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Potential association between serum human epididymis protein 4 and chronic kidney disease in female patients

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Abstract
Background: It has been noted that CKD patients have significantly higher levels of the human epididymis protein 4 (HE4) and beta-2 microglobulin (B2M). Aim of the study: This study aims to determine whether serum HE4 and β2-MG are hold promise as a potential biomarker for chronic kidney disease (CKD) among female patients, this study will provide a new clue for CKD diagnosis.
Method: The kidney transplant centre at Al-Sadr Teaching Hospital conducted a case-controlled research from 17 April 2022 to 4 June 2023. The study comprised 50 chronic renal disease patients and 40 healthy people. The University of Basrah College of Medicine ethical committee approved this study. After the interview, suitable patients were transferred to the kidney transplant centre laboratory in Al-Sadr Teaching Hospital and a private laboratory to assess HE4, B2M, S.cr, and B.Urea in whole blood.
Results: In this study comparing chronic kidney disease (CKD) patients and controls, median ages were 66 and 58, respectively (P=0.127). Significant differences were seen in HE4 and β2 microglobulin (β2m) levels, with patients exhibiting substantially higher medians of 16.78 pmol/l for HE4 and 2.02 mg/dl for β2m compared to the control group (P=0.0001). The patient group had significantly higher medians for blood urea, serum creatinine, and GFR compared to the control group: 94.67 mg/dl for blood urea, 3.13 mg/dl for serum creatinine, and 16 ml/min/1.73m2 for GFR (p<0.0001). Age did not significantly differ between CKD stages (P=0.837), and HE4 and β2m levels did not change either.
Conclusion: In conclusion, HE4 and B2M have a significantly close relationship with CKD. Therefore, providing a possibility for the diagnosis and intervention of CKD.

Keywords: Beta-2 microglobulin, **c**hronic kidney disease; human epididymis protein 4

Introduction
Chronic Kidney Disease (CKD) is defined by kidney function or structural abnormalities lasting for at least three months, with or without reduced Glomerular Filtration Rate (GFR). A GFR of 60 ml/min/1.73 m² or lower for three months, along with signs of kidney damage from blood, urine, or imaging tests, confirms CKD [1]. CKD presents several severe complications, notably heart disease, including heart failure and stroke, which is the leading cause of death in CKD patients [2, 3]. CKD also leads to bone and mineral disorders, anemia, and fluid imbalances, further aggravating cardiovascular risks [4, 5]. Additionally, metabolic issues, such as insulin resistance and dyslipidemia, are common, exacerbating kidney damage and heart disease [6]. The global CKD mortality rate is 12.2 deaths per 100,000 individuals annually, with a high prevalence of 11% [7, 8]. Early detection through proactive screening and diagnosis is critical, especially in developed nations, to prevent complications such as cardiovascular disease and end-stage kidney disease [8]. Effective CKD management includes controlling cardiovascular risk with statins, regulating blood pressure, managing albuminuria with ACE inhibitors or ARBs, avoiding nephrotoxic drugs, and optimizing medication dosages [9]. Regular monitoring for CKD complications such as hyperkalemia, hyperphosphatemia, and secondary hyperparathyroidism is essential, with nephrology referrals recommended for patients with eGFR below 30 ml/min/1.73m² [10]. Early detection via routine GFR reporting is key to improving outcomes. Screening for CKD involves biomarkers like the albumin-to-creatinine ratio (ACR) and serum creatinine. ACR detects proteinuria, a sign of kidney injury linked to cardiovascular risks, while serum creatinine is used to estimate GFR, though it can be influenced by factors like muscle mass [11].

Novel biomarkers like cystatin C, NGAL, and KIM-1 show potential for earlier CKD detection, but practical application is limited by cost and availability [12]. Human Epididymis Protein 4 (HE4), primarily studied in ovarian cancer, has been identified as a promising biomarker in CKD due to its association with renal fibrosis and disease progression [13, 14]. Beta-2 microglobulin (B2M), another emerging biomarker, helps assess renal function and is crucial in dialysis settings for evaluating adequacy and membrane biocompatibility [15, 16]. These markers may enhance early detection and management of CKD. The objective of this study is to determine if blood HE4 and β2-MG show potential as biomarkers for chronic kidney disease (CKD) in female patients. This approach will offer a novel insight for diagnosing CKD.

Method

This case-control study was conducted at the Kidney Transplant Centre in Al-Sadr Teaching Hospital from April 17, 2022, to June 4, 2023, involving 50 female patients diagnosed with chronic kidney disease (CKD) and 40 healthy individuals. The study received ethical approval from the College of Medicine, University of Basrah. The inclusion criteria for patients included female patients aged 19 to 89 years, those with diabetes, hypertension, cardiovascular disease without heart failure, prolonged urinary tract obstruction by stones, obesity, a family history of kidney disease, frequent use of medications (NSAIDs, ACE inhibitors, ARBs, diuretics, antibiotics, antiviral drugs, chemotherapy, PPIs, lithium, and bisphosphonates), and smokers. The exclusion criteria were males, patients younger than 19 years, and patients with urinary tract obstructions caused by cancer. Blood samples (3 ml) were collected from eligible patients, allowed to clot for 10-20 minutes, and centrifuged at 2000–3000 RPM for 20 minutes. Serum was analyzed for HE4, B2M, serum creatinine (S.cr), and blood urea (B. Urea). The equipment used included a Hettich Rotofix 32 A centrifuge, a COBAS Integra 400 Plus analyzer (Roche), and an ELISA Genex MR-100 Microplate Reader. ELISA kits for HE4 (Lot No. YLVRIR44) and B2M (Lot No. YLV82945) were employed, while COBAS kits were used for serum creatinine (Lot No. 57938701) and blood urea (Lot No. 62758901). Data analysis was conducted using SPSS version 26. Quantitative data were tested for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests. Mann-Whitney U test, Kruskal-Wallis H test, and Spearman's correlation were used for statistical analysis, with a significance threshold set at $p<0.05$.

Results

Table 1 shows no significant statistical difference between patient and control groups according to age.

Table 1: Comparison of age between patients and controls

Group		Age (year)
Patient	N	50
	Mean± SD	61.86±15.53
	Median	66
Control	N	40
	Mean± SD	57.58±16.05
	Median	58
Sig.*		0.127

* Mann-Whitney U Test

In (Table 2) clear significant statistical differences were observed in patient and control groups according to HE4 and β2m.

Table 2: Comparison of HE4 and β_2m between patients and controls

Group		HE4 (pmol/ l)	β_2m (mg/ dl)
Patient	N	50	50
	Mean± SD	18.55±11.44	3.78±10.17
	Median	16.78	2.02
Control	N	40	40
	Mean± SD	4.14±2.47	3.77±10.17
	Median	3.38	2.07
P-value*		0.0001	0.0001

* Mann-Whitney U Test

There was a significant statistical association between patient and control groups and the B. urea, S. creatinine and GFR (Table 3).

Table 3: Comparison of blood urea, serum creatinine, and GFR levels between patients and controls

Group		B. urea (mg/ dl)	S. creatinine (mg/ dl)	GFR (ml/ min/ 1.73m^2)
Patient	N	50	50	50
	Mean± SD	111.97±50.25	4.87±3.90	20.18±17.59
	Median	94.67	3.13	16
Control	N	40	40	40
	Mean± SD	21.55±5.49	0.48±0.09	129.57±10.08
	Median	20.25	0.48	127.5
P-value*		0.0001	0.0001	0.0001

* Mann-Whitney U Test

In (Table 4), there was no any significant statistical difference in age among CKD stages.

Table 4: Comparison of age, among CKD stages

CKD stage		Age (Year)
3	N	8
	Mean± SD	67.63±12.28
	Median	68.5
4	N	18
	Mean± SD	60.89±16.71
	Median	65
5	N	21
	Mean± SD	60±16.71
	Median	67
P-value*		0.837

* Kruskal-Wallis H Test

To explore if there is any association between HE4, β_2m among CKD stages, no statistically significant differences were found (Table 5).

Table 5: Comparison of HE4, β_2m, among CKD stages

Stage		HE4/ pmol/ l	B2m/ mg/ dl
3	N	8	8
	Mean ± SD	3.59±0.93	1.84±0.87
	Median	3.52	2.11
4	N	18	18
	Mean ± SD	3.57±1.56	2.55±2.3
	Median	3.2	2.14
5	N	21	21
	Mean ± SD	5.13±3.56	5.8±15.53
	Median	3.89	2.06
P-value*		0.204	0.910
P-value** (3 & 4)		0.317	0.739
P-value** (3 & 5)		0.608	0.678
P-value** (4& 5)		0.086	0.877

* Kruskal-Wallis H Test
** Mann-Whitney U Test

In (Table 6) there was significant statistical association between B. urea, S. creatinine (mg/ dl), GFR and CKD stages.

Table 6: Comparison of blood urea, serum creatinine, and GFR levels among CKD stages

CKD stage		B. urea (mg/ dl)	S. creatinine (mg/ dl)	GFR (ml/ min/ 1.73m^2)
3	N	8	8	8
	Mean± SD	75.65±16.45	1.48±0.25	39.75±7.65
	Median	68.98	1.52	38.5
4	N	18	18	18
	Mean± SD	91.74±25.08	3.03±1.59	19.61±5.67
	Median	89.55	2.78	19
5	N	21	21	21
	Mean± SD	150.62±51.58	8.31±3.57	6.43±3.22
	Median	147.24	7.67	6
P-value*		0.0001	0.0001	0.0001

* Kruskal-Wallis H Test

In (Table 7) although, there were several statistically significant correlations in the control group, all of these correlations disappeared in patient group.

Table 7: Spearman correlations of quantitative variables

Group			HE4 (pmol/ l)	β$_2$m (mg/ dl)	B. urea (mg/ dl)	S. creatinine (mg/ dl)	GFR (ml/ min/ 1.73m^2)
Patient	Age (Year)	R	.127	.255	.069	-.086-	-.009-
		Sig.	.380	.074	.636	.552	.951
		N	50	50	50	50	50
	HE4 (pmol/ l)	R		-.105-	-.134-	-.169-	.137
		Sig.		.467	.354	.241	.342
		N		50	50	50	50
	B2m (mg/ dl)	R			-.015-	-.014-	-.015-
		Sig.			.916	.922	.918
		N			50	50	50
Control	Age (Year)	R	.714**	.554**	.353*	.026	-.710-**
		Sig.	.000	.000	.026	.873	.000
		N	40	40	40	40	40
	HE4 (pmol/ l)	R		.862**	.469**	.212	-.634-**
		Sig.		.000	.002	.189	.000
		N		40	40	40	40
	B2m (mg/ dl)	R			.426**	.069	-.385-*
		Sig.			.006	.672	.014
		N			40	40	40

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Discussion

This study compared the levels of Human Epididymis Protein 4 (HE4) and beta-2 microglobulin (B2M) between female patients with chronic kidney disease (CKD) and a healthy control group. The median age of the control group was 58, while the median age of patients was 66, with no statistically significant difference, aligning with findings by Sedighi O *et al.* [17] but differing from Meng Z *et al.* [18], where a statistically significant age difference was observed. In terms of HE4 levels, the median value in the control group was 3.38 pmol/L, compared to 16.78 pmol/L in the patient group, which was statistically significant. This finding supports previous research by Wan J *et al.* (19) and Meng Z *et al.* [18], which demonstrated elevated HE4 levels in women with impaired renal function, pointing to HE4's association with renal fibrosis, a common CKD complication. HE4 is increasingly recognized as both a mediator and biomarker in kidney fibrosis, with studies showing its elevated expression in fibrotic kidneys, particularly through interactions with proteases that prevent collagen degradation [20]. Chovanec *et al.* [21] highlighted HE4's effectiveness as a CKD biomarker, especially in women, where it was more accurate than traditional indicators such as creatinine, B2M, and cystatin C (CysC). HE4 levels also increased with CKD progression, with the highest levels observed in stage 5 CKD, consistent with Wan J *et al.* [19], who noted a significant rise in serum HE4 across more advanced CKD stages. B2M levels showed no significant difference between the control and patient groups, with median values of 2.07 mg/dL in the control group and 2.02 mg/dL in CKD patients. This finding aligns with Meng Z *et al.* [18] and Foster MC *et al.* [22]. However, B2M levels were highest in stage 4 CKD, as reported by Dajak M *et al.* [23]. Under normal physiological conditions, B2M is generated at a steady rate and cleared through the kidneys. In CKD, impaired renal function leads to elevated B2M levels in the blood [24]. The study also found significant differences in blood urea, serum creatinine, and estimated glomerular filtration rate (eGFR) between the patient and control groups, in line with previous research by Meng Z *et al.* [18] and Sedighi O *et al.* [17]. A direct correlation between HE4 and serum creatinine, as well as B2M, was observed, while an inverse correlation between HE4 and eGFR was noted, supporting the findings of Meng Z *et al.* [18]. Both HE4 and B2M are freely filtered by the glomeruli and reabsorbed in the tubules. When GFR declines, these proteins accumulate in the bloodstream, and their levels have been linked to increased mortality and adverse cardiovascular and kidney outcomes [18]. Serum creatinine levels increased with advanced CKD stages, reaching a median of 7.67 mg/dL in stage 5, with a statistically significant difference, consistent with studies by Wan J *et al.* [19] and Meng Z *et al.* [18]. Similarly, eGFR declined with CKD progression, reaching a median of 6 ml/min/1.73 m² in stage 5, also statistically significant and in agreement with previous studies [18, 19]. While HE4 and B2M levels were not significantly correlated with age or eGFR in this study, HE4 was notably elevated in CKD patients, suggesting its potential as a diagnostic marker. The study underscores the complexity of interpreting these biomarkers and the need for a comprehensive approach when assessing renal function.

Conclusion

The outcomes of this study show that HE4 and B2M have a strong association with CKD. Our study found that while there were no significant age differences between the patient and control groups, there were significant differences in HE4, β2M, blood urea, serum creatinine, and GFR levels. These values may help identify CKD patients from healthy persons. Notably, greater HE4 levels were related with advanced stages of CKD, which is consistent with its involvement in renal fibrosis and suggests that it might be used as a potential biomarker for disease progression.

Conflict of Interest

Not available

Financial Support

Not available

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