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Role of Visfatin in Chronic Kidney Disease: Diagnostic Potential and Association with Hemodialysis

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Abstract

Background: Chronic kidney disease (CKD) is a major cause of morbidity and mortality worldwide, often progressing silently until advanced stages. This study aimed to evaluate the diagnostic potential of serum visfatin levels and Nicotinamide Phosphoribosyl transferase (NAMPT) gene expression in peripheral blood mononuclear cells (PBMCs) among CKD patients, along with their correlation with disease severity and lipid profile.

Methods: A case-control study included 30 CKD patients, divided into two subgroups: 15 end-stage renal disease (ESRD) patients undergoing hemodialysis (HD) and 15 non-dialysis patients. These patients were matched by age and body mass index (BMI) with 30 healthy subjects (HS). Serum visfatin, lipid profile, electrolytes, NAMPT gene expression, and other biochemical markers were measured.

Results: This study showed significantly higher visfatin levels in CKD patients compared to HS, with the highest levels observed in the ESRD group undergoing HD (5.6±1.63 ng/mL compared with 3.5±1.4 ng/mL in CKD without HD, and 2.7±1.1 ng/mL in HS; $p \leq 0.001$). Similarly, NAMPT gene expression was significantly upregulated in CKD patients, with the highest expression in the HD group, correlating strongly with serum visfatin levels ($r = 0.76$, $p \leq 0.001$) and lipid profile markers, including triglycerides ($r = 0.67$, $p=0.002$) and low-density lipoprotein (LDL; $r = 0.61$, $p=0.004$). In CKD patients undergoing HD, visfatin levels showed a positive correlation with triglycerides and LDL levels, suggesting a link with dyslipidemia. No significant correlation was found between visfatin and highly sensitive C-reactive protein (hsCRP), urea, creatinine, or very-low-density lipoprotein (VLDL).

Conclusion: These findings indicate that serum visfatin and NAMPT gene expression could serve as novel biomarkers for assessing CKD severity, particularly in patients undergoing hemodialysis, with potential implications for managing inflammation and cardiovascular risk in CKD.

Keywords: Adipokines, Biological Markers, Inflammation, Dyslipidemias, Kidney Failure, Renal Dialysis.

Introduction

Chronic kidney disease (CKD) is defined as the persistent kidney damage or a sustained reduction in glomerular filtration rate (eGFR) below 60 mL/min/1.73 m² for three months or more, irrespective of the underlying cause.

CKD is a progressive disorder characterized by gradual impairment of kidney function, often leading to end-stage kidney disease (ESKD), at which point renal replacement therapy (RRT), such as dialysis or

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transplantation, becomes necessary for survival. end-stage kidney disease (ESKD) presents a severe stage of CKD, requiring long-term, intensive, and expensive treatments (1). Effective management of ESKD can significantly improve patients' quality of life and extend survival. However, barriers such as limited access to healthcare, a reactive rather than proactive treatment approach, and healthcare disparities among minority populations can hinder optimal patient outcomes. Patients with an eGFR below 15 mL/min/1.73 m^2 face life-threatening complications without timely RRT, which can be administered through hemodialysis, peritoneal dialysis, or kidney transplantation. Dialysis serves as a vital intervention that can temporarily manage kidney failure, either as a bridge to transplantation or as a permanent solution for those who are unable to undergo a transplant.

Regardless of the therapeutic approach, managing CKD and ESKD remains a critical determinant of patients' overall quality of life. In recent years, adipose tissue has come to be recognized not merely as a storage depot for energy but as an active endocrine organ that secretes various biologically active molecules, known as adipokines.

Among these, visfatin, first identified by Fukuhara et al. has garnered significant attention due to its multifaceted roles in the body. Visfatin, a 52-kDa protein primarily derived from visceral fat, is highly conserved across different species, including vertebrates, invertebrates, and even bacteria. Its pleiotropic properties allow it to participate in diverse physiological processes, particularly those involving inflammation, immunity, and metabolism (2, 3).

The primary function of visfatin is as a proinflammatory cytokine. It stimulates the production of inflammatory mediators, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and beta (TNF-β) (4, 5). In patients with CKD, impaired renal function leads to the accumulation of these inflammatory mediators, contributing to a chronic state of low-grade inflammation (6). The interplay between systemic inflammation and atherosclerosis is well-documented in CKD, often resulting in increased cardiovascular disease (CVD) risk, which remains a leading cause of mortality in patients with CKD (7, 8). Thus, measuring blood levels of visfatin could provide valuable insights into the early detection of atherosclerosis and the assessment of cardiovascular risk in individuals with CKD (9, 10). Given the potential importance of visfatin as a marker of inflammation and its association with metabolic dysfunction, this study aims to evaluate serum levels of visfatin and expression of the NAMPT gene in peripheral blood mononuclear cells (PBMCs) from patients with CKD compared to healthy controls. By investigating the correlation between NAMPT gene expression, serum visfatin, and lipid profile markers, the study seeks to elucidate their combined diagnostic and pathophysiological roles in CKD (11). The investigation further seeks to explore the relationship between serum visfatin, inflammatory biomarkers (such as high-sensitivity C-reactive protein (hsCRP)), and metabolic parameters including creatinine clearance (Ccr), serum potassium (K), sodium (Na), and lipid profile markers. Through this approach, the study aims to advance understanding of visfatin's role in the pathophysiology of CKD and its potential utility as a biomarker for assessing disease progression and associated cardiovascular risk.

Materials and Methods

Materials

The kits used in this study for measuring cholesterol, HDL, TG, LDL, VLDL, fasting blood sugar (FBS), C-reactive protein (CRP), urea, creatinine, potassium (K), and sodium (Na) were obtained from Atellica IM by Siemens Healthineers (Germany). The Visfatin kit was purchased from Cusabio (USA) and analyzed using the Huma Reader HS ELISA system (Germany). The kits (Cholesterol, HDL, TG, LDL, VLDL, FBS, CRP, Urea, Creatinine, K, and Na) used in this study were from Atellica IM, by SIEMENS Healthineers (Germany), and the Visfatin kit was from Cusabio (USA)

analyzed using the Huma Reader HS ELISA system (Germany).

Subjects and Study Design

A case-control study was conducted on a total of 60 participants aged between 18 and 90 years. The study included 30 patients diagnosed with CKD, of whom 15 were classified as end-stage renal disease (ESRD) patients undergoing hemodialysis (HD), and 15 were CKD patients not undergoing dialysis. These patients were matched by age and body mass index (BMI) with 30 healthy subjects (HS) who served as the control group. Participants were recruited from Imamian Al-Kadhimein City Hospital over the period from October 2022 to October 2023. All participants were informed about the study objectives, and written consent was obtained prior to enrollment. Inclusion criteria required a confirmed diagnosis of CKD based on the 2022 KDIGO Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Patients with an estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m^2 for at least three months were eligible for inclusion in the study. Exclusion criteria were applied to minimize potential confounding factors and included the presence of malignancies, thalassemia, depression, acute renal failure, cardiovascular diseases, and age below 18 or above 90 years. Control subjects were healthy individuals with no known history of renal problems. Data collected included age, gender, duration of hemodialysis, duration of CKD, cause of hemodialysis, and frequency of dialysis sessions. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Specimen Collection and Methods

Five milliliters (5 ml) of venous blood were drawn under aseptic conditions from each participant, including both patients and healthy subjects (HS). The blood samples were processed by centrifugation at 3000 x g for 10 minutes to separate the serum, which was divided into three aliquots. The first aliquot was used for the analysis of total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), urea, creatinine, potassium (K) and sodium (Na), and fasting blood glucose. The second aliquot was designated for hsCRP analysis and was stored at -20 °C until further use. The remaining serum was stored at -20 °C for the future analysis of visfatin levels using enzyme-linked immunosorbent assay (ELISA) kits. Additionally, PBMCs to peripheral blood mononuclear cells (PBMCs) were isolated for RNA extraction and Nicotinamide Phosphoribosyl transferase (NAMPT) gene expression analysis.

Visfatin Gene Expression Analysis

The expression of NAMPT gene was analyzed to assess its potential role as a molecular marker of CKD progression and its correlation with serum visfatin levels and metabolic dysfunction. Quantitative real-time polymerase chain reaction (qRT-PCR) was conducted to evaluate the expression levels of NAMPT (the visfatin gene) in PBMCs from CKD patients and healthy subjects. Blood samples were collected from three groups: CKD patients undergoing hemodialysis (n=15), CKD patients not undergoing hemodialysis (n= 15), and healthy controls (n= 30). PBMCs were isolated using Ficoll-Paque density gradient centrifugation, as described by Jaatinen et al. Total RNA was extracted from PBMCs using the RNeasy Mini Kit (Qiagen, Hilden, Germany), and cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). The NAMPT gene sequence used in this study corresponds to the NCBI Reference Sequence NM_005746.3. The reference gene GAPDH was selected from the NCBI Reference Sequence NM_002046.7. Quantitative analysis was performed using SYBR Green Master Mix (Thermo Fisher Scientific) on an Applied Biosystems QuantStudio Real-Time PCR System. Primer sequences for NAMPT were as follows: Forward primer 5′-AGCCTAGAGCCACCAACTGC-3′ and Reverse primer 5′-GCCAGCCTTGTACCATCAGC-3′. GAPDH

was used as the reference gene for normalization.

Determination of Glomerular Filtration Rate (eGFR)

The glomerular filtration rate (eGFR) was calculated using the following formula:
eGFR can be calculated from equation GFR=(140-age) ∗ Weight /72 ∗ Creatinine.
BMI was determined following the World Health Organization's international classification system (WHO, 2019), which categorizes individuals as underweight (<18.5 kg/m²), normal weight (18.5-24.9 kg/m²), and overweight (25.0-29.9 kg/m²).

Statistical Analysis

Statistical analysis was conducted using SPSS software (version 25). Data were presented as mean ± standard deviation (SD) for normally distributed numerical variables and as median (25th and 75th percentiles) for non-normally distributed variables. Frequency and percentage were used for categorical variables. Differences between groups were analyzed using independent t-tests and ANOVA for normally distributed variables, while the Mann-Whitney U test and Kruskal-Wallis test were employed for non-normally distributed variables. Categorical data were compared using chi-square and Fisher's exact tests. A p-value ≤ 0.05 was considered statistically significant. Pearson correlation analysis was used to assess the relationship between visfatin levels and other biochemical markers. Additionally, receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic accuracy of serum visfatin as a potential marker for CKD. The ROC curve was used to determine the optimal cut-off value of visfatin concentration to maximize sensitivity and specificity for CKD diagnosis.

Results

The study included 60 participants, comprising 30 patients with CKD and 30 healthy subjects HS. Table 1 presents descriptive data of the study population. The mean age of patients in the CKD groups was 63.47 ± 11.76 years for those with ESRD undergoing HD and 56.20 ± 14.99 years for CKD patients without dialysis.
In comparison, the HS group had a significantly lower mean age of 48.93 ± 13.95 years. Across all groups, males were generally more prevalent, except in the CKD group without dialysis, where the proportion of females was higher. Although male predominance was most notable in the CKD (ESRD undergoing HD) group (73.3% males), the differences in gender distribution between groups were not statistically significant.
Regarding BMI, patients in the CKD (ESRD undergoing HD) and CKD (without HD) groups exhibited higher mean BMIs (28.72 ± 5.89 and 30.16 ± 4.52 kg/m², respectively) compared to the HS group (26.59 ± 2.90 kg/m²). A significant difference was observed between the HS and CKD without dialysis groups. Smoking prevalence was notably higher in both CKD groups (79.9%) compared to the HS group (23.3%), but these differences were not statistically significant.

Table 1. The Anthropometric in CKD with and without dialysis patients and HS groups.

Variables		CKD With dialysis N=15	CKD Without dialysis N=15	HS N=30	P-value
Age (year)		63.47 ± 11.759	56.20 ± 14.99	48.93 ±13.948	0.005
	BMI (Kg/m²)	28.724± 5.88	30.159±4.515 [b]	26.586±2.9	0.027
Gender	**Male**	11 (73.3)	6 (40)	17 (56.6)	0.183
	Female	4 (26.6)	9 (60)	13 (86.6)	
Smoking		8 (53.3)	4 (26.6)	7 (23.3)	0.111
Nonsmoking		7 (4.6)	11 (73.3)	23 (76.6)	

a) The difference between the HS and CKD With dialysis groups.
b) The difference between the HS and CKD Without dialysis groups.
c) The difference between the CKD With dialysis and CKD Without dialysis groups.
All data represent as Mean ±SD and NO (%).

Clinical Characteristics of the Study Population

Patients in the CKD (ESRD undergoing HD) and CKD (without HD) groups exhibited higher fasting blood sugar (FBS) levels (217.27 ± 114.12 mg/dL and 170.47 ± 114.82 mg/dL, respectively) compared to the HS group (142.24 ± 85.51 mg/dL) (Table 2). The difference was statistically significant between the HS and CKD with dialysis groups, aligning with the increased incidence of hyperglycemia in CKD patients, which can exacerbate renal complications. This is consistent with other studies showing a higher risk of glucose metabolism disorders in CKD, particularly in ESRD patients on dialysis (12).

The lipid profile analysis revealed that the mean high-density lipoprotein (HDL) levels were significantly higher in the HS group (41.26 ± 7.95 mg/dL) compared to the CKD (ESRD undergoing HD) group (29.26 ± 9.08 mg/dL) and the CKD (without HD) group (30.04 ± 7.30 mg/dL). Reduced HDL levels are associated with impaired lipid metabolism often seen in CKD, which can contribute to cardiovascular risks. This study's findings align with prior research highlighting the role of HDL in maintaining vascular health and how its dysfunction in CKD can lead to complications (13, 14).

Sodium (Na) levels were lower, while Potassium (K) levels were higher in both CKD groups compared to the HS group, with significant differences observed ($p< 0.05$). These electrolyte imbalances are common in CKD patients and can lead to complications such as volume overload and hyperkalemia. Hyponatremia and hyperkalemia, particularly prevalent in dialysis patients, are often linked to higher mortality rates. Hyponatremia can result from inadequate water removal and excessive water intake, while hyperkalemia results from reduced glomerular filtration and impaired potassium secretion, frequently worsened by high dietary potassium intake (15, 16).

In terms of renal function, patients in the CKD groups had elevated concentrations of urea and creatinine compared to HS. Significant differences were observed between the HS group and both CKD groups, as well as between CKD patients with and without dialysis. Elevated levels of urea and creatinine are indicative of impaired renal clearance, commonly observed in CKD. Hemodialysis helps to remove excess urea, yet the persistently high levels in ESRD patients highlight the inadequacy of dialysis to fully correct metabolic waste retention (17). The mean estimated glomerular filtration rate (eGFR) was lower in the CKD (ESRD undergoing HD) group (51.53 ± 33.5 mL/min) and the CKD (without HD) group (72.8 ± 20.9 mL/min) compared to the HS group (113.56 ± 35.7 mL/min), with statistically significant differences. These results align with the understanding that reduced eGFR is a hallmark of CKD progression, and decreased creatinine clearance reflects worsening kidney function, consistent with findings by (18-20).

Systolic blood pressure (SBP) was notably higher in the CKD (ESRD undergoing HD) group (14.64 ± 2.31 mmHg) and the CKD (without HD) group (13.6 ± 1.91 mmHg) compared to the control group (12.7 ± 1.14 mmHg), showing significant differences. Elevated SBP has been linked to the worsening of renal function and poorer outcomes in CKD patients, particularly those with proteinuria, as higher baseline blood pressure levels can accelerate renal decline (21). Although diastolic blood pressure (DBP) was lower in the HS group (8.3 ± 0.7 mmHg), there was no significant difference between the groups.

The mean serum visfatin levels were highest in the CKD (ESRD undergoing HD) group (5.6 ± 1.63 ng/mL), followed by the CKD (without HD) group (3.5 ± 1.4 ng/mL), and the HS group (2.7 ± 1.1 ng/mL), with significant differences observed between these groups (Fig. 1). Similarly, NAMPT gene expression in PBMCs was significantly elevated in CKD patients, with mean fold changes of 4.76 ± 1.21 in the CKD with HD

group, 3.21 ± 0.98 in the CKD without HD group, and 1.00 ± 0.34 in the HS group ($p< 0.001$) (Fig. 2). These findings are consistent with previous studies demonstrating elevated visfatin in CKD, potentially reflecting its role in systemic inflammation and metabolic dysregulation (22-24).

Patients in the CKD (ESRD undergoing HD) and CKD (without HD) groups exhibited higher fasting blood sugar (FBS) levels (217.27 ± 114.12 mg/dL and 170.47 ± 114.82 mg/dL, respectively) compared to the HS group (142.24 ± 85.51 mg/dL) (Table 2). The difference was statistically significant between the HS and CKD with dialysis groups. The lipid profile analysis revealed that the mean high-density lipoprotein (HDL) levels were significantly higher in the HS group (41.26 ± 7.95 mg/dL) compared to the CKD (ESRD undergoing HD) group (29.26 ± 9.08 mg/dL) and the CKD (without HD) group (30.04 ± 7.30 mg/dL). Sodium (Na) levels were lower, while K levels were higher in both CKD groups compared to the HS group, with significant differences observed ($p< 0.05$). In terms of renal function, patients in the CKD groups had elevated concentrations of urea and creatinine compared to HS. Significant differences were observed between the HS group and both CKD groups, as well as between CKD patients with and without dialysis. The mean eGFR was lower in the CKD (ESRD undergoing HD) group (51.53 ± 33.5 mL/min) and the CKD (without HD) group (72.8 ± 20.9 mL/min) compared to the HS group (113.56 ± 35.7 mL/min), with statistically significant differences. SBP was notably higher in the CKD (ESRD undergoing HD) group (14.64 ± 2.31 mmHg) and the CKD (without HD) group (13.6 ± 1.91 mmHg) compared to the control group (12.7 ± 1.14 mmHg), showing significant differences. Although diastolic blood pressure (DBP) was lower in the HS group (8.3 ± 0.7 mmHg), there was no significant difference between the groups. The mean serum visfatin levels were highest in the CKD (ESRD undergoing HD) group (5.6 ± 1.63 ng/mL), followed by the CKD (without HD) group (3.5 ± 1.4 ng/mL), and the HS group (2.7 ± 1.1 ng/mL), with significant differences observed between these groups.

Table 2. Biochemical test results across study groups.

Variables	CKD With dialysis N=15	CKD Without dialysis N=15	HS N=30	P-value
FBS (mg/dL)	217.266± 114.12 **a**	170.466±114.824	142.24± 85.51	0.071
TC (mg/dL)	142.857±46.8	131.8±31.9	148.82±46.01	0.465
TG (mg/dL)	138.93±50.95	134.5±52.78	118.37±59.191	0.438
HDL-C (mg/dL)	29.26±9.08 **a**	30.04±7.299 **b**	41.26±7.95	0.0001*
LDL-C (mg/dL)	87.3±46.1	84.0±32.8	88.73±17.88	0.953
VLDL-C (mg/dL)	28.64±10.14	29.39±11.5	31.69±12.14	0.660
Na (mmol/L)	125.4±9.7 **a**	126.9±14.4 **b**	139.7±14.2	0.001*
K (mmol/L)	5.99±1.85 **a**	5.47±1.14 **b**	4.66±0.47	0.001*
Urea (mg/dL)	151.68±30.42 **a**	67.83±24.81 **b**	29.02±8.57	0.0001*
Creatinine (mg/dL)	3.02±2.2 **a,c**	1.26±0.497	0.79±0.137	0.0001*
CRP (mg/dL)	7.03 (3.1-24.1)	10 (4.5-27.7)	10.4 (7.1-25.3)	0.612
eGFR (mL/min)	51.526±33.5a	72.8±20.9 b	113.56±35.7	0.0001*
SBP (mmHg)	14.64±2.31 **a**	13.6±1.91	12.7±1.14	0.002*
DBP (mmHg)	8.7±1.39	8.6±1.1	8.3±0.7	0.449
Visfatin (ng/mL)	5.6±1.63 **a,c**	3.5±1.4	2.7±1.1	0.0001*

a) The difference between the HS and CKD With dialysis groups.

b) The difference between the HS and CKD Without dialysis groups.

c) The difference between the CKD With dialysis and CKD Without dialysis groups.

-All data represents as Mean ±SD. Median (IQR) and NO (%).

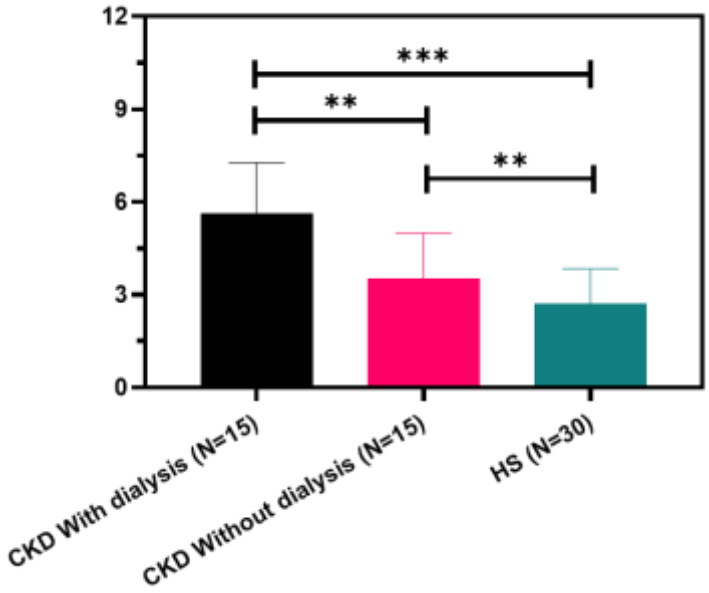

Fig. 1. Comparative visualization of serum visfatin levels across the study population. The differences reflect the potential association between visfatin and disease severity in chronic kidney conditions.

Visfatin Gene Expression Analysis

The analysis of NAMPT (visfatin gene) expression levels revealed significant upregulation in CKD patients compared to healthy controls, with the highest expression observed in patients undergoing hemodialysis. The mean ± SD relative expression levels were 4.76 ± 1.21 in the CKD with hemodialysis group, 3.21 ± 0.98 in the CKD without hemodialysis group, and 1.00 ± 0.34 in the healthy control group ($p< 0.001$) (Fig. 2a). Post hoc analysis confirmed statistically significant differences between each pair of groups, suggesting that the severity of CKD and hemodialysis treatment are associated with increased NAMPT expression. Correlation analysis further revealed a strong positive association between serum visfatin and lipid markers such as triglycerides ($r = 0.67$, $p = 0.002$) and LDL levels ($r = 0.61$, $p = 0.004$). NAMPT expression was also positively correlated with serum visfatin levels ($r = 0.76$, $p< 0.001$), triglycerides ($r = 0.67$, $p = 0.002$), and LDL levels ($r = 0.61$, $p = 0.004$) (Fig. 2b). These results highlight the interconnected roles of NAMPT expression and visfatin in CKD pathophysiology. However, no significant correlations were observed between NAMPT expression and hsCRP, serum urea, or creatinine levels, suggesting that NAMPT expression may be more closely linked to lipid metabolism and inflammatory pathways rather than direct renal function markers.

These findings are consistent with the role of visfatin as a pro-inflammatory adipokine that contributes to systemic inflammation and lipid dysregulation in CKD. Elevated NAMPT expression in PBMCs aligns with higher circulating visfatin levels observed in serum, reinforcing its role in CKD pathophysiology. The observed association between NAMPT expression and lipid disturbances, such as increased triglycerides and LDL suggests that NAMPT may contribute to the metabolic and cardiovascular complications commonly seen in CKD. Prior studies have highlighted the involvement of visfatin in modulating lipid storage and inflammatory pathways, further supporting its role as a biomarker and potential therapeutic target. The significantly higher NAMPT expression observed in CKD patients undergoing hemodialysis compared to those not on dialysis underscores the exacerbation of systemic inflammation and metabolic stress induced by hemodialysis. This finding aligns with reports suggesting that the bioincompatible materials used and oxidative

stress associated with hemodialysis amplify inflammatory responses. These results highlight the potential of NAMPT expression as a biomarker for monitoring disease progression and identifying patients at greater risk of cardiovascular complications.

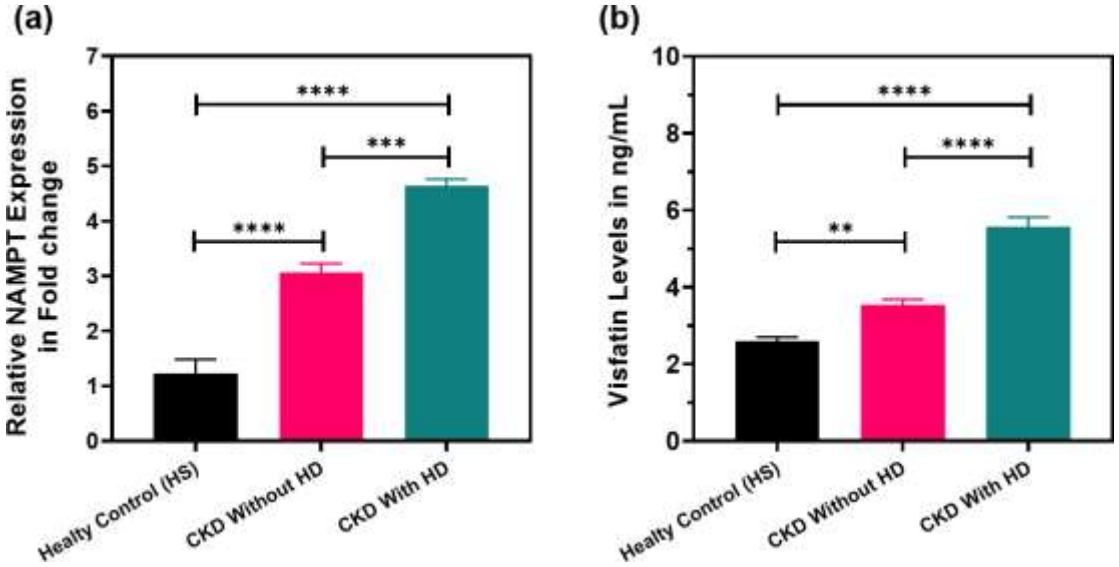

Fig. 2. a: Relative NAMPT Expression in HS, CKD patients without and with HD. b: Serum Visfatin Levels in HS, CKD patients without and with HD.

Correlation Analysis Between Visfatin and Biochemical Parameters

Pearson correlation analysis revealed a strong positive correlation between serum visfatin and triglycerides (TG) (r = 0.669, p = 0.006) as well as low-density lipoprotein (LDL) levels (r = 0.630, p = 0.012) in CKD patients undergoing dialysis (Table 3). Elevated TG and LDL levels alongside high visfatin suggest visfatin's involvement in lipid metabolism and inflammatory processes in CKD, consistent with its proposed role as an adipokine modulating lipid storage and release (25, 26). No significant correlations were observed between serum visfatin and HDL-C, hsCRP, blood urea, serum creatinine, or VLDL-C.

Table 3. Correlation Coefficient between Visfatin with Biochemical test in study groups.

Parameters	CKD With dialysis (r,p)	CKD Without dialysis (r, p)	HS (r, p)
FBS (mg/dL)	-0.006 (0.984)	-0.331 (0.228)	-0.018 (0.925)
TC (mg/dL)	-0.669** (0.006)	0.006 (0.984)	-0.136 (0.475)
TG (mg/dL)	-0.411(0.128)	-0.430 (0.110)	0.030 (0.875)
HDL-C (mg/dL)	0.010(0.973)	0.594* (0.019)	-.147 (0.439)
LDL-C (mg/dL)	-0.630* (0.012)	-0.093 (0.742)	-0.059 (0.756)
VLDL-C (mg/dL)	-0.283 (0.306)	-0.088 (0.754)	-0.016 (0.932)
Na (mmol/L)	-.423 (0.116)	-0.136 (0.630)	-0.239 (0.204)
K (mmol/L)	0.260 (0.349)	-0.273 (0.325)	-0.136 (0.475)
Urea (mg/dL)	0.266 (0.339)	-0.213 (0.447)	0.113 (0.553)
Creatinine (mg/dL)	0.191 (0.495)	0.335 (0.222)	0.263 (0.160)
CRP (mg/dL)	0.072 (0.800)	-0.268 (0.334)	-0.035 (0.855)
SBP (mmHg)	0.094 (0.740)	0.274 (0.322)	0.140 (0.460)
DBP (mmHg)	0.297 (0.283)	0.13 (0.623)	0.213 (0.258)

Diagnostic Value of Visfatin

The eceiver operating characteristic (ROC) curve was utilized to evaluate the diagnostic potential of visfatin in detecting CKD, particularly in patients with ESRD undergoing HD. The area under the curve (AUC) for visfatin was 0.814 (95% CI: 0.693 - 0.903), indicating good diagnostic accuracy ($p< 0.05$) (Fig. 3). Sensitivity and specificity at a cutoff value >2.943 ng/mL were 80% and 70%, respectively. These results suggest that visfatin could be a useful biomarker in differentiating CKD patients, especially those requiring dialysis, from healthy individuals. The findings agree with Axelsson et al., who observed higher visfatin in CKD and its association with other metabolic markers, suggesting potential for future diagnostic use (22).

The area under the receiver operating characteristic (ROC) curve (AUC) was 0.814 with a standard error of 0.0539, indicating good diagnostic accuracy. The 95% confidence interval for the AUC ranged from 0.693 to 0.903, and the significance level was $p< 0.0001$, demonstrating strong statistical significance. The Youden index was 0.5000, with a 95% confidence interval from 0.3333 to 0.6333, reflecting the optimal balance between sensitivity and specificity.

The negative predictive value (NPV) was 77.8% and the positive predictive value (PPV) was 72.7%, with a 95% confidence interval (CI) ranging from 50.6% to 85.3%. The test demonstrated a specificity of 70.0% (95% CI: 61.4% to 92.3%) and a sensitivity of 80.0% at a cutoff criterion of > 2.943.

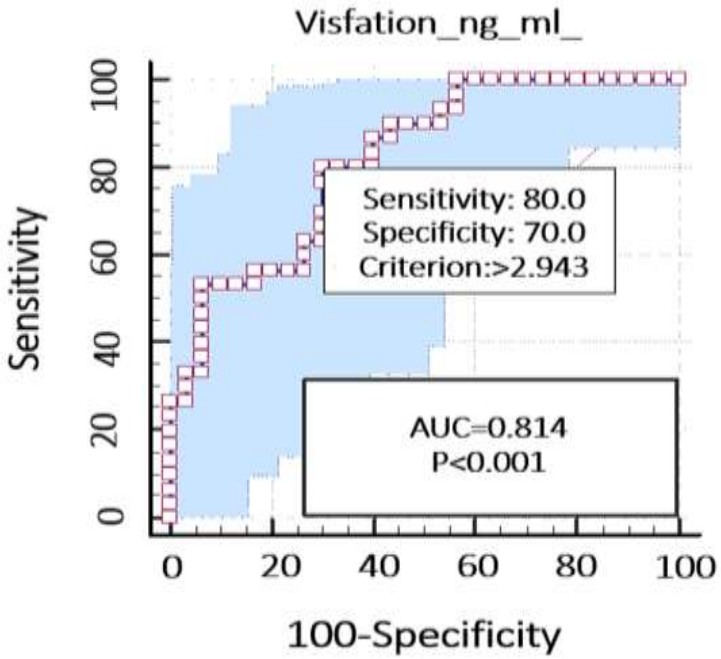

Fig. 3. ROC curve analysis to determine the threshold value of Visfatin. This curve demonstrating the diagnostic utility of serum visfatin levels in identifying CKD, particularly among patients with ESRD undergoing HD. The AUC indicates favorable discriminative ability, supporting visfatin as a potential biomarker in advanced stages of renal disease.

Discussion

This study was designed to explore the diagnostic potential of serum visfatin levels and NAMPT gene expression in peripheral blood mononuclear cells (PBMCs) in patients with chronic kidney disease and their association with disease severity and lipid profiles. The most important result of this study is that serum visfatin levels and NAMPT gene expression were significantly higher in CKD patients compared to healthy controls, with the highest levels observed among patients undergoing hemodialysis. These findings suggest that visfatin and NAMPT expression could be useful biomarkers for the evaluation of chronic kidney disease (CKD)

severity and cardiovascular risk, especially in hemodialysis (HD) patients. These results are in in accordance with previous reports showing high circulating levels of visfatin in CKD patients. For instance, Axelsson et al. (27) have reported that visfatin levels were higher in CKD patients, especially those on dialysis, and related to inflammation and metabolic derangement. Romacho et al. (10) demonstrated that high levels of visfatin correlated with dyslipidemia and enhanced cardiovascular risk in CKD patients. These results confirm our findings and extend them by showing the positive correlation between NAMPT gene expression and lipid profile markers, including triglycerides and low-density lipoprotein, especially in patients on HD.

Such correlations may indicate a link between visfatin, NAMPT expression, and dyslipidemia in CKD patients. The positive correlation found between the levels of visfatin and triglycerides, along with LDL, highlights visfatin as one of the adipocytokines implicated in pathways of both lipid metabolism and inflammatory processes. Dyslipidemia, as previously established, is a proven cardiovascular disease (CVD) risk factor in CKD, and our observation puts visfatin forward as a contributor in its own right. The lack of significant correlations between visfatin levels and hsCRP, urea, creatinine, or VLDL in our study suggests that the role of visfatin in CKD may be more closely related to lipid metabolism and chronic inflammation rather than to direct markers of renal function. Our study also found that NAMPT gene expression was significantly upregulated in CKD patients, especially those undergoing HD, and strongly correlated with serum visfatin levels. The enhanced expression of NAMPT in inflammatory conditions and its relationship with metabolic dysregulation was previously reported by Imai et al. (28). Our results on upregulated NAMPT expression may reveal a role in chronic low-grade inflammation in CKD patients it is a molecular marker of the disease's progression. Interestingly, our study did not find significant associations between the level of visfatin and hsCRP, indicating that visfatin is not likely to be a direct marker of acute inflammation but a marker of chronic metabolic stress and disturbances in lipid metabolism. This finding agrees with the study by Hsu et al. (29) who reported that the levels of visfatin were more closely related to the lipid profile and long-term metabolic changes in CKD patients than with acute inflammatory markers.

In conclusion, the serum levels of visfatin and NAMPT gene in patients with CKD, particularly on hemodialysis (HD), appeared significantly higher and positively related to markers of lipid profile. Such findings indicate that visfatin and NAMPT expression might become biomarkers in assessing the degree of CKD and the possibility of cardiovascular events. Incorporation of visfatin and NAMPT into routine clinical monitoring could be very informative for patient stratification and personalized therapy, with the ultimate goal of better management of CKD and reduction of cardiovascular complications.

Future studies with larger cohorts are needed to validate these findings and further explore the mechanisms through which visfatin influences lipid metabolism and inflammation in CKD. Longitudinal research could also clarify visfatin's role in predicting disease progression and treatment outcomes. Developing standardized protocols for visfatin assessment in clinical practice could enhance its utility as a diagnostic and therapeutic tool, offering new opportunities for early intervention and improved management of patients with CKD.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors Contributions

Conceptualization: M.M., M.H.J.A., M.T., Data curation: H.A.H., D.A.J.S., M.H.J.A., Visualization: M.H.J.A., M.Sh.Kh., M.T., M.M., Investigation: H.A.H., D.A.J.S., M.H.J.A., M.Sh.Kh., M.T., M.M., Validation: H.A.H., D.A.J.S., M.H.J.A., M.Sh.Kh., M.T., M.M., Formal analysis: H.A.H., D.A.J.S., M.H.J.A., N.A.H., Methodology: M.H.J.A., M.T., M.M., M.Sh.Kh., N.A.H., Supervision: M.H.J.A., M.M., M.T., Resources: M.H.J.A., Project administration: M.H.J.A., M.M., Software: H.A.H., D.A.J.S., Writing, original draft: H.A.H., M.Sh.Kh., Writing, review & editing: M.H.J.A., M.Sh.Kh., M.T., M.M.

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