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EVALUATION OF SOME PHYSIOLOGICAL PARAMETERS IN MEN WITH DIABETIC NEPHROPATHY

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Abstract

Background: Nutrition helps the damaged liver to function properly, reduces the risk of infection and The study aims to the possibility of dependence some physiological criteria (renin, angiotensin-converting enzyme, and nitric oxide) as indicators for early diagnosis of diabetic nephropathy in patients with type 2 diabetes and avoiding its development to advanced stages. The study included the follow-up of (90) men, (30) healthy individuals and (60) patients with type 2 diabetes to periodic clinical examination, and evaluation of physiological parameters represented in the estimation of glycosylated hemoglobin ratio (HbA1c), levels of renin, angiotensin-converting enzyme, nitric oxide, and albumin to creatinine ratio(ACR) .The results showed a significant increase ($P< 0.05$) in the glycosylated hemoglobin ratio, the levels of renin, angiotensin-converting enzyme (ACE), monocyte chemoattractant protein and the albumin to creatinine ratio in urine (ACR), and in contrary to a significant decrease ($P < 0.05$) in the level of nitric oxide. The trial also diagnosed a notable effect ($P < 0.05$) for the age groups on the glycosylated hemoglobin ratio and albumin to creatinine ratio (ACR),while no noticeable differences appeared ($P > 0.05$)in levels of renin, angiotensin-converting enzyme ,and nitric oxide, also a significant impact ($P < 0.05$) was observed for the duration of diabetes mellitus on the levels of renin, and the albumin to creatinine ratio in the urine ,whereas no significant differences revealed ($P > 0.05$)in levels of angiotensin-converting enzyme and nitric oxide. In addition to the foregoing, the study recorded a significant effect ($P < 0.05$) of body mass index on the levels of renin, angiotensin-converting enzyme, and the albumin to creatinine ratio in the urine, in contrast no observable differences appeared ($P > 0.05$)in nitric oxide and glycosylated hemoglobin ratio. Moreover to the significant effect ($P < 0.05$) for stages of diabetic nephropathy on glycosylated hemoglobin rate, renin levels, ACE, nitric oxide and the albumin to creatinine ratio (ACR) in urine. It was concluded from the study that increased levels of renin and angiotensin-converting enzyme, and unlike it, decreased levels of nitric oxide is considered important predictive vital markers for progression the diabetic nephropathy to advance stages.

Key words: diabetes , insulin, blood glucose, creatinine, glycosylated hemoglobin.

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1.Introduction

Diabetes mellitus is defined as a group of metabolic diseases characterized by high blood glucose due to defect in insulin secretion, insulin action, or both [1], in 2014 the global prevalence of DM was estimated to be 9% and almost 1.6 million deaths worldwide were caused directly by diabetes mellitus. Diabetes mellitus is associated with increased rates of morbidity resulting from complications such as retinopathy, kidney disease, neuropathy, and cardiovascular disease [2], where these disorders lead to disturbances in metabolism and blood circulation, which results in increased blood glucose, insulin resistance, dyslipidemia, high blood pressure, and immune imbalance in the body [3]. The American Diabetes Association categorizes four basic types, which are type 1 diabetes, which occurs because to insulin deficiency because of a defect in the autoimmune pancreatic beta cells, which are damaged over time. As for type 2 diabetes, it occurs when the body resists insulin with the gradual loss of insulin secretion from the pancreas. There are other special types of diabetes, such as gestational diabetes (GDM), which do not exist before pregnancy, but appear in the second and third trimesters of pregnancy and result from the interaction of several hereditary genetic and environmental factors [4], there are other types that occur for multiple genetic or pathological reasons or because some chemical drugs are taken [5] . Pancreatic alpha cells secrete glucagon hormone at low blood glucose levels and beta cells secrete insulin when the blood glucose is high, with insulin and glucagon working inversely to maintain blood glucose levels within normal limits [6], there are many complications of diabetes which are influenced by several factors including gender, age, genetic status, social culture, duration of diabetes, body mass index, and blood pressure [7].

Diabetic nephropathy is a chronic loss of kidney function and a devastating complication for patients with diabetes are among the leading causes of cardiovascular disease, early death and end-stage kidney disease, its diagnosis depends on the microalbuminuria and the macro albuminuria, it is considered albuminuria early initial sign of microvascular complications that detect the risk of developing to the advanced stages of chronic kidney disease [8,9,10].

Diabetic nephropathy, is characterized by many functional and structural changes in nephron at the glomerulus level, damage to renal tubules and blood vessels, glomerular hyper filtration, thickening of the glomerular basal membrane, and expansion of mesangial cells [11]. The renin angiotensin system is known as, it is a hormonal system that regulates the balance of both blood pressure and water when the blood volume is low sensed by the juxtaglomerular cells(JG) in the kidneys and secrets an enzyme called renin to the blood [12], the renin angiotensin-aldosterone system is associated with kidney and heart disease where the renin in the blood plasma converts the secreted enzyme from the liver to angiotensin I, after which angiotensin I convert into angiotensin II by the converted enzyme that is found in the lung and specifically the lung endothelial membrane, angiotensin II is one of the strongest vasoconstrictors, thus increasing the narrowing of blood vessels, leading to an increase in blood pressure, and stimulating the adrenal cortex to secrete a hormone called aldosterone [13].

Nitric oxide is a free radical gas, a powerful regulator of blood circulation and an internal vasodilator that lowers blood pressure and maintains blood flow and is a neurotransmitter [14], nitric oxide (NO) deficiency is associated with endothelial disorder, hypertension, and atherosclerosis and chronic kidney disease [15], when produced it leads to the phosphorylation of many proteins that cause relaxation of the vascular muscle and control of blood vessels, prevents platelet accumulation and thus clot formation ,as well as increases blood flow to the kidneys, the glomerular filtration rate and urine production ,moreover regulates the immune response [16].

Materials and Working Methods

The study included the follow-up of (90) men, (30) healthy individuals and (60) patients with type 2 diabetes who were attending the diabetes and Endocrinology Center / Al-Sadr Medical City and also Al-Manathira General Hospital / Al-Najaf Governorate, their ages ranged between (30- 60) years old, and the study continued for the period of 1/10/2022 to 31/3/2023 after obtaining the consent of the two study groups, patients with diabetes were excluded who used insulin injections, patients with hypothyroidism, with high blood pressure, and with chronic diseases. The patients were

also divided into subgroups, according to the age; they were divided into three groups (30-39) years, (40-49) years and (50-60) years. While the duration of the diabetes, they were divided into three stages, (1-5) years, (6-10) years, and more than (10) years, they were also divided according to BMI into three groups: normal (20-24.9) kg/m2, overweight (25-29.9) kg/m2, and obesity (30≤) kg/m2, also divided according to the stages of diabetic nephropathy into the normal albuminuria stage (<30), the microalbuminuria stage (30-300), and the macro albuminuria stage (>300) [17].

1-Estimation of glycosylated hemoglobin levels

Evaluation of glycosylated hemoglobin ratio is using the hemoglobin dissolution reagent (tetradecyl trimethyl ammonium bromide) as a detergent to remove the white blood cells and the addition of the antibody reagent to the sample, the reaction between the glycosylated hemoglobin of the sample and the glycosylated hemoglobin of the antibody results in a soluble complex at the antibody site on the HbA1c molecule [18].

2-Estimation of renin levels in serum

The estimation of renin levels in the serum according to the equipment provided by the Chinese Company Bioassay technology laboratory this kit is an enzyme-linked immunosorbent assay (ELISA). The plate has been pre-coated with human REN antibody. Renin present in the sample is added and binds to antibodies coated on the wells, and then biotinylated human renin antibody is added and binds to REN in the sample, after that streptavidin-HRP is added and binds to the biotinylated renin antibody at 450 nm [19].

3-Estimation of angiotensin-converting enzyme (ACE) level in serum

To assess the angiotensin-converting enzyme levels in the serum, the equipment provided by the Chinese Company Bioassay technology laboratory this kit is an enzyme-linked Immunosorbent assay (ELISA). The plate has been pre-coated with Human ACE antibody. ACE present in the sample is added and binds to antibodies coated on the wells, and then biotinylated human ACE antibody is added and binds to ACE in the sample. Then streptavidin-HRP is added and binds to the Biotinylated ACE antibody at 450 [20].

4-Estimation of nitric oxide level in serum

The nitric oxide levels in the serum evaluate according to the equipment purchased from the Chinese Company Bioassay technology laboratory .This kit is an enzyme-linked immunosorbent assay (ELISA). The plate has been pre-coated with human NO antibody. NO present in the sample is added and binds to antibodies coated on the wells, and then biotinylated human NO antibody is added and binds to NO in the sample. After that, streptavidin-HRP is added and binds to the Biotinylated NO antibody at 450 nm [21].

5-Estimation of albumin to creatinine ratio in urine

To measure the level of albumin in the urine , the assessment of immune turbidity used through the interaction that occurs between the albumin antibodies with the antigen present in the sample to produce the antibody-antigen complex at 340 nm using the device Cobas e411that manufactured by a Company Roche [22], also, the enzymatic colorimetric method was used to determine the level of creatinine in the urine using the device Cobas e411 manufactured by a Company Roche[23].

Results

The Physiological Study

A-Comparing of some physiological blood parameters in men between the control group and the group of patients with type 2 diabetes mellitus

The present study showed significant differences (P = 0.001), (P = 0.003) ,(P = 0.001), (P = 0.05) and (P=0.05)in the glycosylated hemoglobin ratio, the level of renin, ACE ,and nitric oxide , as well as albumin to creatinine ratio respectively when the control group and the patients compared with each other , table (1).

Table 1. Comparison of some physiological parameters of blood in men between the control group and group of patients with type 2 diabetes mellitus

Variables	Mean±SE	Mean±SE	p- value
	Control group n=30	Patients with DN n=60	
HbA1C %	6.13±0.06	8.89±0.26	0.001 **
Renin (ng/l)	153.26±9.2	188.73±5.88	0.003**
ACE (ng/l)	70.83±6.78	125.44±4.62	0.001**
Nitric oxide µ mol/l	170.41±15.7	137.2±3.81	0.05*
ACR%	7.1±1.21	164.2±55.12	0.05*

* Significant differences at p-value <0.05 between the averages of the groups.
** Significant differences at p-value <0.001 between the averages of the groups.

B. The effect of age on some physiological parameters in men with type 2 diabetes mellitus

The statistical results of the current study pointed to significant differences (P = 0.017) and (P = 0.04) in the glycosylated hemoglobin and albumin to creatinine ratio, while there were no observable variations (P =0.91), (P =0.8) and (P=0.93) in renin ,ACE and nitric oxide respectively, when comparing the age groups between the patients with type 2 diabetes mellitus ,table (2)

Table 2. The effect of age on some physiological parameters in men with type 2 diabetes mellitus

Variables	Effect of the age			
	30-39 year n=9	40-49 year n=18	50-60 year , n=33	p-value
HbA1C %	7.9±0.31A	8.48±0.39B	9.55±0.31 C	0..017**
Renin (ng/l)	187.91±10.03 A	190.58±9.67 A	194.94±8.67A	0.91
ACE (ng/l)	114.37±12.4 A	122.36±7.80 A	123.39±5.84A	0.8
Nitric oxide µ mol/l	142.21±10.29 A	140.66±6.18 A	137.79±5.44A	0.93
ACR%	11.4±1.98 A	22.56±5.43 A	395.18±135.8 B	0.04*

The different letters mean significant differences between the averages of the groups.
The similar letters mean no significant differences between the averages of the groups.

C. The effect of duration on some physiological parameters in men with type 2 diabetes mellitus

The findings show a significant differences (P = 0.006) and (P =0.01) in the renin and albumin to creatinine ratio ,whereas there were no notable alterations (P=0.11),(P=0.33), (P=0.95) in glycosylated hemoglobin ratio , ACE and nitric oxide levels for the duration of diabetes mellitus, table (3)

Table 3. The effect of duration on some physiological parameters in men with type 2 diabetes mellitus

Variables	Effect of the duration			
	1-5 year N=30	6-10 year N=12	> 10 years N=18	p-value
HbA1C %	8.66±0.29A	9.41 ±0.52A	9.86±0.74A	0.11
Renin (ng/l)	181.71±5.85A	216.8±20.6B	220.57±6.61C	0.006 **
ACE (ng/l)	116.54±7.6A	130.13±7.43A	134.46±7.29A	0.33
Nitric oxide µ mol/l	138.87±5.77A	138.1±6.62A	135.18±8.97A	0.95
ACR%	22.65±3.93A	299.15±88.3B	500±262.66C	0.01*

The different letters mean significant differences between the averages of the groups.
The similar letters mean no significant differences between the averages of the groups.

D. The effect of BMI on some physiological parameters in men with type 2 diabetes mellitus

There was a significant variations (P = 0.007), (P =0.001) and (P=0.006) in renin, ACE and albumin to creatinine ratio, whereas no observable differences (P =0.98) and (P =0.68) in HbA1C and nitric oxide respectively when comparing the body mass index in patients with diabetes mellitus, table (4).

Table 4. The effect of body mass index on some physiological parameters in men with type 2 diabetes mellitus

Variables	Effect of the body mass index			
	Natural 20-24.9Kg/m^2	More 25- 29.9Kg/m^2	Obesity >30 Kg/m^2	p-value
HbA1C %	8.07±0.35 A	8.26±0.47 A	8.25±0.75 A	0.98
Renin (ng/l)	70.98±6.63 A	179.85±6.06 B	214.28 ±11.62 C	0.007**
ACE (ng/l)	109.72±7.84 A	123.91±6 B	169.09±11.7 C	0.001**
Nitric oxide µ mol/l	145.17±9.83 A	138.13±4.66 A	136.38±6.47 A	0.68
ACR%	66.7±19.22 A	229.97±63.8 B	416.25±118 C	0.006**

The different letters mean significant differences between the averages of the groups.
The similar letters mean no significant differences between the averages of the groups.

E. The effect of albumin to creatinine ratio (ACR) on some physiological parameters in men with type 2 diabetes mellitus

The results indicate a significant effect of (P = 0.001) , (P =0.05), (P = 0.002) , (P =0.007) on the renin, glycosylated hemoglobin , ACE and nitric oxide according to the albumin to creatinine ratio (ACR) for the diabetes mellitus patients, table(5)

Table 5. The effect of albumin to creatinine ratio (ACR) on some physiological parameters and among men with type 2 diabetes mellitus

Variables	Effect of the albumin to creatinine ratio (ACR)			
	Normo albuminuria N=21	Micro albuminuria N=19	Macro albuminuria N=20	p-value
HbA1C %	8.97±0.3 A	8.02±0.27 A	10.88±0.43 B	0.001**
Renin (ng/l)	174.2±15.62 A	205.97±13.28 B	220.27. ±6.04 C	0.05*
ACE (ng/l)	127.45±9.17A	140.83±9.47 B	144.11±6.52 B	0.002**
Nitric oxide µ mol/l	134.55±7.49A	126.03±5.19 B	122.72±3.98 C	0.007**
ACR%	21.34±1.24 A	81.43±12.5 B	842.87±271 C	0.001**

The different letters mean significant differences between the averages of the groups.
The similar letters mean no significant differences between the averages of the groups.

Discussion

The current research indicated that there was a significant increase in the glycosylated hemoglobin ratio (HbA1c) in men with type 2 diabetes than in healthy men, and the result agreed with some studies [24,25].The reason for the high glycosylated hemoglobin ratio in the blood may be attributed to the inability of the pancreas to produce insulin or due to the tissues resistance to the hormone insulin, despite the work of the pancreas and its continuation in secreting the hormone, over

time the beta cells are damaged and stop producing insulin completely, so the cells do not get enough energy, which leads to its rise, as recorded by some studies [26]. It was also noted that there was a significant effect of age groups on the glycosylated hemoglobin ratio in patients with type 2 diabetes mellitus was compatible with some studies [27], the findings may attribute to the occurrence of many physiological changes, such as a decrease in the production of insulin by the pancreas, which leads to an increment in insulin resistance in peripheral tissues particularly the liver, muscles, and adipose tissue, and a subsequent decrease in their sensitivity to insulin, as well as the activity of its receptors, furthermore a reduction in muscle tissue mass and glucose consumption with age in general, as indicated by some studies [28].

The study diagnosed an increase in the glycosylated hemoglobin ratio with the progression of the duration of diabetes mellitus, but it did not give a significant difference, and this was agreed with some studies [29]. It was probably due to the metabolic disorders that resulting from two main factors, the first a defect in insulin secretion because of poor functioning of pancreatic beta cells and their damage with an increase in the duration of diabetes mellitus , and the second a decrease in tissues sensitivity to insulin or what is known as insulin resistance in diabetic nephropathy patients [30]. The results of the current study also showed a rise in the glycosylated hemoglobin ratio with an increase in body mass index, but it did not rise to the level of significance and it was similar to some studies [31]. It was likely to be due to the accumulation of fat in the body tissues of patients who suffer from overweight, obesity, or oxidative stress, which leads to muscle tissue resistance to insulin and an increment in the level of glucose in the blood [32].

The results of the present experiment also revealed a significant increase in the levels of renin and ACE in patients with type 2 diabetes than in healthy subjects, this agreed with some studies [33]. Perhaps the reason for this may attribute to the elevation in the level of blood glucose or a decrease in blood volume within the kidneys (renal ischemia) in the afferent arteries to the cells adjacent to the renal glomerulus (Juxtaglomerular cell JG)) as a result, an sensory response occurs through the alpha receptors of these cells to secrete renin, as confirmed by some studies [34], or may be explained to the sodium retention or sympathetic stimulation of the nervous system in the renal circulation, where the enhancement is done directly by activating beta receptors to secrete renin in the kidneys, according to some studies [35]. The current trial also diagnosed a rise in the levels of renin and angiotensin-converting enzyme with advancing of the age group and the duration of diabetes mellitus, but it did not record a significant difference, and this was consistent with some studies [36].The results may be suggested to the progression of the stages of kidney disease with age due to an increase in the level of blood glucose and the rate of albumin excretion in the urine[37]. The statistical analysis of the results revealed a significant increment in the levels of renin and the angiotensin-converting enzyme with an increase in body mass index. This was in agreement with some studies [38], it may be because the obesity, which leads to structural and functional changes in many organs such as the heart , blood vessels, impairment of kidney functions ,damage to its tissues, and consequent abnormal activation of cytokines and adipokines that produced from visceral adipose tissue, which increases insulin resistance and the factors leading to inflammation and activation of the renin-angiotensin system ,as well as elevated levels of blood pressure and oxidative stress accordingly[39]. Besides ,the results of the present study exhibited a significant increase in the levels of renin and angiotensin-converting enzyme with the progression of the stages of diabetic nephropathy , but it did not reach to the level of significance and was compatible with some studies [40],this elevation may be due to a reduction in the rate of glucose uptake because of a rise in the level of blood glucose, thereby changes occur in the histological structure of the renal glomeruli and the basement membrane, moreover an activation in apoptosis, and the mesangial cells expansion as postulated by some studies [41].

Furthermore ,the results of the study appeared a significant decrease in the nitric oxide level in patients with type 2 diabetes in compared to healthy subjects was agreed with some studies [42], may be attributed to a defect in the lining of blood vessels, or in the immune system and

renal ischemia, which may occur as a result of elevated levels of extracellular sodium or changes in cell shape, as indicated by some studies [43]. In addition to what have been preceded ,the results of the current trial also revealed a decrease in the level of nitric oxide with the progression of the age group and the duration of diabetes mellitus, which did not rise to a significant level, and these results were consistent with some studies [44], the cause may be explained to the diabetic nephropathy and damage to renal cells as well as capillaries, which causes organ damage, decreased nitric oxide production and increased oxidative stress in the elderly patients , as proposed by some studies [45]. It was noted from the results of the present study a decline in the level of nitric oxide with an increment in body mass index, but it did not rise to a significant level in men with diabetes mellitus, and the results agreed with some studies [46-49], it possibly due to the oxidative stress that generates pro-inflammatory cytokines and resulting from reduced availability of nitric oxide causing endothelial dysfunction associated with obesity in diabetic patients [50] . The data of the study also showed a significant reduction in nitric oxide levels with the progression of the stages of diabetic nephropathy, and the results similar to some studies [51]. The reason for this may be due to changes in the glomerular capillaries, a decrease in the number of white blood cells, poor control of metabolism, protein kinase activity, an increase in glucose and angiotensin (Ang II) levels, in addition a reduction in nitric oxide leads to the stimulation of the renin-angiotensin system (RAS), which causes an increment in endothelial dysfunction according to some studies [52-54]

On the other hand ,the results recorded a significant elevation in the albumin to creatinine ratio in patients with type 2 diabetes when compared with the healthy subjects, and the results were in agreement with some studies [55-57], this is due to several factors, including increased loss of plasma proteins in the urine because of dysfunction of the renal tubules, which leads to insufficient reabsorption of the filtered protein, and thus substantial excretion of proteins from the tubular epithelial tissues and the occurrence of morphological and histological changes in the size of the glomerulus and basement membrane [58]. The study also diagnosed a significant rise in the albumin to creatinine ratio with age and duration of diabetes mellitus, and this was compatible with some studies ,it probably due to the occurrence of many pathological changes in the tissues of renal glomeruli, fibrosis of the interstitial connective tissue of the renal tubules, and the accumulation of proteins within the tubules, which leads to the secretion of protein in the urine, an increase in blood glucose, and a subsequent decrease in the glomerular filtration rate in elderly patients with type 2 diabetes mellitus, as confirmed by some studies [59]. There were significant differences in the albumin to creatinine ratio according to the body mass index, and the result agreed with some studies [60], the cause may be attributed to obesity, which is associated with a notable increment in body mass index, which leads to the release of adipokines (adipocytes) into the blood circulation, which may cause kidney injury resulting in hyper filtration of the nephrons and increased albuminuria . In addition, the poor control of blood glucose level and weight gain increase the risk of developing the diabetic nephropathy for the patients of type 2 diabetes mellitus , as shown by some studies [61]. The study recorded a substantial increase in the ratio of albumin to creatinine in the urine with the development of the three stages of renal dysfunction [62], where glomerular damage is one of the sensitive biomarker of renal impairment and a strong indicator of renal disorder, moreover, the imbalance in the processes of absorption of filtered proteins is associated with a gradual loss of kidney functions, as indicated by some studies[62].

Conclusions :

The results of the present experiment also revealed a significant increase in the levels of renin and ACE in patients with type 2 diabetes than in healthy subjects, this agreed with some studies . Perhaps the reason for this may attribute to the elevation in the level of blood glucose or a decrease in blood volume within the kidneys (renal ischemia) in the afferent arteries to the cells adjacent to the renal glomerulus (Juxtaglomerular cell JG)

References

1- Verhulst, M. J.; Loos, B. G.; Gerdes, V. E.; & Teeuw, W. J. (2019). Evaluating all potential oral complications of diabetes mellitus. *Frontiers in Endocrinology*., *10*: 56

2- World Health Organization. (2016). *World malaria report 2015*. World Xanthus, A.; Hatzitolios, A.; Koliakos, G.; & Tatola, V. (2007). Advanced glycosylation end products and nutrition—a possible relation with diabetic atherosclerosis and how to prevent it. Journal of food Science., 72(8): R125-R129

3- Tian, L.; Zhao, R.; Xu, X.; Zhou, Z.; Xu, X; Luo, D.; ... & Sun, Q. (2023). Modifying effects of Lactiplantibacillus plantarum on. chronic metabolic diseases. Food Science and Human Health, 12(4): 959-974

4- Triolo, T. M.; & Bellin, M. D. (2021). Lessons from human islet transplantation inform stem cell-based approaches in the treatment of diabetes. *Frontiers in endocrinology*, *12*: 636824.

5- American Diabetes Association. (2020). Classification and diagnosis diabetes: Standards of Medical Care in Diabetes—2020Diab

6- Ojha, A. Ojha, U.; Mohammed, R.; Chandrashekar, A.; & Ojha, H. (2019). Current perspective on the role of insulin and glucagon in the pathogenesis and treatment of type 2 diabetes mellitus. Clinical pharmacology: advances and applications, 57-65.

7- Gebermariam, A. D.; Tiruneh, S. A.; Ayele, A. A.; Tegegn, H. G.; Ayele, B. A.; & Engidaw, M. (2020). Level of glycemic control and its associated factors among type II diabetic patients in debre tabor general hospital, northwest Ethiopia. *Metabolism Open*, *8*: 100056

8- Natesan, V.; & Kim, S. J. (2021). Diabetic nephropathy–a review of risk. factors, progression, mechanism, and dietary management. *Biomolecules & therapeutics*, *29*(4): 365

9- Najjar, Z. S. R.; Jwad, S. M.; & Alkhafaji, R. S. (2020). Evaluation of VDR gene polymorphisms with nephropathy stages in men with type 2 diabetes mellitus. *J. Cardiovasc Disease Res*, *11*(4), 275-279.

10- Jwad, S. M.; & AL-Fatlawi, H. Y. (2022). Diabetic nephropathy and its effect on the physiological factors of the body. *Pharmaceuticals and Nanotechnology*, 4(1):24-30.

11- Erekat, N. S. (2022). Programmed cell death in diabetic nephropathy: A review of apoptosis, autophagy, and necroptosis. *Medical Science Monitor*: *28*.

12- Ames, M. K.; Atkins, C. E.; & Pitt, B. (2019). The renin-angiotensin-aldosterone system and its suppression. *Journal of veterinary internal medicine*, *33*(2): 363-382. care, 43(Supplement

13- Mahmood N A. (2016). Synthesis and Biological Study of Hetero (Atoms and Cycles) Compounds , Der Pharma Chemica 8(6), 40-48.

14- Upadhya, B.; Kozak, P. M.; Stacey, R. B.; & Vasan, R. S. (2022). Drugs to Reduce High Blood Pressure and Mitigate Hypertensive

15- Tejero, J.; Shiva, S.; & Gladwin, M. T. (2019). Sources of vascular nitric oxide and reactive oxygen species and their regulation. Physiological Reviews., 99(1): 311-379.

16- Rinde, N. B.; Enoksen, I. T.; Melsom, T.; Fuskevåg, O. M.; Eriksen, B. O.; & Norvik, J. V. (2023). Nitric-oxide precursors and dimethylarginines as risk markers for accelerated measured GFR. decline in the general population. *Kidney International Reports*

17- Mahmood A M A, Mahmood S J. Estimation of the protective efficacy of the alcoholic extract of Lepidium sativum seeds on the level of erythropoietin and some physiological parameters of albino male rats dosed with acetaminophen., Uttar Pradesh Journal of Zoology, 2022, 43(7): 42-51.

18- Serreli, G.; & Deiana, M. (2023). Role of Dietary Polyphenols in the Activity and Expression of Nitric Oxide

Synthases: A Review. *Antioxidants*, *12*(1): 147

19- Eng, E.; & Quaggin, S. (2022). Putting it All Together: Practical Approach to the Patient with Diabetic Kidney Disease. In Diabetes and Kidney Disease., (pp. 637-659): Springer

20- Nada, A. M.; & Younan, M. A. (2021). Dapagliflozin improves cardiovascular risk factors in Emirati patients with T2DM. *Therapeutic Advances in Endocrinology and Metabolism*, *12*: 2042018821995364.

21- Mowafy, H.; Yehia, H.; Samir, A.; Hazem, E. A.; & Elsisi, M. (2020). The prognostic value of renin in critically Ill patients on mechanical ventilator. The Medical Journal of Cairo University., 88(March): 739-746

22- Mukhlif, M. G.; Hameed, A. S.; & Algburi, F. S. (2021). Design, Synthesis And Molecular Docking Of L-Prolinamide ContainingThiazolidine-4-One Ring System As (ACE Inhibitors). *Nveo-Natural Volatiles & Essential Oils Journal| Nveo*., 15390-15415

23- Mara sing, I. N.; Idris, I.; Sunarno, I.; Arifuddin, S.; Sinrang, A. W.; & Bahar, B. (2021). Comparison of nitric oxide levels, roll over test value, and body mass index in preeclampsia and normotension. Gaceta Sanitaria., *(35)*: S306-S309.

24- Mahmood N A. (2015). Synthesis and Chemical Identification of Macro Compounds of (Thiazol and Imidazol). Research J. Pharm. and Tech 8(1), 78-84., DOI: 10.5958/0974-360X.2015.00016.5.

25- Sumida, K.; Nadkarni, G. N.; Grams, M. E.; Sang, Y.; Ballew, S. H., Coresh, J.; ... & Chronic Kidney Disease Prognosis Consortium‡. (2020). Conversion of urine protein–creatinine ratio or urine dipstick protein to urine albumin–creatinine ratio for use in chronic kidney disease screening and prognosis: An individual participant–based meta-analysis. *Annals of internal medicine*, *173*(6): 426-435.

26- Kashani, K.; Rosner, M. H.; & Ostermann, M. (2020). Creatinine: From physiology to clinical application. European journal of internal medicine., (72) :9-14.

27- Wu, T. E. ; Su, Y. W. ; & Chen, H. S. (2022). Mean HbA1c and HbA1c variability are associated with differing diabetes- related complications in patients with type 2 diabetes mellitus. Diabetes Research and Clinical Practice, 192, 110069.

28- AL-Fatlawi,H.Y..; & Jwad, S. M. (2022). Evaluation of physiological and immunological parameters in men with type 2 diabetic nephropathy. *NeuroQuantology*, *20*(5), 646-657.

29- Liu, J.; Bankir, L.; Verma, A.; Waikar, S. S.; & Palsson, R. (2022). Association of the urine-to-plasma urea ratio with CKD progression. *American Journal of Kidney Diseases*

30- Mahmood A J, Mahmood N A, Jwad S M. Development and Preparation of ciprofloxacin Drug Derivatives for Treatment of Microbial Contamination in Hospitals and Environment. Indian Journal of Forensic Medicine & Toxicology, 2020; 14(2): 1115-1122

31- Mimenza-Alvarado AJ, Jiménez-Castillo GA, Yeverino-Castro SG, Barragán-Berlanga AJ, Pérez-Zepeda MU, Ávila-Funes JA, & Aguilar-Navarro SG (2020). Effect of poor glycemic control on cognitive performance in older adults with type 2 diabetes: the Mexican Health and Aging Study. BMC Geriatrics, 20(1): 1-8.

32- Castaño, C.; Novials, A.; & Párrizas, M. (2019). Exosomes and diabetes. *Diabetes/metabolism research and reviews*, *35*(3): .e3107

33- Haghighatpanah, M.; Nejad, A. S. M.; Haghighatpanah, M.; Thunga, G.; & Mallayasamy, S. (2018). Factors that correlate with poor glycemic control in type 2 diabetes mellitus patients with complications. *Osong public health and research perspectives*, *9*(4):123

34- Galicia-Garcia, U.; Benito-Vicente, A.; Jebari, S.; Larrea-Sebal, A.,Siddiqi, H., Uribe, K. B.; ... & Martín, C. (2020). Pathophysiology of type 2 diabetes mellitus. *International Journal of Molecular Sciences*, *21*(17): 6275.

35- Boye, K. S.; Lage, M. J.; Shinde, S.; Thieu, V.; & Bae, J. P. (2021). Trends in HbA1c and body mass index among individuals with type 2 diabetes: evidence from a US database 2012–2019. *Diabetes Therapy*, *12*(7): 2077-2087

36- Shireen, R. R., Mahmood N A and A. J. Al-Zuhairi. (2020) Guanine substituted heterocyclic derivatives as bioactive compounds., Biochem. Cell. Arch 20(2), 3651-3655, 2020 ., DocID: https://connectjournals.com/03896.2020.20.3651

37- Wondmkun, Y. T. (2020). Obesity, insulin resistance, and type 2 diabetes:associations and therapeutic implications. Diabetes, metabolic syndrome and obesity: targets and Therapy., 13: 3611

38- Dart, A. B.; Wicklow, B.; Scholey, J.; Sellers, E. A.; Dyck, J.; Mahmud, F.; ... & Burns, K. (2020). An evaluation of - renin angiotensin system markers in youth with type 2

39- Senatore, F.; Balakumar, P.;& Jagadeesh, G. (2021). Dysregulation of. the renin-angiotensin system in septic shock: mechanistic insights and application of angiotensin II in clinical management. *Pharmacological Research.*, *174*: 105916

40- Sharma, N.; Anders, HJ, & Gaikwad, A.B. (2019). Villain and friend in the renin-angiotensin system: insight into acute kidney injury. Biomedicine and Pharmacotherapy, 110: 764-774

41- Blum, M. F.; Chen, J.; Surapaneni, A.; Turner, S. T.; Ballantyne, M.; Welling, P. A.; ... & Grams, M. E. (2023). Renin: measurements, correlates, and associations with long-term adverse kidney outcomes. *American Journal of Hypertension*, *36*(1): 42-4

42- Rawal, N.; Mittal, A.; & Khullar, M. (2023). Renin angiotensin system in aging and regeneration. In *the renin angiotensin system in. Cardiovascular Disease* (pp. 275-289). Springer, Cham

43- Galceran, I., & Oliveras, A. (2023). Renin angiotensin system and obesity-related organ damage. In *The Renin Angiotensin System. in Cardiovascular Disease* (pp. 259-273). Cham: Springer International Publishing

44- Ogura, Y.; Kitada M, and Koya D. (2021). Sirtuins and renal oxidative stress. Antioxidants, 10 (8): 1198

45- Liu, D.; & Lv, LL (2019). New understanding of the role of proteinuria the development of chronic kidney disease. Renal Fibrosis: Mechanisms and Treatments, 487-500.

46- Chen, Q.; Zhang, Y.; Meng, Q.; Wang, S.; Yu, X.; Cai, D.; ... & Bian, H. (2020). Liuwei Dihuang prevents postmenopausal atherosclerosis and endothelial cell apoptosis via-medicated αmethylation *Ethno Pharmacology*, *2521125.31*,

47- Abd El Dayem, S. M.; Battah, A. A.; El Bohy, A. E. M.; Ahmed S.; Hamed, M.; & Abd El Fattah, S. N. (2019). Nitric oxide gene polymorphism is a risk factor for diabetic nephropathy and atherosclerosis in type 1 diabetic patients. *Open Access Macedonian Journal of Medical Sciences*, *7*(19): 3132.

48- Ahmad, A.; Dempsey, S. K.; Daneva, Z.; Azam, M.; Li, N.; Li, P. L.; & Ritter, J. K. (2018). Role of nitric oxide in the cardiovascular andrenal systems. *International Journal of Molecular Mciences*, *19*(9): 260

49- Baylis, C. (2012). Sexual dimorphism: the aging kidney, involvement of nitric oxide deficiency, and angiotensin II overactivity. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, *67*(12), 1365-1372.

50- Gao, Z., Zhong, X., Tan, Y. X., & Liu, D. (2021). Apelin-13 alleviates diabetic nephropathy by enhancing nitric oxide production and suppressing kidney tissue fibrosis. *International Journal of Molecular Medicine*, *48*(3): 1-9.

51- Virdis, A.; Masi, S.; Colucci, R; Chiriacò, M.; Uliana, M.; Puxeddu, I.; Taddei, S. (2019). Microvascular endothelial dysfunction in obese patients. Current Hypertension Reports, 21 (4): 1-7.

52- Mengozzi, A., Masi, S., & Virdis, A. (2020). Obesity-related endothelial dysfunction: Moving from classical to

emerging mechanisms. Endocrine and Metabolic Science, *1*(3-4): 100063

53- Prabhakar, S.; Starnes, J.; Shi, S.; Lonis, B.; & Tran, R. (2007). Diabetic nephropathy is associated with oxidative stress and decreased renal nitric oxide production. *Journal of the American Society of Nephrology*, *18*(11): 2945-2952.

54- Hsu, C. N.; & Tain, Y. L. (2019). Regulation of nitric oxide production in the developmental programming of hypertension and kidney. disease. *International Journal of Molecular Sciences*, *20*(3): 681

55- Weaver, RG, James, MT, Ravani, P.; Weaver, CG, Lamb, EJ, Tonelli, M.; ... & Hemmelgarn, BR (2020). Estimation of the ratio of urine albumin to creatinine from the ratio of protein to creatinine: development of equations using same-day measurements. Journal of the American Society of Nephrology, 31(3): 591-601.

56- AL-Fatlawi, H. Y.; & Jwad, S. M. (2022). Assessing the level of a trial natriuretic peptide and some biochemical parameters in men with type 2 diabetic nephropathy. *Uttar Pradesh Journal of Zoology*,43(9): 1-13.

57- Kopp, J. B.; Anders, H. J.; Susztak, K.; Podestà, M. A.; Remuzzi, G.; Hildebrandt, F.; & Romagnani,P.(2020).Podocytopathies . Nature Reviews Disease Primers, 6(1): 68.

58- van der Burgh, A. C.; Rizopoulos, D.; Ikram, M. A.; Hoorn, E. J.; & Chaker, L. (2021). Determinants of the evolution of kidney function with age. *Kidney international reports*, *6*(12): 3054-3063.

59- Raikou, V.D, & Gavriil, S. (2019). Body mass index and risk of albuminuria in hypertensive patients with poor estimated glomerular filtration rate and the possible role of diabetes mellitus. Diabetes Mellitus and Metabolic Syndrome: Research and Clinical Reviews,13(2): 1041-1046

60- Rasoul, W. M. R., Mahmood S J. (2022). Evaluation of the efficacy of Brassica oleracea L. alcoholic extract on the levels of D3 and antioxidants in albino male rats dosed with Paracetamol. Uttar Pradesh Journal of Zoology, 43(6), 72-77. Available at:https://mbimph.com/index.php/UPJOZ/article/view/2984

61- Nakanishi, S.; Hirukawa, H.; Shimoda, M.; Tatsumi, F.; Kohara, K.; Obata, A.; & Kaneto, H. (2019). Comparison of HbA1c levels and body mass index for prevention of diabetic kidney disease: a retrospective longitudinal study using outpatient

62- Najjar, Z. S. R., Jwad, S. M., & Alkhafaji, R. S. (2020). Assessment of 1.25 (OH) 2D3 levels and nephropathy stages in adult male patients with type-2 diabetes mellitus. *J. Crit. Rev*, *7*(13), 1667-1674.