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Evaluation The Serum Level of Gasdermin D in Relation to Pyroptosis in Diabetic Nephropathy

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Abstract

Background Diabetic nephropathy (DN) is a serious consequence of diabetes that can result in end-stage renal failure. The development of this condition is characterized by the activation of inflammatory pathways, in which pyroptosis, a specific type of programmed cell death, plays a vital role. Gasdermin D plays a crucial role in the process of pyroptosis, which is becoming more and more associated with DN. Elevated glucose levels and advanced glycation end-products stimulate the nucleotide-binding oligomerization domain - like receptor protein 3 inflammasome, resulting in the activation of caspase-1 and pyroptosis in different types of kidney cells.

Objective To investigate the role of pyroptosis in DN by evaluation of serum Gasdermin D level and biochemical parameters.

Methods This study was case-control study that had 160 individuals divided into three groups including 40 individuals with DN, 40 individuals with type 2 diabetes mellitus (T2DM), and 80 individuals as control group. The parameters total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), very low-density lipoprotein-cholesterol (VLDL-C), low-density lipoprotein-cholesterol (LDL-C), random blood sugar (RBS), glycated hemoglobin (HbA1c%), urea, and creatinine, GSDMD and IL-18) were examined in this study.

Results The study revealed a strong association between DN and the control group in all investigated parameters. The comparison between DM and the control group did not yield a statistically significant result for GFR, whereas other parameters did reveal significant results. The link between DN and DM is found to be substantial in terms of urea, creatinine, GFR, GSDMD, and IL-18. However, the remaining parameters also exhibit significant results.

Conclusion The significant result in all parameters shown in comparison between DN and control group due to elevated levels of parameters in DN patients. The non-significant result in (GFR) in comparison between DM and control group while other parameter shown significant result due to elevated levels of parameters in DM patients.

Keywords GSDMD, IL-18, DN, T2DM, pyroptosis

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List of abbreviations: DM = Diabetes mellitus, DN = Diabetic nephropathy, GFR = Glomerular filtration rate, GSDMD = Gasdermin D, HDL-C = High-density lipoprotein cholesterol-C, IL-18 = Interleukin 18, LDL-C = Low-density lipoprotein cholesterol-C, NLRP3 = NOD-like receptor protein 3, RBS = Random blood sugar, T2DM = Type 2 diabetes mellitus, TC = Total cholesterol, TG = Triglyceride, VLDL-C = Very low-density lipoprotein cholesterol

Introduction

Diabetic nephropathy (DN) has emerged as the primary cause of end-stage renal failure and cardiovascular mortality,

among other comorbidities. This illness often develops several years after the onset of diabetes [1].

DN, or diabetic kidney disease (DKD), is a significant consequence of diabetes that can develop in patients with either type 1 or type 2 diabetes. The condition is marked by injury to the glomeruli, the kidney's filtration system, resulting in diminished kidney function and the possibility of developing end-stage renal disease (ESRD) [2,3].

Pyroptosis is a specific form of programmed cell death that is different from apoptosis. It is characterized by the quick disintegration of the cell and the release of proinflammatory substances from within the cell. Pyroptosis is frequently initiated by activating inflammatory caspases, including caspase-1, caspase-4, caspase-5, and caspase-11 [4].

Nod-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome activation has a crucial role in causing pyroptosis in DN. Several stimuli, such as elevated glucose levels, oxidative stress, and advanced glycation end-products (AGEs), can activate the NLRP3 inflammasome [5].

The initiation of the NLRP3 inflammasome results in the fragmentation and initiation of caspase-1, which subsequently fragments the premature versions of interleukin 1β (IL-1β) and IL-18, transforming them into their fully developed, proinflammatory versions. These cytokines that cause inflammation can subsequently cause additional harm to cells and contribute to the advancement of diabetic nephropathy [6].

Gasdermin D (GSDMD) plays a crucial role in pyroptosis. it has gained recognition as a significant contributor to the development and advancement of diabetic nephropathy [7].

GSDMD-mediated pyroptosis can result in the depletion of these essential cell types, which can contribute to the structural and functional deterioration of the glomeruli and renal tubules. This process ultimately leads to the formation and advancement of DN [8].

This study aimed to investigate the role of pyroptosis in DN by evaluation of serum GSDMD level and biochemical parameters

Methods

This research was a case-control study conducted from December 2023 to February 2024, 160 participants, aged between 40 and 79 years, were enrolled in this study. They were divided into three groups for comparison:

- The first case group consists of 40 individuals diagnosed with type 2 diabetes mellitus (T2DM) with nephropathy (DN).
- The second case group consisted of 40 individuals diagnosed with T2DM who did not have any problems related to nephropathy.
- The control group consists of 80 sampled individuals.

Inclusion criteria were male and female patients with T2DM with or without nephropathy.

The specialists with specialized expertise at Al-Hassan Medical Center for Endocrinology, located in Al-Hussein Medical City, Kerbala Health Directorates in Iraq, have made the decision to diagnose the patients. The study protocol received approval from the Medical Ethics Committee of the College of Medicine, Al-Nahrain University, according to book number (2/3/319 in 22/2/2023) and the Kerbala Health Directorate, according to book number (1367 in 9/7/2023). After receiving comprehensive information, the participants in this study gave their signed consent. Patients' privacy was safeguarded, and the entire treatment was conducted with express written consent.

The levels of various parameters including total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), very low-density lipoprotein-cholesterol (VLDL-C), low-density lipoprotein-cholesterol (LDL-C), random blood sugar (RBS), glycated hemoglobin (HbA1c%), urea, and creatinine were assessed using the COBAS INTEGRA® 400 system manufactured by Roche in Germany.

Additionally, the levels of GSDMD and IL-18 biomarkers were analyzed using an ELISA kit produced by Biont in China. The GFR was calculated using the provided equation [9]:

eGFR = 142 × min(S. Cr/κ,1)α × max(S. Cr/κ,1)−1.200 × 0.9938Age × 1.012 (if female)
(S. Cr is serum creatinine in mg/dL, κ=0.7 for females and 0.9 for males, α = −0.329 for females and −0.411 for males, min=the minimum of S/κ or 1, and max=the maximum of S/κ or 1)

The exclusion criteria were patients with inflammation, patients with neuropathy and patients with cancer.
Five milliliters of blood were drawn by venipuncture from all participants. The blood samples were divided into three parts:

1. Two milliliters collected in EDTA tubes for HbA1c, and random blood sugar testing.
2. Three milliliters placed in gel tubes for freezing and subsequent analysis of GSDMD protein, IL-18, urea, creatinine, GFR, and lipid profile. Samples were transported in ice packs to the laboratory for analysis.

The data were presented as the mean±standard deviation (SD). The student t-test was employed to compute the likelihood. The PAST version 4.04, which was released in 2020, was utilized to compute the probability value (P value) and evaluate biochemical parameters within the study groups. The significance level in all statistical research is set at a threshold of P ≤0.05.

Results

Comparative in biochemical parameters between patients and healthy control groups

The results of this study are summarized in table (1), comparing three groups: diabetic patients with nephropathy (DN), diabetic patients without nephropathy (DM), and healthy controls. Group 1: DN vs. DM, significant differences were found for: Blood urea (DN: 59.1±12.8 vs. DM: 98.6±11.9, P ≤0.05), serum creatinine (DN: 1.4±0.3 vs. DM: 0.7±0.2, P ≤0.05), GFR (DN: 55.5±14.6 vs. DM: 98.6±11.9, P value ≤0.05), GSDMD (DN: 484.5±137.6 vs. DM: 711.7±478.4, P ≤0.05), IL-18 (DN: 16.02±6.4 vs. DM: 56.2±13.3, P value ≤0.05). Non-significant differences were observed in TC, TG, HDL-C, VLDL-C, LDL-C, RBS, and HbA1c. Group 2: DN vs. Healthy Control, significant differences were found in all parameters: TC, TG, HDL-C, VLDL-C, LDL-C, RBS, HbA1c, blood urea, creatinine, GFR, GSDMD, and IL-18 (all P value ≤0.01). Group 3: DM vs. Healthy Control, non-significant difference in blood urea (P = 0.97) and all other parameters showed significant differences in (P value ≤0.05).

Distribution of study participants by age group

The age distribution across different groups shown in figure (1) indicates that the majority of DN patients were concentrated in the 50-59 years age group, which is consistent with the age-related progression of nephropathy. Diabetic patients without nephropathy (DM) also show a similar age distribution but with a higher proportion in the younger age group (40-49 years). Healthy controls, on the other hand, are predominantly found in the 40-49 years age group. The statistical analysis confirms that these age distributions are significantly different across the groups (P ≤0.05), emphasizing the age-related risk factors in diabetic nephropathy development.

Sex distribution among study participants

The sex distribution across DN, DM, and healthy control groups shows a higher proportion of females in both DN and DM patient groups compared to males. In figure (2), the number of females in the healthy control group is also higher than males. This distribution suggests a potential gender-related predisposition or susceptibility to developing diabetic nephropathy. The Chi-square test confirms that the gender distribution is significantly different across the groups (P ≤0.01).

Table 1. Comparative biochemical parameters between patients and healthy control groups

Parameters	Mean±SD		P value	Mean±SD		P value	Mean±SD		P value
	DN patients n. (40)	DM patients n. (40)		DN patients n. (40)	Healthy control n. (80)		DM patients n. (40)	Healthy control n. (80)	
TC (mg/dL)	212.1±41.9	202.8±51.5	0.38	212.1±41.9	102.9±21.1	≤ 0.05	202.8±51.5	102.9±21.1	≤ 0.05
TG (mg/dL)	255.6±115.1	229.5±83.01	0.25	255.6±115.1	90.2±22.5	≤ 0.05	229.5±83.01	90.2±22.5	≤ 0.05
HDL-C (mg/dL)	33.3±5.8	34.4±4.4	0.34	33.3±5.8	39.7±7.9	≤ 0.05	34.4±4.4	39.7±7.9	≤ 0.05
VLDL-C (mg/dL)	51.1±23.03	459±16.6	0.25	51.1±23.03	18.03±4.5	≤ 0.05	459±16.6	18.03±4.5	≤ 0.05
LDL-C (mg/dL)	127.4±37.2	122.6±49.97	0.6	127.4±37.2	45.1±22.3	≤ 0.05	122.6±49.97	45.1±22.3	≤ 0.05
RBS (mg/dL)	251.8±112.7	265.6±100	0.56	251.8±112.7	89.8±11.9	≤ 0.05	265.6±100	89.8±11.9	≤ 0.05
HbA1c (%)	9.2±1.7	9.5±1.8	0.4	9.2±1.7	5.1±0.4	≤ 0.05	9.5±1.8	5.1±0.4	≤ 0.05
Blood Urea (mg/dL)	59.1±12.8	25.45±6.2	≤ 0.05	59.1±12.8	25.4±7.3	≤ 0.05	25.45±6.2	25.4±7.3	≤ 0.05
Creatinine (mg/dL)	1.4±0.3	0.7±0.2	≤ 0.05	1.4±0.3	0.6±0.2	≤ 0.05	0.7±0.2	0.6±0.2	≤ 0.05
GFR (ml/min/1.73 m^2)	55.5±14.6	98.6±11.9	≤ 0.05	55.5±14.6	109.9±13.9	≤ 0.05	98.6±11.9	109.9±13.9	0.97
GSDMD (pg/mL)	1980.6±230	1806±259.2	≤ 0.05	1980.6±230	977.5±334.7	≤ 0.05	1806±259.2	977.5±334.7	≤ 0.05
IL-18 (ng/mL)	102.2±22.9	81.2±23.6	≤ 0.05	102.2±22.9	61.01±15.6	≤ 0.05	81.2±23.6	61.01±15.6	≤ 0.05

*DN: Diabetic nephropathy; DM: Type 2 diabetes mellitus without nephropathy; TC: Total cholesterol; TG: Triglyceride; HDL-C: High-density lipoprotein – cholesterol; VLDL-C: Very low-density lipoprotein – cholesterol; LDL-C: Low-density lipoprotein – cholesterol; RBS: Random blood sugar; GSDMD: Gasdermin D; IL-18: Interleukin 18. *The statistical analysis was used is (t test)

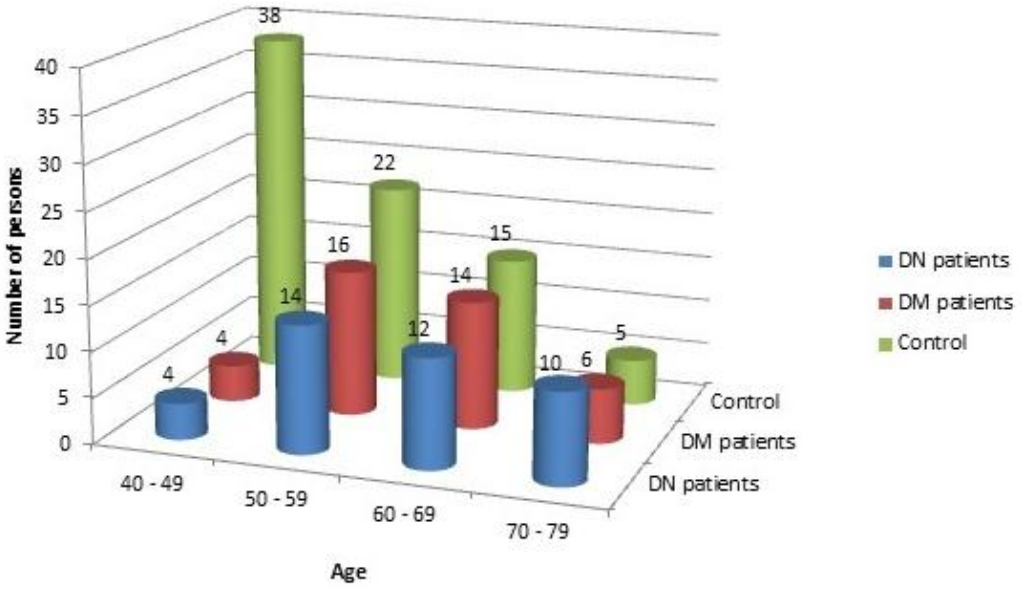

Figure 1. Distribution of study participants by age group

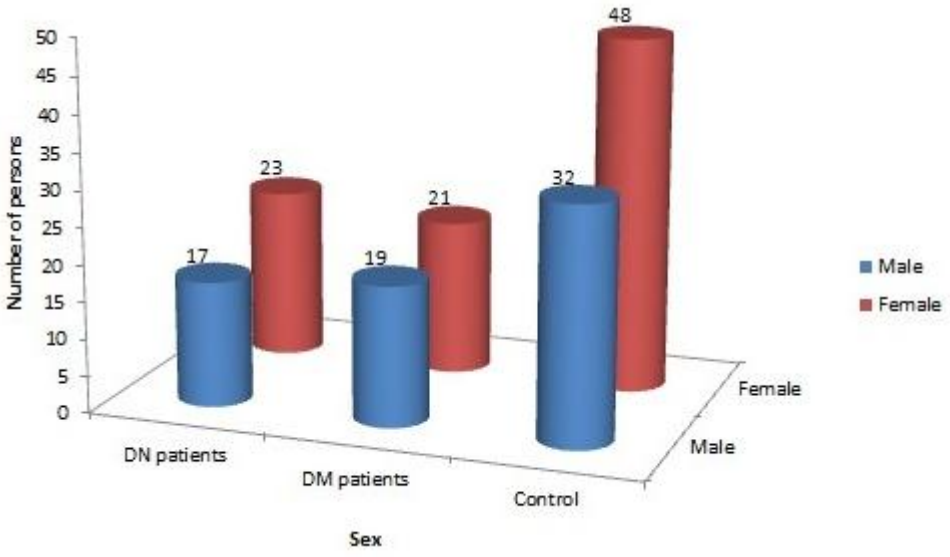

Figure 2. Sex distribution among study participants

Stages of diabetic nephropathy in study participants

The classification of DN patients shown in figure (3) divided into different stages (S1, S2, S3) reveals that the majority of DN patients are in the advanced stage (S3). There are no DN patients in Stage 1, while a significant number of DM patients are in Stage 1, and the majority of controls are also in Stage 1. The classification criteria were strictly adhered to, and the differences between stages among the groups are statistically significant ($P \leq 0.05$).

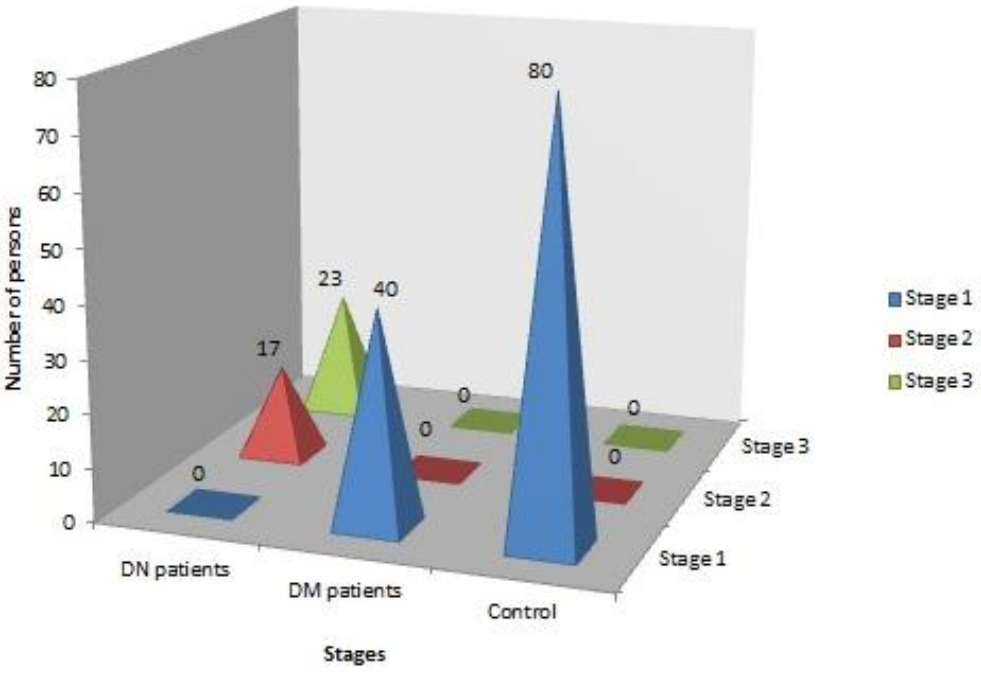

Figure 3. Stages of diabetic nephropathy in study participants

ROC curve analysis

ROC curve analysis between diabetic nephropathy and healthy control groups

The results in current study in table (2) and figure (4) were appeared ROC curve analysis between DN and healthy control groups. The result showed high sensitivity for (GSDMD and IL-18) (97% and 92%) respectively, the area under the curve for (GSDMD and IL-18) is (0.895 and 0.865) respectively.

Table 2. ROC analysis of Gasdermin D and interleukin-18 between diabetic nephropathy and healthy control group

Parameters	Sensitivity	Specificity	AUC	95% CI	Cut-off value
GSDMD	97%	65%	0.895	0.84-0.94	1455
IL-18	92%	64%	0.865	0.80-0.92	71

GSDMD: Gasdermin D; IL-18: Interleukin 18; AUC: Area under the curve; CI: Confidence interval

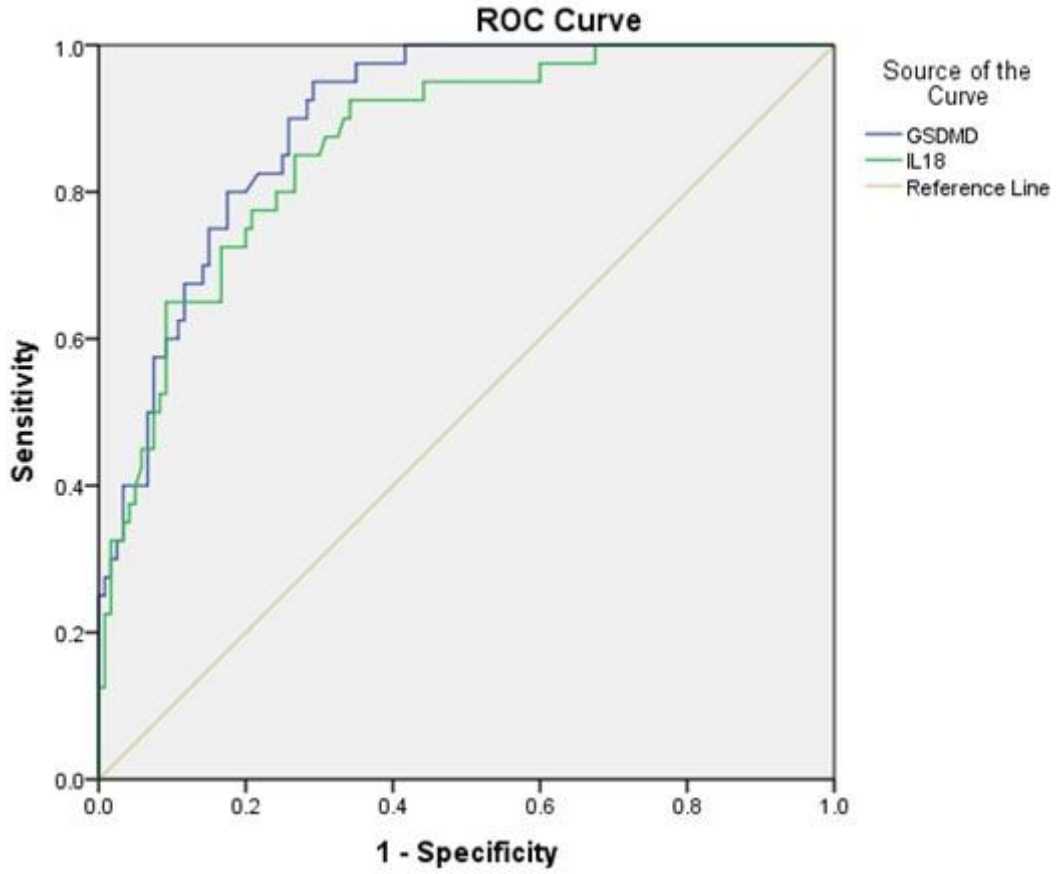

Figure 4. ROC curve analysis of Gasdermin D and interleukin-18 between diabetic nephropathy and healthy control group

ROC curve analysis between diabetic without nephropathy and healthy control groups

The current study presented the findings of the ROC curve analysis comparing the diabetic without nephropathy group with the healthy control group. These results can be found in table (3) and figure (5). The sensitivity of GSDMD was found to be high, with values of

(92%) and acceptable result for IL-18 with value (55%). The area under the curve for GSDMD and IL-18 was 0.739 and 0.583 respectively.

Table 3. ROC analysis of Gasdermin D and interleukin-18 between diabetic without nephropathy and healthy control group

Parameters	Sensitivity	Specificity	AUC	95% CI	Cut-off value
GSDMD	**92%**	**56%**	**0.739**	**0.66-0.81**	**1425**
IL-18	**55%**	**58%**	**0.583**	**0.48-0.68**	**75**

GSDMD: Gasdermin D; IL-18: Interleukin 18; AUC: Area under the curve; CI: Confidence interval

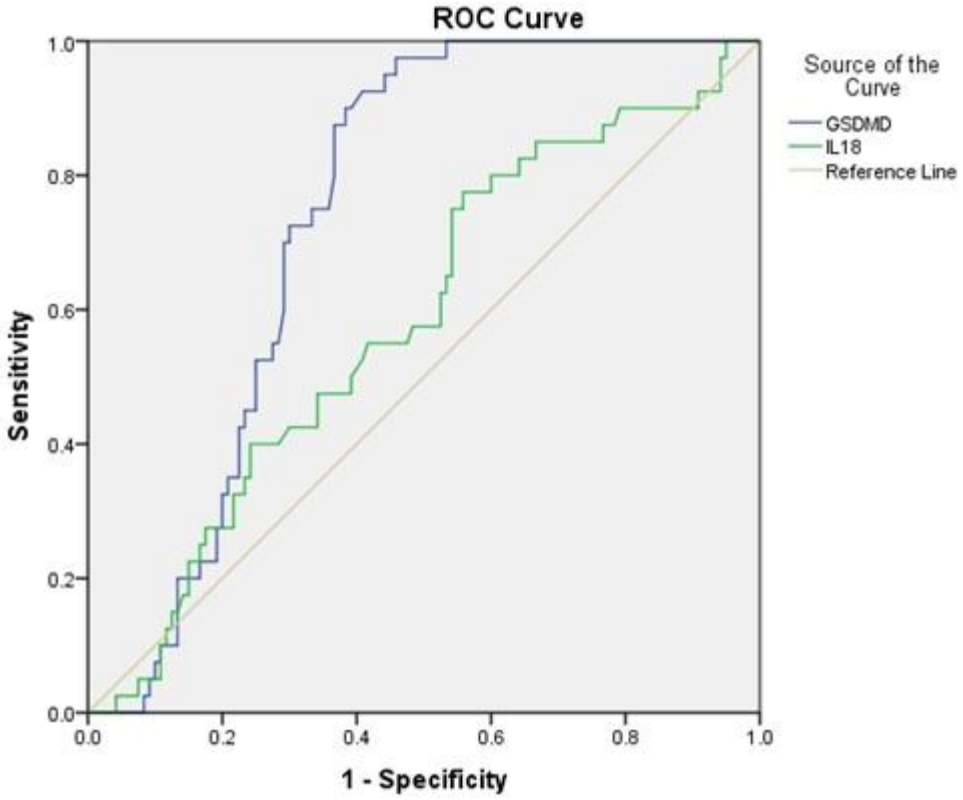

Figure 5. ROC curve analysis of Gasdermin D and interleukin-18 between diabetic without nephropathy and healthy control group

Discussion

The clinical and biochemical features demonstrated substantial variations among the three compared groups in several parameters, including (TC, TG, HDL-C, VLDL-C, LDL-C, RBS, HbA1c, blood urea, creatinine, GFR, GSDMD and IL-18).

RBS: diabetic patients exhibit chronic hyperglycemia caused by insulin resistance, which results in decreased responsiveness of peripheral tissues to insulin activity. Most cells in the body require insulin to facilitate the entry of glucose into them. However, insulin resistance reduces glucose absorption by cells, particularly muscle cells and adipocytes, by affecting the movement of glucose transporter type 4 (GLUT-4). This resistance also triggers glucose production in the liver cells through gluconeogenesis and glycogenolysis, ultimately

resulting in high blood sugar levels (hyperglycemia) [10].

Lipid profile: The current study demonstrates notable variations in the average lipid profile values (TC, TG, LDL-C, and HDL-C) among the three comparable groups. In individuals with T2DM, both TC, TG, and LDL-C levels were elevated. In contrast, HDL-C levels are reduced in T2DM without nephropathy and T2DM with renal problems compared to the control group. Dyslipidemia, an abnormal lipid profile, occurs in T2DM due to insulin resistance. Elevated levels of LDL-C and decreased levels of HDL-C can lead to an increased risk of cardiovascular disease in individuals with diabetes [11,12].

HbA1c: The current investigation revealed notable variations in the average levels of HbA1c% among the three compared groups: control; the typical HbA1c level in uncontrolled diabetes is below 6%. Measuring HbA1c in patients with DN is limited due to renal failure and erythropoietin shortage. In the present investigation, it was found that the level of HbA1c in patients with T2DM was higher than in those with T2DM and nephropathy problems [13,14].

Renal function tests: There is a notable disparity in the levels of urea, creatinine, and GFR among the three groups. The DN group exhibited the highest average amounts of urea and creatinine compared to the DM and control groups. Urea is the final byproduct of protein metabolism; it is synthesized in the liver from amino acids derived from protein. Creatinine is a byproduct that is generated by the breakdown of muscle creatine. Urea and creatinine levels are used to evaluate renal function. Due to the primary method of waste disposal being excretion in urine, individuals with renal failure experience higher amounts of these chemicals in their plasma. Estimating GFR is considered the most valuable overall indicator for assessing the extent of renal impairment. A reduction of 75% in renal tissue leads to a decrease in GFR of 50% (less than 60 ml/min.1.73 m^2). As the glomerulus's function worsens, substances typically eliminated by the kidneys, such as urea and creatinine, build up in the blood [15].

IL-18: The three comparable groups yielded statistically significant results (P value ≤0.01). IL-18 appears to have a more precise association with DN. Increased concentrations of IL-18 in the bloodstream are linked to DN. The upregulation of IL-18 serves as the primary predictive indicator for developing DN in individuals with diabetes. Both urinary and serum levels of IL-18 are positively associated with the progression of renal injury and early renal dysfunction in individuals with T1DM and T2DM [16]. The increase of IL-18 was directly correlated with the level of urine albumin excretion, which serves as a diagnostic for DN. The increase in IL-18 in the tubular epithelial cells of individuals with diabetes may result from the activation of mitogen-activated protein kinase (MAPK) pathways dependent on transforming growth factor beta (TGF-β). IL-18 is present in various renal cell types, including tubular and epithelial cells, intercalated cells, tubulointerstitial cells, glomerulus, and renal tubular cells. Its expression in these cells may not rely on macrophages. The expression of IL-18 was higher in the renal biopsies of diabetic individuals, namely in the proximal, tubular, and epithelial cells. Additionally, the levels of IL-18 in the serum of diabetes individuals were greater than those of healthy individuals [17].

GSDMD: The three comparable groups yielded statistically significant results (P value ≤0.01). Pyroptosis, also known as GSDM-mediated programmed necrotic cell death, is heavily influenced by the GSDMD protein [18]. DN is typically characterized by aseptic inflammation, kidney damage, and loss of renal parenchymal cells. The involvement of pyroptosis signaling pathways in the progression of DN has garnered significant interest among academics and physicians. Recent data suggests that pyroptosis plays a role in the development of DN [19]. A study conducted by Li et al. has demonstrated an increase in the expression of pyroptotic-associated proteins, such as GSDMD, NLRP3, caspase-1, and IL-1β, in renal tubules [20]. Recent investigations have shown that pyroptosis contributes to the development of diabetic late complications, such as DN. Li et al. conducted a study where they observed that the GSDMD protein molecule enhances

pyroptosis in mouse podocytes treated with high glucose (HG) [21].

GSDMD-mediated cell death promotes renal inflammation, suggesting that GSDMD-dependent pyroptosis plays a critical role in the progression of DN pathogenesis. It was previously observed that HG circumstances significantly enhance caspase-11 and GSDMD-N protein expression levels in podocytes. Pyroptosis has lately been recognized as a crucial element in various kidney-related clinical disorders [22].

Pyroptosis is a primary pro-inflammatory process that involves cell death dependent on GSDMD. Recent investigations have demonstrated that pyroptosis plays a definitive function in promoting DN. Furthermore, ongoing investigations have discovered that the activation of NLRP3 inflammasome plays a role in promoting pyroptotic cell death, which in turn contributes to the development of DN [23].

In this study, most patients with DN were found in the age category of 50-59 years, while the same age group also had the highest number of patients with DM. The lowest number of patients was seen in the age group of 40-49 years for both DN and DM. The prevalence of DN in the majority of individuals with T2DM at any given moment is estimated to be around 30-50%. This statistic was reported specifically among people in the United States who have diabetes, with over 90% of them having T2DM. The prevalence of this condition varied from 25% in patients under the age of 65 to about 50% in patients beyond the age of 65 [22]. In younger individuals, microalbuminuria is more common, but in older individuals, lower GFR becomes increasingly widespread in cases of DN [24]. Among patients diagnosed with T2DM between the ages of 20 and 59, there was a considerably larger proportion of men. There was no notable disparity in the gender distribution among patients diagnosed at or above the age of 60 years [25].

The results of present study were revealed that female number were higher in the three study groups; in general, adult males possess more visceral fat that is hormonally active and promotes insulin resistance, which is a precursor to metabolic syndrome and diabetes. After the age of 60, there were no longer any gender differences in the age at which diabetes was diagnosed. This suggests that the risk factors for T2DM were equally distributed between men and women at this age [26].

The sex disparities in relation to BMI were also identified. Adult males, while being on average overweight or obese, had significantly lower BMI values compared to adult females. This means that T2DM appeared in men at relatively lower BMI values than in females, suggesting differences in the BMI risk profiles between males and females [27].

The impact of sex on diabetic renal disease is subject to great debate. Several research indicates that being male is a risk factor for DKD, while others suggest that women have a higher likelihood of acquiring the ailment. Additionally, some investigations have identified no significant difference in risk between men and women [28]. A recent study aimed to evaluate the impact of sex on the occurrence of chronic kidney disease (CKD) in diabetic patients. The findings revealed that diabetic women had a considerably higher rate of CKD. In addition, women with diabetes had a slightly increased occurrence of microalbuminuria, although the difference was not statistically significant. This study encompassed participants diagnosed with both T1DM and T2DM. In a separate investigation utilizing the pathways investigation database, it was found that women had a comparable occurrence of DKD as men but had a higher occurrence of advanced DKD [29]. In general, CKD appears to be more common in women than in males. Nevertheless, an extensive analysis comprising numerous studies revealed that 38 studies reported a higher frequency of CKD in women, whereas only 13 studies reported the same among males. It is worth noting that sex hormones have a significant role in the pathophysiology of diabetes and its consequences, particularly in women with DM [30].

In the current study, most patients with DN were in stage 3. DN in Class II is divided into subcategories based on the extent of

mesangial growth. Class IIa is defined by mesangial expansion of 25% or less, whereas Class IIb comprises more than 25% mesangial expansion. Kidney enlargement is clinically observed when the mesangial matrix, glomeruli, and kidney volume are augmented. Typically, kidneys measure 11 cm or more on kidney ultrasound. Patients with this condition often experience elevated levels of urine albumin excretion [31].

Class III DN is characterized by an augmentation in the mesangial matrix, which is subsequently accompanied by mesangial sclerosis. The characteristic abnormality observed on a kidney biopsy is the presence of nodular glomerulosclerosis, also known as Kimmelstiel-Wilson nodules. The presence of Kimmelstiel-Wilson nodules on kidney biopsy is associated with the onset of diabetic retinopathy, indicating the activation of shared pathogenic pathways such as vascular endothelial growth factor (VEGF) [32].

GSDMD has been shown to be highly specific towards certain cell types in the kidneys, such as glomerular and tubular epithelial cells, which are typically affected in DN. The activation and pore-forming activity of GSDMD in these cell types can lead to their dysfunction and death, contributing to the development and progression of the disease [33].

Elevated glucose levels can trigger the activation of inflammatory pathways, including the GSDMD-mediated pyroptosis pathway, leading to increased cell death and tissue damage in the kidneys [8].

The sensitivity of GSDMD to oxidative stress further contributes to its specificity in the context of DN, where elevated oxidative stress is a key pathogenic factor [34].

In patients with DN, IL-18 levels are significantly elevated compared to healthy individuals or diabetic patients without nephropathy. This upregulation of IL-18 is a specific response to the pathological processes occurring in the kidneys during the development and progression of diabetic nephropathy [35]. IL-18 has a high degree of specificity towards certain renal cell types, such as glomerular and tubular epithelial cells, which are the primary targets of damage in diabetic nephropathy. The binding of IL-18 to its receptor on these cells can trigger a cascade of inflammatory and pro-apoptotic signaling pathways, leading to cell dysfunction and death [36]. Elevated levels of IL-18 have been shown to correlate with the severity of kidney damage and the rate of progression of DN. The high specificity of IL-18 in this context is evidenced by its ability to predict the risk of developing ESRD in patients with DN [37].

GSDMD-mediated pyroptosis in various cell types, such as adipocytes and hepatocytes, can lead to the development of insulin resistance, a key feature of diabetes mellitus. The high specificity of GSDMD in this context is linked to its ability to disrupt insulin signaling pathways and promote the production of proinflammatory cytokines that further exacerbate insulin resistance [38].

In conclusion, significant differences were observed in various biochemical parameters between DN patients, DM patients, and healthy controls, with elevated levels of TC, TG, HDL-C, LDL-C, VLDL-C, RBS, HbA1c, blood urea, creatinine, GSDMD, and IL-18. The GFR parameter was an exception in DM patients. Significant findings were also noted in the age and sex distributions, particularly among the 50-59 age group and highly specificity for GSDMD and IL-18 in comparison between DN, DM and Healthy control groups.

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Author contribution

All authors have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Conflict of interest

The authors declare there is no conflict of interest.

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