (Ang II) by renin that found in the tubules and the angiotensin converting enzyme (ACE) [18].

Aims of the study

1- Diagnosis of the effect of diabetes mellitus on the functional performance of the kidney and its ability to secrete angiotensinogen, by estimating the levels of angiotensinogen in urine in diabetics of men and women whom infected with the disease and compare with the healthy controls of both genders too.

2- Investigation of the levels of angiotensinogen in urine in patients with diabetes after diagnosing their diabetic nephropathy stage by evaluating the ratio of albumin to creatinine in urine (ACR) to determine the impact of the disease on the levels of angiotensinogen .

3- Study of the relationship between the imbalance in the level of angiotensinogen in urine in patients with diabetes and other factors such as age of the patient , duration of disease, BMI, gender, and some of the physiological and biochemical properties of blood that included in the current study .

Materials and methods:

1. Patients and the study place

The study included the follow-up of (30) healthy people of both sexes as control group in equal numbers and (60) patients of men and women with type 2 diabetes in equal numbers also reviewed the Diabetes and Endocrine Center in Al-Sadr Medical City / Najaf. The age of two groups ranged between 30-66 years.

The study began in March 2017 and continued until March 2018. The group of patients was divided according to albumin / creatinine ratio in urine into three subgroups:

- 1- The first group included type II diabetic patients in the normoalbuminuria stage.
- 2- The second group was diabetic type II in the stage of microalbuminuria.
- 3- The third group included type II diabetic patients (macroalbuminuria) (clinical nephropathy).

The group of patients was also divided into three age groups included , (30-40) years, (41-52) years, and the last group was between (53-66 years), as well as dividing them by the duration of the disease into a group of (1-5) years, and a group of (6 - 10), and the last group was 10 years or more. In addition, the group of patients also divided into three subgroups according to the body mass index (BMI), normal weight (18.5-25) kg/m², Overweight (25-30) kg/m², Obesity (30-40) kg/m². Body mass index had been calculated according to the following equation [19] :

$$\text{BMI} = \frac{\text{Body weight (kg)}}{\text{Length (m}^2\text{)}}$$

The collected information included name, gender, age, duration of diabetes, type of treatment, weight, height, blood pressure and chronic diseases such as hypertension and smoking. The study excluded patients with high blood pressure, thyroid disease,

smokers, and those who received injecting diabetes treatments as well as different types of diuretic drugs.

2. Collection of the blood samples

5 ml of the venous blood of the two groups (healthy and diabetic) was taken between 8:30 am and 10:30 am using a syringe, and 1 ml of blood was transferred to tubes containing an ethylen diamine tetracetate (EDTA) to evaluate percentage of the glycosylated hemoglobin in the blood (HbA1c), 4 ml of blood was put in serum tubes and left at room temperature for 30 minutes, then centrifuged at 4000 cycles / min for 10 minutes to separate the serum from other ingredients and then stored at temperature $(-20)^{\circ}\text{C}$ until the biochemical tests of blood are done.

3. Collection of the urine samples:

Urine samples collected from the two groups immediately after the blood samples were taken and placed in sterile plastic containers to estimate the albumin to creatinine ratio in urine. The samples were also stored in Eppendorf tubes with a capacity of 2 ml at -80°C for the ELISA test to detect angiotensinogen level.

1-3. Evaluation of albumin / creatinine ratio (ACR)

1-3-1. Evaluation of microalbumin level in the urine

The Pyrogallol method was used (Stable liquid reagent) to estimate the amount of albumin in human urine, equipped by Syrbio Company, Syria, the absorbance was read at wavelength (598) nm by using the spectrophotometer [20].

1-3-2. Evaluation of creatinine level in the urine

Colorimetric method with deproteinization was used to estimate the amount of creatinine in human urine, supplied by Randox Company, United Kingdom, the absorbance was read at wavelength (520) nm by the spectrophotometer [21].

2-3. Evaluation of angiotensinogen level in the urine

The Enzyme – Linked Immunosorbent assay (ELISA) was used to estimate the level of angiotensinogen in urine, supplied by IBL-International GMBH, Germany, at a wavelength of (450) nm [22].

4. Study of some physiological parameters of the blood

1-4. Evaluation of fasting blood glucose

According to the method that mentioned by [23], the fasting blood glucose assessed, the kits purchased from Spinreact Company, Spain, at (490-550) nm.

2-4. Evaluation of glycosylated hemoglobin percentage in the blood:

The rapid ion exchange separation method was used, and the kits supplied by Stanbio Company, America. The absorbance of the standard solution and the sample read at the wavelength (415) nm by the spectrophotometer [24].

5. Study of some biochemical parameters of the blood

1-5. Evaluation of urea level in the serum

The urea is hydrolyzed in the presence of water and urease enzyme to produce ammonia and carbon dioxide, then ammonium ions interact with salsalite and hypochlorite to form a green complex (2,2 dicarboxylindophenol), the level of urea was read by using the spectrophotometer for each sample at a wavelength of (600) nm [25].

2-5. Evaluation of albumin level in the serum

As regard to the serum albumin level, the colorimetric method used, which is called Bromocresol green method (BCG), the kits supplied by Biolabo Company, France. The mixture absorption of the sample and the standard solution was tested against the blank detector by using the spectrophotometer at a wavelength (630) nm [26].

6. Statistical analysis of the study

Statistical analysis carried out by using the Statistical Package for Social Sciences (SPSS) version 21. In addition, the Chi-Square, the T test, the analysis of variables ANOVA and LSD were also used for the experimental group comparison. The significant differences calculated at the probability level ($P < 0.05$) [27].

Results:

1- The effect of age, duration of disease, and stage of nephropathy on angiotensinogen levels in male diabetic patients

The current study recorded significant differences ($P < 0.05$) in angiotensinogen levels according to the age group, duration of disease and stage of nephropathy in male diabetic patients, as shown in table (1).

Table (1): effect of age group, duration of disease, and stage of nephropathy in angiotensinogen levels in male diabetic patients

Variables	Stages of nephropathy in male diabetic patients			P value
	Normo (n=10)	Micro(n=10)	Macro(n=10)	
Age / year	38.75±4.71	49.10±4.52	60.88±5.00	0.659
Duration of disease / year	1-5	6-10	10 or more	0.674
Angiotensinogen levels ng / ml	10.60 ±0.41 a	29.46±1.36 b	276.0±10.47 c	<0.001

The different letters refer to significant differences between the averages at $P < 0.05$

2- Effect of age, duration and stage of nephropathy on angiotensinogen levels in female diabetic patients.

From the statistical analysis of the results of the current study, there were noticeable differences ($P < 0.05$) in angiotensinogen levels according to the age, duration of disease and stage of nephropathy in female diabetic patients, as shown in table (2).

Table (2): effect of age, duration of disease and stage of nephropathy in angiotensinogen levels in female diabetic patients

Variables	Stages of nephropathy in female diabetic patients			P value
	Normo (n=10)	Micro(n=10)	Macro(n=10)	
Age / year	37.93±4.42	49.07±4.69	60.19±4.61	0.998
Duration of disease / year	1-5	6-10	10 or more	0.454
Angiotensinogen levels ng / ml	10.77 ±0.47 a	27.78±1.70 b	269.14±14.4 c	<0.001

The different letters refer to significant differences between the averages at $P < 0.05$

3 - The effect of body mass index on angiotensinogen levels in diabetic patients of males and females

From the findings of the current study, the levels of angiotensinogen were significantly increased ($P < 0.05$) according to BMI in diabetic males as well as females with the same disease as illustrated in table (3).

Table (3): the effect of BMI on angiotensinogen levels in diabetic patients of males and females

		Angiotensinogen levels in male diabetic patients ng / ml	Number of samples	Angiotensinogen levels in female diabetic patients ng / ml	Number of samples	P value
BMI Kg / m ²	Normal	10.60±0.41 a	5	10.77±0.47 a	3	0.442
	Over weight	29.46±1.36 b	12	27.18±1.70 b	10	
	Obesity	276.0±10.47 c	13	269.14±14.4 c	17	
Total			30		30	

The similar letters indicate that there were no significant differences between the averages $P < 0.05$

The different letters refer to significant differences between the averages at $P < 0.05$

4- The effect of gender in diabetic patients on angiotensinogen levels and some other vital criteria

Table (4) has been revealed significant differences $P < 0.05$ in angiotensinogen levels and some other vital criteria when compared between males and females of diabetic patients.

Table (4): effect of gender in diabetic patients on angiotensinogen levels and some other vital criteria

Variables	Male patients (n= 30)	Female patients (n=30)	P value
	Average ± standard error	Average ± standard error	
ACR Ratio mg/g	324.39±93.30*	299.32±113.02	0.867
Angiotensinogen level ng/ml	132.55±27.29*	109.12±23.52	0.519
Fasting blood glucose mg/dl	235.16±19.18*	215.44±13.52	0.397
HbA1c value%	9.62±0.33*	8.10±0.33	0.240
Urea level mg / dl	43.08±2.27*	37.32±1.96	0.214
Albumin level g/dl	5.24±0.15*	4.01±0.14	0.139
Creatinine level mg / dl	0.69±0.03*	0.50±0.02	<0.001

* Indicates to significant differences between the two groups at $P < 0.05$

5 - The effect of diabetes mellitus on angiotensinogen levels and some other vital criteria

The present research reported significant differences $P < 0.05$ in angiotensinogen levels and some other vital criteria when comparing diabetic patients of both sexes with healthy males and females as shown in table (5).

Table (5): comparison between diabetic patients of both sexes with healthy males and females in angiotensinogen levels and some other vital criteria

Variables	Controls (n= 30)	Patients (n=60)	P value
	Average \pm standard error	Average \pm standard error	
ACR Ratio mg/g	15.87 \pm 1.66	311.12 \pm 73.51*	<0.001
Angiotensinogen level ng/ml	4.27 \pm 0.429	119.93 \pm 17.76*	<0.001
Fasting blood glucose mg/dl	100.25 \pm 1.67	224.72 \pm 11.48*	<0.001
HbA1c value%	5.60 \pm 0.12	8.92 \pm 0.24*	<0.001
Urea level mg / dl	25.42 \pm 1.79	41.05 \pm 1.49*	<0.001
Albumin level g/dl	3.11 \pm 0.10	5.01 \pm 0.24*	<0.001
Creatinine level mg / dl	0.23 \pm 0.03	0.66 \pm 0.02*	0.074

*Indicates to significant differences between the two groups at $P < 0.05$

6-Comparison between male diabetic patients & healthy males in angiotensinogen levels and some other vital criteria

There were observable differences ($P < 0.05$) in the levels of angiotensinogen and some other vital criteria when comparing diabetic males with healthy individuals of the same gender.

Table (6):comparison between male diabetic patients & healthy males in angiotensinogen levels and some other vital criteria

Variables	Male Controls (n= 15)	Male diabetic Patients (n=30)	P value
	Average \pm standard error	Average \pm standard error	
ACR Ratio mg/g	17.17 \pm 2.60	324.39 \pm 93.30*	0.027
Angiotensinogen level ng/ml	3.33 \pm 0.66	132.55 \pm 27.29*	0.002
Fasting blood glucose mg/dl	98.75 \pm 2.86	235.16 \pm 19.18*	<0.001
HbA1c value%	5.49 \pm 0.20	9.62 \pm 0.33 *	<0.001
Urea level mg / dl	29.58 \pm 2.66	43.08 \pm 2.27*	0.001
Albumin level g/dl	3.12 \pm 0.17	5.24 \pm 0.15*	0.508
Creatinine level mg / dl	0.35 \pm 0.06	0.69 \pm 0.03*	0.417

* Indicates to significant differences between the two groups at $P < 0.05$

7- Comparison between female diabetic patients & healthy females in angiotensinogen levels and some other vital criteria

Significant variations ($P < 0.05$) were observed in the levels of angiotensinogen and some other vital criteria when comparing diabetic females with healthy individuals of the same gender, table (7).

Table (7): comparison between female diabetic patients & healthy females in angiotensinogen levels and some other vital criteria

Variables	Female controls (n= 15)	Female diabetic Patients (n=30)	P value
	Average \pm standard error	Average \pm standard error	
ACR Ratio mg\g	14.90 \pm 2.2	299.32 \pm 113.02*	<0.001
Angiotensinogen level ng\ml	4.98 \pm 0.51	109.12 \pm 23.52*	<0.001
Fasting blood glucose mg\dl	101.37 \pm 2.03	215.44 \pm 13.52*	<0.001
HbA1c value%	5.68 \pm 0.15	8.10 \pm 0.33 *	<0.001
Urea level mg \ dl	22.31 \pm 2.17	37.32 \pm 1.96*	<0.001
Albumin level g\dl	2.99 \pm 0.14	4.01 \pm 0.14*	<0.001
Creatinine level mg \ dl	0.27 \pm 0.02	0.50 \pm 0.02*	0.014

*Indicates to significant differences between the two groups at $P < 0.05$

Discussion:

1- The effect of age, duration of disease and stage of nephropathy on angiotensinogen levels in diabetic patients

The data of the current study indicated to notable differences in the level of angiotensinogen in different age groups, duration of disease and stages of nephropathy in patients with diabetes. This finding differed with the results of some studies, which confirmed that there is no relationship between the level of angiotensinogen and the age of diabetic patients [28], but agreed with another study has been demonstrated that the level of angiotensinogen significantly increased with the age in compared with the younger age groups [29].

The result possibly attribute to the fact that as diabetic patients (both genders) progressed in age, this may have negatively impacted the kidney functions, which in turn reflected on the levels of angiotensinogen and led to increase its excretion in the urine , which is likely to indicate progression of nephropathy stages in diabetic patients of both males and females .

A previous study has shown that the most important risk factors that play a major role in the development of diabetic nephropathy are ageing, hyperglycemia, hyper filtration and male gender. A significant correlation was noted with progression of age and increasing the rate of urinary albumin excretion in large amounts [30].

It is worth mentioning that according to the results of the current study, diabetic patients who developed diabetic nephropathy to the stage of microalbuminuria were of the older age groups in compared to younger diabetic patients who have been diagnosed in the stage of normoalbuminuria, [31] has proved that the arrival of diabetic patients to microalbuminuria stage was associated with an increment in mortality risk in older people to about 50% .

In patients with type II diabetes, the two factors age and duration of disease raise the risk of having proteinuria , and more than 45% of patients with type II diabetes of

older age groups diagnosed that they have high glomerular filtration rate (GFR) were twice as high as their peers in the same age group but they had no diabetes and their body mass index was normal [32].

In addition to what have been preceded, patients with type II diabetes with high age groups had been recorded of a big probability of adverse changes occur in the blood vessels, indicating atherosclerosis, which negatively affected the glomerular filtration rate and the size of renal glomeruli [33].

Diabetic nephropathy has been defined as diabetes with the presence of albuminuria or disturbance in glomerular filtration rate or both. The prevalence of diabetic nephropathy in the United States of the adult population aged about 20 years and above was 3.3%, while the noticeable increase to infect with diabetic nephropathy have been recorded in patients aged about 65 years or older [33].

Moreover, diabetic nephropathy is also a major threat to the health of the older people, and taking care with chronic kidney diseases in older people with diabetes is weak. One of the most important challenges for older people with diabetic nephropathy is that they may develop more complications, specially heart diseases, eye, peripheral blood vessels, a report of US in 2011 about diabetes facts has been mentioned that in 2004 heart diseases and stroke had recorded the values 68% and 16% respectively of diabetes-related deaths in patients with 65 years of age or more, as well as many older patients with diabetes complain of a significant immune deficiency and are at high risk for the development of many common aging syndromes such as depression, impaired cognition, incontinence, and persistent pain according to some studies [34]. This probably due to the fact that the vast majority of patients included in the current study may have been subjected to extreme stress, exhaustion and fatigue because the lack of a balanced diet, as well as the possibility of not doing useful exercise or may result in not taking enough rest and psychological calm.

Or likely to be belonged to the lack of good blood sugar control that stimulates or increases the lipid peroxidation process and the consequent rise in the rate of harmful free radicals, which in turn attacks sensitive biomolecules such as carbohydrates, proteins, fats and possibly the nitrogenous bases of the DNA, resulting in partial or total depletion of the enzymatic and non-enzymatic antioxidants within the cells, especially the glutathione molecules, some study confirmed that reactive oxygen species (ROS) cause damage to all cells of the body and cause atherosclerosis, which results in the cover of the renal arteries with a substance that affects the kidneys and their arteries, as well as capillaries. However, the antioxidants found in fruit and vegetables can reduce the detrimental free particles and prevent the damage it can cause to the body and the diet which is rich in antioxidants is important to the health of the heart and blood vessels, which can be obtained by eating a healthy and good diet, as postulated by some studies [35].

It should be noted that some patients had been long-term infected with diabetes and with good control of blood glucose level, they did not have complications of diabetes, while those complications appeared in a number of patients when diagnosed with diabetes, which formed in diabetic nephropathy that results from infected with diabetes and that may suggest to the presence of a period without symptoms before the diagnosis of diabetes, as confirmed by some studies [36].

With regard to the duration of disease, the current study found that the diabetic patients who have been infected with the disease for ten years and more from both genders have recorded the highest observable rise in levels of angiotensinogen in

urine compared with their peers of diabetes patients with the least durations of disease , this was compatible with the study of [28], who noticed a positive association between the angiotensinogen levels and duration of disease. This may be explained by the fact that the longer period of diabetes, the more negatively affected on the functions of the kidneys, including increasing the excretion rate of biomolecules such as creatinine and albumin, which caused the occurrence of nephropathy and its progress to advanced stages and thus angiotensinogen excretion rate increased in urine subsequently.

The current study also indicated that there was a significant effect to the duration of infection in type II diabetes with the progression of diabetic nephropathy stages (normoalbuminuria , microalbuminuria and macroalbuminuria) of both sexes compared to healthy of both genders . The finding of this study showed an agreement with the study of [33], it also agreed with a study of researchers [37], and with [28], which indicated to a positive correlation relationship between the rise of angiotensinogen level in urine and the duration of diabetes, as well as the stage of nephropathy. This is likely to explain that the longer period of diabetes, the more body's tissues are exposed to the various pathological influences and complications of the blood vessels, in both types micro and macro vessels, especially renal tissues, because its responsible for the filtration and re-absorption processes ,as well as secretion of various biological molecules such as food, different kinds of enzymes , hormones and medical drugs , therefore, the occurrence of these tissues under the toxicity of the deadly sugar molecules, especially when the sugar levels are high, which reflected negatively on the functional performance of those sensitive tissues and cause the arrival to progress stages of nephropathy .

It is important to mentioning that there have been many pathological changes in renal glomerular tissues in patients with diabetes who have been infected for a long time before being diagnosed with microalbuminuria stage, which is characterized by the thickness of the glomerular basement membrane (GBM) , the expansion of glomerular mesothelioma – the Kimilstil-Wilson change , diffusion glomerulosclerosis, the fibrosis of the interstitial tissue of renal tubules ,arteriosclerosis and glaucoma of blood vessels of the kidney, the severity of glomerular damage corresponds to the rate of glomerular filtration and the duration of diabetes ,as well as the ability to regulate the level of sugar in blood [33].

The statistical analysis of the results of the present study showed remarkable differences in levels of the angiotensinogen hormone in patients with type II diabetes of both sexes with the progression of the stage of nephropathy. The finding comes along with some studies [28], which indicated that renal excretion of angiotensinogen may be a marker or indicator of intrarenal angiotensin activity and it is closely associated with a decrease in the rate of renal functions as well as different stages of nephropathy in patients with diabetes. This may be attributed to the increased of the secretion activity of the adrenal cortex, or perhaps the thyroid gland or both, or to increase the secretion of estrogen or possibly to stimulate the synthesis of angiotensin II .

Moreover, may be to the activation of the intrarenal renin - angiotensin system (RAS) which plays an important role in increasing the excretion of angiotensinogen in urine in the early stages of diabetic nephropathy [28].

The angiotensinogen, which is studied in high-sensitivity ELISA, and the specialty of diagnosis the diabetic nephropathy in patients with type II diabetes, is significantly higher in type II diabetic patients in the normoalbuminuria stage of both genders,

when compared with healthy individuals. These data show that urinary angiotensinogen is an index to the injury of renal tubules; it is also a biomarker to diagnosis of early stages of diabetic nephropathy. The intrarenal RAS plays a vital role in the development of diabetic nephropathy [38], and the only and unique substrate for renin is angiotensinogen, also the changes in angiotensinogen levels could be control on the RAS activity [39].

In addition, the increment in intrarenal angiotensinogen levels have been observed in multiple tests for hypertension [40,41] and kidney diseases that including diabetic nephropathy [42,43], furthermore, the levels of angiotensinogen in the proximal renal tubules cells in rats indicate that the development of albuminuria and the activity of intrarenal RAS increased the tubular fibrosis in diabetics [44].

A subsequent study reported that the elevated angiotensinogen levels in urine due to increase in its excretion when infect in diabetic nephropathy were significantly associated with decreases in the rate of renal functions [45].

Some studies have suggested that angiotensinogen levels in urine may be an indicator of damage in filtrated barriers rather than the activity of intrarenal RAS. However, the excretion of angiotensinogen in urine showed a significant positive relationship with levels of albuminuria in both genders [46].

Another study found that high levels of angiotensinogen excreted in urine in patients with chronic kidney diseases caused an increased in the rate of renal injury [43].

2- The effect of body mass index on angiotensinogen levels in diabetic patients

According to the results of the present study, there was a significant effect of BMI on the levels of angiotensinogen excreted in urine in both genders of diabetic patients, and this was incompatible with some studies have been demonstrated that there was no correlation between levels of angiotensinogen in urine and the body weight [28], but this agreed with another study has been indicated that patients with diabetes and chronic kidney diseases who had a high BMI had recorded a significant increase in levels of angiotensinogen in urine [29]. This is likely to be explained to the appearance of risk factors in diabetic patients with high BMI, especially those who are obese, including high levels of total cholesterol, triglycerides, low-density lipoproteins, very low-density lipoproteins, in the opposite, the decreased levels of high density lipoprotein, which may cause an increase in the activity of intrarenal renin-angiotensin system (RAS) and consequent increment in the biological synthesis of intrarenal angiotensinogen in renal glomeruli and proximal renal tubules as well as rise its excretion in the urine.

Moreover, the high levels of angiotensinogen in diabetic patients with high-body mass index may also stimulate lipid peroxidation processes in the body's tissues, resulting in increased production of various reactive oxygen species, such as hydroxyl, hydrogen peroxide, superoxide anion radicals, and others that cause noticeable tissue damage particularly the renal tissues, due to its highly sensitivity, which may have caused an increase in the excretion rate of angiotensinogen in urine.

Many studies have proved that BMI is higher in patients with type II diabetes of males and females, and leptin levels also have a significant raise [47,48,49], as well as another study indicated that patients with diabetes and obese revealed a observable increase in levels of leptin compared to healthy individuals, it is a hormone with a protein structure of 167 amino acid has significant effects on regulation of the body weight, metabolism and reproductive functions [50].

In addition, several studies have confirmed a positive correlation between BMI and leptin levels, which suggested that to raise the intensity of adipose tissues in obese individuals compared to their non-obese counterparts [51, 52, 53]. Therefore, the recorded significant increment in angiotensinogen levels during the current study may explain the increase in the fatty tissues and the consequent increment in the rate of biosynthesis of this hormone. It is worth mentioning that the liver is the main source of angiotensinogen followed by adipose tissue, according to some sources [54].

The high levels of angiotensinogen are likely to be attributed to the presence of a positive correlation coefficient for this hormone and leptin hormone, which is mainly produced from fatty tissue as noted by some studies[55]. A latter study was found a close relationship between levels of Leptin hormone and the amount of fat stored in the bodies of type II diabetic patients[56].

The angiotensinogen-increased levels may be explained by a lack of adherence to quality and well-integrated diets. A balanced and varied diet, as well as decreases body weight, reduce the rate of protein output in the urine and improve kidney functions. In the contrast, weight gain is a risk factor for diabetic nephropathy, as many studies have confirmed [32].

The development of an organized diet program and exercise to control the level of blood sugar facilitates the reduction of hypertension cases and reduces the accumulation of fat that causes weight gain, which controls the distribution and spread of obesity around the world many factors including genetics, age, food, geographic area, climate, psychological state and exposure to chemical substances [57]. Moreover, the psychological factors related to the security situation or trauma resulting from a sudden event are one of the catalysts for increasing blood sugar levels to increase the secretion of other hormones such as glucocorticoids, catecholamines and growth hormone, which reverse insulin in their work [58].

A study found rise in the levels of glucose, lipids and lipoproteins in the serums of type 2 diabetic patients compared to their levels in the control group, the reason for the high level of glucose in people with type II diabetes was the lack of insulin secretion, or a defect in its receptors in the body, or disorder in the work of the pancreas and metabolism, especially sugars in the liver, leading to accumulation of fat and obesity in type II diabetic patients.

Both diabetes mellitus and obesity are associated with the filtration of large phagocytic cells into the fatty and kidney tissues, these cells then become a source of stimulation for the production of primary inflammatory cytokines. Moreover, weight gain leads to the release of adipokines into the bloodstream, which in turn may cause renal injury. Continued high blood sugar also activates effective hormonal pathways in the vessels, including the RAS and endothelin. Furthermore, kidneys in individuals who suffer from obesity often suffer from an increase in the thickness of lipid layers on the mesangial renal glomerular cells, which is a major cause of induction of renal toxicity. Data from clinical studies indicate that obesity, high blood pressure, high blood sugar, hyperlipidemia and other metabolic syndromes are strongly associated with the development of diabetic nephropathy, so the prevention and treatment of obesity, metabolic syndromes and diabetes helps to reduce kidney damage [34]. Overall, these factors may have played a key role in an increment the BMI significantly in diabetic patients of both sexes, causing an increase of excretion of angiotensinogen in urine.

3 - The effect of gender in diabetic patients on angiotensinogen levels and other vital criteria

The current study showed significant alterations in the levels of angiotensinogen and some other vital parameters when comparing between diabetic males and females, the recorded remarkable increase in albumin to creatinine (ACR) ratio ,levels of angiotensinogen in urine , urea, albumin and creatinine in males serum compared to females may be explained by affecting the intrarenal renin-angiotensin system in males more than females, which caused a decline in the functions of renal tissues, or the possibility of infection of these tissues with partial or total damage, some studies indicate that the renin - angiotensin system (RAS) is notably activated in diabetic males compared to diabetic females [60].

It is likely that the observed raise in the ratio of albumin to creatinine in urine , as well as levels of creatinine, urea and albumin in serum in diabetic males than in females with the same disease to the large size of muscle mass and high rate of bio-production of various proteins by the liver, resulting in high levels of total proteins in the serum and different body tissues ,and thus increase albumin molecules, which constitute the largest proportion of those proteins in males than in females.

The result may be suggested to the male sex hormones, especially the testosterone hormone, in addition, the sex hormones may also affect the glomerular filtration rate, therefore, the potential physiological effects of these hormones may increase the ratio of albumin to creatinine in urine and angiotensinogen levels in urine ,as well as albumin, creatinine and urea levels in serum .

It is probably that the results of the current study regarding the high level of fasting sugar and the percentage of glycosylated hemoglobin in males than in females to the nature of work performed by men or the quality of food or non-adherence to diet or beneficial exercises, which reflected negatively on kidney functional performance and therefore, the ratio of albumin to creatinine in the urine ,levels of angiotensinogen in urine and levels of albumin, creatinine and urea in the serum significantly increased, some studies have confirmed that high blood sugar in diabetic patients of males and females contributes substantially to the development of glomerular hyperfiltration through mechanisms similar to those that occur in the case of obesity [34].

4- The effect of diabetes mellitus on albumin/creatinine ratio in the urine

There is a significant difference in the ratio of albumin to creatinine (ACR) when comparing diabetes patients with healthy ones. This increase may be due to the possibility of histological changes in the basal membrane of the renal glomeruli, or may result from the dilatation of mesangial cells, or because the loss of the negative charge of the basal membrane surrounding the renal glomeruli due to a lack of production of the components carrying the negative charge, which is Glycosaminoglycan of the walls of capillaries, which leads to an increase in the excretion rate of albumin in urine , as demonstrated by some studies [61].

On the other hand, it is probably due to the occurrence of an imbalance in the process of reabsorption of the filtrated proteins to proximal tubules, which leads to the release of the albumin in urine [62].

In addition to what have been mentioned, diabetes patients with diabetic nephropathy may be exposed to many factors, including long-term standing by nature of work , which increases the rate of renal filtration of albumin in urine , or exposure to stress and fatigue, or most patients did not follow a diet which may mean the intake of proteins is abundance, which would lead to a high level of total proteins in the blood and thus increase in the excretion of those molecules, especially albumin in the

urine. The preservation of albumin level within normal limits is an important factor for its vital functions which is formed in preserving of the body fluids and preventing their exit from the blood vessels, as well as its essential for transferring of vital compounds such as hormones, fatty acids and amino acids to the target organs, scientists set this protein as a piece of sponge that take all excess water from the normal value in the body tissues to be excreted through the kidneys, and regulation of osmotic pressure, moreover work on metabolism of minerals and toxins, as confirmed by [63].

Furthermore, the high ratio of albumin to creatinine in urine perhaps explain to the increase in the levels of total cholesterol, triglycerides, low-density lipoproteins and very low-density lipoproteins, in contrast the decreased levels of high-density lipoproteins in the serums of diabetic patients compared to the levels in healthy individuals and the resulting of stimulation of the lipid peroxidation processes, oxidative stress and the subsequent increase of the deteriorative free radicals that cause degeneration or necrosis in the various body's tissues, particularly the renal tissues, because they are very sensitive.

It can also prevent the occurrence of nephropathy in patients with diabetes or reduce the damage caused by infection through good control of the levels of glucose in the blood and follow a diet that enables the patient to back off from eating foods that increase the levels of albumin and urea in the body, also doing sports, smoking cessation and regulation of blood pressure, as both of diabetic nephropathy and increase the ratio of albumin to creatinine in urine is a micro-vascular complications that affect the health of the patient with diabetes, in addition the increment in the excretion of albumin in urine is linked with the occurrence of renal impairment, and the excretion of more than 2 g of albumin within 24 hours is closely associated with the arrival to the end-stage renal disease (ESRD) [30].

Patients with diabetes in the stage of microalbuminuria have a lower glomerular filtration rate compared to their peers who are in the stage of normoalbuminuria, and decrement of the glomerular filtration rate refers to nephropathy [32], as well as the stage of microalbuminuria is considered the first clinical stage, which gives a specific indicator of the glomerular damage and usually occurs between 5-10 years of diabetic nephropathy, about 40% of diabetics reach this stage. Furthermore, glomerulonephritis depends on glomerular filtration rate, duration of diabetes and the ability of the blood glucose regulation [33].

In the chronic kidney failure (CKF), and the albuminuria stage, the glomerular filtration rate is less than 60 ml / min, while the blood pressure increased above the normal level. As regard with the terminal kidney failure (TKF), the glomerular filtration rate becomes (15 ml / min), and about 50% of patients who arrived to this stage need peritoneal dialysis and renal transplantation (renal replacement) [33].

Finally, the rise in the ratio of albumin to creatinine in urine may be due to damage or destruction in the glomerular filtration barriers, therefore, the glomerular filtration rate will increase significantly. In addition, the inflammation that associated with elevated in blood glucose level and oxidative stress, which is noticeably stimulated by diabetes may cause weakened in the albumin reabsorption process in the proximal convoluted tubules.

5 - The effect of diabetes mellitus on angiotensinogen levels in the urine

The findings of the current study revealed a significant increment in the level of angiotensinogen in urine when comparing diabetic patients with the healthy individuals, and these data are similar to many studies [28,64,65]. The result may

explain to high sugar levels in diabetic patients have stimulated the processes of lipid peroxidation (oxidative stress), which has been reflected in increased gene expression of angiotensinogen in renal tissues.

It is also suggested to the intrarenal RAS activation in patients with diabetes compared to healthy ones and resulting in increasing the rate of angiotensinogen renal output in urine.

As well as, the high levels of blood glucose in patients with diabetes may be caused many patho-histological changes in the kidneys such as cellular hypertrophy or hyperplasia, especially the tissues responsible for angiotensinogen producing, which was significantly reflected in the increased rate of angiotensinogen excretion in urine.

6- The effect of diabetes mellitus on fasting blood glucose and glycosylated hemoglobin levels

The results of the statistical analysis showed a significant difference in the level of glycosylated hemoglobin in patients with type II diabetes when compared with the healthy group and this was agreed with some studies [66], as well as with the study of [37], which reported that the estimation of HbA1c can provide important evidence for the blood glucose control and the detection of people with the primary stage of diabetes (prediabetic) .

It also an agreement with the study of [67], who found that glycosylated hemoglobin is an important test for diagnosis of diabetes or not, and with [68], which confirmed that the level of fasting blood glucose is considered a risk factor for diabetes, raises with increasing the glycosylated hemoglobin levels.

In addition, it was agreed with [69], who studied the second type of diabetes in Japan and found that the level of glycosylated hemoglobin increased by about 10 times with fasting blood glucose in patients who have been diagnosed with diabetes for seven years.

The result may be explained to absence of the good control of glucose metabolism in patients with type 2 diabetes because insulin is not enough to regulate the level of glucose in the blood, or it is secreted in sufficient quantities, but it does not exert its physiological functions correctly due to the occurrence of resistance to insulin in patients with diabetes, as well as glucose molecules can be produced or manufactured from non-carbohydrate sources.

The increase in glycosylated hemoglobin levels in diabetic patients is likely to attribute to poor control of glucose levels in the blood, resulting from patients' lack of adherence to balanced diet, or inadequate treatments that taken by diabetics, which may have adversely affected the blood glucose level and the level of glycosylated hemoglobin increased accordingly.

The increment in levels of glycosylated hemoglobin and fasting blood glucose in diabetic patients may be explained by oxidative stress and resulting in stimulating the production of reactive oxygen species that may have been attacked the body's cells, particularly beta cells in the pancreas, causing low insulin production or lack of its secretion when that cells damage completely.

In addition, obesity and its associated high levels of total cholesterol, low-density lipoproteins and very low-density lipoproteins may have the greatest negative effects on increased fasting blood glucose levels and glycosylated hemoglobin in patients with diabetes compared with healthy people.

Moreover, the elevated levels possibly suggest to the dysfunction of hepatic tissues, which has a negative effect on raising the levels of fasting blood glucose and glycosylated hemoglobin for the essential and effective role of these tissues in

regulating the levels of the biological molecules in the body, including sugars. The increase in the percentage of glycosylated hemoglobin has a direct relationship with the level of glucose in the blood during the period of 6-8 weeks [70], because this percentage is constantly changing according to the change in blood glucose level, and on this basis, the rise in the level of glucose in the blood due to increase the association of glucose molecules with hemoglobin, followed by high levels of glycosylated hemoglobin in the bloodstream [71].

Some studies reported that the blood glucose levels increased significantly with progression of the disease, which was explained by an increment in the insulin resistance in diabetics [37]. A previous study indicated that the reason for the high level of glucose in patients with type II diabetes is due to the lack of insulin secretion or the emergence of resistance by its receptors in the body, or dysfunction of the pancreas and metabolism, especially glucose in the liver [72].

In addition, the rise in blood glucose levels is also one of the most important factors leading to the development of diabetic nephropathy in patients with type II diabetes, moreover, the risk of progression and development of the albuminuria stage could be significantly reduced by improving blood glucose control [30].

7 - The effects of diabetes mellitus on urea level in the serum

The present study indicated to a remarkable increase in the serum urea level in patients with diabetes compared with healthy individuals. These results have been agreed with many studies [12, 73, 74].

This is likely to be explained by many factors, including eating high-protein diets, or decreasing the rate of urea excretion out of the body, as in the case of hypovolaemia, which leads to increase the reabsorption process by renal tubules, as was noted by [75].

Or perhaps because of the low efficiency of insulin secreted by beta cells, which caused an increment in the metabolism rate of proteins in the body and according to that the levels of plasma amino acids increased subsequently, which either be used directly to produce energy or in direct as a substrate for the glucose production.

Finally, the elevated urea levels in serum probably suggest to the adverse effects of oxidative stress that induced in liver and pancreatic tissues, which may have been largely stimulated because of diabetes mellitus.

8 - The effect of diabetes mellitus on albumin level in the serum

Concerning to the results of the current study ,there was a significant increase in serum albumin levels in patients with diabetes compared to healthy individuals , and this was agreed with many studies [76,77], but differed with another study [78], which demonstrated no observable change in serum albumin level when comparing diabetic patients with healthy subjects .

This may be explained by the fact that people with diabetes have a notable decrement in the level of insulin hormone secreted from beta cells ,or the secretion of this hormone may not be affected, as happens when the resistance of insulin and in both cases the level of albumin significantly elevated in the serum.

Or is likely to be attributed to other factors, such as the lack of a balanced diet or eating diets rich in proteins, which reflected negatively in increasing the level of albumin in serum as it is the most abundant protein among other plasma proteins ,it accounts about 60% of the total protein level, and any change in its level, results in a remarkable alteration in the total protein levels in serum [61]. Besides , another research has been indicated a high level of total protein in diabetic patients who safer

from diabetic nephropathy because of the noticeable rise in albumin level in serum[79] .

In addition, diabetes mellitus is characterized by increasing the levels of glucose in plasma, which in turn mutates plasma proteins through a non-enzymatic reaction called diabetes decomposition, which leads to the formation of toxic molecules (final products of biodegradable diabetes), and the accumulation of these molecules lead to accelerate diabetes and development and contribute to Pathogenesis of complications of diabetes [76,77].

In addition ,the increased serum albumin levels may be due to other factors including diet, lifestyle, inflammations, diseases, intake of high-fat foods, weight gain, lack of exercise, disturbances or dysfunction of thyroid and adrenal glands, and taking some medications such as antidepressants and contraceptives (For women) and other reasons.

9 - The effect of diabetes mellitus on creatinine level in the serum

In relation to the serum creatinine levels, there was a significant variation in this biomarker when comparing diabetes with healthy individuals and the finding compatible with several studies [80,81,82].

The significant difference in the level of creatinine between the two groups may explain to the completely or partially kidney dysfunction, in other words, the rate of glomerular filtration has been adversely affected, and which was inferred by the remarkable excretion of albumin in urine. The decrease in the rate of glomerular filtration to 50% is the main reason for the high level of creatinine in the serum [75] .

The reason for the high level of creatinine in the serum perhaps due to the arrival of patients with diabetes to advanced stages of nephropathy, or some diabetes drugs may contain a high amount of harmful materials or it lead to form secondary metabolic end products which were possibly accumulate in kidney tissues and caused in high levels of creatinine.

Moreover, it may be because of ulcers, or alienations in the internal kidney tissues, or decreases the level of kidney blood perfusion, or blockages in various areas of the urinary tract due to diabetes mellitus.

Furthermore, it probably due to a shrinkage in the mesangial cells because of the damaging free radicals that may be stimulated in results to oxidative stress or the potent activation of lipid peroxidation processes, which leads to decrease in the glomerular filtration rate and significant elevation in serum creatinine levels consequently.

Conclusions:

- 1- Diabetic nephropathy had effected on the functional performance of the kidney that caused an increment in excretion of angiotensinogen in urine.
- 2- The excreted angiotensinogen in urine could be one of the important vital indices to diagnose the stages of diabetic nephropathy, it is appears in the urine of diabetic nephropathy patients before the albuminuria stage.
- 3- The test of angiotensinogen that excreted in urine in ELISA method could be useful as an early biomarker to detect the activation of renin-angiotensin system in the diabetic nephropathy patients.
- 4- When the both genders of diabetic patients progress in age, the excreted angiotensinogen in urine is increased in compared to the young patients of the same disease.
- 5- The taken urine samples of both sexes of diabetic patients who had the duration of the disease about ten years or more had recorded the highest increasing of the

excreted angiotensinogen in urine in comparison to the patients with low duration of the disease.

- 6- The BMI had a negative effect on increase the level of urine angiotensinogen in patients with diabetes mellitus of males and females.
- 7- The albumin to creatinine ratio (ACR), the levels of angiotensinogen in urine, the levels of fasting blood glucose, glycosylated hemoglobin percentage, urea, albumin, and creatinine levels in serum were higher in diabetic males than in diabetic females.

Recommendations

- 1- Submitting the diabetic patients of both genders especially those in elevated stages of diabetic nephropathy for the periodical evaluation of angiotensinogen levels in urine to get the required medical care and prevention of development of nephropathy to dangerous stages or the renal failure.
- 2- Observation of angiotensinogen levels in diabetic patients with advanced age groups from both genders.
- 3- Make a regulated test of angiotensinogen levels of diabetic patients from both genders whom infected with this disease more than ten years.
- 4- Underscoring on the necessity of balanced diet with doing useful exercise regularly for both genders of diabetic patients ; especially patients whom complain of obesity because the current study proved that angiotensinogen levels were the highest for them , in addition to the importance of taking their urine samples for the continues estimation to the levels of this marker.
- 5- Assessment of vitamin D3 levels in patients with diabetes mellitus of males and females, particularly those with advanced age groups and high body weights ,as well as who have been infected for a long time (more than 10 years).

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