Uttar Pradesh Journal of Zoology

Volume 44, Issue 5, Page 42-54, 2023; Article no.UPJOZ.2485
ISSN: 0256-971X (P)

Evaluation of Some Immunological Parameters in with Men Diabetic Nephropathy

Senan Abd Al-Razaq Al-Haddad [a] and Saher Mahmood Jwad [a++*]

[a] Department of Biology, College of Education for Girls, University of Kufa, Iraq.

Authors' contributions

This work was carried out in collaboration between both authors. Authors SMJ and SAARAH managed the research. Authors SMJ and SAARAH done the research and wrote the main manuscript text. Authors SMJ and SAARAH prepared tables and wrote a part of manuscript text. Both authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2023/v44i53441

Editor(s):
(1) Dr. Osama Anwer Saeed, University of Anbar, Iraq.
Reviewers:
(1) Zagrai Gavrilă, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania.
(2) Kiranmayi S Vinapamula, Sri Venkateswara Institute of Medical Sciences, India.

Original Research Article

Received: 30/01/2023
Accepted: 01/04/2023
Published: 06/04/2023

ABSTRACT

The Aim: The study aims to the possibility of depending on some immunological criteria (monocyte chemoattractant protein (MCP-1, MCP-3) and interleukin-18).
Study Design: The study is indicators for early diagnosis of diabetic nephropathy in patients with type 2 diabetes and avoiding its development to advanced stages.
Place and Duration of Study: 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 from 1/10/2022 to 31/3/2023 after obtaining the consent of the two study groups.

[++] Professor;
**Corresponding author: Email: sahermahmood45@gmail.com;*

Methodology: The study included the follow-up of (90) men, (30) healthy individuals and (60) patients with type 2 diabetes mellitus to periodic clinical examination ,the estimation of glycosylated hemoglobin ratio (HbA1c) and evaluation of immunological parameters represented in levels of monocyte chemoattractant protein (MCP-1 , MCP-3) and interleukin-18 .
Results: The study diagnosed a significant increase ($P < 0.05$) in glycosylated hemoglobin ratio, monocyte chemoattractant protein MCP-1, MCP-3) and interleukin-18 ,and in contrast to a significant decrease ($P < 0.05$) in the glomerular filtration rate (GFR) in men with diabetes mellitus type II compared to healthy men.
The study also showed a significant effect ($P < 0.05$) for the age groups on the glycosylated hemoglobin ratio and monocyte chemoattractant protein (MCP-3), and conversely a significant decline in the glomerular filtration rate (GFR) ,while no significant differences appeared ($P > 0.05$) in levels of monocyte chemoattractant protein (MCP-1) and interleukin-18. Also, a notable impact ($P < 0.05$) was observed for the duration of diabetes mellitus on the levels of monocyte chemoattractant protein (MCP-1 , MCP-3),whereas no substantial differences appeared ($P > 0.05$) in levels of glycosylated hemoglobin ratio, glomerular filtration rate (GFR) and interleukin-18.
In addition to the foregoing, the trial recorded a significant influence ($P < 0.05$) of body mass index on the levels of monocyte chemoattractant protein (MCP-3),while no noticeable differences appeared ($P > 0.05$)in, the glycosylated hemoglobin ratio , glomerular filtration rate(GFR),and monocyte chemoattractant protein (MCP-1).
Moreover, a significant effect ($P < 0.05$) for stages of diabetic nephropathy was diagnosed on the glycosylated hemoglobin ratio, monocyte chemoattractant protein (MCP-1, MCP-3), interleukin-18 and glomerular filtration rate (GFR).
Conclusion: It is possible to depend on the increased levels of some immunological indicators especially MCP-1, MCP-3 and interleukin-18 as predictive biomarkers to diagnose the progression to advance stages of the diabetic nephropathy if this coincides with a decrease in the glomerular filtration rate.

Keywords: GFR; Interlink-18; MCP-1; MCP-3; diabetic nephropathy.

1. INTRODUCTION

Diabetes mellitus (DM) is defined as a metabolic disease characterized by chronic hyperglycemia as a result of a deficiency of the hormone insulin or a decrease in the sensitivity of body tissues to insulin, or both, and the global incidence of it increases annually. Diabetes mellitus (DM) is one of the chronic diseases that accompany the patient, with the exception of gestational diabetes, and it occurs when the pancreas is unable to produce insulin in sufficient quantity, and the hormone insulin is the main factor responsible for regulating the level of glucose in the blood [1].

Diabetes is classified according to the American Diabetes Associations diabetes, type 1 diabetes (T1D) is an autoimmune disease that leads to the destruction of pancreatic beta cells by cellular immunity, which leads dependence on external insulin to lifelong [2] , diabetes type 2 (T2DM) is a complex chronic disease that, affects patient as a result of the body's resistance to the insulin hormone or a decrease in the effectiveness of the insulin hormone by pancreatic beta cells, and it is characterized by high blood glucose, which leads to disorders in the blood circulation, the nervous system, and immune system [3]. As for the third type, gestational diabetes, which is caused by high blood glucose, and is considered one of the most common complications during pregnancy [4], and it is diagnosed in the second or third trimester of pregnancy, there are other types of diabetes resulting from other causes, for example, monogenetic diabetes, such as diabetes (in newborns), diabetes mellitus that begins at maturity in young people, and external diseases of the pancreas (cystic fibrosis) [5].

Complications of type 2 diabetes including acute complications particularly diabetic ketoacidosis, hypoglycemia, and hyperosmolar coma [6], while the chronic complications that include types of disorders such as diabetic nephropathy, neuropathy, and retinopathy [7,8,9].

There are many mechanisms that lead to the development of diabetic nephropathy and kidney failure, including elevated blood glucose and systemic high blood pressure, which negatively affects the blood vessels, as when it rises, it elevates the flow of large amounts of blood into the renal blood vessels, and this in turn increases the narrowing of blood vessels [10].

One of the predictive markers for the progression of renal disease is the increment in the levels of monocyte chemoattractant protein MCP-1 and MCP-3, which are major chemicals that belong to small molecular weight cytokines and have a role in the selective stimulation of monocytes, neutrophils and lymphocytes [11], MCP-1 is associated with inflammation and increases the risk of coronary artery disease .Moreover, the resulting inflammation contributes to the formation of sclerotic areas in the arteries and leads to their rupture, platelet accumulation, and clot formation [12], as well as elevated levels of MCP-3 stimulate the inflammation, and the development of diabetic kidney disease due to the renal fibrosis through accumulation of macrophages, infiltration of immune cells, and inflammatory ROS production consequently [13].

Interleukin-18 also possesses multidirectional immune regulation capabilities with other cytokines to regulate immune responses, as it was found that elevated (IL-18) levels in blood, urine, and plasma are vital predictive indices for the progression of diabetic nephropathy in patients with type 2 diabetes mellitus [14].

2. MATERIALS AND METHODS

The present 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 from 1/10/2022 to 31/3/2023 after obtaining the consent of the two study groups. The patients were also divided into numerous 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 mellitus, 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) [15], and finally according to the glomerular filtration rate GFR it is divided into three phases: the first phase (<90 mg/min/1.73), the second phase (60-90 mg/min/1.73), and the third phase (45-60 mg/min/1.73).

1- Estimation of glycosylated hemoglobin levels

Evaluation of glycosylated hemoglobin ratio is using the hemoglobin dissolution reagent (Tetradecyl trim ethyl ammonium bromide) as a deterrent 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 [16].

2- Estimation of monocyte chemoattractant protein MCP-1 levels in serum

The MCP-1 levels were assessed 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 antibody. MCP-1 present in the sample is added and binds to antibodies coated on the wells, and then biotinylated human MCP-1 antibody is added and binds to MCP-1 in the sample. After that, streptavidin-HRP is added and binds to the biotinylated MCP-1 antibody at 450 nm [17].

3- Estimation of monocyte chemoattractant protein MCP-3 levels in serum

The assessment of MCP-3 levels in the serum were according to the equipment that 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 antibody. MCP-3 present in the sample is added and binds to antibodies coated on the wells, and then biotinylated human MCP-3 antibody is added and binds to MCP-3 in the sample, after that streptavidin-HRP is added and binds to the biotinylated MCP-3 antibody at 450 nm [18].

4- Estimation of Interleukin-18 (IL-18) level in serum

As for the levels of IL-18 in the serum, were evaluated 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 IL-18 antibody. IL-18 present in the sample is added and binds to antibodies coated on the wells, and then

biotinylated human IL-18 antibody is added and binds to IL-18 in the sample. Then streptavidin-HRP is added and binds to the Biotinylated IL-18 antibody at 450 [19].

5- Calculation of glomerular filtration rate

The glomerular filtration rate was calculated according to the Cockcroft-Gault equation. Using this equation is more accurate in estimating the glomerular filtration rate calculation.

GFR (male) ml/min = 140-age (years) × bodyweight (kg)/ (72×serum creatinine) (mg/dl) [20].

3. RESULTS

3.1 The Immunological Study

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

The present study showed significant differences (P = 0.001), in the glycosylated hemoglobin ratio, MCP-1*,* MCP-3, and IL-18 respectively, and in contrast, a significant decrease in glomerular filtration rate (P = 0.001) between the two groups of study (healthy and patients), Table (1).

B- The effect of age on some immunological parameters in men with type 2 diabetes mellitus

The results of the current study recorded statistically significant differences (P = 0.017) and (P = 0.029) in the glycosylated hemoglobin ratio and MCP-3 level, contrary to a significant decrease in the glomerular filtration rate (P = 0.001).While there were no observable variations (P =0.11), (P =0.46) in MCP-1 and IL-18 respectively, when comparing the age groups of diabetes mellitus patients with each other, Table (2).

C- The effect of duration on some immunological parameters in men with type 2 diabetes mellitus

It was noted from the results of the current study a significant increment (P = 0.015) in MCP-1 and (P =0.04) in MCP-3, whereas there were no significant differences (P=0.11),(P=0.142), (P=0.27) in glycosylated hemoglobin ratio, the level of IL-18 and glomerular filtration rate for the duration of diabetes mellitus, Table (3).

D- The effect of BMI on some immunological parameters in men with type 2 diabetes mellitus

A substantial effect (P = 0.05) was indicated in MCP-3, while there were no observable variations (P =0.98), (P =97), (P =0.33), (P =0.76) in glycosylated hemoglobin ratio, MCP-1,IL-18 and glomerular filtration rate respectively when comparing the body mass index of diabetes mellitus patients as in Table (4).

E- The effect of glomerular filtration rat (GFR) on some immunological parameters in men with type 2 diabetes mellitus

The findings of the statistical analysis of the current study revealed a significant elevation (P = 0.02) in glycosylated hemoglobin ratio, (P = 0.001) in levels of (MCP-1, MCP-3) and (P = 0.05) in IL-18, in contrast a notable decrease (P = 0.001) was diagnosed in the glomerular filtration rate (GFR), Table (5).

Table 1. Comparison of some immunological 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
	Healthy control n=30	Patients with DN n=60	
HbA1c %	6.13±0.06	8.89±0.26	0.001 **
MCP-1 (ng/l)	134.1±3.16	261.66±11.03	0.001**
MCP-3 (ng/l)	165.1±2	230.75±2.5	0.001**
Interleukin;-18(ng/l)	20.14±0.54	27.32±0.52	0.001**
GFR (mg*min/1. 73)	179.62.±6.27	80.4±3.8	0.001**

** 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*

Table 2. The effect of age on some immunological 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±0.31C	0. 017**
MCP-1 (ng/l)	237.53±30.05A	246.0±10.21A	291.67±14.75A	0.11
MCP-3 (ng/l)	216.44±6.92A	222.12±4.54B	235.25±3.24C	0.029*
Interleukin-18 (ng/l)	26.05±0.52A	26.2±0.64A	27.63±0.8A	0.46
GFR (mg*min/1. 73)	115.22±9.04A	94.31±7.71B	68.59±3.93C	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

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

Variables	Effect of the duration of diabetes mellitus			
	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
MCP-1 (ng/l)	249.71±31.88A	248.15±22.6A	315.64±22.75B	0.015 *
MCP-3 (ng/l)	223.35±3.37A	235.6±5.04B	235.94±4.22B	0.04*
Interleukin-18 (ng/l)	26.52±0.58A	26.8±1.5A	28.96±1.2A	0.142
GFR (mg*min/1. 73)	85.54±11.96A	81.40±10.7A	70.6±6.65A	0.27

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

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

Variables	Effect of 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±0.35A	8.26±0.47A	8.25±0.75A	0.98
MCP-1 (ng/l)	239.3±23.2A	262.33±14.42A	266.0 ±18.32A	0.97
MCP-3 (ng/l)	221.00±4.5A	232.41±3.31B	238.85±5.27C	0.05*
Interleukin-18 (ng/l)	26.09±0.72A	27.41±0.73A	28.44±1.25A	0.33
GFR (mg*min/1. 73)	82.62±7.28A	76.3±4.75A	75.9±9.96A	0.76

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

Table 5. The effect of glomerular filtration rate (GFR) on some immunological parameters in men with type 2 diabetes mellitus

Variables	Glomerular filtration rate			
	>90mg* min /1.73 n= 23	(60- 90)mg* min /1.73 n= 21	(45- 60)mg* min /1.73 n=16	p-value
HbA1c %	8.83±0.36A	8.49±0.33B	10.0±0.43C	0.02*
MCP-1 (ng/l)	238.47±10.34A	255.7±14.75A	317.1 ±18.94C	0.001**
MCP-3 (ng/l)	215.92±3.25A	230.40±3.39B	249.65±3.10C	0.001**

Variables	Glomerular filtration rate			
	>90mg* min /1.73 n= 23	(60- 90)mg* min /1.73 n= 21	(45- 60)mg* min /1.73 n=16	p-value
Interleukin-18 (ng/l)	25.43±0.57A	27.07±0.99B	29.54±0.98C	0.05*
GFR (mg*min/1. 73)	114.81±3.58A	73.94±1.87B	46.2±1.93C	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

4. DISCUSSION

1- Comparison of HbA1C ratio between healthy and patients groups with type 2 diabetes mellitus, demographic parameters and some biochemical biomarkers

The current research indicated that there was a significant increase in the glycosylated hemoglobin ratio (HbA1c) in men with type 2 diabetes mellitus than in healthy men, and the study agreed with some studies [21,22,23].

The reason for the high glycosylated hemoglobin ratio in the blood, may be attributed to the oxidation of proteins, nucleic acids and fats, which leads to increased oxidative stress, which causes poor functioning of pancreatic beta cells and a lack of insulin production, according to some studies [24]. The study also showed that there was a significant effect of age groups on the glycosylated hemoglobin ratio in patients with type 2 diabetes mellitus [25], the reason may be due to cognitive impairment in the elderly patients and the inability to regulate blood glucose levels (lack of good control) [26].

The data of the current study showed an increment in glycosylated hemoglobin ratio with the length of the duration of diabetes mellitus, but it did not rise to the level of significance, and some studies recorded this [27], it may be explained to the complications resulting from long-term diabetes mellitus, leading to damage to pancreatic beta cells, increased insulin resistance, and vascular disease [28].

It was also observed from the findings of the this study 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 agreed with some studies [29]. It likely to be due to excessive obesity in patients with diabetes mellitus, resulting from the accumulation of fat in the visceral tissues, leading to increase in the blood glucose levels and insulin resistance subsequently [30].

The results also pointed to a significant increment in the glycosylated hemoglobin ratio with a decrease in the glomerular filtration rate, and this was compatible with some studies [31] , it may be suggested to the occurrence of a physiological defect in the renal tissues resulting from damage to the glomerular filtration barriers and the accumulation of proteins in the renal tubules, leading to a reduction in the glomerular filtration rate and an increase in the secretion of albumin in the urine, as indicated by some studies [32].

2- Comparison of MCP-1 levels between healthy and patients groups with type 2 diabetes mellitus, demographic parameters and some biochemical biomarkers

The results of the current study showed that there was a significant increase in the level of the monocyte chemoattractant protein MCP-1 in men with type 2 diabetic men in compared to the healthy men, and this result in agreement with many studies [33], and this may attribute to the high level of glucose in the blood which causes stimulation of the production of chemoattractant protein for monocytes (MCP-1) from tubular, mesenteric and foot epithelial cells ,as well as renal smooth muscle cells, which activates macrophages and releases varies reactive oxygen species(ROS) substantially, as confirmed by some studies [34] .

The study revealed a rise in the level of monocytes chemoattractant protein (MCP-1) with advancing age, but it did not rise to a significant level in patients of diabetic nephropathy, the result was consistent with some studies [35]. It possibly explain to the renal tissue aging, which resulting from immunodeficiency in the elderly, and this may lead to changes in the structural and functional performance of the kidneys

gradually, increase catabolism process and inhibition of growth factors [36].

In addition, it was also observed that there was a significant increment in the level of the MCP-1 in patients with diabetic nephropathy and long duration of diabetes mellitus, and this was in agreement with some studies [37]. The data may be attributed to the fact that chronic inflammation is stimulated with the long duration of diabetes mellitus, leading to the development of the disease, as these infections cause many pathological and histological changes, causing a defect in the functions of the glomeruli, and thus a decrease in the size and number of nephrons over time and an increase in proteinuria [38].

Besides, the results of the statistical analysis noticed an elevation in the level of MCP-1 with an increment in body mass index, but it did not give significant differences in patients with diabetic nephropathy, and the results were compatible with some studies [39], might due to the obesity and its association with muscle hypertrophy and multiple factors such as inflammation, insulin resistance, nutrition, lack of physical activity, increased inflammatory cytokines and fat mass, as well leptin that secreted by adipocytes and white blood cells in adipose tissue, as found by some studies [40].

Furthermore, the results showed a significant increase in the level of monocyte chemoattractant protein (MCP-1) with the decreasing of the glomerular filtration rate, and the result agreed with some studies [41].

This can suggest to the fact that the elevated production of MCP-1 indicates poor renal performance due to glomerular changes in particular increased basement membrane thickness and the expansion of mesangial cells , which affect the glomerular filtration rate and lead to hardening of the glomeruli due to the high level of glucose in the blood, which results in elevated excretion of proteins in the urine [42].

3- Comparison of MCP-3 levels between healthy and patients groups with type 2 diabetes mellitus, demographic parameters and some biochemical biomarkers

The present trial showed a significant increment in the level of monocyte chemoattractant protein (MCP-3) in men with type 2 diabetes mellitus when compared with healthy subjects and agreed with some studies [43].

It is likely that this is due to the poor performance of renal functions because of the occurrence of infections; particularly the inflammation which leads to tissue injury and the secretion of (MCP-3) chemoattractant protein that mainly recruit immune cells, which are monocytes, macrophages and T lymphocytes from the blood to the damaged site, according to some studies [44].

The current experiment also showed a significant increase in the level of (MCP-3) monocyte chemoattractant protein with age, and it was consistent with the study of [45]. This may attribute to a decline in immune functions and increased inflammations in the elderly, which indicated by a decreasing in the number of T cells (immune tissue aging) that leads to a weakening of the acquired immune responses and hence increment in the secretion of tumor necrosis factor-alpha (TNF-α), interleukin (IL-6), and the level of inflammation subsequently [46].

In addition, the current study identified a significant rise in the level of MCP-3 with the length of the duration of diabetes mellitus. This is explained by the fact that the long duration of diabetes activates chronic infections, the development of complications of the disease, and the deterioration of kidney functions associated with increased renal fibrosis due to the production of inflammatory cytokines and interleukins that stimulate inflammation, as postulated by some studies [47].

Moreover, the statistical analysis also noted that there was a significant effect of BMI on the level of (MCP-3) monocyte chemoattractant protein in patients with diabetic nephropathy. The results of the study agreed with some studies [48].

A significant increase in the level of monocyte chemoattractant protein (MCP-3) was diagnosed with a decrement in the glomerular filtration rate, and this probably due to the negative changes that occur in the glomerulus structure, including mesangial dilatation, capillary surface reduction, loss of foot cells, and renal fibrosis [49].

4- Comparison of (Interluiken-18) levels between healthy and patient's groups with type 2 diabetes mellitus, demographic parameters and some biochemical biomarkers

There was a significant increment in the levels of interleukin-18 in patients with diabetic nephropathy compared to healthy men, and the result agreed with many studies [50], This is due

to the high level of blood glucose in patients who suffer from impaired glucose tolerance, as the increase in the glycosylated hemoglobin ratio weakens the functions of tubular cells and causes glomerular sclerosis ,as well as interstitial fibrosis, or due to high levels of triglycerides and increased insulin resistance, as confirmed by some studies [51]. Furthermore, the trial showed an elevation in the level of interleukin-18 with age, but it is not significant, and this may be because the diseases which associated with the elderly, especially atherosclerosis, diabetes mellitus, and stimulation of inflammatory pathways that are more common with age and affect IL-18 levels, as indicated by some studies [52].

It was noted from the results of the statistical analysis a rise in the level of interleukin-18 with the long duration of diabetes mellitus, but it did not reach the level of significance, and this could attribute to the long period of diabetes which causes various common complications especially, atherosclerosis, diabetic nephropathy, damage to the capillaries in the glomeruli and substantial elevation in the secretion of proteins in the urine, which affects the production of inflammatory cytokines, as indicated by some studies [53]. The study diagnosed an increment in the level of interleukin-18 with an increase in body mass index, but it was not significant and agreed with the study of [54], and this may explain to the chronic inflammation that caused by cytokines activating inflammatory pathways in patients with obesity-related metabolic syndrome, and they have elevated levels of interleukin-18, as well as may be attributed to the high levels of oxidized low density lipoproteins that activate inflammations [55]. The present experiment showed a significant increase in the levels of interleukin-18 in the serum with the reduction of the glomerular filtration rate, and the results were similar to some studies [56], This may be explained by a decrease in the rate of filtration efficiency ,hyperplasia, and renal fibrosis with a gradual loss of functions of the kidneys due to a high level of glucose in the blood, particularly in the final stages of diabetic nephropathy, as demonstrated by some studies [57].

5- Comparison of GFR between healthy and patient's groups with type 2 diabetes mellitus, demographic parameters and some biochemical biomarkers

The study revealed a significant decrease in glomerular filtration rate in patients with type 2 diabetes mellitus comparing with healthy subjects, and the results consistent with some studies [58,59]. It may be due to diabetic nephropathy, which is one of the most common complications of type 2 diabetes as a result of the high level of glucose in the blood and the occurrence of histological changes in the small blood vessels in the kidneys, which leads to numerous alterations in the levels of salts that present in the proximal tubules and the re-absorption of fluids, which stimulate oxidative stress, tubular fibrosis, and a decrease in glomerular filtration rate, as confirmed by some studies [60]. The data also recorded a significant decline in the glomerular filtration rate with the advanced age group. The results similar to the study of [61] and it possibly due to aging associated with structural and physiological changes in the kidneys and muscle mass that may affect the glomerular filtration rate, because of a large loss of nephrons with age and there is no compensation by the remaining glomerular nephrons [62-67].

In addition ,the study diagnosed a reduction in the glomerular filtration rate with the long duration of diabetes mellitus did not reach a significant level, and the results of the study agreed with the study of [68], This may be attributed to diabetic nephropathy associated with the loss of kidney functions, as patients who have a long duration of diabetes have severe damage glomeruli, which is represented by infection of the foot cells, thickening of the glomerular basement membrane, expansion of the mesangial, damaged renal tubules, and infiltration of inflammatory cells, which in turn causes a decrease in the glomerular filtration rate as indicated by some studies [69]. A decrement was observed in the glomerular filtration rate with an increment in body mass index, but it did not give a significant difference. The results of the current study were in agreement with some studies [65], and this may be because the high levels of cholesterol and triglycerides in the blood, as well the obesity in the abdominal region, besides impaired glucose metabolism, which are associated with chronic kidney disease, insulin resistance, and metabolic syndrome [70].

4. CONCLUSIONS

The study also showed a significant effect ($P < 0.05$) for the age groups on the glycosylated hemoglobin ratio and monocyte chemoattractant protein (MCP-3), and conversely a significant

decline in the glomerular filtration rate (GFR) ,while no significant differences appeared (P > 0.05) in levels of monocyte chemoattractant protein (MCP-1) and interleukin-18. Also, a notable impact (P < 0.05) was observed for the duration of diabetes mellitus on the levels of monocyte chemoattractant protein (MCP-1 , MCP-3),whereas no substantial differences appeared (P > 0.05) in levels of glycosylated hemoglobin ratio, glomerular filtration rate (GFR) and interleukin-18.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ning C, Jiao Y, Wang J, Li W, Zhou J, Lee YC, et al. Recent advances in the managements of type 2 diabetes mellitus and natural hypoglycemic substances. Food Sci Hum Wellness. 2022;11(5):1121-33.
   DOI: 10.1016/j.fshw.2022.04.004
2. Mallone R, Eizirik DL. Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type diabetes? Diabetologia. 2020;63(10):1999-2006.
   DOI: 10.1007/s00125-020-05176-7, PMID 32894310.
3. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of type 2 diabetes mellitus. Int J Mol Sci. 2020;21(17):6275.
   DOI: 10.3390/ijms21176275, PMID 32872570.
4. Anastasiou E, Farmakidis G, Gerede A, Goulis DG, Koukkou K, A, Stefos T. Clinical practice. guidelines on diabetes mellitus and pregnancy: I. Pre-existing type 1 and type 2 diabetes mellitus. Hormones. 2020;19(4):593-600.
5. American Diabetes Association. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes. Diabetes Care, 43(Supplement_1). 2020. 2;2020:S14-31.
6. Balaji R, Duraisamy R, Kumar MP. Complications of diabetes mellitus: a review. Drug Invent Today. 2019;12(1).
7. Yadav R. A case study on complication of diabetes nephropathy, neuropathy, retinopathy and diabetic foot ulcer [international journal]. 2019;2(4):99.
8. Najjar ZSR, Jwad SM, Alkhafaji RS. Evaluation of VDR gene polymorphisms with nephropathy stages in men with type 2 diabetes mellitus. J Cardiovasc Dis Res. 2020;11(4):275-9.
9. Jwad SM, Fatlawi HY. Diabetic nephropathy and its Effect on the physiological factors of the body. Pharmaceutical and Nanotechnology. 2022;4(1):24-30.
10. Vaidya SR, Aeddula NR. Chronic renal failure. StatPearls [Internet]; 2021.
11. Yoshimura T. The production of monocyte chemoattractant protein-1 (MCP-1) /CCL2intumor oenvironments. Cytokine. 2017;98:71-8.
    DOI: 10.1016/j.cyto.2017.02.001
    PMID 28189389.
12. Blanco-Colio LM, Méndez-Barbero N, Pello Lázaro AM, Aceña Á, Tarín N, Cristóbal C, et al, J.. MCP-1 Predicts recurrent cardiovascular events in patients with persistent inflammation. J Clin Med. 2021; 10(5): 1137:MCP-1.
    DOI: 10.3390/jcm10051137
    PMID 33803115.
13. Srivastava SP, Hedayat AF, Kanasaki K, Goodwin JE. microRNA crosstalk affects transitions from epithelial to mesenchyme, endothelial to mesenchyme, and macrophage to mesenchyme in the kidney. Front Pharmacol. 2019;10:904.
    DOI: 10.3389/fphar.2019.00904
    PMID 31474862.
14. Mahmood AMA, Mahmood SJ. 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 J Zool. 2022;43(7):42-51.
15. Cavalcante JEA, de Sousa ELH, de Oliveira Rodrigues R, de Almeida Viana G, Duarte Gadelha D, de Carvalho MMD, et al. Interleukin-18 promoter- 137/C polymorphism (rs187238) is associated with biochemical markers of renal function

and cardiovascular disease in type 2 diabetes. patients. Clin Biochem. 2020; 80:1-7.
DOI: 10.1016/j.clinbiochem.2020.03.011, PMID 32209332.

16. Eng E, Quaggin S. Putting it all together: Practical approach to the patient with diabetic kidney disease. In: Diabetes and kidney disease. Springer. 2022;637-59.
17. Nada AM, Younan MA. Dapagliflozin improves cardiovascular risk factors in Emirati patients with T2DM. Ther Adv Endocrinol Metab. 2021;12: 2042018821995364.
DOI: 10.1177/2042018821995364, PMID 33796252.
18. Akhter N, Wilson A, Thomas R, Al-Rashed F, Kochumon S, Al-Roub A, et al, S. ROS/TNF-α Crosstalk triggers the expression of IL-8 and MCP-1 in human monocytic THP-1 cells via the NF-κB and ERK1/2 Mediated Signaling. Int J Mol Sci. 2021;22(19):10519.
DOI: 10.3390/ijms221910519
PMID 34638857.
19. Rasoul WMR, Mahmood SJ. 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 J Zool. 2022;43(6):72-7.
20. Hassan AM, Kata FS. The role of the biomarkers interleukin-18 and transforming growth factor-beta-1 in predicting and detecting diabetic nephropathy in patients with type 2 diabetes. J Pharm Neg Results. 2022;243-9.
21. Hirooka Y, Nozaki Y. Interleukin-18 in inflammatory kidney disease. Front Med (Lausanne). 2021;8:639103.
DOI: 10.3389/fmed.2021.639103
PMID 33732720.
22. Šečić D, Turohan A, Begić E, Rebić D, Pepić E, Begić Z, et al, J. *Serum creatinine* versus corrected Cockcroft-Gault Equation according to Poggio reference values in patients with arterial hypertension. Int J Appl Basic Med Res. 2022;12(1): 9-13.
DOI: 10.4103/ijabmr.ijabmr_301_21
PMID 35265474.
23. Wu TE, Su YW, Chen HS. Mean HbA1c and HbA1c variability are associated with differing diabetes- related complications in patients with type 2 diabetes mellitus. Diabetes Res Clin Pract. 2022;192: 110069.
DOI: 10.1016/j.diabres.2022.110069,
PMID 36067915.
24. AL-Fatlawi HY, Jwad DSM. Evaluation of physiological and immunological parameters in men with type 2 diabetic nephropathy. Neuro Quantology. 2022; 20(5):646-57.
DOI: 10.14704/nq.2022.20.5.NQ22220
25. Najjar ZSR, Jwad SM, Alkhafaji RS. Assessment of 1.25 (OH) 2D3 levels and nephropathy stages in adult male patients with type-2 diabetes mellitus. J. Crit Rev. 2020;7(13):1667-74.
26. Flier JS. Starvation in the midst of plenty: reflections on the history and biology of insulin and leptin. Endocr Rev. 2019; 40(1):1-16.
DOI: 10.1210/er.2018-00179
PMID 30357355.
27. Mimenza-Alvarado AJ, Jiménez-Castillo GA, Yeverino-Castro SG, Barragán-Berlanga AJ, Pérez-Zepeda MU, Ávila-Funes JA, et al. Effect of poor glycemic control on cognitive performance in older adults with type 2 diabetes: the Mexican Health and Aging Study. BMC Geriatr. 2020;20(1):1-8.
28. Liu Z, Dai X, Zhang H, Shi R, Hui Y, Jin X, et al. Gut microbiota mediates intermittent-fasting alleviation of diabetes-induced cognitive impairment. Nat Commun. 2020; 11(1):855.
DOI: 10.1038/s41467-020-14676-4, PMID 32071312.
29. Haghighatpanah M, Nejad ASM, Haghighatpanah M, Thunga G, Mallayasamy S. Factors that correlate with poor glycemic control in type 2 diabetes mellitus patients with complications. Osong Public Health Res Perspect. 2018; 9(4):167-74.
DOI: 10.24171/j.phrp.2018.9.4.05
PMID 30159222.
30. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al, C. Pathophysiology of type 2 diabetes mellitus. Int J Mol Sci. 2020;21(17):6275.
DOI: 10.3390/ijms21176275, PMID 32872570.
31. Boye KS, Lage MJ, Shinde S, Thieu V, Bae JP. Trends in HbA1c and body mass index among individuals with type 2 diabetes: evidence from a US database 2012-2019. Diabetes Ther. 2021; 12(7):2077-87.
DOI: 10.1007/s13300-021-01084-0
PMID 34076849.

32. Mahmood N. A, Nemah SM. Chemo – spectral and biological studying of new ligands, research journal of pharmaceutical, biological and chemical sciences. RJPBCS. 2017;8(3):674.
33. Wondmkun YT. Obesity, insulin resistance, and type 2 diabetes: Associations and therapeutic implications. Diabetes Metab Syndr Obes. 2020;13:3611-6.
DOI: 10.2147/DMSO.S275898
PMID 33116712.
34. Atkin SL, Butler AE, Hunt SC, Kilpatrick ES. The retinopathy-derived HbA1c threshold of 6.5% for type 2diabetes also captures the risk of diabetic nephropathy in HANES. Diabetes Obes Metab. 2021; 23(9):2109-15.
DOI: 10.1111/dom.14449
PMID 34033191.
35. Natesan V, Kim SJ. Diabetic nephropathy–A review of risk. Factors, progression, mechanism, and dietary management. Biomol Ther (Seoul). 2021;29(4):365-72.
DOI: 10.4062/biomolther.2020.204
PMID 33888647.
36. Scurt FG, Menne J, Brandt S, Bernhardt A, Mertens PR, Haller H, et al. Monocyte chemoattractant protein-1 predicts the development of diabetic nephropathy. Diabetes Metab Res Rev. 2022;38(2):e3497.
DOI: 10.1002/dmrr.3497, PMID 34541760.
37. Mahmood NA. Farhan ZM. Egypt J Chem. Anticancer study of innovative macrocyclic formazan compounds from trimethoprim drug. 2023;66:1.
DOI:10.21608/EJCHEM.2022.132514.5852
38. Raina P, Sikka R, Gupta H, Matharoo K, Bali SK, Singh V, et al. Association of eNOS and MCP-1genetic variants with type 2 diabetes and diabetic nephropathy genetics, susceptibility: A Case–Control and Meta-Analysis Study. Biochemical. 2021;59(4):966-96.
39. Mahmoud AA, Soliman MS, Moustafa A. Evaluation of monocyte chemoattractant protein 1 (MCP-1) as a predictor of complications in type 2 diabetes mellitus in Zagazig University Hospital. Egypt J Hosp Med. 2021;83(1):995-1001.
DOI: 10.21608/ejhm.2021.160038
40. Chi M, Tian Zi, Ma K, Li Y, Wang L, Nasser MI, Liu C. Diseased kidneys: Aging and immune aging. Immunity and Aging. 2022;19(1):1-12.
41. Sanner H, Schwartz T, Flatø B, Vistnes M, Christensen G, Sjaastad I. Increased levels of eotaxin and MCP-1 in juvenile dermatomyositis median 16.8 years after disease onset; associations with disease activity, duration and organ damage. PLOS ONE. 2014;9(3):e92171.
DOI: 10.1371/journal.pone.0092171, PMID 24647150.
42. Wei SY, Pan SY, Li B, Chen YM, Lin SL. Rejuvenation: turning back the clock of aging kidney. J Formos Med Assoc. 2020;119(5):898-906.
DOI: 10.1016/j.jfma.2019.05.020
PMID 31202499.
43. Das R, Lee N, Y, Mukherjee S, Oh S. Relationship of body mass index with diabetes and breast cancer biomarkers. J Diabetes Manag. 2019;9(1):163-8.
44. Lim JP, Leung BP, Ding YY, Tay L, Ismail NH, Yeo A et al. Monocyte chemoattractant protein-1: A proinflammatory cytokine elevated in sarcopenic obesity. Clin Interv Aging. 2015;10:605-9.
DOI: 10.2147/CIA.S78901
PMID 25848236.
45. Satirapoj B, Dispan R, Radinahamed P, Kitiyakara C. Urinary epidermal growth factor, monocyte chemoattractant protein-1 or their ratio as predictors for rapid loss of renal function in type 2 diabetic patients with diabetic kidney disease. BMC Nephrol. 2018;19(1):246.
DOI: 10.1186/s12882-018-1043-x
PMID 30241508.
46. Siddiqui K, Joy SS, George TP, Mujammami M, Alfadda AA. Potential role and excretion level of urinary transferrin, KIM-1, RBP, MCP-1 and NGAL markers in diabetic nephropathy. Diabetes Metab Syndr Obes. 2020;13:5103-11.
DOI: 10.2147/DMSO.S282166
PMID 33408494.
47. Huang J. Comparing biomarkers for COVID-19 disease with commonly associated preexisting conditions and complications. MedRxiV. 2020;2020-10.
48. Gonzalez J, Mouttalib S, Delage C, Calise D, Maoret JJ, Pradère JP, et al. Dual effect of chemokine CCL7/MCP-3 in the development of renal tubulointerstitial fibrosis. Biochem Biophys Res Commun. 2013;438(2):257-63.
DOI: 10.1016/j.bbrc.2013.07.025
PMID 23872063.

49. Larsson A, Carlsson L, Gordh T, Lind AL, Thulin M, Kamali-Moghaddam M. The effects of age and gender on plasma levels of 63 cytokines. J Immunol Methods. 2015;425:58-61.
DOI: 10.1016/j.jim.2015.06.009, PMID 26080062.
50. Kim HO, Kim HS, Youn JC, Shin EC, Park S. Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassays. J Transl Med. 2011;9(1): 1-7.
51. Yang C, Jeong S, Ku S, Lee K, Park MH. Use of gasotransmitters for the controlled release of polymer-based nitric oxide carriers in medical applications. J Control Release. 2018;279:157-70.
DOI: 10.1016/j.jconrel.2018.04.025, PMID 29673643.
52. Huber J, Kiefer FW, Zeyda M, Ludvik B, Silberhumer GR, Prager G, et al. CC chemokine and CC chemokine receptor profiles in visceral and subcutaneous adipose tissue are altered in human obesity. J Clin Endocrinol Metab. 2008;93(8):3215-21.
DOI: 10.1210/jc.2007-2630, PMID 18492752.
53. Gilbert RE. Proximal tubulopathy: prime mover and key therapeutic target in diabetic kidney disease. Diabetes. 2017;66(4):791-800.
DOI: 10.2337/db16-0796, PMID 28325740.
54. Suri S, Mitra P, Abhilasha A, Saxena I, Garg MK, Bohra GK, et al. Role of interleukin-2 and interleukin-18 in newly diagnosed type 2 diabetes mellitus. J Basic Clin Physiol Pharmacol. 2021;33(2): 185-90.
DOI: 10.1515/jbcpp-2020-0272, PMID 33711216.
55. Zheng H, Cao JJ. Angiotensin-converting enzyme gene polymorphism and severe lung injury in patients with disease 2019. Am J Pathol. 2020;190(10):2013-7.
DOI: 10.1016/j.ajpath.2020.07.009, PMID 32735889.
56. Qin Z, Li H, Jiao P, Jiang L, Geng J, Yang Q, et al, B. The value of urinary interleukin-18 in predicting acute kidney injury: a systematic review and meta-analysis. Ren Fail. 2022;44(1):1717-31.
DOI: 10.1080/0886022X.2022.2133728, PMID 36259446.
57. Mir M, Rostami A, Hormozi M. Comparison of serum levels of IL-18 in peripheral blood of patients with type II diabetes with nephropathy clinical protests and patients with type II diabetes without nephropathy clinical protests. Diabetes Metab Syndr. 2017;11(4):245-50.
DOI: 10.1016/j.dsx.2016.08.018, PMID 27663212.
58. Zaharieva E, Kamenov Z, Velikova T, Tsakova A, El-Darawish Y, Okamura H. Interleukin-18 serum level is elevated in type 2 diabetes and latent autoimmune diabetes. Endocr Connect. 2018;7(1):179-85.
DOI: 10.1530/EC-17-0273, PMID 29217651.
59. Yasuda K, Nakanishi K, Tsutsui H. Interleukin-18 in health and disease. Int J Mol Sci. 2019;20(3):649.
DOI: 10.3390/ijms20030649, PMID 30717382.
60. Hrp RSA, nst, AT, Ganie RA. Differences in interleukin 18 levels in diabetic nephropathy and non-diabetic nephropathy patients; 2020.
61. Sun YM, Su Y, Li J, Wang LF. Recent advances in understanding the biochemical and molecular mechanism of diabetic nephropathy. Biochem Biophys Res Commun. 2013;433(4):359-61.
DOI: 10.1016/j.bbrc.2013.02.120, PMID 23541575.
62. Carrara F, Ruggenenti P, Perna A, Iliev IP, Gaspari F, Ferrari S, Parvanova A. Glomerular resistances predict. Decreased long-term glomerular filtration rate in patients with type 2 diabetes without overt nephropathy: A longitudinal subgroup analysis of the DEMAND trial. Acta Diabetol. 2022;59(3):309-17.
63. AL- Fatlawi HY, Jwad SM. Uttar Pradesh J Zool. Assessing the level of a trial natriuretic peptide and some biochemical parameters in men with type 2 diabetic nephropathy. 2022;43(9):1-13.
64. Radcliffe NJ, Seah JM, Clarke M, MacIsaac RJ, Jerums G, Ekinci EI. Clinical predictive factors in diabetic kidney disease progression. J Diabetes Investig. 2017;8(1):6-18.
DOI: 10.1111/jdi.12533, PMID 27181363.
65. Delanaye P, Jager KJ, Bökenkamp A, Christensson A, Dubourg L, Eriksen BO et al. CKD: A call for an age-adapted definition. J Am Soc Nephrol. 2019;30(10):1785-805.
DOI: 10.1681/ASN.2019030238, PMID 31506289.

66. Szczepanska-Sadowska E, Czarzasta K, Cudnoch-Jedrzejewska A. Dysregulation of the renin-angiotensin system and vasopressinergic system interactions in cardiovascular disorder. Curr Hypertens Rep. 2018;20:1.
67. Nata N, Rangsin R, Supasyndh O, Satirapoj B. Impaired glomerular filtration rate in type 2 diabetes mellitus subjects: A Nationwide Cross-Sectional Study in Thailand. J Diabetes Res. 2020;2020:6 353949.
DOI: 10.1155/2020/6353949
PMID 32855974.
68. de Ponte MC, Cardoso VG, Gonçalves GL, Costa-Pessoa JM, Oliveira-Souza M. Early type 1 diabetes aggravates ischemia/reperfusion-induced acute kidney injury. Sci Rep. 2021;11(1):19028.
DOI: 10.1038/s41598-021-97839-7
PMID 34561469.
69. Zhang Y. Role of follistatin-like 1 (FSTL1) in kidney fibrosis. The Chinese University of Hong Kong. Hong Kong; 2019.
70. Lo JC, Go AS, Chandra M, Fan D, Kaysen GA. GFR, body mass index, and low high-density lipoprotein concentration in adults with and without CKD. Am J Kidney Dis. 2007;50(4):552-8.
DOI: 10.1053/j.ajkd.2007.07.011
PMID 17900454.