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Study of Irisin in End Stage Renal Disease on Hemodialysis

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Abstract

Irisin is a myokine that controls energy metabolism by making adipose tissue brown. The present goal in doing this research was to determine how irisin concentration relates to other biochemical markers of disease. Hemodialysis (HD) for chronic kidney failure. The study included 30 individuals with end-stage renal disease on HD and 30 healthy subjects as the control group. The ages of all patients and the control group ranged from (25 to 60) years. The excluded criteria included patients with viral hepatitis and diabetes. Serum irisin concentration and the level of fasting serum glucose (FSG), urea, creatinine (Cr), total protein (TP), albumin (Alb), albumin to creatinine ratio (ACR), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), sodium (Na^+), potassium (K^+), calcium (Ca^{+2}), phosphorus (PO_4), and vitamin D were measured. The results showed significantly reduced serum irisin, vitamin D, Na^+, K^+, Ca^{+2}, PO4, TC, ALT, AST, glomerular filtration rate (eGFR), total protein TP, Alb, ACR, weight, height, and a significantly increased level of urea, Cr, and ALP. in patients compared with the control group. Also, the results showed significant negative correlations between irisin and age, weight, height, FSG, Alb, urea, AST, ALP , ALT, Na, and vitamin D in the HD group. While significant positive correlations were found between irisin and body mass index, Cr, Ca, Po4, Tp, TC, and K, as well as significant negative correlations between vitamin D and weight, hight, BMI, FSG, Cr, Ca, PO_4, TP, Alb, urea, ALP, ALT, and Na, While significant positive correlations were found between vitamin D age, AST, and K, In conclude, it has been found that serum irisin levels and vitamin D levels both fell considerably in uremic patients receiving frequent HD. Irisin has a perfect cut-off value of 78% sensitivity and 99.9% specificity, according to a ROC analysis, suggesting it is a reliable diagnostic marker.

Keywords: End stage renal disease, hemodialysis, irisin, vitamin D, electrolytes.

1. Introduction

In 2012, Bostrom *et al*., discovered irisin (derived from the Greek goddess Iris, which means rainbow and messenger of the god) and considered it a novel cytokine. Irisin is a glycosylated protein hormone with a molecular weight of about 12 KDa, composed of 112 amino acid residues [1]. The kidneys, are a pair of reddish-brown bean-shaped organs located on 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 [2]. The kidney's primary functional and filtration unit is the nephron. Around a million nephrons may be found in each kidney. There are two primary structures in every nephron: the glomerulus and the tubule [3]. The 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 (CKD) and determining the severity of the condition [4]. With a GFR of 15 mL/min or below, people with stage 5 CKD are considered to have end-stage renal disease (ESRD). Because the kidneys have lost almost all of their function at this point, dialysis or a transplant is necessary for survival [5].

When the kidneys are unable to filter out waste and excess fluids, toxins accumulate in the blood and contribute to a general feeling of illness. As a result, the kidneys will lose their capacity to regulate blood pressure, produce a hormone that's essential for making red blood cells, and activate vitamin D, which is necessary for healthy bones. The enzyme CYP27B1 (1-hydroxylase of 25-hydroxyvitamin D) catalyzes this conversion. Genetic deficiency in CYP27B1 leads to a scarce autosomal recessive condition characterized by the onset of rickets at an extremely young age [6]. Dialysis is a therapy for people with stage 5 CKD in which toxins are removed from the blood, and drugs are utilized to restore the kidneys' functions. Kidney transplantation is an alternative therapy for end-stage renal disease [7]. Hemodialysis is a process wherein blood is filtered and reintroduced to the body through a membrane. Due to the dialysate fluid covering the membrane and maintaining a concentration gradient, substances are able to cross from the dialysate to the blood [8]. Patients at increased risk of bleeding may undergo dialysis, even if they are on anticoagulation. Although hemodialysis is the most effective therapy for ESRD [9], the kidney's 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 [10].

2. Materials 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 chosen as a control group without any chronic diseases. The study included 30 individuals with ESRD on hemodialysis (HD) and 30 subjects as a control group. The ages of all patients and the control group ranged from 25 to 60 years. The excluded criteria included patients with viral hepatitis and diabetes. Blood samples were taken from study groups (HD patients and controls). Blood samples were collected between 11:00 and 8:30 using a 5 mL syringe into a gel tube and allowed to clot at room temperature. Then, we centrifuged the sample at a speed of 3000 degrees per minute for 10 minutes to separate the serum. About 2 mL was used to determine the body mass index (BMI) [11], albumin [12], urea [13], creatinine [14],fasting serum glucose (FSG) [15], total cholesterol

(TC) [16], alanine aminotransferase (ALT) [17], aspartate aminotransferase (AST) [18], alkaline phosphatase (ALP) [19], sodium (Na^+), and potassium (K^+) [20], calcium (Ca^{+2}) [21] and phosphorus (PO_4) [22]. The residues were transferred to an Eppendorf tube and stored in a deep freezer (-20 °C) to be used to determine the levels of serum irisin [23] and vitamin D [24].

The level of plasma 25(OH)D was measured using the Enzyme-Linked Fluorescent Assay (ELFA) method by Biomerieux; Marcy-I'Etoile, France kit on a small VIDAS Biomerieux automated immunological analyzer.

2.1 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 > 0.05 and ≤ 0.05 were regarded as statistically non-significant and significant, respectively. The link between the several parameters was analyzed, and the correlation coefficient (r) was used to characterize it. Using statistical software for the social sciences (SPSS) version 23.0 and Microsoft Office 2007, this study 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 Discussion

Anthropometric and clinical features of the HD and control groups in the study are listed in **Table 1**. There was a significant increase ($p \leq 0.05$) in age, SBP, and DBP, a significant decrease ($p \leq 0.05$) in height and weight, and a non-significant decrease ($p \geq 0.05$) in BMI in the HD group as compared to the control group.

Table 1. Anthropometric and clinical features of the HD and control groups.

Parameters	Means ± SD		*p*-value
	HD (n= 30)	Control (n= 30)	
Age (years)	44.8±17.9	37.5±13.8	0.05
Height (cm)	165.1± 6.3	170.5±10.2	0.05
Weight (kg)	68.6±12.8	75.5±10.3	0.05
BMI (kg/m^2)	25.2±4.4	26.1±3.2	0.30
SBP (mmHg)	155.79 ± 8.57	119.60 ± 2.44	0.001
DBP (mmHg)	90.17 ± 3.0	78.67 ± 2.34	0.001

$p \leq 0.05$: significant, $p \leq 0.001$:high- significant, $p \geq 0.05$: non-significant.

Millions of people throughout the globe are living with CKD, making it a significant issue in public health. Importantly, hypertension (HTN) is a predictor of CKD and its ultimate ESRD manifestation. As a result, several recommendations stress the need for diagnosing and treating HTN early to prevent the disease's worsening and its associated problems in both sexes [25].

Many pathways contribute to HTN in patients with CKD, illustrating the complexity of the disease's pathogenesis. Sodium dysregulation increased sympathetic nervous system activity, and variations in renin, angiotensin, and aldosterone system activity are all examples of these pathogenic pathways [26]. There is a growing consensus throughout the globe that the ageing of the population poses significant health risks. CKD, 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 is for Kidney Transplantation (dialysis or kidney transplant) [27].

The examination of kidney function in HD and control groups in the study is listed in **Table 2**. There was a significant decrease ($p \leq 0.05$) in albumin and albumin to creatinine ratio (ACR), a substantial increase ($p \leq 0.05$) in urea, creatinine, and eGFR, and a significant decrease in Tp in the HD group as compared to the control group.

Table 2. Kidney function of the HD and control groups.

Parameters	Means ± SD		*p*-value
	HD (n= 30)	Control (n= 30)	
eGFR (ml/min/1.73m^2)	15.40±0.45	110.23±4.45	0.001
Albumin (g/L)	3.70±0.40	4.10±0.40	0.001
Urea (mg/dL)	128.70±35.40	25.90±6.90	0.001
Creatinine (mg/dL)	9.70±2.40	0.70±0.20	0.001
ACR (mg/g)	0.38±0.16	5.85 ± 2.0	0.001

$p \leq 0.05$: significant, $p \leq 0.001$: high- significant, $p \geq 0.05$: non-significant.

Chronic renal failure patients have an 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 [28, 29].

Examinations of liver function, total cholesterol, and FSG in HD and control groups in the study are listed in **Table 3**. There was a significant decrease ($p \leq 0.05$) in TC, AST, and ALT, as well as a significant increase in ALP and a non-significant decrease in FSG in the HD group as compared to the control group.

Table 3. Biochemical parameters of the HD and control groups.

Parameters	Means ± SD		*p*-value
	HD (n= 30)	control (n= 30)	
FSG (mg/dL)	96.20±24.30	96.40±10.10	0.50
TC (mg/dL)	166.18 ± 20.03	178.6±32.70	0.05
AST (U/L)	12.90±3.60	28.80 ±5.60	0.001
ALT (U/L)	15.70±5.70	32.70±8.80	0.001
ALP (U/L)	284.90±202.30	80.6±18.80	0.001

$p \leq 0.05$: significant, $p \leq 0.001$:high- significant, $p \geq 0.05$: non-significant.

Monitoring hepatic enzyme levels has been found to be a valuable prognostic predictor in CKD and ESRD [30, 31]. Many studies have shown that serum aminotransferase levels frequently decrease in CKD patients. Inhibition of tyrosine kinase activity in endothelial cells, which in turn impairs endothelial NO synthase function; promotion of high production of reactive oxygen species (ROS); and apoptosis due to increased degradation of pyrophosphate, which promotes atherosclerotic lesions in the vascular wall, are all possible mechanisms connecting ALP with endothelial dysfunction [32, 33].

High levels of ALP in CKD patients may be used as an indicator of declining kidney function, as shown by the research of Angela [36], who discovered a strong inverse correlation between eGFR and ALP [30].

Table 4. Level of Na, K, Ca, and iPO4 in HD and control groups.

Parameters	Means ± SD		*p*-value
	HD (n= 30)	control (n= 30)	
Irisin	17.5±16.10	32.0±7.0	0.001
Vitamin D	174.8±52.20	229.8±69.0	0.001

$p \leq 0.05$: significant, $p \leq 0.001$:high- significant, $p \geq 0.05$ non-significant.

The levels of serum electrolytes (Na^+, K^+, Ca^{+2}, and iPO_4) in the HD and control groups in the study are listed in **Table 4**. There was a significant decrease ($p \leq 0.05$) in Na, K, and Ca and a non-significant decrease in iPO4 in the HD group as compared to the control group.

According to a study by Luo *et al*., patients with CKD had abnormally low levels of K^+ in their blood. Some HD patients (eGFR 60 or below) had their K^+ ion levels analyzed to learn more about the nuances of their blood potassium levels. Luo *et al*., identified a strong correlation between an increase in body fluids and a higher mortality rate due to K^+ ions, suggesting that a slow GFR raises blood K^+ levels, putting patients at risk of dying [35]. Since the dialysis machine flushes extra K^+ from the kidneys, blood K^+ levels drop after treatment. Another major contributor to low K^+ levels is vomiting, which causes the mineral to be lost from the digestive system [36].

The activation of vitamin D during dialysis promotes the re-absorption of Ca^{+2} lost into the urine by the kidney and tubes, and the transfer of Ca^{+2} from the dialysate to the patient's blood also contributes to an increase in the patient's ionized calcium levels after dialysis [37].

Table 5 lists the levels of serum irisin and vitamin D in the HD and control groups. There was a significant decrease in serum irisin and vitamin D in the HD group compared to the control group.

Table 5. Serum Irisin and vitamin D levels of HD and control groups.
$p \leq 0.05$: significant, $p \leq 0.001$: high- significant, $p \geq 0.05$: non-significant.

Parameters	Means ± SD		*p*-value
	HD (n= 30)	Control (n= 30)	
Na^+	108.2±17.40	141.1±1.40	0.001
K^+	3.4±1.10	4.0±0.40	0.05
Ca^{+2}	4.4±1.60	5.0±1.50	0.05
iPO_4	3.4±0.80	3.6±0.50	0.30

Patients on HD may have reduced muscle volume compared to those with CKD, suggesting that this might be a reason for the decrease in irisin level. Previous studies have demonstrated that the amount of muscle mass influences the production of irisin by muscles [38].

Another study 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 irisinemia. 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 [39].

When renal function falls, so does the body's capacity to convert 25(OH)D to 1,25$(OH)_2$D (1,25 dihydroxy vitamin D or calcitriol). It is also possible that the kidneys' capacity to absorb 25 (OH)D might be compromised, leading to insufficient levels of the vitamin [40].

There were significant negative correlations between serum irisin and age, weight, height, FSG, Alb, urea, AST, ALP, ALT, Na, and vitamin D levels in the HD group. At the same time, significant positive correlations were found between irisin and BMI, Cr, Ca, PO_4, TC, and K^+ levels in the HD group, as shown in **Table 6**.

Table 6. Correlation between serum irisin with other parameters in control.

Groups	Irisin	Correlation coefficients(r)		*p*-value	
Parameters		**Control**	**HD**	**Control**	**HD**
Age		- 0.01	-0.01	0.000	0.000
Weight		- 0.19	-0.04	0.000	0.000
Height		0.11	-0.09	0.000	0.000
BMI		- 0.33	0.003	0.000	0.000
FSG		- 0.20	-0.12	0.000	0.000
Cr		0.03	0.01	0.000	0.000
Ca^{+2}		0.14	0.53	0.000	0.000
PO_4		0.07	0.13	0.000	0.000
Alb		- 0.17	-0.21	0.000	0.000
Urea		0.125	-0.05	0.000	0.000
AST		0.211	-0.13	0.000	0.000
ALP		0.08	-0.17	0.000	0.000
ALT		0.04	-0.04	0.000	0.000
Na^+		0.06	-0.17	0.000	0.000
K^+		-0.29	0.04	0.000	0.000
Vitamin D		-0.006	-0.24	0.000	0.000

There were significant negative correlations between vitamin D and weight, height, BMI, FSG, Cr, Ca^{+2}, PO_4, Alb, urea, ALP, ALT, and Na levels in the HD group. While significant positive correlations were found between vitamin D and age, AST, and K^+ levels in the HD group as shown in **Table 7**.

In this study, there is no significant correlation between the duration of dialysis and the level of vitamin D. The current findings were in agreement with those of those who measured 25-hydroxyvitamin D levels in 120 HD and 31 peritoneal dialysis patients and found that there was no correlation between vitamin D levels and the duration of dialysis. Moreover, it showed that there is no significant correlation between the duration of dialysis and the level of vitamin D [41]. The ROC test for irisin showed a perfect cutoff value with 78% sensitivity and 99.9% specificity, which indicates it is considered a good diagnostic marker. The cutoff value is lower than 23.2 representatives of patients. The result of Irisin was interesting as compared to the results of urea and creatinine, as shown in **Table 8**, **Figures 1** and **2**.

Table 7. Correlation between serum vitamin D with other parameters in HD and control groups.

Groups	Vitamin D	Correlation coefficients (r)		*p*-value	
Parameters		**Control**	**HD**	**Control**	**HD**
Age		0.21	0.26	0.000	0.000
Weight		0.11	-0.08	0.000	0.000
Height		-0.12	-0.20	0.000	0.000
BMI		0.24	-0.02	0.000	0.000
FSG		0.16	-0.11	0.000	0.000
Cr		0.29	-0.17	0.000	0.000
Ca^{+2}		0.28	-0.16	0.000	0.000
PO4		-0.53	-0.02	0.000	0.000
Alb		-0.35	-0.05	0.000	0.000
Urea		0.08	-0.09	0.000	0.000
AST		0.14	0.24	0.000	0.000
ALP		-0.11	-0.20	0.000	0.000
ALT		-0.13	-0.02	0.000	0.000
Na^{+}		0.12	-0.06	0.000	0.000
K^{+}		0.08	0.25	0.000	0.000

Table 8. The ROC for HD and control groups**.**

Test Result Variable (s)	Area%	Sensitivity %	Specificity %	Cut-off value	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
Irisin	91%	78%	100%	16.5	0.00	0.83	0.99
Vitamin D	69%	65%	99%	187	0.01	0.55	0.83
Urea	100%	100%	100%	53.8	0.00	1.0	1.0
Cr	100%	100%	1005	2.5	0.00	1	1

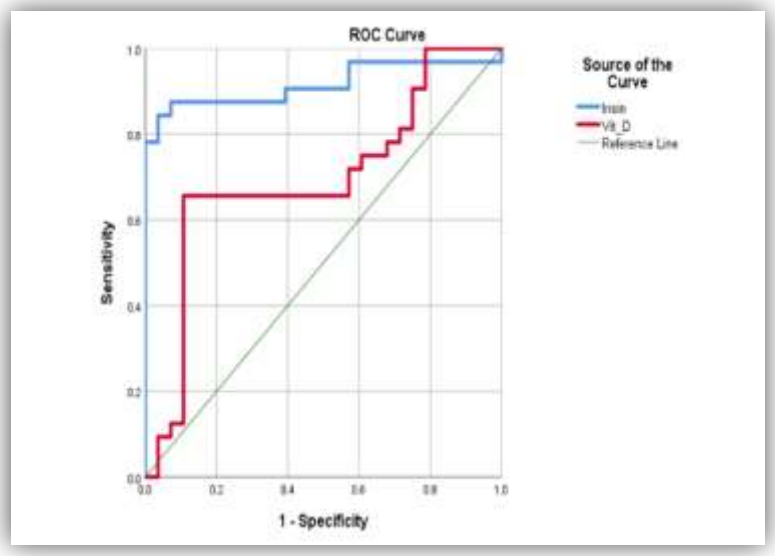

Figure 1. The ROC of the ability for irisin as a good diagnostic marker**.**

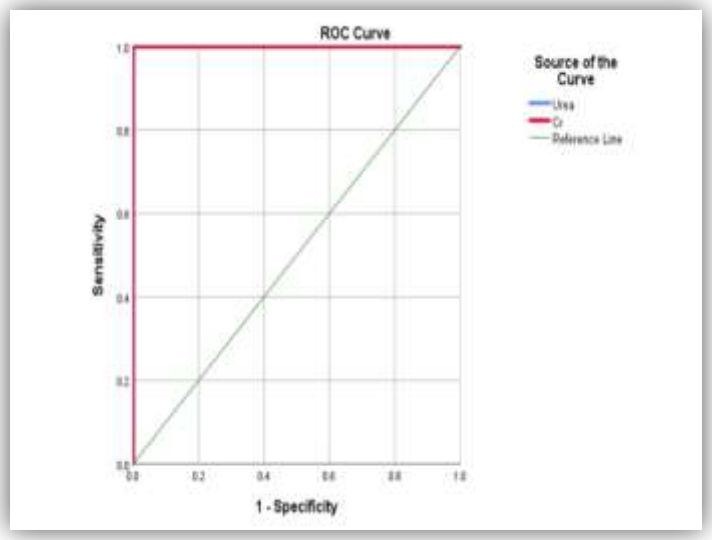

Figure 2. The ROC of urea and creatinine**.**

4. Conclusion

Serum irisin levels were shown to be lower in our research among uremic patients receiving routine HD; also, vitamin D levels significantly decreased. The ROC test for irisin showed a perfect cutoff value with 78% sensitivity and 99.9% specificity, which indicates it is considered a a good diagnostic marker. The cutoff value is lower than 23.2 representatives of patients. The result of irisin was interesting as compared to the results of urea and creatinine.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

The samples were gained according to Local Research Ethics Committee approval in Iraqi Ministry of Health No.339 on 25/8/2022.

References

1. Leustean, L.; Preda, C.;Teodoriu, L.; Mihalache, L.; Arhire, L.; Ungureanu, M. Role of irisin in endocrine and metabolic disorders-possible new therapeutic agent. *Applied Sciences* **2021**, *11(12)*, 5579. https://doi.org/10.3390/app11125579.
2. Koeppen, B.; Stanton, B. Renal physiology 5th ed., *Elsevier* **2015**, *18*,15-20. https://doi.org/10.1016/C2009-0-62255-8.

3. Preston, R. ; Wilson ,T. Physiology. Lippincott Williams and Wilkins. *Canadian Medical Association Journal* **2012**, *21*, 313-327. https://books.google.iq/books/about/Physiology.html?id=vpFoCWvjr6EC&redir_esc=y.
4. Garasto, S.; Fusco, S.; Corica, F.; Rosignuolo, M.; Marino, A., Montesanto, A., De Rango, F.; Maggio, A.; Mari, V.; Corsonello, A.; Lattanzio, F. Estimating glomerular filtration rate in older people, *BioMed Research International* **2014**, *2014*, 916542. https://doi.org/ 10.1155/2014/916542.
5. Levin, A.; Hemmelgarn, B.; Culleton, B.; Tobe, Sh.; McFarlane, P.; Ruzicka, M.; Burns, K.; Manns, B.; White, C.; Madore, F.; Moist, F.; Klarenbach, S.; Barrett, B.; Foley, R.; Jindal, K.; Senior, P.; Pannu, N.; Shurraw, S.; Akbari, A.; Cohn, A.; Reslerova, M.; Deved, V.; Mendelssohn, D.; Nesrallah, G.; Kappel, J.; Tonelli, M.; Canadian Society of Nephrology. Guidelines for the management of chronic kidney disease. *Canadian Medical Association Journal* **2008**, *179(11)*,1154-1162. https://doi.org/10.1503/cmaj.080351.
6. Ahmed, S.Y.A. ; Albayaty, N.K. A study of hepcidin levels and other biochemical parameters in woman with osteoporosis with type 2 diabetes mellitus. *Ibn AL-Haitham Journal For Pure and Applied Sciences* **2022**, *35(4)*,183–193. https://doi.org/10.30526/35.4.2867.
7. Unruh, M.L; Hess, R. Assessment of health-related quality of life among patients with chronic kidney disease. *Adv Chronic Kidney* **2007**, *14(4)*,345-352. https://doi.org/ 10.1053/j.ackd.2007.07.011.
8. Beers, M.H.; Porter, R.S.; Jones, T.V.; Kaplan, J.L.; Berkwits, M. Renal replacement therapy. The Merck Manual of Diagnosis and Therapy, 18th ed., Whitehouse Station, *NJ: Merck Research Laboratories*, **2006**; pp.1989-1996. http://www.mentalhealthpromotion.net/?i=training.en.bibliography.1397.
9. Tortora GJ; Derrickson, B . The urinary system. In: Principles of Anatomy and Physiology. 11th ed., Hoboken, *NJ: John Wiley & Sons*. **2006**; Ch.*14*, pp. 992-1035.
10. Boga, MS.; Sönmez, MG. Long-term renal function following zero ischemia partial nephrectomy, *Res Rep Urol*. **2019**,*11*,43-52. https://doi.org/ 10.2147/RRU.S174996.
11. Kasper, D.; Fauci, A.; Hauser, S.; Longo, D.; Jameson, J.; Loscalzo, J. Harrison's principles of internal medicine, New York, NY, USA, *Mcgraw-hill* **2012**, Ch. 1, pp.1813-1814. https://doi.org/10.1001/jama.308.17.1813-b.
12. Doumas, BT;Watson ,WA; Biggs, HG. Albumin standard and the measurement of serum Albumin with Bromocresol green, *Clin Chim Acta*. **1971**,*31*,87-96. https://doi.org/10.1016/0009-8981(71)90365-2.
13. Chaney, A.L.; Marbach, E.P. Modified reagents for determination of urea and ammonia,*Clinical chemistry* **1962**, *8*,130-132. https://doi.org/10.1093/clinchem/8.2.130.
14. Allen, L.C.; Michalko, K. More on cephalosporin interference with creatinine determinations. *Clinical Chemistry* **1982**, *28*, 555-556. https://doi.org/10.1093/clinchem/28.3.555.
15. Massod, M.F. Nonparametric percentile estimates of clinical normal ranges, *The American journal of medical technology* **1977**,*43*(*3*),243-252. PMID: 848498.
16. Nayak, S. Manipal manual of clinical biochemistry. *Jaypee Brothers Publishers* **2007**,*15*,155.
17. Bergmeyer, H. U.; Herder, M. International federation of clinical chemistry (IFCC). *J. Clin. Chem. Clin. Biochem*. **1986**, *24(7)*,497-510. https://doi.org/10.1515/9783110863697-002.
18. Winn-Deen, E. S.; David, H.; Sigler, G.; Chavez, R. Development of a direct assay for alpha-amylase. *Clinical Chemistry* **1988**, *34(10)*,2005-2008. https://doi.org/10.1093/clinchem/34.10.2005.
19. Belfield, A.; Goldberg, D. Colorimetric determination of alkaline phosphatase activity. *Enzyme* **1971**, *12*(*5*), 561-568.
20. Stove, V.; Slabbinck, A.; Vanoverschelde, L.; Hoste, E.; De Paepe, P.; Delanghe, J. How to solve the underestimated problem of overestimated sodium results in the hypoproteinemic patient. *Critical Care Medicine* **2016**, *44(2)*,83-88. https://doi.org/10.1097/CCM.0000000000001304.

21. Thyer, B.A. Bibliography of randomized controlled experiments in social work (1949-2013): Solvitur ambulando, *Research on Social Work Practice* **2015**, *25(7)*,290. https://doi.org/10.1177/1049731515599174.
22. Drewes, P.A. Direct colorimetric determination of phosphorus in serum and urine. *Clinica Chimica Acta* **1972**, *39(1)*,81-88. https://doi.org/10.1016/0009-8981(72)90302-6.
23. Wrann, C.D.; White, J.P.; Salogiannnis, J.; Laznik-Bogoslavski, D.; Wu, J.; Ma, D.; Spiegelman, B. M. Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. *Cell Metabolism* **2013**, *18(5)*, 649-659. https://doi.org/10.1016/j.cmet.2013.09.00.
24. Wang, T.; Bengtsson, G.; Kärnefelt, I.; Björn, L.O. Provitamins and vitamins D_2 and D_3 in Cladina spp. over a latitudinal gradient: possible correlation with UV levels. *Journal of Photochemistry and Photobiology B: Biology* **2001**, *62(1-2)*,118-122. https://doi.org/ 10.1016/s1011-1344(01)00160-9.
25. Weldegiorgis, M. ; Woodward, M. The impact of hypertension on chronic kidney disease and end-stage renal disease is greater in men than women: a systematic review and meta-analysis. *BMC* **2020**, *21(1)*, 506. https://doi.org/10.1186/s12882-020-02151-7.
26. Pugh, D.; Gallacher, P.J.; Dhaun, N. Management of hypertension in chronic kidney disease. *Drugs* **2019**, *79(4)*,365–379. https://doi.org/10.1007/s40265-019-1064-1.
27. Hussein, N.A.; Alaa, T.; Shafeeq, N.K. Determination of Glucagon-Like Peptide-1 and Dipeptidyl Peptidase-4 Levels in Diabetic Nephropathy Patients. *Indian Journal of Public Health Research* **2019**, *10(2)*,843. https://doi.org/10.5958/0976-5506.2019.00400.5.
28. Carrero, J.J.; Stenvinkel, P.; Cuppari, L.; Ikizler, T.A.; Kalantar-Zadeh, K.; Kaysen, G.; Mitch, W.E.; Price, S.R.; Wanner, Ch.; Wang, A.Y.M.; ter Wee, P.; Franch, H.A. Etiology of the protein-energy wasting syndrome in chronic kidney disease: a consensus statement from the International Society of Renal Nutrition and Metabolism (ISRNM). *J Ren Nutr* **2013**, *23(2)*,77-90. https://doi.org/ 10.1053/j.jrn.2013.01.001.
29. Al-Taiee, T.A.K.; Al-Shammaa, N.M.; Aljber, A A. Study of the anti-diuretic hormone (ADH) on end stage renal failure disease (ESRD) pre-hemodialysis in Iraqi patients. *Ibn AL-Haitham Journal For Pure and Applied Sciences* **2019**, *32(2)*,30–37. https://doi.org/10.30526/32.2.2135.
30. Ray, L.; Nanda, S.K.; Chatterjee, A.; Sarangi, R.; Ganguly, S. A comparative study of serum aminotransferases in chronic kidney disease with and without end-stage renal disease: Need for new reference ranges. *Int J Appl Basic Med Res* **2015**, *5(1)*,31. https://doi.org/ 10.4103/2229-516X.149232.
31. Oyelade, T.; Alqahtani, J.; Canciani, G. Prognosis of COVID-19 in patients with liver and kidney diseases: An early systematic review and meta-analysis. *Trop Med Infect Dis* **2020**, *5(2)*,80. https://doi.org/10.3390/tropicalmed5020080.
32. Wu Z, Yang D. A meta-analysis of the impact of COVID-19 on liver dysfunction. *Eur J Med Res* **2020**, *25(54)*,1-9. https://doi.org/10.1186/s40001-020-00454-x.
33. Romanelli, F.; Corbo, A.; Salehi, M.; Yadav, M. C.; Salman, S.; Petrosian, D.; Savinova, O.V. Overexpression of tissue-nonspecific alkaline phosphatase (TNAP) in endothelial cells accelerates coronary artery disease in a mouse model of familial hypercholesterolemia. *PLOS One* **2017**, *12(10)*, e0186426. https://doi.org/10.1371/journal.pone.0186426.
34. Sciacqua, A.; Tripepi, G.; Perticone, M.;Cassano, V.; Fiorentino, T. V.; Pititto, G. N.; Perticone, F. Alkaline phosphatase affects renal function in never-treated hypertensive patients: Effect modification by age. *Sci Rep* **2020**, *10(1)*,9748. https://doi.org/10.1038/s41598-020-66911-z.
35. Farhan, L.O.; Taha, E.M.; Farhan, A.M. A case control study to determine macrophage migration inhibitor, and N-telopeptides of type I bone collagen Levels in the sera of osteoporosis patients. *Baghdad Science Journal* **2022**, *19(4)*,0848. https://doi.org/10.21123/bsj.2022.19.4.0848.
36. Dolson, G.M.; Ellis, K.J.; Bernardo, M.V.; Prakash, R.; Adrogué, H. Acute decreases in serum potassium augment blood pressure. *Am J Kidney Dis* **1995**, *26(2)*,321–326. https://doi.org/ 10.1016/0272-6386(95)90652-5.

37. Khaled, M.D.; Faiz, A.; Abdelgader, A.T.; Bioprabhu, S.; Elshafie, E.I. Effect of haemodialysis on some metabolic products of ckd patients in Libya. *WJPPS* **2015**,*4*,45-54. https://storage.googleapis.com/journal-uploads/wjpps/article_issue/1448862833.pdf.
38. Liu, J.J.; Wong, M.D.; Toy, W.C.; Tan, C.S.; Liu, S.; Ng, X.W.; Lim, S.C . Lower circulating irisin is associated with type 2 diabetes mellitus. *J Diabetes Complications* **2013**, *27(4)*,365-369. https://doi.org/10.1016/j.jdiacomp.2013.03.002.
39. Rodríguez-Carmona, A.; Fontán, M.P.; Alvarellos, S.S.; Falcón, T.G.; Bello, M.L P.; Muniz, A.L. Serum levels of the adipomyokine irisin in patients with chronic kidney disease. *Nefro* **2016**, *36(5)*, 496-502. https://doi.org/ 10.1016/j.nefro.2016.05.019.
40. Christodoulou, M.; Aspray, T.; Schoenmakers, I. Vitamin D supplementation for patients with chronic kidney disease: A systematic review and meta-analyses of trials investigating the response to supplementation and an overview of guidelines, *Springer* **2021**, *109(2)*,157-178. https://doi.org/ 10.1007/s00223-021-00844-1.
41. El-Arbagya, A.; El-Zorkanya, K.; Helwab,M.; El-Khalifac, E. Assessment of vitamin D in hemodialysis patients. *Menoufia Medical Journal* **2020**, *33(1)*,122-126. https://doi:10.4103/mmj.mmj31718.