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Study of Homocysteine, SDMA, ADMA, UMOD, AVP and KIM-1 in serum of chronic renal disease patients suffering from Type-2 diabetes in Basra Province

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Chronic kidney disease (CKD) is a reduction in renal function manifested by a GFR of less than 60 mL/min per 1.73 m2 or kidney damage marker, or maybe both, last about 3 months, regardless of actual cause. Diabetes mellitus (DM) seem to be the causative factors of CKD in all high- middle-income regions, as well as in numerous low country income. Mellitus accounts for 30–50% of all CKD and affects 285 million (6.4%) individuals globally. A case-control study included 30 CKD patients with T2DM and 30 healthy subjects as a control group who visited Al-Basrah Teaching Hospital in Al-Basrah province between October 2021 and February 2022. The Age average for study population was (25-60) years. Serum levels of human AVP, ADMA, KIM-1, HCY, UMOD, and SDMA were measured by a sandwich-ELISA technique. The results revealed a highly significant increase in the levels of homocysteine, SDMA, ADMA, AVP, and KIM-1 in CKD-diabetic patients ($P < 0.05$) and a highly significant decrease in the level of UMOD ($P<0.05$) compared to control. According to the results, we conclude: Hyperhomocysteinemia occurs in chronic and end-stage kidney diseases. A potential indicator of renal health, uromodulin allows for the early identification of CKD. This tubular secretion marker may possibly represent intrinsic "kidney function" and residual nephron mass in addition to glomerular filtration. The oxidative stress markers ADMA and SDMA are both known to contribute significantly to the emergence of endothelial dysfunction. Increased kidney damage molecule-1 and arginein vasopressin levels suggest that these molecules may be involved in the etiology of declining renal function.

Keywords: Chronic Kidney Disease; Diabetes Mellitus Oxidative Stress; End Stage Renal Disease.

Chronic kidney disease (CKD) is a reduction in renal function manifested by a GFR of less than 60 mL/min per 1.73 m^2 or a kidney damage marker, or maybe both, that lasts about 3 months, regardless of the actual cause[1].

Based on available global health estimates, this disorder was responsible for 864,226 mortalities (or 1.5 percent of all mortality globally) in 2012. Chronic kidney disease was the 14th most common cause of death, accounting for 12.2 deaths per hundred thousand people[2].

Diabetes mellitus (DM) and hypertension seem to be the causative factors of CKD in all high- and middle-income regions, as well as in numerous low-income countries. Mellitus accounts for 30–50% of all CKD and affects 285 million (6.4%)

individuals globally. By 2030, this estimate is set to grow by 69 and 20% in high- and low-income countries, respectively[2].

Nevertheless, not every diabetic will acquire renal damage. But it might be difficult to spot at-risk people, and the present biomarkers don't always signal a decline in renal function[3].

Type 2 diabetes mellitus (T2DM) is a pro-inflammatory circumstance accompanied by high oxidative stress, predisposing to macro- and micro-vascular complications. Diabetic nephropathy (DN), a microvascular outcome of lengthy uncontrolled DM, is the commonest cause of end-stage renal disease (ESRD), accounting for roughly 40% of suspected cases of ESRD each year[4].

In human medical studies, arginine, a new screening tool for the timely identification of kidney failure, is routinely used. Symmetric dimethylarginine **(**SDMA) is an intrinsically methylated style of arginine that's also transported in the blood during usual protein breakdown[5].

Asymmetrical dimethylarginine (ADMA) is a nitric oxide synthase endogenous inhibitor. ADMA is considered a serious risk factor, along with CVD, atherosclerosis, pulmonary hypertension, atrial fibrillation, stroke, peripheral vascular diseases, and diabetes[6–8].

Homocysteine (HCY) is a non-proteinogenic amino acid. Hyperhomocysteinemia is classified as an indicator of CVD, most probably via atherogenesis, which can result in ischaemia.

Uromodulin (UMOD), a urinary glycoprotein known to affect the formation of the kidney's calcium calculus[9],

A type I membrane protein, Kidney Injury Molecule-1 (KIM-1), that was explored in 1998, also plays a role in CKD diagnosis.

The study aimed to shed light on the investigation of end-stage renal disease patients by using homocysteine and uromodulin and revealing the critical role of oxidative stress biomarkers (ADMA and SDMA) and other unusual markers in chronic kidney disease.

MATERIALS AND METHODS

Participants in this study were 30 CKD patients with T2DM and 30 healthy subjects as a control group who visited Al-Basrah Teaching Hospital in Al-Basrah province between October 2021 and February 2022. The study population ranged in age from 25 to 60 years.All patients in this study were examined by hospital specialists. The practical study portion was completed at Southern Technical University/Basrah's Department of Medical Laboratory Technology.

The blood samples were drawn from both patients and controls, emptied into sterilized test tubes, and then left to coagglutinate at RT for 30 min. By centrifugation at 3000 rpm for 15 minutes, the blood sample was separated, and the serum was stored at -20 °C until use.

Too many individuals were excluded because they did not meet the inclusion criteria, such as patients with acute and chronic diseases or diseases other than kidney disease, such as hypertension, and all patients with hormonal imbalance.

GFR Calculation

GFR was calculated by (MDRD) equation (Chen et al., 2016)

GFR (mL/min/1.73 m2) = 186 × Serum Cr-1.154 × age-0.203 × 1.212 (if patient is black) × 0.742 (if female).

Serum levels Human AVP, ADMA, KIM-1, HCY, UMOD, SDMA was measured by A Sandwich-ELISA technique

Statistical Analysis

Statistical analyses were done in a (SPSS) version 22. Means and SD were used for data representation. ANOVA was used for assessing a significance difference in the mean of normality distributed variables and Kruskal Wallis test for non-normaly distributed. P-values ($P \leq 0.05$) are significant.

RESULTS

Table (1) for differences in the measurements of (Homocysteine, SDMA, ADMA, UMOD, AVP and KIM-1) between unusual bio-chemical markers in Control and CKD-diabetic show there are highly significant increase in the levels of (Homocysteine, SDMA, ADMA, AVP and KIM-1) with CKD-diabetic patients ($P < 0.05$) and highly significant decrease in the level of UMOD ($P < 0.05$).

Table 1. Unusual bio-chemical markers in Control and CKD with Diabetes

Unusual Markers	Control N= 30 Mean	SD	CKD diabetic N= 30 Mean	SD	P value*
Homocysteine	1.404	0.450	1.914	0.928	0.032*
SDMA	0.450	0.314	0.870	0.388	0.0001*
ADMA	10.426	5.661	17.469	8.944	0.004*
UMOD	2.261	0.983	1.227	1.228	0.001*
AVP	165.75	140.42	243.39	70.03	0.008*
KIM-1	1424.1	746.1	2332.9	593.4	0.001*

DISCUSSION

Homocysteine levels in the blood were significantly higher in DN patients in comparison to T2DM patients and were associated with the degree of renal dysfunction.

After adjusting for various progression markers, H. Wang *et al*. (2015) looked into the connection between homocysteine and the onset of early nephropathy in T2DM patients. They found that homocysteine was significantly associated with a decline in eGFR[10].

The findings showed that, in T2DM patients, Hcy was a distinct risk factor and an early indicator of the development of DN[11]. Additionally, homocysteine was found to be connected to the onset of nephritis and may be used as a biomarker for the early identification of DN[12].

According to[13-16], CKD-diabetic patients appear to have a significant increase in SDMA and ADMA, which is consistent with current observation.

Our study agrees with Tanhäuserová *et al*. (2012) that SDMA and GFR have a strong association, showing that SDMA is virtually totally removed by the kidneys and that its plasma level predominantly reflects renal function[15]. The findings are consistent with a major meta-analysis that established SDMA as a reliable indicator of renal function[14]. Similarly, ADMA levels are associated with GFR, a finding that supports prior research on T2DM patients[17]. The strong association between ADMA and GFR appears to contradict the fact that ADMA is thought to be removed mostly via enzymatic degradation (dehydrogenase docosahexaenoic acid (DDAH)), implying that it cannot be regarded as a generic measure of renal function[18]. [19-22]found a significant drop in serum uromudulin in CKD-diabetic patients, which is consistent with the current study. In more advanced stages of CKD, uromodulin concentrations were shown to be lower in CKD patients than in healthy controls, according to recent studies on the substance[22].

The link between uromodulin and CKD is likely best explored in diabetic nephropathy, according to El-Achkar & Wu's 2012 research. Uromodulin outflow is higher in those with diabetes and early renal disease but does not significantly lower GFR. This might be related to diabetes' hyperfiltration stage. Baseline uromodulin levels may help determine bad outcomes[19].

The epithelial cells of the thick ascending limb (TAL) of Henle's loop and distal tubule only generate uromodulin. TAL structural disorders are related to low uromodulin amounts[23]. In T2DM, metabolic alterations, such as hyperglycemia, increase interstitial inflammation, leading to interstitial fibrosis and tubular atrophy, and cause the tubular cells to undergo hyperplasia and hypertrophy[24]. As diabetic nephropathy worsens, the tubules are damaged before the glomerulus. Early stages of diabetic nephropathy are marked by decreased serum uromodulin levels[21].

The results from this research are in line with earlier results from various studies, including those by Meijer *et al*. (2011), Bankir *et al*. (2013), and Tasevska *et al*. (2016), which found a significant rise in the outcomes of AVP with CKD-diabetic patients[25–27].

Our research supports Meijer *et al.'s* findings from 2011 that vasopressin regulates free water clearance and is essential for maintaining water homeostasis. Despite its crucial function for

healthy physiology, mounting evidence indicates that vasopressin also has a role in the development of CKD[25].

Plasma vasopressin levels rose in CKD animal studies, as well as diabetic and non-diabetic nephropathies.Vasopressin activity is suppressed in CKD animals, which promotes glomerulosclerosis and tubulointerstitial fibrosis[25].

Despite the fact that elevation of AVP, as evaluated by copeptin, predicted CKD and reduction of eGFR in our general population with T2DM, irrespective of other traditional risk variables, it is uncertain if the relationships we discovered are causal. Nevertheless, there is evidence in the literature, gathered from patient intervention studies and animal research, that suggests high AVP may be directly related to subpar renal function. In animal tests, fusion of V2R agonists in mice was linked to increased fatality and proteinuria[27].

All of the following[28-30] found a significant increase in the result of KIM-1 in CKD-diabetic patients, which is in agreement with this study.

According to Khan et al., 2019, KIM-1, a relatively new marker for diabetic nephropathy, has relevance. The length of diabetes was associated with higher KIM-1 levels. This symptom of detectable serum KIM-1 levels demonstrates unequivocally that as diabetes worsens and lasts longer, renal function is disrupted and the severity of renal inflammation rises[29].

Participants in this study who had diabetes for a shorter period of time had higher levels of KIM-1 six months later, demonstrating the evolution of kidney impairment over time. We were able to show that KIM-1 levels rise with the progression of renal disease even when all other indicators, such as BUN or Cr levels, remain within the normal ranges by monitoring rates in study participants at two different times and with varying degrees of symptom severity. Kidney dysfunction is linked to diabetes and is frequently left untreated. This research shows that KIM-1 levels can be utilized to detect DN in diabetic individuals at an early stage and that more KIM-1 is released as kidney damage advances[29].

The amount of fatty acids in the tubular lumen rises as a result of glomerular damage, which encourages the tubular epithelium to absorb additional fatty acids. The authors speculate that KIM-1 could function in this process as both a sentry and a sentinel. The current work inspires further investigation into the role of the renal tubule in the pathogenesis of a condition that was previously thought to be a glomerulopathy and sheds light on fresh strategies for preventing progressive and lasting damage to the tubule and interstitium in DKD and other kidney diseases[30].

The causes of tubular damage are complicated mechanisms[31]. Patients with diabetes who have tubular reabsorption of glucose are continuously exposed to hyperglycemic situations. Chronic hypoxia and the local renin-angiotensin system also play a role in the progression of tubular damage. Additionally, albuminuria might directly injure renal tubular cells, causing fibrosis and inflammation. 24 Particularly susceptible to these metabolic and hemodynamic variables is the proximal tubule[32].

CONCLUSION

Hyperhomocysteinemia occurs in chronic and end-stage kidney diseases. Uromodulin is a reliable biomarker for renal health that enables the early detection of CKD. In addition to glomerular filtration, this marker of tubular secretion may also indicate residual nephron mass and intrinsic "kidney function." Both ADMA and SDMA are recognized as indicators of oxidative stress and play a significant part in the development of endothelial dysfunction. Increasing of arginein vasopressin and kidney injury molecule-1 suggesting their role in the pathogenesis of deterioration of renal function.

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