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Research Article

Evaluation of Novel Biomarkers Fetuin-A and KIM-1 and Bacterial Isolation in Early Diagnosis of Chronic Kidney Disease in Iraq

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Abstract

This study evaluates the role of novel biomarkers Fetuin-A and Kidney Injury Molecule-1 (KIM-1) in early chronic kidney disease (CKD) diagnosis. It identifies bacterial isolates associated with CKD patients in Iraq. One hundred-five samples (60 CKD patients and 45 healthy controls) were analyzed using ELISA for biomarker levels, and bacterial isolation was performed using standard culture methods. Results showed significantly elevated levels of Fetuin-A and KIM-1 in CKD patients compared to controls, correlating with disease progression stages. Commonly isolated bacteria included *Klebsiella*, *E. coli*, and *Staphylococcus aureus*, highlighting potential infection risks in CKD. These findings suggest that Fetuin-A and KIM-1 are valuable biomarkers for early CKD detection, and bacterial infections are prevalent among CKD patients, emphasizing the need for early screening and targeted interventions. The novel biomarkers and high levels of renal function (urea and creatinine) are related.

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INTRODUCTION

Chronic kidney disease (CKD) is a significant public health concern globally[1]. Because they are organs involved in excretion, biosynthesis, and metabolism, the kidneys are essential for maintaining a healthy physiology. Dialysis can take over some of the activities of the kidney, but it cannot duplicate the metabolism and biosynthesis of a healthy kidney[2, 3]. A worldwide statistic stated that approximately 10% of the world's population are at risk of chronic kidney failure, making it one of the major issues facing medicine in the twenty-first century. Additional data verified that, as of 2017, the global infection rate of chronic kidney disease patient was approximately 843.6 million[4]. In the United States, around one in nine individuals suffers with chronic kidney disease (CKD), whereas a lesser proportion have end-stage renal disease (ESRD), which is the disease's deadly consequence[5]. As a result, any kidney impairment impairs the kidney's ability to fulfill its activities, which causes a fault in the body's internal environment and interferes with the organs' ability to function as a whole[6]. Due to the high cost of dialysis in its latter stages, chronic renal disease must be diagnosed in its early stages before it advances to its last phases. The biggest challenge is that most individuals do not have clinical symptoms in the early stages of the illness; it is only when the disease has advanced to a large degree that it is found in them. It is difficult to manage the sickness. Early detection of chronic kidney disease has attracted the interest of many medical professionals and researchers because it may reduce death rates, stop the disease's progression in its early stages, and reduce the number of patients needing dialysis and associated medical costs[7]. Estimating albuminuria and glomerular filtration rate is a common clinical practice for diagnosing chronic kidney disease[8]. Glomerular filtration rate estimates are mostly linked to kidney impairment, An adult patient is diagnosed with CKD if their glomerular filtration rate (GFR) is less than 60 ml/min/1.73 m^2, or if their GFR is greater than 60 ml/min/1.73 m^2 .[5] severity of chronic kidney disease (CKD) measured by Glomerular filtration rate [9] Early-stage CKD is defined as stages 1–3. People in the early stages of the disease are usually asymptomatic, and diagnosis is made through laboratory tests or imaging. KDIGO revised CKD staging in 2013 to take into account both 5 stages of GFR[10] approximately 11.1% (22.4 million) of adults have stage 1 to 3 CKD, and prevalence appears to be increasing, particularly for stage 3 CKD[11]. One-half of people with CKD have stage 1 or 2 CKD (increased albuminuria with normal GFR), and the other half have stage 3 CKD (low GFR, with one-third having increased albuminuria and two-thirds having normal albuminuria). [12]whereas albuminuria establishes whether renal damage is present. Still, the prevalence of these conventional biomarkers keeps rising. kidney damage reaches an advanced stage when the filtration capacity is greatly reduced. Early identification of chronic kidney disease not only improves patient survival and reduces comorbidities, but it also aids in disease prediction and prevents the condition from growing worse. Early biomarkers are therefore necessary to achieve this[13]. Fetuin-A is a circulating serum glycoprotein with an estimated molecular mass of 60 KDa. Fetuin-A, like serum albumin, is primarily derived from the liver (>95%). During fetal development, extrahepatic fetuin-A expression can occur in the kidney, the choroid plexus, and all major organs[14]. Fetuin A is a naturally occurring calcium antagonist. Low circulating fetuin A levels have been linked to vascular calcification in both human and animal studies, and may be an independent risk factor for premature death in CKD patients [15]. Kidney injury molecule-1 is a type 1 transmembrane protein (T-cell immunoglobulin; mucin-containing molecule) [16]. After ischemic or toxic injury, KIM-1 has been shown to be up-regulated in dedifferentiated proximal tubule epithelial cells in the kidney)[17]. It is not found in healthy kidneys or urine. KIM-1 upregulation

is a known result of proximal tubular damage in the nephron)[17]. The serum concentration of urea or creatinine is the easiest and simplest method of estimating glomerular function. These concentrations are determined by the rate of production of each substance The normal percentage of urea ranges from 15mg/dL to 45 mg/dL and creatinine 0.4 mg/dL to 1.3 mg/dL)[18]. The current study is to explore potential associations between the incidence of chronic kidney disease and various biomarkers, including Fetuin-A and Kidney injury molecule-1, as well as indices of renal function.

MATERIAL AND METHOD

Sample Collection

One hundred five blood samples were taken from patients; 60 of these were chronic kidney disease patients who came to Imam Hussein Medical City and Imam Hassan Al-Mujtaba Hospital in the esoteric halls after being diagnosed by a specialist physician of different ages; the remaining 45 samples came from healthy individuals because syringes were used to take blood samples from patients. Five milliliters of the serum were extracted and put into a gel tube. After coagulation, the gel tube's samples were centrifuged for 20 minutes at 3000 revolutions per minute. The serum was transferred into Eppendorf tubes and frozen at -20 °C after extraction. I overlooked the other components of the blood.

Furthermore, 105 urine samples were obtained from identical patients from whom blood samples were obtained. 45 samples were from healthy individuals, and 60 samples were from an infected individual. Urine samples were taken in specialized plastic cups, and the types of bacteria found in the samples were identified by cultivating them on growth media.

- **Inclusion sample**

By the diagnosis of an internal medicine physician specializing of artificial kidney who suffer from chronic kidney diseases after taking samples from patient and diagnosing them in the laboratory .

- **Exclusion sample**

People over 80 years old and people under 10 years old

Biomarker test

Human Fetiun-A and Kidney injury molecule-1 tested procedure by ELISA Kit

1- All reagents, standard solutions and samples were prepared according to the instructions. All reagents were at room temperature before use. The test was performed at room temperature.
2- The number of strips required for the test was determined. The strips were inserted into the frames for use.
3- 50 µl of standard solution was added to the standard well. Note: Biotin antibodies are not added to the standard well because the standard solution contains biotin antibodies.
4- 40 µl of sample was added to the sample wells, then 10 µl of anti-NPhs1 antibody was added to the sample wells, then 50 µl of streptavidin-HRP was added to the sample wells and standard wells (not the blank control well). Mix well. The plate was then covered with a sealant. Incubation was carried out for 60 min at 37°C.
5- The blocking agent was removed and the plate was washed 5 times with wash solution. The wells were soaked with 300 µl wash solution for 30 s to 1 min per wash. For automatic washing, each well was emptied and washed 5 times with wash solution. We wiped the plate on paper towels or other absorbent material.
6- We added 50 µl of substrate A solution to each well and then 50 µl of substrate B solution to each well. The plate covered with fresh blocking agent was incubated for 10 min at 37 °C in the dark.
7- 50 µl of stop solution was added to each well, the color changed blue to yellow immediately.
8- The optical density (OD value) of each well was determined immediately using a

microplate reader set to 450 nm within 10 min after adding the stop solution

Biochemical test

Determination Urea and creatinine

Serum creatinine was measured using a modified version of Jaffe's method[19]. At the same time, the blood urea was measured using the method described [20]. The methods used colorimetric measurement of creatinine concentrations with the RANDOX Reagents (USA) creatinine Assay Kit in compliance with the manufacturer's instructions. RANDOX Reagents (USA) supplied the Urea Assay Kit, which was used to measure the urea levels. Utilizing the spectrophotometer-300, the absorbance of urea at 580 nm and creatinine at 510 nm were measured. The Modification of Diet in Renal Disease equation was used to compute estimated Glomerular Filtration rate (eGFR) for patients who were 18 years of age or older, while the Schwartz equation was used for those who were younger.[21].

BACTERIA DIGNOSIS

The bacteria present in urine were diagnosed using traditional culture methods for diagnosis Which includes:-

1- culturing urine samples on Blood Agar and MacConkey Agar media
2- incubating at 37°C for 24 hours
3- Selecting colonies and then spreading them by plotting. (The process was repeated three times) to purify the colonies.
4- Diagnosis by microscopic and biochemical examinations.
   in addition to using the VITEK 2 system (BioMerieux, USA ;Version VT-R01.02) method to confirm the diagnosis of common types of bacteria.

Ethics approval

The Declaration of Helsinki's ethical criteria were followed in the conduct of this investigation. Prior to sample collection, the patient's consent was obtained verbally and in writing following the local ethics committee's review and approval of the study protocol and subject data, as verified by number 2665 (which includes the date and number in 1/8/2023) to obtain this approval.

Statistical Analysis

The standard deviation (SD) and mean were used to summarize the study variables. An analysis of variance (ANOVA) was performed to analyze the differences in biochemical measurements across the various patient groups. To investigate the relationship between biological indicators and Human Fetiun-A and kidney damage molecule-1, several linear regression models were built. Every test was run as a two-sided test with a 0.05 significance level. SAS software was utilized to conduct all analyses

RESULTS

New biomarker of chronic kidney disease (CKD)

In our study, Table (1) shows that 40% were male patients with 37.77% normal males, and 60% of the patients were female patients with 62.23% normal females. There were no significant ($p \geq 0.05$) differences between the groups.

Table 1: Distribution of studded groups in our study				
Groups	Gender	No.	(%)	*p-value*
Patient No.(60)	Male	24	40%	P= 0.817
	Female	36	60%	
Normal No.(45)	Male	17	37.77%	
	Female	28	62.23	
	No	18	30%	

* Significant differences (P< 0.05); χ^2= 0.05: Chi-square test; Df= Degree of freedom

Renal function test (RFT) results

Table (2) renal function test (RFT) results in our study			
Parameter	Patient Mean± SD	Normal Mean± SD	P value
Urea mg/dl	109.11 ± 12.39	37.59 ± 11.94	0.021*
Creatinine mg/dl	3.24 ± 0.59	0.62 ± 0.15	0.017**
eGFR ml/mi/1.73m2	27.91 ± 6.96	131.73 ± 29.91	0.0014**

* Significant differences in P< 0.05; ** significant differences in P< 0.0

in Table (2) the renal function parameter shows the significant increases ($p \leq 0.05$) in urea and shows the significant increases ($p \leq 0.01$) in creatinine when patient groups compared with healthy groups, in GFR shows the significant decreases ($p \leq 0.01$) in patient groups compared with healthy groups.

Table 3: Biomarker Levels in Chronic kidney disease Group vs. Control Groups			
Parameter	Patient Mean± SD	Normal Mean± SD	P value
Kidney injury molecule-1(ng/ml)	4.73 ± 1.32	0.578 ± 0.09	0.0001**
FETUIN-A(ng/ml)	488.51 ± 339.73	544.81 ± 236.11	0.031*

* Significant differences in P< 0.05; ** significant differences in P< 0.01

In Table (3), The results of the current study showed that kidney injury molecule-1 levels significantly increased ($p \leq 0.05$) in CKD patients compared to controls, as shown in Table (3), while the study showed that Fetuin-A levels significantly decreased ($p \leq 0.05$) in CKD patients compared to controls., as shown in Table (3)

Table 4: Kidney injury molecule-1 Biomarker Levels in Chronic kidney disease stages:-

Parameters	-	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Statistical analysis
Kidney injury molecule-1(ng/ml)	N	0	4	20	17	19	0.00018*
	Min	-	1.102	1.6	0.4	6.4	
	Max	-	1.6	3.101	6.102	12.213	
	Mean	-	1.38 c	2.18 c	3.89 b	8.89 A	
	S.D.	-	0.21	0.45	1.136	1.93	

*Anova multiple comparsion / Tukeys parwise

The current study showed that there was a significant increase($p \leq 0.05$) in the concentration of Human Kidney injury molecule-1(KIM-1) When the stage of chronic kidney disease rises, as shown in the Table (4)

Table 5: Fetuin-A Biomarker Levels in Chronic kidney disease stages

Parameters	-	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Statistical analysis
FETUIN-A(ng/ml)	N	0	4	20	17	19	0.017*
	Min	-	269.70	319.81	223.19	428.74	
	Max	-	841.85	2780.76	598.67	514.75	
	Mean	-	635.16 a	465.30 B	462.37 b	391.13 c	
	S.D.	-	55.09	90.46	39.12	36.85	

*Anova multiple comparsion / Tukeys parwise

Same letter means no significant differences; different letters means significant differences

The current study shows no significant ($p \geq 0.05$) differences in the concentration of fetuin_A between patients with stages 3,4, while it has been demonstrated significant ($p \leq 0.05$) differences among stages 3,4 and 2,5 as shown in Table (5)

Sensitivity and specificity of the new biomarker of chronic kidney disease in the CKD patients

Sensitivity and specificity of the Fetuin-A in chronic kidney disease

The result of statistical analysis by using ROC analysis curve of Fetuin-A biomarker show that the specificity is more than the sensitivity in chronic kidney disease (CKD) patients .

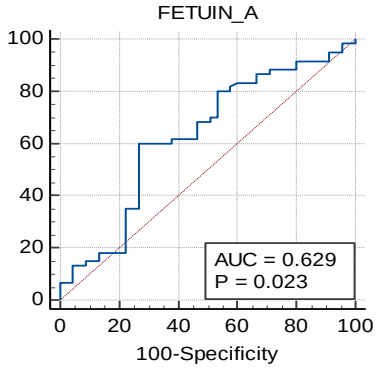

Figure 1: The sensitivity and Specificity of the new biomarker (Fetuin-A)of chronic kidney disease in the CKD patients .

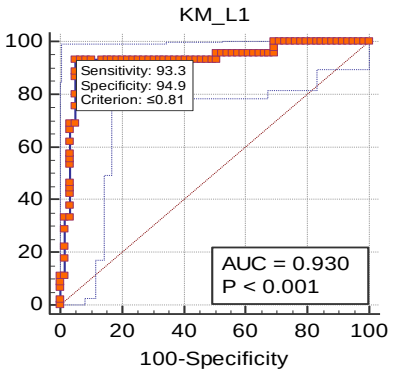

Figure 2:The sensitivity and Specificity of the new biomarker Human Kidney injury molecule (KM_L1) of chronic kidney disease in the CKD patients.

Sensitivity and specificity of the Human Kidney injury molecule(KM_L1) in chronic kidney disease:-

The result of statistical analysis by using ROC analysis curve of Human Kidney injury molecule (KM_L1) biomarker show that the specificity is more than the sensitivity in chronic kidney disease (CKD) patients.

Correlation between CKD and biomarker that diagnosis the chronic kidney disease depending on the stages:-

Correlation between the chronic kidney disease and biomarker in stage 2:-

The result show non correlation between the parameter of chronic kidney disease in stage 2

Table 6: The correlation between the biomarker of CKD in stage 2.					
	urea	CRI	GFR	FET-A	KIM-L1
urea		0.77	0.56	0.99	0.42
CRI	0.22		0.09	0.04	0.07
GFR	-0.43	-0.90		0.12	0.09
FET-A	-0.68	-0.61	0.88		-0.79
KIM-1	-0.12	-0.57	0.22	0.20	

Yellow color negative correlation
Red color positive correlation

Green color mean significant differences between two parameters

Correlation between the chronic kidney disease and biomarker in stage 3:-

The result show positive correlation and significant differences between two parameters the GFR and creatinine , this show in Table (8)

Table 7: The correlation between the biomarker of CKD in stage 3.					
	urea	CRI	GFR	FET-A	KIM-L1
Urea		0.34	0.74	0.02	0.56
CRI	0.23		0.00	0.52	0.43
GFR	-0.08	-0.73		0.74	0.69
FET-A	0.11	-0.44	0.37		0.08
KIM-1	-0.14	0.19	-0.10	0.07	

Yellow color negative correlation
Red color positive correlation

Green color mean significant differences between two parameters

Correlation between the chronic kidney disease and biomarker in stage 4:-

The result shows a negative correlation. There were significant differences between GFR and creatinine, urea, as shown in Table 9.

Table 9: The correlation between the biomarker of CKD in stage 4.					
	urea	CRI	GFR	FET-A	KIM-L1
Urea		0.07	0.31	0.92	0.30
CRI	0.45		0.001	0.84	0.68
GFR	-0.26	-0.84		0.53	0.88
FET-A	-0.60	0.08	-0.03		0.07
KIM-L1	-0.31	-0.43	0.35	0.07	

Yellow color negative correlation
Red color positive correlation

Green color mean significant differences between two parameters

Correlation between the chronic kidney disease and biomarker in stage 5:-

Table 10: The correlation between the biomarker of CKD in stage 5.					
	urea	CRI	GFR	FET-A	KIM-L1
Urea		0.64	0.92	0.18	0.08
CRI	-0.11		0.001	0.03	0.87
GFR	-0.02	-0.77		0.15	0.52
FET-A	0.08	0.29	-0.14		0.92
KIM-L1	-0.20	0.13	-0.10	0.92	

Yellow color negative correlation
Red color positive correlation

Green color mean significant differences between two parameters

Isolation and diagnosis of bacteria associated with chronic kidney disease

After culturing the samples of urine which were obtained from patients with chronic kidney disease on blood agar and MacConkey agar media, 42 bacterial isolates were obtained, with 32 Gram-negative isolates being obtained from the total number of isolated bacteria, as well as 10 Gram-positive isolates. Based on the diagnostic results, which will be mentioned later, the isolation process resulted in obtaining 13 isolates of *Klebsiella*, 5 isolates of *Enterobacter*, one isolate of *Pseudomonas*, 7 isolates of *E. coli*, 6 isolates of *Proteus*, in addition to 10 isolates of *S. auras* , as shown in the Figure (3)

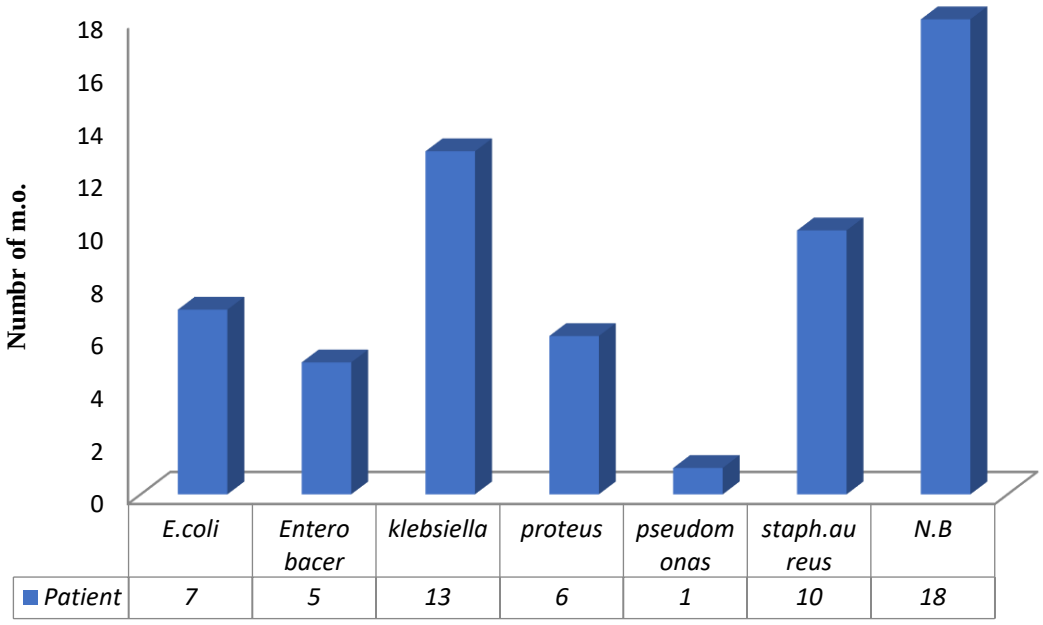

Figure 3: Number and types of bacteria isolated in this study.

DISCUSION

The clinical usefulness of Fetiun-a and kidney Injury molecule-L1 as indicators in individuals with chronic kidney diseases is being assessed for the first time in this investigation. Reliable indicators of how well the kidneys are functioning are creatinine and urea. Increased and decreseas blood concentrations of these substances signify renal illness. It is important to remember, though, that these levels can also be caused by other factors, including high protein intake, shock, and gastrointestinal hemorrhage. The new study's findings show that the patient group had greater levels of urea and creatine than the healthy population, which suggests a problem with kidney function. The results of the current study are consistent with the study conducted by Kamal[22] They discovered that the levels of urea and creatinine in the blood serum of patients with renal failure were noticeably elevated. [23] The body naturally excretes urea and creatinine through urine, therefore blood serum levels of these chemicals are higher in kidney failure patients. When renal function is impaired or deficient, as occurs in kidney failure, waste products are not excreted as much, which causes them to build up and become more concentrated in the blood serum. [24]. found that a lower level of KIM-1 is expressed in normal kidneys compared to diseased kidneys[25]. Hant et al shown a significant increase in KIM-1 expression in kidney biopsy specimens from individuals who had an acute tubular necrosis pathology diagnosis. Additionally, raised KIM-1 levels were detected in the urine within 12 hours of the initial ischemic renal insult, before casts started to develop in the urine[26] . Studies have shown that patients with modestly elevated serum levels of fetuin A may have a survival benefit compared to those with low levels of fetuin A, according to observational studies suggesting that low serum fetuin A may be an independent risk factor for death. Early onset in patients with chronic kidney disease. [27]. As for the isolated bacteria as Jalil *et al* (2015) were also able to isolate *E. coli*, *klebsiella*, *Enterobacter*, and *Pseudomonas* were isolated from patients with kidney failure, with rates reaching 8, 34.32%, 6.71%, and 4.47%, while the Gram-positive bacteria, Staphylococcus

aureus, were isolated at a rate of 13.41%. Referring to Figure (3), it is clear that the predominance of *Klebsiella* and *E. coli* from patients' infections. Kidney failure. These results were in agreement with what was indicated by many previous studies, as this type of bacteria is prevalent in chronic kidney disease infections.

CONCLUSION

According to the result of the present study were concluded the follow in

1-Individuals diagnosed with chronic kidney disease exhibit decreased glomerular filtration rates and elevated amounts of urea and creatinine.

2-The concentrations of kidney injury molecule biomarkers for tubular damage diagnosis, rise as the stage of chronic kidney diseases

3-The concentration of Fetuin-a biomarkers for tubular damage diagnosis, decrease as the stage of chronic kidney disease increases

examined by the biomarkers mentioned in our study.

Recommendation

1- We recommend conducting the study using larger samples.

2- We recommend a molecular study using the PCR technique for samples that were

3- We recommend that each stage of kidney failure be studied separate

Conflicts of interest

There are no conflicts of interest.

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