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Assessing the Reliability of Serum Macrophage Migration Inhibitory Factor as a Marker for Diabetic Nephropathy Prediction in Type 2 Diabetes Patients and the Effect of ACE Inhibitors on its Level

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Abstract

Diabetic nephropathy (DN) is a prevalent chronic microvascular diabetic complication. The macrophage migration inhibitory factor (MIF), a versatile proinflammatory cytokine, appeared to play a critical function in inflammatory responses in various pathologic situations like DN since inflammation plays a crucial role in the genesis and progression of DN. The aim of study is to assess serum levels of MIF in a sample of Iraqi diabetic patients with nephropathy supporting its validity as a marker for predicting nephropathy in type 2 diabetes mellitus (T2DM) patients. In addition, to evaluate the nephroprotective effect of angiotensin-converting enzyme (ACE) inhibitors in terms of their influence on MIF levels. This study is a case-control study involving ninety subjects categorized into three groups: twenty apparently healthy control group and seventy patients with T2DM divided into two equal groups according to the presence of diabetic nephropathy that has been further divided into two groups according to the use of ACE inhibitors or not. Serum MIF, glycemic indices, urea, creatinine, and urinary albumin to creatinine ratio (ACR) were measured for each subject. Serum MIF's the highest levels were observed in the diabetic nephropathy patients (24.9 ng/ml), followed by the T2DM group (14. 1 ng/ml), with the lowest level observed in the control group (4.8 ng/ml). There was a remarkable relation between MIF levels and ACE inhibitors (p-value <0.05) with reduced MIF levels in ACE inhibitors users. The receiver operator curve (ROC) showed that MIF has a good performance in disease prediction. These findings support the reliability of MIF as a biomarker for predicting diabetic nephropathy and the possible reducing effect of ACE inhibitors on MIF levels.

Keywords: T2DM, Diabetic nephropathy, MIF, ACE inhibitors.

تقييم مستوى العامل المثبط لهجرة البلاعم في المصل و مدى صلاحيته كموشر حيوي لاعتلال الكلية في مرضى داء السكري من النوع الثاني و ثاثير مثبطات الانزيم المحول للانجيوتنسين على مستواه

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الخلاصة

اعتلال الكلية السكري هو احد مضاعفات السكري المزمنة السائدة في الاوعية الدموية الدقيقة و نظرا لان الالتهاب يلعب دورا حيويا في تطور و تقدم المرض فان العامل المثبط لهجرة البلاعم و هو سيتوكين متعدد الوضائف و مسبب للالتهاب اظهر انه يلعب دورا حيويا في الاستجابات الالتهابية للامراض المختلفة مثل اعتلال الكلية االسكري. هدفت هذه الدراسة إلى تقييم مستويات العامل في مصل عينة من مرضى السكري من النوع الثاني العراقيين المصابين باعتلال الكلية مما يدعم صلاحيته كمؤشر حيوي لاعتلال الكلية بالإضافة إلى ذلك ، لتقييم التأثير الكلوي لمثبطات الإنزيم المحول للأنجيوتنسين على مستويات العامل في المصل. هذه دراسة حالة وضبط تشمل تسعين شخصًا تم تقسيمهم إلى ثلاث مجموعات: عشرين شخص سليم كمجموعة ضابطة وسبعون مريضًا يعانون من داء السكري من النوع ٢ مقسمة إلى مجموعتين متساويتين وفقًا لوجود اعتلال الكلية السكري ام عدمه الذين تم تقسيمهم إلى مجموعتين حسب استخدام مثبطات الإنزيم المحول للأنجيوتنسين. تم قياس مستوى كل من السكر, الهموكلوبين السكري, اليوريا , الكرياتين ومؤشر كتلة الجسم و نسبة الالبومين البولي لكل شخص بالاضافة لقياس مستوى العامل المثبط لهجرة البلاعم. لوحظت أعلى مستويات للعامل في مصل مرضى اعتلال الكلية السكري (٢٤,٩ نانوغرام / مل) يليهم مرضى السكري(١٤,١ نانوغرام / مل) مع أدنى مستوى لوحظ في المجموعة الضابطة (٤,٨ نانوغرام /مل).و كانت هناك علاقة معنوية بين مستويات العامل واستخدام مثبطات الإنزيم المحول للأنجيوتنسين مع انخفاض مستوى العامل في مستخدمي مثبطات الانزيم المحول للانجيوتنسين. تدعم هذه النتائج موثوقية العامل المثبط لهجرة البلاعم كمؤشر حيوي للتنبؤ باعتلال الكلية السكري والتأثير المحتمل لمثبطات الإنزيم المحول للأنجيوتنسين على مستوياته

الكلمات المفتاحية: داء السكري النوع الثاني, اعتلال الكلية السكري, العامل المثبط لهجرة البلاعم, مثبطات الانزيم المحول للانجيوتنسين

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Introduction

Diabetic nephropathy (DN) is a prevalent chronic microvascular diabetes sequela and a significant contributor to end-stage renal disease (ESRD) and cardiovascular complications, particularly in patients with type 2 diabetes mellitus (T2DM), is also known as a diabetic kidney disease (DKD), is a pathophysiologically complicated and poorly understood. Even though oxidative stress, hyperglycemia, and renin-angiotensin-aldosterone system (RAAS) are the primary causes, a growing body of data suggests that inflammation (through chemokines, cytokines, and intracellular signaling pathways) has a critical influence on the development and progress of DN[1].

Macrophage migration inhibitory factor (MIF), a versatile proinflammatory cytokine, possesses a chemokine-like action. It stimulates the guided migration and mobilization of leukocytes towards infectious and inflammatory areas and prevents migration outside the inflammatory site. Another physiologic activity of MIF was to refute glucocorticoid suppression of immune cell reaction, which is essential for controlling the biological inflammatory response in conditions such as intense stress or acute sickness. By suppressing activation-induced apoptosis, MIF plays a critical function in immune cell survival, which is responsible for both optimum and excessive inflammatory responses in various pathologic situations[2].

MIF is the innate immune system mediator that encourages the expression of many cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and prostaglandin E2. The cluster of differentiation74 (CD74) is a MIF-binding receptor (a type II transmembrane protein) that accelerates leukocyte recruitment into inflammatory areas, boosting the innate response and spreading an adaptive response in a chemokine-like manner. The chemokines CXCR2 and CXCR4 receptors also bind MIF aiding in its immune-mediated mechanism[3].

Excessive MIF expression by glomerular and tubule-interstitial cells linked to significant macrophage and T-cell accumulation leads to localized glomerular and tubule-interstitial damages, especially glomerular crescent development, and these results in progressive renal dysfunction such as proteinuria, raised serum creatinine, and a decline in glomerular filtration rate(GFR)[4]. Although the pathogenic significance of MIF overexpression in the development of DN is yet unknown, the primary mechanism suggests that persistent hyperglycemia plays a major role in increasing MIF expression in podocytes, causes severe proteinuria and glomerulosclerosis, eventually leading to end-stage kidney disease[5].

The utilization of angiotensin-converting enzyme (ACE) inhibitors as the primary treatment for proteinuric DN is supported by several studies as they show additional blood pressure-independent renoprotective properties[6]. However, administering an ACE inhibitor will not entirely halt DN progression. Angiotensin II (Ang II) is known to cause renal cellular changes by releasing cytokines such as tissue growth factor-β (TGF-β), IL10, TNF-α, Monocyte chemoattractant protein-1 (MCP-1) and MIF, and glomerular hypertension. As Ang II is also demonstrated to cause podocyte death, tubular microvessel loss, and hypoxia, the use of ACE inhibitors delays the course of DN. Despite these findings, the role of an ACE blockade in diabetics and DKD is yet unknown[7].

Patients with DN are more likely to develop ESRD, cardiovascular complications, and mortality. Early identification and new effective therapies that delay the course of DN or lower cardiovascular risk have had a long-term influence on improving the disease prognosis. In addition, the global prevalence of DN in T2DM patients is steadily rising, resulting in increased morbidity and mortality, as well as adding significant socioeconomic burdens on global healthcare systems[8]. Using estimated glomerular filtration rate (eGFR) with albuminuria as diagnostic modules to diagnose and monitor DN is globally expressed, but these indicators have numerous limitations[9]. The primary rationale for the current study is the hunt for novel biomarkers critical to providing successful DN care and finding a unique mechanism that may be targeted to delay disease development and progression.

The aim of the study is to assess serum levels of MIF (as inflammatory cytokine mediates DN progression) and its relation to glycemic indices, kidney function, and ACE inhibitors in a sample of Iraqi diabetic patients with nephropathy supporting its validity as a marker for predicting nephropathy in T2DM patients. In addition, the nephroprotective effect of ACE inhibitors was evaluated in terms of their influence on MIF levels.

Subjects and Method

This is a case-control study involving ninety subjects recruited by the researcher during their visit to the privet endocrinologist and nephrologist's clinics in Al-Kut City/ Wasit government/ Iraq from November 2021 to February 2022. The participants were divided into three groups: twenty apparently healthy in the control group and seventy type 2 diabetic patients divided into thirty-five patients without nephropathy and thirty-five patients with nephropathy. To investigate the nephroprotective role of ACE inhibitors in terms of their effect on the level of MIF, T2DM patients in each group were then subdivided into two groups according to the use of ACE inhibitors.

All participants included in this study were aged between (20 and 65 years) of both genders. Diabetic patients were selected by a

specialized endocrinologist and diagnosed with type 2 diabetes according to the 2019 American Diabetes Association (ADA) guideline [10]. While Diabetic patients with nephropathy have been selected by the professional consultant nephrologist and diagnosed based on the urinary albumin to creatinine ratio (ACR) [ACR> 3 mg/mmol] [11] or based on eGFR (<60 ml /min/1.73 m^2) with and without renal damage for at least three months [12] with a DM duration (since diagnosis) of at least five years or more. The patients were treated with ACE inhibitors for at least three months. The healthy participants for the control group were randomly selected (they should be of comparable age, sex, and BMI to the two studied patient groups). Excluding patients with concurrent infection, debilitating illness, autoimmune diseases, metabolic disease, pregnant and lactating women, or patients using concurrent medications thought to affect serum levels or give misreading for MIF assay (e.g., angiotensin receptor blockers[13], chemotherapy[14]). In addition, patients who provide inaccurate information on the questionnaire will also be banned from the study.

After the patient rest for 5 min at a private laboratory, blood pressure, body mass index (computed by dividing the weight in kilograms (kg) by the square of the height in meters (m^2)) [15], and detailed history were obtained by the researcher using a patient data collecting sheet was explicitly made for the research purpose.

Then an eight milliliters blood sample obtained by a vein puncture was collected from the three groups of participants; two milliliters of the sample were preserved in an ethylene diamine tetraacetic acid (EDTA) tube for glycated hemoglobin (HbA1c) measurements while the rest of the blood let to be clotted at room temperature for 5-10 min then centrifuged to obtain the serum that has been divided into two parts, one for immediate measurements of serum creatinine, serum urea and random blood sugar (RBS) using the Cobas c111 autoanalyzer by Roch® Diagnostics, USA. At the same time, the other part is stored in an Eppendorf tube and refrigerated at -20 °C to measure MIF levels by sandwich enzyme-linked immunosorbent assay (ELISA) test [16]after all samples needed for the study are collected. Random spot urine samples were collected from each participant in a suitable urine container and used immediately for measurements of urine Albumin-Creatinine Ratio (ACR) [17] by urine analyzer system "Combilayzer 13" using "Combina 13" urine test strip licensed by Human® Diagnostic, Germany. The modification of diet in renal disease (MDRD) equation was used to calculate the eGFR. [18].

Statistical analysis

The statistical package for social science (SPSS) version 25 was utilized for all graphs and statistical analysis. Categorical data were summarized in numbers, while continuous data were expressed in median and interquartile ranges. Nonparametric tests were applied since the data were not normally distributed. The degree of significance between every two continuous variables was obtained using the Mann-Whitney U test. In contrast, the Kruskal-Wallis test was used to determine the difference between three continuous variables. For categorical data comparisons, Chi-square was employed, but Fisher's exact test was utilized if the first was not appropriate. The association between the biomarker and the various variables was assessed using the Spearman correlation. The diagnostic performance of the biomarker for predicting nephropathies by employing the receiver operator curve (ROC). A p-value of less than 0.05 indicates statistical significance.

Results

Concerning the participant sociodemographic characteristics, there was any notable variance in BMI, gender, smoking habit, and living place between the three study groups (p-value>0.05). Still, there was a difference in age between the control group and the two diabetic patient groups (p-value<0.05) with no considerable variations between the two diabetic patient groups. The duration of T2DM shows notable differences between the three groups, with the DN group having the most extended duration. Serum levels of HbA1c, RBS, urea, creatinine, and eGFR show remarkable variance between the three studied groups (p-value<0.05). The HbA1c and RBS the highest levels were observed within the DM group while serum urea and creatinine levels were higher in the DN patient group than in both the control and DM groups while eGFR showed the lowest levels in the DN group. Patient distribution to the ACR three stages were considerably different between the groups with the majority of control and DM group with A1 stage, and most DN patients were at A2 stage. As illustrated in Table (1).

Table 1. Participant sociodemographic characteristics

Character	Group 1 (n=20)	Group 2 (n=35)	Group 3 (n=35)	p-value
Age (year)	50±13	56±12	56±8	0.015*[b]
Gender (male\female)	10\10(50.0\50.0%)	15\20(42.9\57.1%)	17\18(48.6\51.4%)	0.679
Living place (City\Village)	12\8(60.0\40.0%)	21\14(60.0\40.0%)	23\12(62.2\37.8%)	0.862
BMI (kg/m^2)	27.45±6	29.10±8	27.30±6	0.269
Smoking (smoker\nonsmoker)	5\15(25.0\75.0%)	10\25(28.6\71.4%)	11\24(28.9\71.1%)	0.879
T2DM duration (year)	-	10.0±6	13±5	0.008*[c]
S B\P (mmHg)	12.5±1.9	14.0±2	14.0±4	0.018[b]
D B\P (mmHg)	8±0.9	8±1.5	8±1.0	0.424
HbA1c %	4.350±1.5	8.7±3.1	7.2±2.7	0.000*[a b c]
RBS (mg/dl)	105.0±49	227.0±191	200.0±102	0.000*[a b]
Urea (mg/dl)	26.0±10.2	33.4±10.5	71.3±67.6	0.000*[a b c]
Creatinine (mg/dl)	0.67±0.18	0.70±0.19	1.7±1.4	0.000*[b c]
eGFR (mL/min/1.73 m2)	103.55±18.72	89.10±26.6	38.70±34.4	0.000*[a b c]
ACR A1 (<3 mg/mmol) **A2 (3 -30 mg/mmol)** **A3 (> 30 mg/mmol)**	20 0 0	23 10 2	6 21 8	0.000*[a b c]

(Group 1: healthy participant control group, Group 2: type 2 diabetes mellitus patient, Group 3: type 2 diabetes mellitus patient with nephropathy, BMI: body mass index, T2DM: type 2 diabetes mellitus, S B\P: systolic blood pressure, D B\P: diastolic blood pressure, HbA1c: glycated hemoglobin, RBS: random blood sugar, eGFR: estimated glomerular filtration rate, ACR: urinary albumin-creatinine ratio) Continuous variable expressed as median ± IQR and categorical variable defined as a number and percent. (*: significant difference between the three groups, a: significant difference between group1&2, b: significant difference between group1&3, c: significant difference between group2&3) The Chi-square test was used to assess the statistical significance between categorical variables, while for assessing the difference between continuous variables and groups, Kruskal Wallis and Man Whitney tests were used.

Figure (1) demonstrates the serum levels of MIF and shows a notable variation between the three groups (p-value<0.05). Serum MIF's highest levels were observed in the diabetic nephropathy patients (24.9 ng/ml), followed by the T2DM group (14. 1 ng/ml), with the lowest level observed in the control group (4.8 ng/ml).

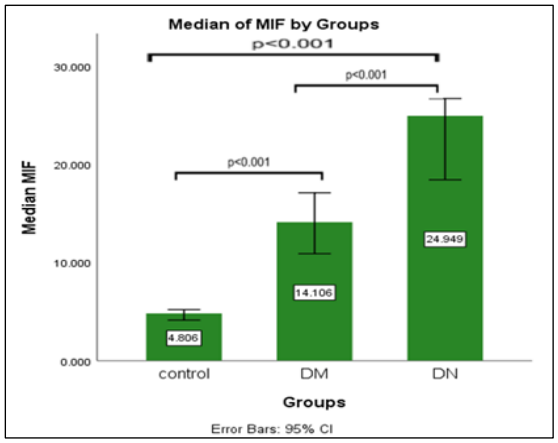

Figure 1. The median serum MIF level between the study three groups

(DM: T2DM patient group, DN: diabetic nephropathy patient group); for assessing the difference between serum level and groups Kruskal Wallis and Man Whitney tests were used.

Serum MIF has a positive correlation with systolic blood pressure, HbA1c, RBS, serum urea and creatinine, and T2DM duration (p-value<0.05), in addition to a negative correlation with eGFR, while there was no correlation between serum MIF and the smoking habit, age, gender, BMI, and diastolic blood pressure (p-value>0.05) as shown in Table (2).

Table 2. Correlation of MIF with different study variables

MIF	r	p-value
Age	0.194	0.066
Gender	-0.007	0.946
Smoking	0.046	0.665
BMI	-0.020	0.849
S B\P	0.250*	0.018
D B\P	0.178	0.94
HbA1c	0.427**	0.000
RBS	0.457**	0.000
Urea	0.385**	0.001
Creatinine	0.450**	0.000
eGFR	-0.573**	0.000
ACR	0.564**	0.000
T2DM duration	0.629**	0.000

(BMI: body mass index, T2DM: type 2 diabetes mellitus, S B\P: systolic blood pressure, D B\P: diastolic blood pressure, HbA1c: glycated hemoglobin, RBS: random blood sugar, eGFR: estimated glomerular filtration rate, ACR: urinary albumin to creatinine ratio) * p-value <0.05, ** p-value < 0.01, r: spearman correlation coefficient.

Diabetic patients were further divided into four subgroups according to their ACE inhibitors use. Serum levels of MIF show no significant differences between the DM subgroups (p-value >0.05) and a considerable difference between DN patients using and not using ACE inhibitors (p-value <0.05), as shown in Figure (2) .

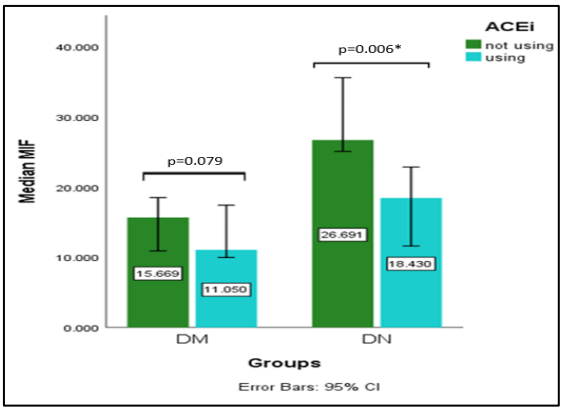

Figure 2. comparison of MIF levels between patients using and not using ACE inhibitors.

(DM: T2DM patient group, DN: diabetic nephropathy patient group) for assessing the difference between serum levels and groups Man Whitney test was used.

According to the ROC curve, MIF shows good diagnostic reliability for predicting diabetic kidney disease in diabetic patients, as shown in Figure (3). MIF shows good sensitivity and specificity at the chosen optimal cut-off point with a p-value <0.001.

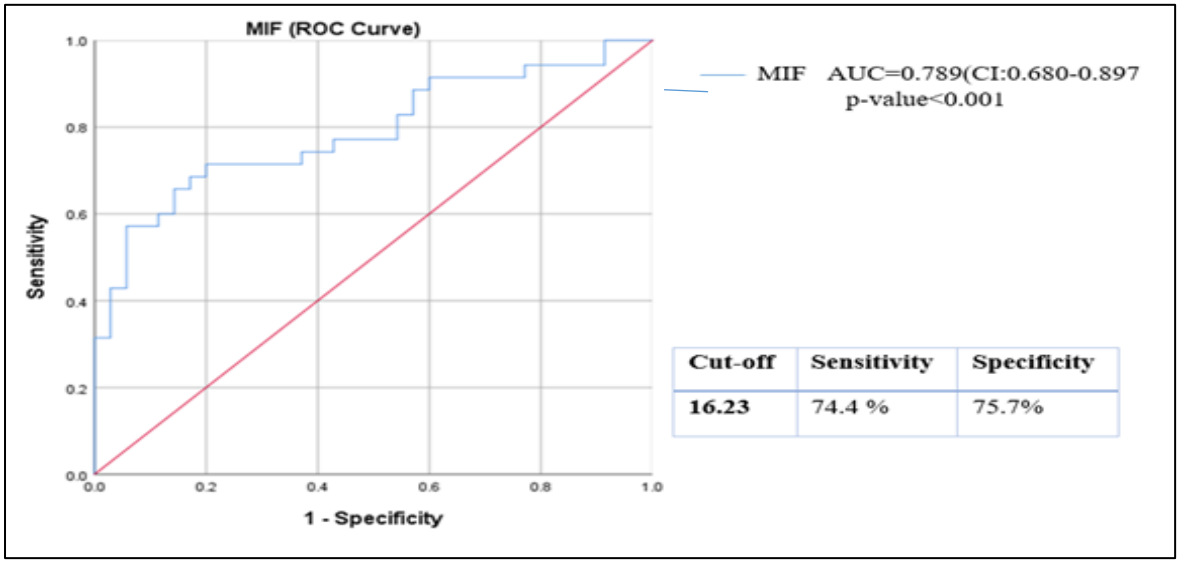

Figure 3. diagnostic reliability of MIF for predicting diabetic kidney disease in diabetic patients.

(AUC: area under the curve, MIF: macrophage migration inhibitory factor, CI: confidence interval)

Discussion

According to the recent research, various cytokines and inflammatory mediators have been raised in DN patients, providing a solid indicator for DN prognosis and supporting their reliability as markers for the prediction of nephropathy in diabetic individuals[19][20].

In the present study, in T2DM patients, MIF levels were substantially higher than in the control group. This is in accordance with Yuriko et al.[21] findings demonstrating that MIF is a polytrophic agent of pancreatic cells and proinflammatory cytokines that has a role in diabetes as well as in the early stages

of renal illness and predisposing factors, including obesity.

The main finding of the current study was that serum MIF concentrations in the DN were considerably higher than in the DM and control groups. Serum MIF levels were also positively correlated with HbA1c, RBS, ACR, urea, and creatinine levels and negatively correlated with eGFR. On the other hand, the association between MIF and eGFR or urea and creatinine in DN is described as a condition of chronic inflammation and hastened nephropathy with significant morbidity and mortality consequences irrespective of other risk factors. This could be explained by the fact that MIF is a proinflammatory cytokine of the innate immune system, and circulating MIF has been linked to renal dysfunction. Given that DN is linked to inflammation, it is not surprising that MIF was significantly elevated in this population. This is in harmony with prior DKD research that found GFR to be a predictive factor for circulating proinflammatory cytokines such as IL-10, IL-6, TNF-α, and MIF[22–24]. This was shown to be true for MIF and suggests that impaired renal function may be the primary source of elevated serum MIF in this group. This raises the possibility of MIF role in DM-related vascular disease. Alternatively, these findings do not answer the question of whether MIF is involved in the onset of diabetic podocytopathy or the development of DN. Still, they do suggest that MIF-mediated damage in DN might be targeted. Most diabetic patients may benefit not only from therapy options that target glucose management but as well as MIF[25].

In addition, Liu et al.[26] found that MIF levels were considerably higher in DN patients, and mosial et al.[27] found that MIF was higher in individuals with a variety of glomerular and tubular renal disorders (serum MIF was also linked to creatinine clearance). These two studies' findings are consistent with the present study's findings regarding that there were no considerable variations between age, gender, BMI, and MIF mean levels. However, there was a strong and direct association between systolic blood pressure, urea and creatinine levels, disease duration, RBS and HbA1C, and MIF levels.

Another research suggests that higher MIF levels have been observed in people with T2DM and linked to coronary diseases in these patients[28]. Khalilpour et al. found that treating diabetic rats with the MIF inhibitor reduced blood glucose levels and albuminuria, indicating that MIF inhibition might be a feasible therapeutic method in diabetic nephropathy[29]. This can be partially revealed by that MIF is the first molecule to reach the site of inflammation and is thought to determine the severity of cellular inflammation and play a significant role in local macrophage proliferation in renal inflammation, in addition to being a macrophage chemoattractant. As a result, increased MIF levels have been recognized as a potential additional mechanism of diabetic kidney macrophage accumulation caused by prolonged hyperglycemia. In addition, the influence of persistent hyperglycemia on the development of oxidative stress (OS) may result in a high MIF level as a vicious circle binds hyperglycemia to OS and lead to microvascular complications in T2DM such as DN[30]. That might explain why the DN group had significantly greater blood urea and creatinine levels as well as a lower eGFR than the DM group. Indeed, MIF might be used as a biomarker for renal disease[31].

In the DN group, there was a notable correlation between MIF levels and the use of ACE inhibitors. The idea that addresses this is that RAAS plays a pathogenic role in immune- and nonimmune-mediated renal disorders in human and animal models[32,33]. Following a renal insult, local synthesis of Ang II by mesangial cells or macrophages may lead to MIF release from tubular epithelial cells, enhancing macrophage and T cell activation and promoting renal damage[33]. These findings are consistent with Rice et al.'s [34] findings, which show that ACE inhibition reduces MIF levels, which correlates with lower macrophage and T-cell infiltration, suggesting that Ang II may cause renal damage indirectly through MIF.

Serum MIF was determined to have good sensitivity and specificity based on the ROC curve study. Although MIF has a sensitivity of about 75%, MIF's reliability as a marker is still within a reasonable range for diagnosing DN in diabetic patients; this is in line with Morsi et al.[35] findings.

Conclusion

This study points to the possible function of MIF in DN and its role as a predictor of metabolic abnormalities that induce vascular problems in T2DM patients. The results of MIF may pave the way for future risk classification and therapy options to lower the incidence of DN in diabetic patients, resulting in enhanced quality and duration of life for individuals with the disease.

The usage of ACE inhibitors has been linked to the suppression of high MIF levels produced by Ang II. As a result, the MIF might be a new therapeutic target for diabetes and DN.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

Ethics statement

The University of Baghdad's College of Pharmacy ethics committee granted the study permission (2615. 23\3\2022), and each subject gave their informed consent Prior to participating in the study.

Author Contribution

Sumaya B. Abdulrahman contributed to the conception and design of the study, performed the statistical analysis, revised and drafted the manuscript, organized the database, performed the practical work and the statistical analysis.

Eman S. Saleh contributed to the conception and design of the study, provided comments, revised the manuscript and supervised the whole research.

Sabah M. Saeedi helped to enroll patients and were involved in the experiments.

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