Original Article

Growth Differentiation Factor-15 as a Marker for Assessment of Anemia in Chronic Kidney Disease Patients with Type II Diabetes Mellitus

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ABSTRACT

Objective: To assess the GDF-15 in conjunction with a number of biochemical markers in individuals with advanced chronic kidney disease and either type 2-diabetes or no diabetes at all.

Study Design: Case-control study

Place and Duration of Study: This study was conducted at the Al-Yarmouk and Baghdad Teaching Hospitals in Baghdad, Iraq from 1st January 2024 to 30th June of 2024.

Methods: One hundred and fifty participants were enrolled and they were divided into three groups. One group consisted of 50 individuals with late-stage chronic kidney disease (27 men and 23 women) who also had type 2 diabetes. There were three groups: one with fifty healthy people (28 men and 22 males) and another with fifty people who were in the last stages of chronic kidney disease (24 men and 26 females) but did not have type 2 diabetes.

Results: A significant difference was found in the age group of 50–59 years when comparing CKD patients (with and without T2DM) to the control group ($p < 0.05$), while gender distribution showed no significant variation ($p > 0.05$).In CKD patients with T2DM, GDF-15 correlated significantly with serum creatinine and glomerular filtration rate (GFR), whereas in CKD patients without T2DM, it was associated with iron levels ($p < 0.05$).ROC analysis showed that GDF-15 had a sensitivity of 97% and a specificity of 100%, highlighting its potential as a diagnostic biomarker.

Conclusion: Growth differentiation factor-15 as a key factor in the development of anaemia in patients with T2DM and chronic kidney disease.

Key Words: Chronic kidney disease (CKD), Type 2 diabetes mellitus (T2DM), Growth differentiation factor-15 (GDF-15), Glomerular filtration rate (GFR), Biochemical parameters

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INTRODUCTION

Chronic kidney disease (CKD) is a worldwide public health challenge and results in substantial morbidity, mortality, and health care costs in adults. CKD is diagnosed based on the presence of abnormalities of kidney structure or function (i.e. abnormal albuminuria or glomerular filtration rate [GFR] less than 60 mL/min per 1.73m^2) for more than 3 months, with implications for health.[1]

If untreated, the condition can progress to renal failure, making it a potentially lethal illness. The likelihood of effective therapy and the length of the patient's life are both enhanced by early and accurate predictions.

Poor clinical outcomes and a diminished quality of life have been associated with anaemia, a prevalent presenting feature in persons with chronic kidney disease (CKD). Decreased erythropoietin synthesis and reticuloendothelial iron blockage are two of the mechanisms that cause anaemia in chronic kidney sickness.[2]

A biomarker of anaemia as well as cardiovascular and chronic inflammatory illnesses, growth differentiation factor 15 (GDF-15) is an important protein in the human body. Growth differentiation factor-15, macrophage inhibitory cytokine-1, non-steroidal anti-inflammatory drug-inducible gene (NAG)-1, and MIC-

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1 are some of its alternate names. The GDF-15 super family includes transforming growth factor β and other divergent members which highly expressed in the heart, liver, kidney, intestine, lung, placenta and the prostate gland.[3-5]

GDF15 gene is located on chromosome 19p12-13.1 and consists of two exons separated by intron. The release of GDF-15 is stimulated by various growth factors and cytokines, including transforming growth factor beta (TGF-β), tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), macrophage colony-stimulating factor (M-CSF), angiotensin II, and p53.[6,7]

It's mainly improves the function of kidneys in CKD and plays an important role in the prediction of CKD progression[8] so when it's level increased linked to an increased risk of incident chronic kidney disease. Under physiological conditions, the placenta is the only tissue that expresses high levels of this protein, with levels peaking during the third trimester of pregnancy.[4]

Also, the expression of this stress-responsive cytokine increases in many pathological conditions such as injury, ischemia, and other forms of oxidative and/or metabolic stress, sparking interest in its potential utility as a biomarker in human disorders cancer, cardiovascular disease, diabetes mellitus, chronic obstructive pulmonary disease.[6,9] Moreover, it is one of the regulators of hepcidin synthesis and thus participates in iron homeostasis. It has been shown that high concentration of GDF-15 is responsible for the reduced synthesis of hepcidin.[10,11]

METHODS

This case-control study was conducted at Al-Yarmouk and Baghdad Teaching Hospitals in Baghdad, Iraq from 1st January 2024 to 30thJune of 2024. Out of a total of 150 participants, the study separated them into three groups. One group consisted of 50 individuals with late-stage chronic kidney disease (27 men and 23 women) who also had type 2 diabetes. There were three groups: one with fifty healthy people (28 men and 22 males) and another with fifty people who were in the last stages of chronic kidney disease (24 men and 26 females) but did not have type 2 diabetes. The researchers used sterile disposable syringes to draw 8 mL of blood from the subjects using vein puncture. Separating serum for biochemical testing and haemoglobin measurement required dividing each sample in half, placing them in separate gel tubes, and then centrifuging the tubes. We determined haemoglobin using the automated CBC equipment (Human/Germany). The GDF-15 concentration (normal range = 494-654 pg/ml) was measured using the ELISA technique. The biochemical tests were also determined using the automated chemistry auto-analyzer (Cobas C311) and the Cobas e411 (Roche, Japan). The MDRD GFR equation was used to predict the glomerular filtration rate (GFR). This equation took creatinine and patient demographics like gender and age into account. The present data was analyzed using the SPSS-24. Receiver operating characteristic (ROC), Pearson's correlation, one-way analysis of variance (ANOVA), and independent T-tests were also utilized in our research. It was deemed significant when the significance criterion was less than 0.05.

RESULTS

The gender distribution between the two CKD groups was not statistically significant. However, there was a significant difference in the age group (50-59 years) between the control group and CKD patients with or without T2DM (Table 1). The levels of serum FBS (mg/dl), urea (mg/dl), creatinine (mg/dl), ferritin (ng/ml), iron (µg/dl), and GDF-15 (pg/ml) were higher in CKD patients as compared to the healthy control group, as indicated in table (2). There was a significantly significant statistical difference between these two groups (P<0.01). (Table 2).

Based on the receiver operating characteristic (ROC) curve of the level between the patient groups and the control group, the cut-off value for GDF-15 was 684, indicating 100% specificity and 97% sensitivity. In addition, the AUC of 0.97 was extremely significant (P<0.0001) (Table 3, Fig. 1).

Results from CKD patients with type 2 diabetes show a robust relationship between GDF-15, serum creatinine, and glomerular filtration rate (GFR). Iron and GDF-15 were also significantly associated with CKD individuals who did not have type 2 diabetes (Table 4).

Table No.1: Distribution of study groups according to age and gender

Variable	CKD with diabetes mellitus (N=50)	Without diabetes mellitus (N=50)	Controls (N=50)	p-value
Age (years)				
30-39	5 (10%)	17 (34%)	5 (10%)	0.1NS
40-49	5 (10%)	-	17 (34%)	0.2NS
50-59	16 (32%)	22 (44%)	15 (30%)	0.01S
60-70	24 (48%)	11 (22%)	13 (26%)	0.06NS
Gender				
Females	23 (46.00%)	26 (52.00%)	22 (44.00%)	0.307 (NS)
Males	27 (54.00%)	24 (48.00%)	28 (56.00%)	0.294 (NS)

NS: Non-significant (P>0.05 P<0.05: Significant

Table No.2: Distribution of biomarkers among diabetic CKD patients, non-diabetic CKD patients and the control group (one-way ANOVA test)

Parameters	Control Group	CDK groups		P value
		WithT2DM	Without T2DM	
FBS (mg/dl)	85.52 ±1.87	212.30 ±7.70	87.68 ±1.30	0.0001
Urea(mg/dl)	20.16 ±0.44	153.54 ±7.87	150.88 ±5.14	0.0001
Creatinine(mg/dl)	0.674 ±0.02	9.09±0.32	8.06 ±0.27	0.0001
GFR(ml/min)	110.70 ±1.66	7.17 ±0.46	6.09 ±0.31	0.0001
Ferritin (ng/m)	124.38 ±9.57	788.16±34.60	737.58 ±37.91	0.0001
Hb(mg/dl)	12.83 ±0.10	8.69 ±0.22	8.94 ±0.17	0.0001
Iron(µg/dl)	63.90 ±2.06	53.08 ±2.12	57.20 ±2.18	0.0082
GDF-15(pg/ml)	539.76 ±7.52	1883.34±68.18	1196.04 ±34.04	0.0001

CKD: Chronic kidney disease, T2DM: Type 2 *Diabetes mellitus*, GDF-15: Growth differentiation factor-15, GFR: Glomerular filtration rate, Hb: Haemoglobin, FBS: fasting blood sugar, SD: standard deviation

Table No.3: ROC curve for GDF-15 (receiver operating characteristic curve test)

Markers	AUC	p-value	Cut-off Point	Sensitivity	Specificity
GDF-15(pg/ml)	0.97	<0.0001	684	97%	100%

ROC: receiver operating characteristic, AUC: area under the curve

Table No.4: Relationship between GDF-15 and some biomarkers in CKD patients with or without T2DM

Markers	GDF-15 in CKD patients with T2DM		GDF-15 in CKD patients without T2DM	
	Pearson's correlation	p-value	Pearson's correlation	p-value
Hemoglobin (g/dl)	-0.07	0.6 (NS)	-0.06	0.6 (NS)
Urea(mg/dl)	0.22	0.1 (NS)	0.03	0.8 (NS)
Creatinine(mg/dl)	0.39	0.004 (S)	0.19	0.1 (NS)
GFR(ml/min)	-0.51	0.000 (S)	-0.11	0.4 (NS)
FBS(mg/dl)	0.05	0.7 (NS)	-0.08	0.5 (NS)
Ferritin(ng/ml)	0.16	0.2 (NS)	0.01	0.9 (NS)
Iron(µg/dl)	-0.05	0.7 (NS)	-0.32	0.02 (S)

NS = Not significant

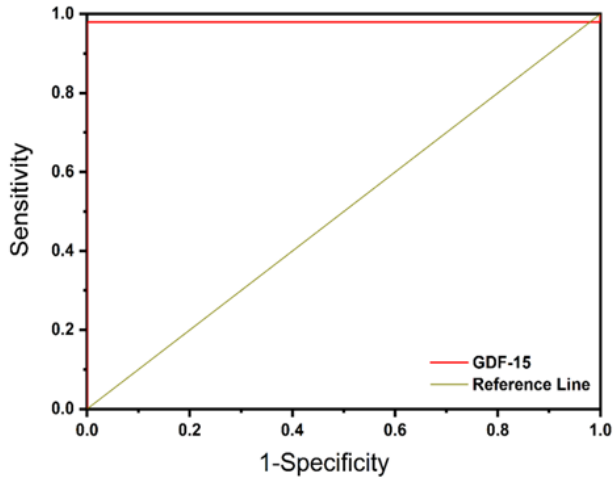

Figure No. 1: Receiver operating characteristic curve for GDF-15

DISCUSSION

One of the leading causes of death globally is chronic renal disease. This research has demonstrated a strong link between chronic renal disease and aging. The elevated occurrence of chronic kidney disease was attributed to decline in glomerular filtration associated with aging. Thus, a crucial strategy for improving results was to conduct screenings for chronic kidney disease in older adults, this result consistent with.[12] Researchers found no statistically significant difference in the gender distribution of the control group, diabetic CKD patients, or non-diabetic CKD patients. Findings from this investigation corroborated those of an earlier study by the same authors. Consistent with other studies, the results demonstrated that individuals with diabetic CKD had poorer renal outcomes and started dialysis earlier than CKD patients without diabetes.[13] Compared to healthy persons, CKD patients with or without T2DM had a much higher amount of urea. The findings aligned with those of another recent study.[14] Kidney function gradually declines during chronic kidney disease (CKD). Kidney function is compromised, making it harder for the kidneys to remove urea and other waste from the blood. These

waste products build up in the circulation when kidney function decreases, which causes blood levels to rise.

Creatinine levels rise above normal in diabetic individuals with chronic kidney disease (CKD) for a number of reasons, including reduced kidney function and the metabolic consequences of diabetes. These findings corroborated those of previous research.[15] Low glomerular filtration rate (GFR) is the primary reason for elevated creatinine in patients with chronic renal impairment. The kidneys' filtering capacity declines with the progression of chronic renal disease.[16,17]

Inflammation and disturbances in iron metabolism are common symptoms of chronic kidney disease (CKD), which may explain the dramatically elevated ferritin levels seen in this research. Ferritin is an acute-phase reactant, meaning that its levels increase in response to inflammation. Inflammation stimulates the liver to produce more ferritin, leading to higher blood ferritin levels.[18]

A significant drop in hemoglobin was discovered in the present investigation. Additionally, compared to non-diabetics, the prevalence of anaemia was greater in all CKD patients, especially diabetic patients. In individuals with chronic kidney disease (CKD), diabetes impacts iron utilization and erythropoiesis.[19,20]

In the past, chronic kidney disease (CKD) was often associated with anaemia because impaired kidney function lowered erythropoietin production.[21]

Malnutrition and inflammation are two of the many potential causes of the greater iron deficit seen in CKD diabetes patients compared to non-diabetic CKD patients in the research.[22]

Our findings corroborated those of previous research that found elevated GDF-15 levels in diabetic individuals with chronic kidney disease and were predictive of faster disease progression and higher mortality.[23,24]

In addition, GDF-15 demonstrated excellent sensitivity and specificity in ROC analysis. Due to its significance for disease progression, inflammation, and clinical outcomes, the link between GDF-15 and blood creatinine in CKD patients with diabetes was considered significant.[25] High GDF-15 implies higher renal damage and inflammation, which correlates with lower GFR and more severe CKD[26], further supporting the significance of the link between GDF-15 and GFR in diabetic individuals with chronic kidney disease. Faster development of chronic renal disease and impairment of kidney function are predicted by elevated GDF-15 levels. More thorough cardiovascular surveillance and care should be implemented for patients with high GDF-15 and low GFR since they are at a higher risk for cardiovascular events.[23]

The cytokine GDF-15 controls iron metabolism plays a role in the immune response, and regulates inflammation. When cellular stress and inflammation, both of which are prevalent in chronic kidney disease, are present, it rises. Elevated GDF-15 levels are linked to chronic illness anaemia and reduced iron utilization.[10] Since GDF-15 influences iron control, which contributes to the functional iron shortage, the association between GDF-15 and iron in non-diabetic CKD patients was substantial. Enhanced anaemia owing to impaired iron utilization was similarly linked to higher GDF-15 levels, according to prior research.[27]

CONCLUSION

The GDF-15 biomarker has strong relationships with other biochemical indicators. It has been linked to anaemia in chronic kidney disease (CKD) patients, whether or not they have diabetes. As a result, this biomarker can be utilized to diagnose anaemia in these individuals.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Hanadi Hendi Khudair, Shatha Hamed Jwaid
Drafting or Revising Critically:	Shatha Hamed Jwaid, Nisreen Sherif Alyasiri
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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