Evaluation of Physiological and Immunological Parameters in Men with Type 2 Diabetic Nephropathy

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Abstract

All men in the present research (15 healthy and 75 patients) have undergone clinical examination as well as other physiological and immunological tests, such as glycosylated hemoglobin test, hemoglobin level, platelets count, albumin to creatinine ratio (ACR), glomerular filtration rate (GFR), monocyte chemoattractant protein-1 (MCP-1 level), white blood cell count (WBC) and neutrophil to lymphocyte ratio (NLR). The results showed significant effect of ($P<0.05$) for the age groups and duration of diabetes mellitus on the hemoglobin level. However, there were no notable differences ($P>0.05$) in the glycosylated hemoglobin ratio and platelet counts when the age groups and duration of diabetes mellitus for men with type 2 diabetes are compared with each other. Moreover, the study revealed an observable impact of ($P<0.05$) for the albumin to creatinine ratio on the hemoglobin level and number of platelets in the blood. Yet, no significant differences ($P>0.05$) was noticed in the glycosylated hemoglobin ratio. For the other parameters, a significant effect of ($P<0.05$) was observed for the glomerular filtration rate on the glycosylated hemoglobin ratio, hemoglobin level and platelets count. In relation with the immunological criteria, a noticeable influence of ($P<0.05$) was shown for age groups, duration of diabetes mellitus, albumin to creatinine ratio and glomerular filtration rate on the MCP-1 level in the serum, and the neutrophils to lymphocytes ratio in patients with type 2 diabetes. However, no significant variations ($P>0.05$) were recorded in the number of white blood cells. The study aims to diagnose whether it is the possibility of using the physiological and immunological criteria under study to predict reaching the final stages of diabetic nephropathy in order to prevent renal failure.

Key Words: Immunology, Albumin, Hemoglobin, MCP-1.

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Introduction

Diabetes mellitus is a metabolic disease characterized by high blood sugar resulting from defects in insulin secretion or insulin function or both, high sugar levels in diabetic patients are associated with long term damage (American Diabetes 2010), dysfunction, and failure in various organs, especially the kidneys (Galicia-Garcia, U 2020), nerves, heart, blood vessels as well as the eyes. According to the American Diabetes Association, diabetes can be divided into four main types, but type 2 is the most common in adults, this type occurs as a result of inadequacy of insulin secretion by pancreatic beta cells, insulin resistance in tissues, and insufficient response to insulin secretion, these factors combined lead to uncontrolled blood glucose level (Tripathi, B.K.; & Srivastava, A.K. 2016). People with type 2 diabetes do not depend on exogenous insulin (insulin injections), but they may need a mechanism to control blood glucose levels if this is not achieved through diet alone or with oral hypoglycemic agents (Astatkie, B.G. et al 2018).

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There are many diabetes complications, which are affected by several factors such as the duration of diabetes, diet, cardiac activity, gender, age, body mass index (BMI), blood pressure, education, marital status, income, alcohol consumption, family history of diabetes, and preventive care services (Alemu, H.; Hailu, W.; & Adane, A. 2020).

Diabetic nephropathy is a common complication of diabetes; about 40% of diabetic nephropathy patients develop chronic nephropathy, which in turn increases the risk of cardiovascular disease and early death (Xue, R.; 2017). Since the kidneys are highly vital organs, high blood sugar affects the small blood vessels, leading to vascular sclerosis and fibrosis of these vessels and damage to the renal mitochondria; this in turn generates a failure in providing energy to the renal tubular epithelial cells (Alicic, R.Z. et al 2017). In addition, the metabolic changes that accompany diabetes lead to glomerulosclerosis, inflammation of the tubulointerstitial nephritis and fibrosis, besides subendothelial deposits of plasma proteins, which lead to osmotic dysfunction, these proteins form positive acidic deposits that accumulate in the branches of small arteries, microvasculatures, and glomerular capillaries, and thus cause atherosclerosis subsequently, such deposits appear under the epithelial cells of the proximal renal tubules and Bowman's capsule In some diabetic patients, glomerulonephritis caused by glomerulosclerosis is observed, and after diabetic glomerulosclerosis, the progression of nephropathy becomes rapid, consequently causing renal failure (Ko, Y.S et al 2016).

Depending on the glomerular filtration rate, there are five stages of diabetic nephropathy; (G1-G5). These stages are: the glomerular hyper-filtration stage (GFR ≥ 90ml/min/1.73 m2), early glomerular damage stage (GFR = 60-89ml /min/1.73m2, microalbuminuria stage (30-300 mg/day) and GFR (30-59 ml/min/1.73m), macroalbuminuria stage characterized by irreparable proteinuria with GFR = (15-29)ml/min/1.73m2 (Chen, T et al 2019), and the renal failure stage with (GFR≤15ml/min/1.73) where a large number of renal glomeruli are destroyed and the remaining glomeruli are highly permeable to plasma proteins, this leads to a decrease in colloid osmotic (or oncotic) pressure in the plasma, resulting in edema and hypertension due to salt and water retention, patients also suffer from uremia, acidosis and hyperkalemia, which is more serious than uremia since a patient sometimes goes into a coma, and may consequently need dialysis (Sulaiman, M.K 2019). The glycosylated hemoglobin test is essential in the diagnosis and control of blood glucose level in diabetic patients, it is an indicator of the average blood glucose for the past three months and provides an estimation of the long-term blood sugar (Rawal, G..et al 2016). International Diabetes Federation (IDF) defined the value of glycosylated hemoglobin as less than 6.5%, and it was determined as less than 7.0% by the American College of Endocrinology (ACE) [13]. The World Health Organization added that the diagnostic value of glycosylated hemoglobin in diabetic patients is more than 6.5% and that (5.7 -6.4%) is the pre-diabetic stage, where the test should be re-confirmed the next day (Leow, M.K.S. 2016).

The glycosylated hemoglobin is formed glucose binds to the valinal amino end in the beta chain of hemoglobin by covalent bonds through a non-enzymatic reaction that results in the Schiff base, after molecular rearrangement of the Schiff base, ketamine products called Amadori or glycosylated hemoglobin are formed mostly known as HbA1C, whose turnover rate depends on the age of red blood corpuscles (Taderegew, M.M. et al 2021), therefore hemoglobin is exposed to glucose for 90-120 days, after the old red blood corpuscles hemolysis and leaving the blood circulation, HbA1C is likely to give a representation of the glucose level in the blood for the past period (Nabih, M.I.; et al 2013). On the other hand, when hemoglobin level decrease in blood of diabetic nephropathy patients, anemia develops earlier than in patients with chronic kidney disease for other reasons and is usually associated with advanced tubular and cellular lesions, as well as the development of cardiovascular disease and finally the mortality (Fan, F et al 2017). Concern to the platelets count,is a simple and inexpensive test and can be obtained through routine complete blood count test, it can also provide information that is important for evaluating complications associated with type 2 diabetes, such as microvascular complications, including retinopathy and diabetic nephropathy (Liu, J. et al 2018).

The chemokines are a group of cytokines that are essential in the selective activation of monocytes, neutrophils, and lymphocytes, they also have a major role in stimulating chemotaxis by monocyte chemoattractant protein-1, MCP-1 is a chemokine that regulates the sequencing and migration of monocytes, and this is necessary for immune surveillance and the inflammation response

(Forget, P et al 2014). Hence, in response to many stimuli, monocyte chemoattractant protein-1 (MCP-1) is produced from tubuloepithelial cells, endothelial cells, fibroblasts, keratinocytes, smooth vascular cells, podocytes, and mesangial cells; the response to attraction is through interaction with cell surface receptors. In places where atherosclerosis occurs, the expression level of MCP-1 increases, activating the monocytes and macrophages and urging them to migrate to the vascular wall, this develops coronary atherosclerosis, weakens the endothelium of blood vessels, and contributes to their instability and subsequent rupture. In a study conducted on mice, it was indicated that renal tissue inflammation is caused by MCP-1 because it accumulates in the kidneys affected with diabetic nephropathy, and increases the complications of morbidity in those mice (Wan, H. et al 2020.; Akase, T. et al 2020). Moreover, the inflammatory factors contribute to the development of kidney disease, however, the relationship between white blood cells and impaired kidney function is unclear, despite the availability of many indications, they inconsistent, and the association among them may be affected by race and gender (Qiu, Y. et al 2020). It is established that the final products are partly related to the stimulation of white blood cells, and these activated cells produce some induced-inflammatory cytokines that cause renal tissue damage (Abusaib, M. et al 2020).

In recent years, the neutrophil to lymphocyte ratio (NLR) has been used as an efficient indicator of the inflammatory response and a biomarker of pathogenesis in many diseases [24]. The normal ratio of (NLR) in adults and non-elderly people ranges between (0.78) and (3.53) (Choi, K., & Kim, Y.B. 2010). Chronic inflammation is usually associated with diabetic nephropathy, as it contributes to the development of tubulointerstitial fibrosis in patients with kidney disease and eventually leads to the progression of the disease; in addition, there are several factors that lead to the development of inflammation in particular acidosis, oxidative stress and increased cytokine production. The role of neutrophils is opposite to that of lymphocytes, as neutrophils damage endothelial cells, while lymphocytes have an anti-sclerotic role (Arkew, M et al 2021). A study performed in China showed a close relation between the neutrophils to lymphocytes ratio and impaired as well as deteriorated kidney function in patients with diabetes (Angelousi, A., & Larger, E. 2015). Furthermore, it gives a sensitive indicator for predicting mortality in adult patients undergoing kidney transplantation with severe pneumonia (Adane, T., et al 2021). Another study conducted on 54 patients with rapidly progressing glomerulonephritis concluded that the NLR could be a good predictor of mortality in these patients and could be considered a measure of glomerular inflammation case (Elbadri, E.Y et al 2020).

Materials and Working Methods

The current research was conducted on 90 men, including 60 men with type 2 diabetes at the Diabetes and Endocrinology Center in Al-Sadr Medical Hospital in Holy Najaf Governorate, 15 men hospitalized at the Dialysis Center in the same hospital, and 15 men as a control group. Their ages ranged from 30 to 80 years. The specimens were collected during the period from 1/3/2021 to 1/10/2021. Patients who use insulin injections, thyroid patients, hypertensive patients, or those who suffer from any disease or use any type of treatment were excluded.

The patients were also divided into groups. According to age, they were divided into five groups (30-39) years, (40-49) years, (50-59) years, (60-69) years and older than 70 years. While,the duration of the diabetes, they were divided into five stages (1-5) years, (6-10) years, (11-15) years, (16-20) years and more than 21. In relation with albumin to creatinine ratio (ACR), they were divided into normal albuminuria stage <30, microalbuminuria stage from (300-30), the macroalbuminuria stage (> 300). According to the stages of diabetic nephropathy and depending on the glomerular filtration rate, the patients were divided into five stages, stage 1 (>90 mg / min / 1.73), stage 2 (60-89 mg/minute/1.73), stage 3 (30-59 mg/minute/1.73), stage 4 (15-29 mg/minute/1.73) and stage 5, which is the end stage or renal failure stage (<15 mg/min/1.73).

Results

The Physiological Study

A-Comparing some Physiological Blood Parameters in Men between the Control Group and the Group of Patients with type 2 Diabetes

The present study showed significant differences (P = 0.0001), (P = 0.006) and (P = 0.042) in the glycosylated hemoglobin, hemoglobin level and platelets count respectively, 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

Variables	Mean±SE		p- value
	Healthy control n=15	Patients DN n=75	
HbA1C %	4.16±0.19	7.14±0.18 *	0.0001
Hb (mg/dl)	13.95±0.40	12.45±0.33 *	0.006
PLAT (X103)	221.0±9.59	246.3±4.94*	0.042

*Significant differences at p value <0.05. T- independent test for comparison of two groups.

B. The Effect of Age Groups on some Physiological Parameters among Men with Type 2 Diabetes

The results of the current research revealed significant differences (P = 0.0001) in the level of hemoglobin in the blood, while there were no observable variations (P =0. 893) and (P =0.103) in glycosylated hemoglobin and platelets count (Zierk, J et al 2020), respectively, when comparing the age groups of men with type 2 diabetes, as in table (2).

Table 2. The effect of age groups on some physiological parameters among men with type 2 diabetes

Variables	Mean±SE					p-value
	30-39 year n=8	40-49 year n=14	50-59 year n=21	60-69 year n=20	≥70 year n=12	
HbA1C %	6.71±0.25	7.14±0.41	7.10±0.24	7.37±0.42	7.12±0.65	0.893
Hb (mg/dl)	14.48±0.51 A	13.37±0.71 A	13.53±0.48 A	11.26±0.65 B	10.12±0.68 B	0.0001
PLAT (X10³)	259±7.57	248.29±15.07	236.29±9.71	224.48±7.7	218.33±11.9	0.103

The different letters significant differences at p-value <0.05. ANOVA and Post Hoc Tests of Tukey (HSD) Multiple Comparisons.

C. The Effect of Duration of Type 2 Diabetes on some Physiological Parameters among men with type 2 Diabetes

Table (3) shows a significant effect of (P = 0.0001) for the duration of diabetes mellitus on the level of hemoglobin in the blood, whereas there were no significant differences (P = 0.493) and (P =0.072) in glycosylated hemoglobin and platelets count (Verga- Falzacappa et al 2007), respectively.

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

Variables	Mean±SE					p-value
	1-5 year N=9	6-10 year N=10	11-15 year N=23	16-20 year N=16	> 21 years N=17	
HbA1C %	6.82±0.22	7.16±0.19	7.60±0.24	7.00±0.48	6.74±0.56	0.493
Hb (mg/dl)	14.43±0.3 A	14.85±0.32 A	13.58±0.48 A	10.88±0.61 B	9.63±0.56 B	0.0001
PLAT (X10³)	254.44±17.6	242.6±14.22	235.30±8.16	212.47±9.58	216.1±9.85	0.072

The different letters significant differences at p-value <0.05. ANOVA and Post Hoc Tests of Tukey (HSD) Multiple Comparisons.

D. The Effect of Albumin to Creatinine Ratio (ACR) on some Physiological Parameters of Men with Type 2 Diabetes

The results indicated a notable effect of the albumin to creatinine ratio on hemoglobin level (P = 0.0001) yet there were no significant differences (P =0. 324) in the glycosylated hemoglobin and (P = 0.034) platelets count, as in table (4).

Table 4. The effect of albumin to creatinine ratio (ACR) on some physiological parameters and among men with type 2 diabetes

Variables	Mean±SE			p-value
	Normoalbuminuria N=14	Microalbuminuri N=30	Macroalbuminuria N=31	
HbA1C %	6.76±0.20	7.45±0.10	6.97±0.42	0.324
Hb (mg/dl)	14.54±0.32 A	14.10±0.26 A	9.74±0.41 C	0.0001
PLAT (X103)	239.29±14.19 A	240.50±7.81 A	214.1±6.25 B	0.034

The different letters significant differences at p-value <0.05. ANOVA and Post Hoc Tests of Tukey (HSD) Multiple Comparisons.

E. The Effect of Glomerular Filtration Rate on some Physiological Parameters in Men with Type 2 Diabetes

The findings of the current study showed a remarkable differences (**Mimenza-Alvarado** et al. 2020) of the five stages of nephropathy on the glycosylated hemoglobin (P = 0.0001), hemoglobin level (P = 0.0001), and platelets counts (P = 0.015), as shown in table (5).

Table 5. The effect of glomerular filtration rate on some physiological parameters among men with type 2 diabetes

Variables	Mean±SE					p-value
	Stage 1 N=15	Stage 2 N=15	Stage 3 N=15	Stage 4 N=15	Stage 5 N=15	
HbA1C %	6.76±0.20 B	7.14±0.12 B	7.76±0.11 B	8.63±0.50 A	5.42±0.37 C	0.0001
Hb (mg/dl)	14.54±0.32 A	14.91±0.20 A	13.29±0.37 A	11.00±0.58 B	8.56±0.40 C	0.0001
PLAT (X103)	239.3±14.19 A	254.27±9.41 B	226.73±11.7 C	225.9±6.49 C	202.94±9.9 D	0.015

The different letters significant differences at p-value <0.05. ANOVA and Post Hoc Tests of Tukey (HSD) Multiple Comparisons.

2. Immunological Study

A. Comparing the Immunological Parameters in men between the Control Group and Group of Patients with Type 2 Diabetes

The data demonstrated that there were significant differences of (P = 0.0001) in the MCP-1 level and the neutrophils to lymphocytes ratio, while there were no significant differences (P = 0.320) in the number of white blood cells in men of the two groups, control and patients with diabetes type 2, when the two groups are compared.

Table 6. Comparison of immunological parameters in men between the control group and the group of type 2 diabetes patients

Variables	Mean±SE		p- value
	Healthy control n=15	Patients DN n=75	
MCP-1 (pg/ml)	140.2±1.93	202.3±6.30 *	0.0001
WBC (X103)	7.45±0.31	7.81±0.18	0.320
Neutrophil/ Lymphocyte ratio	1.58±0.07	3.28±0.20 *	0.0001

*Significant differences at p value <0.05. T- independent test for comparison of two groups.

B. Effect of Age Groups on some Immunological Parameters in Men with Type 2 Diabetes

The results of the current research showed a significant increase of (P = 0.0001) in MCP-1 level and the neutrophils to lymphocytes ratio with aging, but no notable effect(P=0.763) on the number of white blood cells was observed in men with type 2 diabetes, as shown in table (7).

Table 7. The effect of age groups on some immunological parameters in men with type 2 diabetes

Variables	Mean±SE					p-value
	30-39 year n=8	40-49 year n=14	50-59 year n=21	60-69 year n=20	≥70 year n=12	
MCP-1 (pg/ml)	159±4.68 B	176.27±10.6 B	193.21±15.07 A	231.30±10.9 A	239.08±13 A	0.0001
WBC (X103)	8.25±0.32	7.39±0.39	7.94±0.37	7.80±0.26	7.83±0.71	0.763
Neutrophil / Lymphocyte ratio	1.86±0.14 C	3.09±0.48 B	2.35±0.36 B	4.26±0.33 A	4.46±0.41 A	0.0001

The different letters significant differences at p-value <0.05. ANOVA and Post Hoc Tests of Tukey (HSD) Multiple Comparisons.

C. The Effect of Diabetic Duration on some Immunological Parameters in Men with Type 2 Diabetes

Table (8) revealed a significant effect of (P = 0.0001) for the duration of diabetes mellitus on MCP-1 level in serum and the neutrophils to lymphocytes ratio, while there was no noticeable variation (P = 0.429) in the number of white blood cells.

Table 8. The effect of diabetic duration on some immunological parameters in men with type 2 diabetes

Variables	Mean±SE					p-value
	1-5 year N=9	6-10 year N=10	11-15 year N=23	16-20 year N=16	> 21 years N=17	
MCP-1 (pg/ml)	137.89±1.78 C	151.7±3.52 C	186.61±8.33 B	241.00±11.22 A	256.65±7.92 A	0.0001
WBC (X103)	8.33±0.62	8.24±0.40	7.75±0.23	7.23±0.36	7.86±0.54	0.429
Neutrophil / Lymphocyte ratio	1.58±0.09 C	1.48±0.09 C	2.81±0.30 B	4.41±0.35 A	5.04±0.29 A	0.0001

The different letters significant differences at p-value <0.05. ANOVA and Post Hoc Tests of Tukey (HSD) Multiple Comparisons.

D. The Effect of Albumin to Creatinine Ratio (ACR) on some Immunological Parameters in Men with Type 2 Diabetes

According to table (9), there were significant differences of (P = 0.0001) in the levels and ratios of the immunological parameters under study, except for the number of white blood cells (P =0. 486), which was not affected by the increase of albuminuria.

Table 9. The effect of albumin to creatinine ratio (ACR) on some immunological parameters in men with type 2 diabetes

Variables	Mean±SE			p-value
	Normoalbuminuria N=14	Microalbuminuria N=30	Macroalbuminuria N=31	
MCP-1 (pg/ml)	139.71±1.8 C	169.03±1.99 B	265.9±3.15 A	0.0001
WBC (X103)	8.24±0.40	7.61±0.23	7.79±0.34	0.486
Neutrophil to Lymphocyte ratio	1.54±0.07 C	2.09±0.11 B	5.34±0.10 A	0.0001

The different letters significant differences at p-value <0.05. ANOVA and Post Hoc Tests of Tukey (HSD) Multiple Comparisons.

E. The Effect of Glomerular Filtration Rate on some Immunological Parameters in Men with type 2 Diabetes

Regard to table (10), there were observable differences of (P = 0.0001) in the immunological parameters under study, while no significant alterations (P = 0.347) were revealed in the number of white blood cells with progression of the diabetic nephropathy stages.

Table 10. The effect of glomerular filtration rate on some immunological parameters in men with type 2 diabetes

Variables	Mean±SE					p-value
	Stage 1 N=15	Stage 2 N=15	Stage 3 N=15	Stage 4 N=15	Stage 5 N=15	
MCP-1 (pg/ml)	139.7±1.8 E	159±1.07 D	179.07±0.9 C	253.3±3.4 B	277.6±3.1 A	0.0001
WBC (X103)	8.24±0.40	7.94±0.37	7.28±0.26	8.18±0.53	7.42±0.44	0.347
Neutrophil to Lymphocyte ratio	1.54±0.07	1.67±0.07	2.52±0.13	5.08±0.12	5.58±0.13	0.0001

The different letters significant differences at p-value <0.05. ANOVA and Post Hoc Tests of Tukey (HSD) Multiple Comparisons.

Discussion

1. The Physiological Study

The results of the current trial recorded a significant increased glycosylated hemoglobin ratio in men with type 2 diabetes over healthy men, which is consistent with many studies (Meneilly, G. S et al 2018). This is because the high glycosylated hemoglobin ratio is a sign of diabetes, as confirmed by the World Health Organization. Hence, the diagnostic value of glycosylated hemoglobin ratio in diabetic patients is more than 6.5%, and for the pre-diabetes stage, the ratio is (5.7-6.4%). In addition, there are several reasons for the high glycosylated hemoglobin ratio, including a decrease in the pancreatic insulin secretion, and the use of glucose by cells, or an imbalance between glucose production in the liver and its absorption as well as utilization in the tissues (Strain, W.D et al 2021). This increase in patients with type 2 diabetes results from the absence of control on glucose levels due to the weak response of the liver, muscles and adipose tissues to insulin stimulation also known as insulin resistance which may occur as a result of malabsorption by cells due to a mistransmission of insulin signal or dysfunction of glucose transmitter (Lutz, C.; & Cho, H.J. 2016).

Also, the findings of the experiment showed significant decrement in the level of hemoglobin in the blood of patients with type 2 diabetes over healthy group, which agrees with several studies. It is likely to be due to the age of red blood corpuscles decreases when the blood sugar level is not controlled because the change in the permeability of the cellular membrane of these corpuscles, distortion of its shape and the increase in sorbitol then its destruction. Moreover, the membrane of red blood corpuscles in patients with diabetes contain a high phosphatidylserine ratio, which increases its recognition and destruction by the reticuloendothelial system (Donnelly, L.A. et al 2020). It may also be attributed to the fact that high blood sugar level leads to an increase in prion-inflammatory cytokines that alter the response of erythropoietin. It also promotes the programmed death of immature red blood cells, which leads to a decreasing them and, which in turn reduce the level of hemoglobin in the blood (Shimizu, M et al 2021).

In the current study, a significant increment is also shown in the platelets count, which does not agree with the results of the study of. However, the results compatible with many studies. The reason for the high platelets count in diabetic patients may be suggested to the fact that diabetes mellitus is usually associated with atherosclerosis and blood clots, because the difference in the osmotic pressure of the blood caused by the increase in the level of glucose in the blood which stimulates the platelets to gather, these scleroses begin with the formation of sclerotic plaques in the lining of blood vessels, this process occurs after the platelets have activated and interacted with the white blood cells and the lining of the blood vessel, furthermore to complete this process a rise in the number and activity of platelets is required, which is also explained in some studies (Kebede, S.A. et al 2021).

The present research indicated a significant decline in the level of hemoglobin in the blood with aging. These results similar to numerous studies. Anemia in elderly groups is attributed to many factors in particular the defect that may occur in bone marrow tissue in the elderly, or partial loss of kidney functions with age. Such functions include secretion of erythropoietin, which is necessary in forming red blood cells, or possibly lack of nutrition in the elderly, which may affect the low level of hemoglobin in the blood, this was postulated by some studies (Najjar, Z.S.R.; Jwad, S.M. 2020), who established that inflammation indirectly contributes to causing anemia, because inflammatory factors increase the production of hepcidin in the liver, especially in patients with nephropathy which negatively affects the formation of red blood cells through iron retention in the reticuloendothelial system (RES), and weakens the response of erythropoietin to form red blood cells. Hepcidin also reduces iron absorption in the duodenum and iron release from macrophages, as it is the main responsible for iron metabolism. Anemia may be explained to the effect of age on the levels of some hormones, including estrogen and testosterone, which directly increase the hepcidin expression in the liver (Han, J.S et al 2015).

The statistical analysis of results revealed that the age groups have no impact on the glycosylated hemoglobin ratio and blood platelets, which is not consistent with some studies. Yet, the results agree with (Almaawi, A.K.M. 2021), who proposed that age groups have no effect on the glycosylated hemoglobin ratio because unlike younger patients, older patients usually have more control over their blood sugar through their intensive commitment to regular treatments for the level of sugar and their concern for diet. Or, it could be due to the insufficient treatment taken, which negatively affects the glycosylated hemoglobin level, or some

drugs such as sulfonylureas used by some diabetics may have a role in lowering the level of sugar in the blood. In addition, the results of the study clearly indicates an influence of the duration of diabetes mellitus on the reduction in the level of hemoglobin, but it does not affect the rest of the physiological parameters under study, some studies had the same result regarding the level of hemoglobin in the blood [50]. Severe or mild diets probably by many diabetics, as well as the frequent blood tests and self-examination of glucose for a long time may have an adverse effect on the iron level and thus lowers the level of hemoglobin in the blood (Buades, J.M. et al 2021).

Besides, it could be because the level of hemoglobin is associated with complications of diabetes as a result of long-term inflammations, which may prevent bone marrow cells from responding to erythropoietin by inactivating a specific receptor related with the differentiation of red blood cell progenitors, moreover the inflammation directly suppresses these progenitors, this happens in patients with chronic kidney disease. Furthermore, some long-period medicines in particular metformin, may have an effect on lowering the packed cell volume and hemoglobin levels in the blood. Anemia may attribute to the decreased packed cell volume or increased plasma levels because of fluid retention and blood dilution, or both in patients with diabetic nephropathy. Additionally, anemia is also justified by kidney damage due to the long-duration of diabetes mellitus, the renal interstitial fibrosis and tubular atrophy may affect hemoglobin levels in blood substantially (Abe, M.; & Kalantar-Zadeh, K. 2015).

The statistical analysis showed significant impact of albumin to creatinine ratio on the level of hemoglobin and platelet counts. Hence, they decrease when albuminuria increase, which is in line with many studies. Many studies indicated that the increase of protein in the urine and thus the elevation in the (ACR) occur with the stage progression of nephropathy and the impairment of kidney functions. Therefore, anemia is more recurrent in diabetic patients with albuminuria than those without albuminuria. In addition, albuminuria is a vital indicator of complications of nephropathy, since anemia occurs after losing large amounts of transferrin in the macroproteinuria, which leads to iron deficiency and thus anemia. It may as well be a result of a defect in the lining of the renal tubule, which is believed to be the cause of albuminuria, as well as damage to the small blood vessels of the kidneys, which results in weak production and release of erythropoietin, and thus formation of ineffective red blood cells (Shen, Y. et al 2021).

The findings recorded a significant increase in the glycosylated hemoglobin ratio with a decrease in the glomerular filtration rate in its four stages. However, in the last stage, which is the stage of renal failure in patients undergoing hemodialysis, the glycosylated hemoglobin ratio returned to the normal limit, which is consistent with many studies. This is because the low glomerular filtration rate is among the first appearing complications of diabetes; not only does it lead to glomerular damage, but rather affects the interstitial, tubular and renal vascular tissues. The low glomerular filtration rate and glycosylated hemoglobin ratio may be due to tissue injury in the walls of glomerular blood vessels. The rise in blood glucose causes alterations in the renal circulation and the glomerular filtration rate because it leads to a disorder in the lining of large and small blood vessels. In addition to an increment in the level of glucose is usually associated with an increase in systemic inflammation and infiltration of white blood cells in the lining of the urinary tubules, leading to cell death and dysfunction (Kwon, E.; & Ahn, C. 2012).

As for renal failure patients undergoing hemodialysis, the reduction in the glycosylated hemoglobin ratio may be due to the loss of glucose during dialysis. The dialysis used liquid contains glucose at 5.55 mmol/L, if the plasma glucose level is greater than this level during the dialysis, then the glucose will be diffused from blood to the dialysis fluid, which leads to a decrease in the blood sugar level after the dialysis. After repeating this process two or three times a week for long periods, the level of glycosylated hemoglobin becomes normal in these patients. The glomerular filtration rate also had a significant effect on the level of hemoglobin and platelets count in the blood, particularly at the stage of renal failure, compared with the previous stages. The results in agreement with some studies [68], and other study, who proved that the hemoglobin level in the blood decreases by 1 g/dL in each stage of chronic nephropathy. This may be attributed to the relationship of hemoglobin deficiency in patients with diabetic nephropathy, especially in the third and fourth stages or even the fifth stage with impaired kidney functions, this results in a reduction in the erythropoietin secretion, which is

necessary for red blood cell formation [70]. In addition, the reason may associate to several factors, including increasing inflammatory activity in diabetic nephropathy patients, increment in advanced glycation end products (AGEP), the effects of oxidative stress, or because of blood sugar regulators, as confirmed by some studies (Tsai, S.F. & Tarng, D.C. 2019).

2. The Immunological Study

The present research showed an increment in the MCP-1 level in patients with diabetic nephropathy with elders and long duration of diabetes mellitus, which agrees with some studies. A rise in the neutrophils to lymphocytes ratio (NLR) was also observed in the older age groups, which was consistent with many studies. Possibly, because of the aging is one of the underlying mechanisms of inflammation, and these inflammations deteriorate the functions of the body systems, leading to cardiovascular diseases. This is considered a risk factor for kidney disease and many other diseases through the inhibition of growth factors, increased catabolic processes, and negative intrusion with homeostasis signals in the body (Mahmoud, A.A et al 2021).

The duration of diabetes mellitus in the current study recorded a remarkable effect on increasing NLR, which was compatible with many studies. The result could be attributed to the length of the duration of diabetes mellitus which stimulates chronic inflammation and complications of the disease through the final stages of diabetic nephropathy. Another study demonstrated that the NLR had a positive relation with the advanced stages of diabetic nephropathy. In addition, proteinuria is associated with the duration of diabetes mellitus and the increase in the neutrophils to lymphocytes ratio, since the proteinuria is caused by inflammation that causes glomerular dysfunction and the permeability of the endothelium of blood vessels. With Regard to an increase in the level of MCP-1 which was in proportion to the decrease in the glomerular filtration rate and the increment in albuminuria. The current result was in agreement with many studies.It likely to be due to MCP-1 has an effect on the function of the glomerular filtration barriers, including podocytes. A study noted that inhibiting the action of MCP-1 leads to a decrement in albuminuria. Some studies also stated that the increased secretion of MCP-1 indicates structural and functional weakness of renal glomeruli. Besides, some renal structural abnormalities, including mesangial expansion and thickening of the basement membrane affect the glomerular capillary filtration area, leading to the excretion of large amounts of protein in the urine. This may also be because the elevation in the level of this protein increases the production of cytokines and interleukins, which stimulate the inflammation and adhesive cells that in turn enhance the inflammation of the renal tubules and glomeruli (Mahmoud, A.A et al 2021.; Öztürk, Z.A. et al 2019).

Finally, the results diagnosed a gradual rise in the neutrophils to lymphocytes ratio with a decrease in the glomerular filtration rate, and conversely an increase in albuminuria in patients with diabetic nephropathy. The stage of renal failure showed the highest ratio. These results agree with most studies. This may be attributed to the quick response of neutrophils in the bloodstream to the inflammatory stimuli, and subsequent increment in interleukins activates the formation of lymphocytes, thus increasing the ratio between them in general. In addition, these immunological factors indicate inflammations related to the dysfunction in the endothelial cells of the renal glomeruli, which leads to an increase in the transmission of proteins into the urine. Hence, the chronic inflammation and high white blood cells count play a major role in small and large blood vessel complications in diabetic patients. A study demonstrated that the neutrophils to lymphocytes ratio is inversely proportional to the glomerular filtration rate, and this signifies a deterioration in the normal functions of renal tissues.A later study confirmed that this high ratio is positively associated with tubular atrophy and renal interstitial fibrosis, and thus negatively with the glomerular filtration rate in diabetic nephropathy patients. Also, it may be explained to the oxidative stress and glomerulosclerosis as a result of elevated blood sugar (Duman, T.T.; Aktas et al 2019).

Conclusions and Recommendations

The researchers of the current study conclude that the glomerular filtration rate inversely proportional with age, duration of diabetes mellitus, and stages of diabetic nephropathy. The diabetic nephropathy progression had an adverse influence on anemia infection; glycosylated hemoglobin can go back to its normal level in patients with renal failure undergoing dialysis. Moreover, MCP-1 level and the neutrophils to

lymphocytes ratio can be adopted as a subtle immunological biomarkers to predict the transition to the final stages of diabetes and thus kidney failure. The researchers recommend a study on patients with diabetic nephropathy using other immunological factors such as IL-6 and tumor necrosis factor TNFa, also known as Cachexin, which may be associated with insulin resistance since it affects the liver, in order to control and prevent the deterioration up to the renal failure.

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