Evaluation Irisin Level and other Biochemical Parameters in Chronic Kidney Patients Before hemodialysis

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Abstract

Background: The browning of adipose tissue is regulated by the myokine irisin, which plays a role in energy metabolism. This research set out to determine whether there was a correlation between patients' irisin levels and their pre-dialysis biochemical parameters in stage 5 chronic kidney disease (CKD). Methods: The study included 30 individuals with CKD in stage 5 before dialysis and 30 subject as control group, the ages of all patients and the control group ranged from (25-60) years. Excluded criteria included patients with viral hepatitis, diabetes.. Serum irisin concentration and the level of glucose (Glu), urea, creatinine (Cr) ,albumin (Alb) , ACR ,TC, ALT,AST,ALP , Electrolytes ,Ca, PO4 and Vit D were measured. Results: In the present study, the results showed significantly reduced in serum irisin , Vit D, Na , K. Ca.,AST ,ALT, eGFR ,Tp , Weight and a significantly increased in Urea ,Cr, ALP. PO4 of patients was compered with control group, also results showed a significant negative correlations between serum Irisin and FSG ,Cr,Ca,PO4,Alb,Urea,ALT levels in CKD group. While, significant positive correlations were found between serum Irisin and Age, Weight, Hight ,BMI, Tp, Chol, AST, ALP, Na, K, Vit D levels in CKD as well as, a significant negative correlations between Vit D and FSG, Cr, PO4,Alb,Urea, Chol., ALT, ALP ,Na levels in CKD group . While, significant positive correlations were found between Vit D and Age, Weight ,Hight ,BMI, Ca, Tp, ,AST, K levels in CKD group. Conclusion: eGFR, body mass index, calcium, sodium, potassium, aspartate aminotransferase, tyrosine phosphatase, irisin, and vitamin D all rose dramatically as CKD advanced. Urea, creatinine, alkaline phosphatase, and phosphorous all went down. These results raise the possibility that irisin has a role in controlling biochemical factor levels in people with CKD. The ROC curve for irisin demonstrated a flawless cut off value, with a sensitivity of 87% and a specificity of 99.9%, indicating its potential as a diagnostic marker. For patients, a number less than 23.2 is considered indicative of poor health. As compared to the outcomes of urea and creatinine, Irisin's stood out as particularly intriguing.

Keywords: *Chronic kidney disease, Irisin, Stage.*

1-INTRODUCTION

The kidneys, also called the renal, are a pair of reddish-brown bean-shaped organs located in the lower right and left sides of the back, respectively. Typically, the liver will push the right kidney down, making it sit lower than the left. The average adult has kidneys that are each about 3 centimetres thick, 6 centimetres wide, and 12 centimetres long.[Koeppen B. et al,2015] Kidney function primarily involves the generation of urine and the regulation of the rate and capacity of its secretion, with the goal of flushing waste products out of the circulatory system. Renal competence includes the ability to maintain a healthy blood pressure, maintain a healthy fluid balance, and maintain a healthy electrolyte balance. [Wingerd B, 2013, Lovisa, S,2016] Fluid flow capacity between

glomerular capillaries and Bowman's capsule is measured as the glomerular filtration rate (eGFR). A renal function test is the gold standard for diagnosing chronic kidney disease and determining the severity of the condition .[Sabrina Garasto et al.,2014] A glomerular rate (GFR) of less than 60 ml/min/1.73 m2 with an abnormally high creatinine levels for more than 3 months has been characterized as chronic Kidney disease (CKD) [Versino, E et al.,2019] The kidney function was first evaluated by measuring the serum concentration of urea. The kidneys eliminate almost 90% of the by-product urea produced during protein synthesis [Boga MS,et al.,2019] Serum creatinine is an insensitive measure of GFR in the first stages of CRD [Traynor J,et al.,2006] Albumin is a marked to heart diseases and blood vessels or renal disease because renal disease patients have large amounts of albumen in their urine. Urinary protein loss affects albumin levels because protein in urine is the result of the balance between tubular reabsorption and glomerular filtration (GF). [Rieko Eriguchi ,et al.,2017] The kidneys are crucial in controlling the concentration of phosphorus ions in the blood. An imbalance of this ion, which tends to build up when renal function is compromised, is slowed down. Abnormally high blood phosphorus levels are associated with the development of renal disease and may also be linked to cardiovascular and skeletal issues. [Calvo MS,et al.,2013] The kidneys play a vital role in controlling blood Ca2+ levels. [Judith Blaine,2015] The kidneys are responsible for controlling the concentration of chloride ions in the blood very precisely. [Craig Knox,et al.,2018] Salt has a number of vital tasks in the body, but in renal patients, its high levels and poor quality may be harmful because the kidneys are unable to excrete the extra fluid and sodium that causes them to aggregate in the circulation and raise blood pressure. It is well known that hypernatremia or hyponatremia, depending on the severity, result from the presence of any problem in renal function. [Braun,et al.,2015] The kidneys filter the blood and get rid of any extra potassium that the body doesn't need. Hyperkalemia develops when the kidneys are unable to excrete the excess potassium from the blood, as is the case in people with renal illness. An individual's kidneys are responsible for recycling the body's surplus potassium back into the circulatory system. [Packham,et al.,2015] In 2012 Boström et al. was discovered irisin (derived from Greek goddess Iris, means rainbow and a messenger of the god) and considered as a novel cytokine. Irisin is a glycosylated protein hormone with a molecular weight about 12 KDa, composed of 112 amino acids residues(Leustean,et al.,2021) The widespread prevalence of vitamin D deficiency (VDD) is a major issue for public health. In recent years, researchers have paid more attention to it because of its high frequency and likely significance in the onset of various chronic illnesses. (Salman Jasim, H et al ,2022) Vitamin D has massive role in calcium regulation and homeostasis which aids calcium level to maintain in the body and bone health (Matsui,et al.,2020). The purpose of this research was to examine the connection between irisin concentration and pre-dialysis biochemical parameters in individuals with stage 5 chronic kidney disease (CKD).

2-Material and Methods

This study included patients who were admitted to Al-Yarmouk Teaching Hospital and the Center for Diseases and Kidney Transplantation at Ghazi Hariri Hospital in the Medical City for the period from October 2022 to January 2023

Blood samples were collected from chronic kidney patients before dialysis, in addition to healthy individuals as a control group chosen without any chronic diseases. The study included 30 individuals with CKD and 30 subject as control group ,the ages of all patients and the control group ranged from (25-60)

years. Excluded criteria included patients with viral hepatitis, diabetes.

Blood samples were taken from studies groups (Chronic Kidney Disease and control). Blood samples were collected between (11:00-8:30) using a 5ml syringe into a gel tube and allowed to clot at room temperature. Then centrifuged (3000 degrees per minute) for 10 minutes to separate the serum. 2ml was used to determine the levels of (glucose, creatinine, urea, phosphorus, albumin, calcium, total protein, sodium, potassium). The residues were transferred to an Eppendorf tube and stored in a deep freezer (-20 °C) to be used to determine the levels of (Irisin, vitamin D).

Anthropometric and Biochemical measurements:

2.1-Measurement of Body Mass Index (BMI) [Kasper,et al.,2015]

2.2-Determination of Serum Albumin [Doumas BT,et al., 1971]

2.3- Determination of serum Urea: [Chaney,et al.,1962,Searcy,et al.,1967]

2.4- Determination of serum Creatinine : [Allen,et al.,1982, Tanganelli,et al.,1982]

2.5- Measurement of serum glucose (SG) [Massod,et al.,1977]

2.6- Determination of Cholesterol (TC): [Nayak,et al.,2007]

2.7- Determination of serum ALT: [Bergmeyer,et al.,1986]

2.8- Determination of serum AST [Winn-Deen,et al.,1988].

2.9- Determination of serum ALP [Belfield,et al.,1971]

2.10- Determination of serum Electrolytes (Na+, K+):[Roche ISE indirect,et al.,2016]

2.11 -Determination of serum Calcium: [Connerty,et al.,1966]

2.12- Determination of serum Phosphorus: [Drewes PA,1972]

2.13- Determination of serum irisin [Wrann CD,et al.,2013]

2.14- Determination of DHVD3 Levels in Blood Serum [Ting Wang,2001]

The quantities of plasma 25(OH)D were determined using an enzyme-linked fluorescence assay (ELFA) method and a small VIDAS Biomerieux automated immunological analyzer (Biomerieux, Marcy-I'Etoile, France).
2.15-. Statistical Analysis:

A mean ± standard deviation was used to represent the outcomes. A t-test was used to compare the significance of the differences between the groups. P-values of ($p > 0.05$), and ($p \leqslant 0.05$) were regarded statistically non-significant and significant, respectively. The link between the several parameters was analysed, and the correlation coefficient (r) was used to characterise it. Using statistical programme for the social sciences (SPSS) version 23.0 and Microsoft office 2007, we determined the cutoff value, sensitivity, and specificity using a Receiver Operating Characteristics (ROC) curve. Where the p-value was less than 0.05, the findings were judged to be statistically significant.

3-Results and Discission:

Anthropometric and clinical features CKD patients and control groups in the study are listed in Table (1). There were significant increases ($p \leqslant 0.05$) in age , SBP, DBP, and non -significant decrease height , BMI and significant decrease weight in CKD patient group as compared to the controls:

Table 1: Anthropometric and clinical features of CKD and control groups:

Parameters	Means ± SD	Means ± SD	*p*-value
	CKD (n= 30)	Control (n= 30)	
Age (years)	47.8±15.0	37.5±13.8	0.05
Height (cm)	168.1 ±7.7	170.5±10.2	0.26
Weight (kg)	71.7±15.2	75.5±10.3	0.05
BMI (kg/m2)	25.3±4.9	26.0 ±3.2	0.2
SBP (mmHg)	135.76 ± 8.25	119.60 ± 2.44	0.001
DBP (mmHg)	80.83 ± 2.29	78.67 ± 2.34	0.001
Duration of CKD (Years)	5 ±1.0	-	

$p \leqslant 0.05$: Significant, $p \leqslant 0.001$: high- Significant, $p \geqslant 0.05$ non-Significant

Hypertension occurs in CKD for several reasons. Hypertension in CKD is caused in part by an increase in sympathetic tone caused by afferent signals produced by kidneys with deteriorating function. The renin-angiotensin-aldosterone system (RAAS), which encourages salt and water retention, is upregulated in response to a decrease in eGFR. Along with this, high blood pressure has become more sensitive to salt. It is well-established that endothelial dysfunction is associated with hypertension, and this is a hallmark of severe chronic kidney disease (eGFR 30 mL/min/1.73 m2). [DanPugh,etal.,2019]

There is a growing consensus throughout the globe that the ageing of the population poses significant health risks. Acute chronic kidney disease, like many other age-related chronic diseases including dementia, has been demonstrated to increase in prevalence with age. This finding suggests that the elderly population could be growing in size, since acute chronic renal disease is a prelude to kidney failure. Urgent Need for Kidney Transplantation (dialysis or kidney transplant) [Pietro Ravani,et al.,2020]

Examination of the kidney function in CKD and control groups in the study are listed in Table (2). There was a significant increases ($p \leqslant 0.05$) in Urea, Creatinine and a significant decrease ($p \leqslant 0.05$) in eGFR, and non-significant increase ($p \geqslant 0.05$) in albumin ,ACR and non-significant decrease in Tp in CKD group as compared to the control group:

Table 2: kidney function of the CKD and control groups

Parameters	Means ± SD	Means ± SD	*p*-value
	CKD (n= 30)	Control (n= 30)	
eGFR (ml/min/1.73m^2)	30± 2.4	110.23±4.45	0.001
Tp (mg/dL)	7.62±0.82	7.76±0.54	0.22

Albumin (g/L)	4.71 ± 4.74	4.1 ± 0.4	0.5
Urea (mg/dL)	98±59.8	25.9±6.9	0.05
Creatinine (mg/dL)	4.2±2.7	0.7±0.2	0.05
Albumin/Creatinine ACR (mg/g)	13.3±3.6	1.9±6.3	0.4

$p \leqslant 0.05$: Significant, $p \leqslant 0.001$: high- Significant, $p \geqslant 0.05$ non-Significant

Chronic renal failure, characterized by a low glomerular filtration rate and/or the presence of protein in the urine (a condition known as albuminuria), is widely recognized as a major public health issue because of its rising prevalence around the world and its independent relationship with cardiovascular mortality and, more generally, with all-cause mortality. Chronic renal failure patients may have abnormal metabolic rates [Joachim Jankowski,et al.,2021].

Chronic renal failure patients have energy expenditure that is not well regulated, although the particular processes responsible for this are not well known. Impaired glucose metabolism, metabolic acidosis, micro-inflammatory responses, and altered cellular protein turnover are only some of the metabolic changes brought on by renal insufficiency. [Carrero JJ,et al.,2013]

Examination of the liver function , total cholesterol and fasting glucose sugar in CKD and control groups in the study are listed in Table (3). There were a significant decrease ($p \leqslant 0.05$) in ALT, AST and a significant increases in ALP and non-significant decrease in FSG, TC in CKD group as compared to the control group:

Table 3: Biochemical parameters of the CKD and control groups

Parameters	Means ± SD	Means ± SD	*p*-value
	CKD (n= 30)	control (n= 30)	
FSG (mg/dL)	95.9±11.6	96.4±10.1	0.1
TC (mg/dL)	168.1±40.8	178.6±32.7	0.1
AST (U/L)	17.8±5.8	28.8 ±5.6	0.001
ALT (U/L)	26.1±11.0	32.7±8.8	0.05
ALP (U/L)	123.0±36.4	80.6±18.8	0.001

$p \leqslant 0.05$: Significant, $p \leqslant 0.001$: high- Significant, $p \geqslant 0.05$ non-Significant

Several studies have shown the usefulness of measuring hepatic enzyme levels as a prognostic predictor in chronic kidney disease and end-stage renal disease. [Ray L, et al., 2015; Oyelade T, et al., 2020] It has been determined via a number of investigations that Serum aminotransferase levels tend to drop in CKD patients, but the underlying patho-physiological mechanism remains unclear. Possible reasons include UV absorption materials, high concentrations of uremic toxins, and a decrease in the coenzyme of aminotransferase known as pyridoxal-5-phosphate. [Ray L. et al. 2015] Some other

explanations include reduced production and inhibition of release of AST and ALT by hepatocytes, or sped-up clearance of these proteins from the serum. According to many studies [. Goicoechea M,et al.,2020- Sabouri S et al.,2020] In CKD patients, decreased serum amino-transferase levels may also result from fluid retention and hemodilution. (Ray L., et al., 2015)

The levels of Serum electrolytes (Na, K, Ca, inorganic phosphorus) in CKD and control groups in the study are listed in Table (4). There was a significant increase in K, iPO4 and a significant decrease in Na, Ca in CKD group as compared to the control group:

Table 4: levels of Serum electrolytes of CKD and control groups

Parameters	Means ± SD	Means ± SD	*p*-value
	CKD (n= 30)	control (n= 30)	
Na	125.6±19.7	141.1±1.4	0.001
K	4.7±0.7	4.0±0.4	0.001
Ca	3.3±1.0	5.0±1.5	0.001
iPO4	4.6±1.5	3.6±0.5	0.001

$p \leqslant 0.05$: Significant, $p \leqslant 0.001$: high- Significant, $p \geqslant 0.05$ non-Significant

A higher concentration of phosphorus was seen in the CKD group as compared to the healthy control group. The concentration of phosphorus in the cells is inversely proportional to the concentration of calcium in the cells, therefore an increase in phosphorus causes a reduction in calcium in the blood and consequently weak bones. [Glenn T. Nagami,2016]

Patients with renal failure tend to have high blood phosphorus levels because of a combination of factors, including kidney disease that reduces urine production and the inability of the hypoparathyroid gland to secrete enough parathyroid hormone to maintain normal blood calcium levels. Low levels of phosphorus reabsorption in the kidneys have been linked to calcium absorption disturbances, vitamin D overdose, and hyperthyroidism, [Hyperchloremia,2017]

Patients at CKD stage 5 were found to have higher than normal AMH levels, although the difference between their levels and those of healthy people was not statistically significant, according to the results of another research.compared with subjects without kidney disease [Luaibi, N. M,2021]

The levels of serum Irisin and Vit D in CKD and control groups are listed in Table (5). There was a high significant decrease in irisin and Vit D in CKD group as compared to control group:

Table 5: levels of Serum Irisin, Vit D of CKD and control groups

Parameters	Means ± SD	Means ± SD	*p*-value
	CKD (n= 30)	control (n= 30)	
Irisin	23.3 ±13.8	32.0±7.0	0.001
Vit D	133.3±25.9	229.8±69.0	0.001

$p \leqslant 0.05$: Significant, $p \leqslant 0.001$: high- Significant, $p \geqslant 0.05$ non-Significant

Boström discovered irisin in 2012, suggesting it is an exercise-induced myokine that induces the brown-fat-like conversion of white adipose cells. This finding has far-reaching consequences for metabolic and energy balance. [Kelly DP,2012, Liu JJ,et al.,2013]

An additional research found a direct connection (r-adjusted = 0.277) between irisin levels and GFR among 532 CKD patients (including 169 on HD). This work validates earlier investigations that showed a clear association between GFR and irisinaemia. It also found an inverse correlation between age and serum irisin, which, remarkably, appeared to decrease in individuals with lower levels of GFR. In this setting, it is not surprising that CKD patients have reduced irisin levels compared to healthy controls. [Carmona A,et al.,2016]

Whilst Wen et al. found significantly decreased plasma irisin levels in 38 CKD patients compared to 19 age- and sex-matched normal controls, they were unable to demonstrate an independent link of irisin with CKD.. [Wen MS,et al.,2013]

The strong form of vitamin D known as 1,25-dihydroxyvitamin D3 (Calcitriol) is required for calcium absorption and bone mineralization. It refers to a steroid that is either consumed in the form of food or synthesised by the skin when exposed to UV radiation. The liver may then metabolise this vitamin into 25-hydroxyvitamin D3. This intermediate is then further hydroxylated into the active metabolite DHVD3 by the 1α hydroxylase enzyme in the proximal tubular epithelial cells of the kidney. (Tamara ,A. et al ,2021)

The ability to convert 25(OH)D to 1,25(OH)2D (1,25 dihydroxy vitamin D or calcitriol) decreases when renal function declines, leading to a corresponding decrease in plasma 1,25(OH)2D. The kidneys' ability to absorb 25(OH)D might be impaired as well, leading to a shortage of the vitamin. It is believed that plasma 25(OH)D concentrations in healthy individuals must be between 15 and 40 nmol/L to prevent substrate constraint for renal 1,25(OH)2D synthesis. Those with chronic kidney disease may need greater doses .[Christodoulou M,et al.,2021

The present research, like the one by [Wang et al,2021], found a slight positive connection between eGFR and vitamin D. This data suggests that individuals with more advanced CKD had a lower concentration of vitamin D in their blood. Vitamin D deficiency may be caused by a lack of exposure to sunshine, a poor diet, or both [Ravani P,et al.,2009]. It was challenging to analyse the effect of the aforementioned variables in vitamin D insufficiency due to the constraints of the study design in this research. Due to the fact that vitamin D shortage has been linked to adverse outcomes in CKD patients, such as an increased risk of death [Jing J,et al., 2016], it is crucial that doctors stress the necessity of managing vitamin D concentration in their patients withCKD.

Although while previous research found a trend towards higher blood 1,25(OH)2vitamin D3 levels in CKD patients compared to normal healthy people at baseline, the present study found that CKD patients' 1,25(OH)2vitamin D3 levels were substantially elevated (p0.001). [Radeef, M., 2020]

Table (6): correlation between Irisin with other parameters in Control,CKD,

Groups	Irisin	Correlation coefficients(r)		P-value	
Parameters		Control	CKD	Control	CKD
Age		- 0.01	0.03	0.000	0.000
Weight		- 0.19	0.11	0.000	0.000
Hige		0.11	0.10	0.000	0.000
BMI		- 0.33	0.06	0.000	0.000
Sag		- 0.20	-0.31	0.000	0.000
Cr		0.03	-0.05	0.000	0.000
Ca		0.14	-0.08	0.000	0.000
PO4		0.07	-0.09	0.000	0.000
Alb		- 0.17	-0.10	0.000	0.000
TP		-0.01	0.39	0.000	0.000
Urea		0.125	-0.03	0.000	0.000
Chol		0.216	0.07	0.000	0.000
AST		0.211	0.19	0.000	0.000
ALP		0.08	0.10	0.000	0.000
ALT		0.04	-0.11	0.000	0.000
Na		0.06	0.08	0.000	0.000
K		-0.29	0.30	0.000	0.000
Vit D		-0.006	0.35	0.000	0.000

There were significant negative correlations between serum Irisin and FSG ,Cr,Ca,PO4,Alb,Urea,ALT levels in CKD group. While, significant positive correlations were found between serum Irisin and Age,

Weight, Hight, BMI, Tp, Chol, AST, ALP, Na, K, Vit D levels in CKD group

Table (7): correlation between Vit D with other parameters in Control, CKD

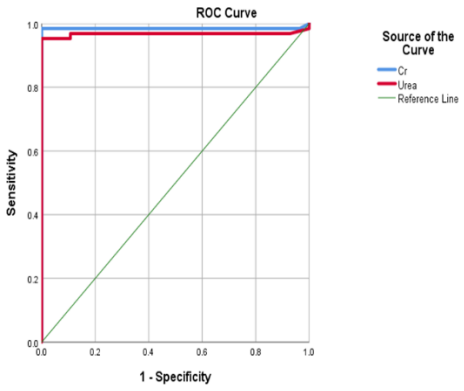
Groups	VIT D	Correlation coefficients(r)		P-value	
Parameters		Control	CKD	Control	CKD
Age		0.21	0.05	0.000	0.000
Weight		0.11	0.08	0.000	0.000
Hige		-0.12	0.007	0.000	0.000
BMI		0.24	0.07	0.000	0.000
Sag		0.16	-0.25	0.000	0.000
Cr		0.29	-0.33	0.000	0.000
Ca		0.28	0.02	0.000	0.000
PO4		-0.53	-0.09	0.000	0.000
Alb		-0.35	-0.12	0.000	0.000
TP		-0.19	0.13	0.000	0.000
Urea		0.08	-0.29	0.000	0.000
Chol		-0.28	-0.003	0.000	0.000
AST		0.14	0.17	0.000	0.000
ALP		-0.11	-0.09	0.000	0.000
ALT		-0.13	-0.02	0.000	0.000
Na		0.12	-0.19	0.000	0.000
K		0.08	0.07	0.000	0.000

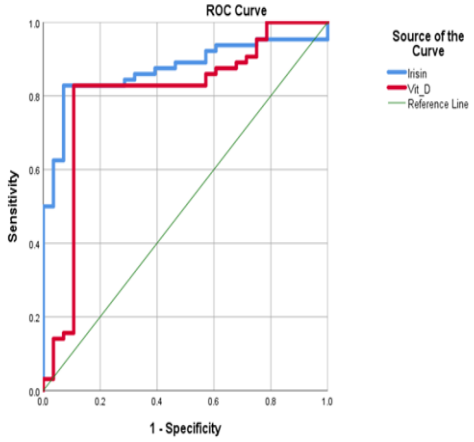
There were significant negative correlations between Vit D and FSG,Cr, PO4,Alb,Urea, Chol., ALT, ALP ,Na levels in CKD group. While, significant positive correlations were found between Vit D and Age, Weight ,Hight ,BMI, Ca, Tp, ,AST, K levels in CKD group.

Roc for patients and control:

Test Result Variable(s)	Area%	Sensitivity %	Specificity %	Cut-off value	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
Irisin	87%	82%	99.9%	23.27	0.00	0.79	0.94
Vit D	80%	83%	99%	195	0.000	0.69	0.91
Urea	96%	95%	100%	38.5	0.000	0.92	1
Cr	98.5%	98%	100%	1.35	0.000	0.95	1

ROC test for Irisin showed perfect cut off value with 87% sensitivity and 99.9% specificity, that indicates considered as a good diagnostic marker. The cutoff value lower than 23.2 representatives of patient. The result of Irisin was interest as compared to the results of Urea and creatinine

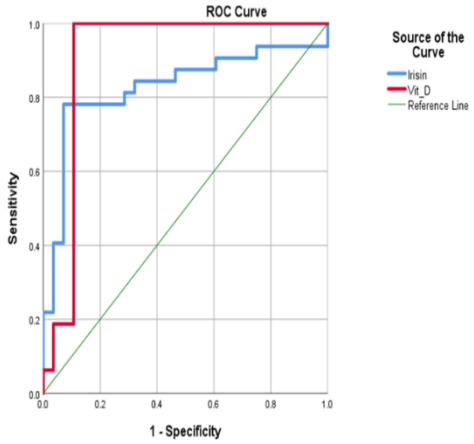


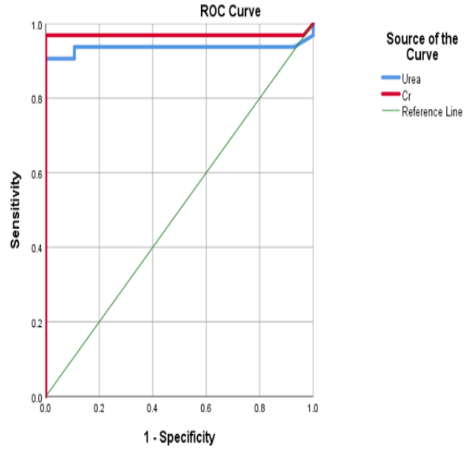


ROC CKD and Control:

Test Result Variable(s)	Area%	Sensitivity %	Specificity %	Cut-off value	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
Irisin	83%	78%	99%	23.1	0.00	0.71	0.94
Vit D	90%	100%	99%	195	0.000	0.80	1

Urea	93%	90%	100%	38.5	0.000	0.85	1
Cr	97%	96%	100%	1.35	0.000	0.91	1





4-Conclusion

With the progression of CKD parameters such as eGFR, weight, Ca, Na ,K,AST ,ALT ,Tp , irisin and Vit D levels significantly increased. Inversely, factors such as Urea, Cr, ALP. PO4 levels significantly decreased. These findings suggest that irisin may be involved in the regulation of biochemical factor levels in CKD patients ROC test for Irisin showed perfect cut off value with 87% sensitivity and 99.9% specificity, that indicates considered as a good diagnostic marker. The cutoff value lower than 23.2 representatives of patient. The result of Irisin was interest as compared to the results of Urea and creatinine.

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