Evaluation of IL-6 and TNFα in Iraqi Patients with Chronic Renal Failure

Athraa Zaidan Hassan *[1], Najah Ali Mohammed [2] and Ibrahim jawad kadhem[3]

[(1)] Medical Laboratory Science Technology / Middle Technical University - College of Health & Medical Technology.
[(2)] Immunology /Middle Technical University / Medical Technical Institute / Baghdad.
[(3)] Medical Laboratory Technicians / Ministry of Health / Baghdad Health AL-Karkh.
Corresponding author E-mail(*) :azh.mousawi@gmail.com

Abstract

Chronic kidney disease (CKD) is a major social health problem. CKD is ranked –5rd amongst life threatening disease in Iraq, the death rate in 2015 of about 6879 patients because of renal failure according to the statistics of the Republic of Iraqi-Ministry of Health/Environment. We aimed to evaluated (IL-6 & TNF-α) in Iraqi patients with chronic renal failure and assess some biochemical parameters include (urea, creatinine, sodium, potassium and calcium).This study included 60 cases and classified as 30 patients with chronic renal failure, these patients with age range (15years and above) who were admitted to the Al-kadimyiah Teaching Hospital, Baghdad Teaching Hospital during the period from December 2020 to March 2021and compared with 30 apparent healthy controls with age range (15-65). Laboratory was depended on using spectrophotometry for detection biochemical tests. IL-6 & TNFα levels were estimation by Enzyme Linked Immuno-sorbent assay (ELISA). It was observed that majority of patients with chronic renal failure within age group (40-60) years that account (60%), most of these patients were females that constituted (73.3%). Furthermore, preponderance diseases associated with chronic renal patients were associated with Diabetes Mellitus (23.33%).

Regarding biochemical parameters, it was observed that serum urea (S.urea), serum creatinine (S.creatinine) ,serum potassium (S. K) were increased in chronic renal cases and decrease in Serum calcium (S. Ca,),Serum sodium (S. Na) in comparison with healthy control group. Considering some cytokines (IL-6, TNFα) revealed increase levels of IL-6 &TNFα significantly (26.26 ±1.48),(92.96 ±1.80) respectively in chronic renal patients when compared with control group(13.02 ±0.34),(66.55 ±0.45) respectively. Highly significant elevated of pro-inflammatory cytokines which include IL-6&TNFα in chronic renal diseases indicated these cytokines participate in the pathophysiology of reduced renal function and role of these cytokines as principle mediators of inflammatory reaction in renal damage and these cytokines could be potential therapeutic targets.

Keywords: *Chronic renal disease, Biochemical tests, TNFα, IL-6.*

P-ISSN: 2664-0562
E-ISSN:2664-0554

تقييم انتر لوكين 6 و عامل التنخر الورمي الفا في المرضى العراقيين المصابين بالفشل الكلوي المزمن

أ.م.د. عذراء زيدان حسن[1] ، أ.د. نجاح علي محمد[2] وَ تقني مختبرات ابراهيم جواد كاظم [3]

الخلاصة

مرض الكلى المزمن (CKD) هو مشكلة صحية اجتماعية كبرى. احتل مرض الكلى المزمن المرتبة الخامسة بين الأمراض التي تهدد الحياة في العراق ، حيث بلغ معدل الوفيات في عام 2015 حوالي 6879 مريضاً بسبب الفشل الكلوي حسب إحصائيات جمهورية العراق- وزارة الصحة / البيئة. هدفنا إلى تقييم (TNF-α & IL-6) في المرضى العراقيين المصابين بالفشل الكلوي المزمن وتقييم بعض المعايير البيوكيميائية تشمل (اليوريا ، الكرياتينين ، الصوديوم ، البوتاسيوم والكالسيوم). اشتملت هذه الدراسة على 60 حالة مصنفة على أنها 30 مريضا يعانون من الفشل الكلوي المزمن ، هؤلاء المرضى من الفئة العمرية (15 سنة فأكثر) الذين تم إدخالهم إلى مستشفى الكاظمية التعليمي ، مستشفى بغداد التعليمي خلال الفترة من كانون الأول 2020 إلى آذار 2021 ومقارنة بـــ 30 اشخاص ظاهريا يبدو اصحاء مع الفئة العمرية (15-65). اعتمد التشخيص المختبري على استخدام القياس الطيفي للكشف عن الاختبارات البيوكيميائي وتم تقدير مستويات IL-6 و TNFα بواسطة مقايسة المواد الماصة المناعية المرتبطة بالإنزيم (ELISA). لوحظ أن غالبية مرضى الفشل الكلوي المزمن ضمن الفئة العمرية (40-60) سنة والتي تمثل (60٪) وان معظم هؤلاء المرضى هم من الإناث بنسبة (73.3٪). علاوة على ذلك ، ارتبطت أمراض المصاحبة لمرضى الكلى المزمن بمرض السكري (23.33٪). فيما يتعلق بالمعايير الكيميائية الحيوية ، لوحظ زيادة اليوريا (S.urea) ، والكرياتينين (S.creatinine) ، والبوتاسيوم (S) والصوديوم (S. Na) بالمقارنة مع مجموعة السيطرة. بالنظر إلى بعض السيتوكينات (IL-6، TNFα) كشفت عن زيادة مستويات IL-6 و TNFα بشكل ملحوظ (26.26 ± 1.48) ، (92.96 ± 1.80) على التوالي في مرضى الكلى المزمنين بالمقارنة مع مجموعة السيطرة (13.02 ± 0.34) ، (66.55 ± 0.45)) على التوالى. تشير الزيادة الكبيرة في السيتوكينات المؤيدة للالتهابات والتي تشمل IL-6 و TNFα في أمراض الكلى المزمنة إلى أن هذه السيتوكينات تشارك في الفيزيولوجيا المرضية لوظيفة الكلى المنخفضة ودور هذه السيتوكينات كوسيط رئيسي للتفاعل الالتهابي في التلف الكلوي ويمكن أن تكون هذه السيتوكينات اهداف علاجية محتملة .

الكلمات المفتاحية : *مرض الكلى المزمن, الفحوصات الكيمائية, عامل التنخر الورمي الفا,انترلوكين -6.*

Introduction

Chronic Renal Failure (CRF) also called chronic kidney disease (CKD) is defined as the presence of kidney damage or an estimated glomerular filtration rate (GFR) less than 60 ml/min, persisting for 3 months or more. It is a progressive loss of kidney function ultimately resulting in the need for renal replacement therapy (dialysis or transplantation) [1] Kidney damage refers to pathologic abnormalities either suggested by imaging studies or renal biopsy, abnormalities in urinary sediment, or increased urinary albumin excretion rates [2]. Worldwide, CKD accounted for three million and over two million of life-years lost in 2012 [3]. Various symptoms and disorders including water electrolyte balance disorders, metabolic acidosis, anemia, hypertension, hypophosphatemia with bone disease [4].

P-ISSN: 2664-0562
E-ISSN:2664-0554

Risk factor for kidney disease include race, gender, age, and family history are highly important. Moreover, smoking, hypertension, and diabetes mellitus, obesity can also lead to kidney disease [5,6]. CKD presented with elevated blood urea and creatinine are found during routine examination [7,8]. Chronic inflammation is prevalent in patients with chronic renal failure [9]. Numerous studies have demonstrated that chronic inflammation may contribute to the morbidity and mortality among dialysis patients [10]. Indeed, deterioration of renal function in uremia increases risk to infection and various abnormalities of the immune system [11] In addition, the repeated dialysis treatments in patients lead to leucocyte activation and consequently the production of cytokines. [12].

The upregulation and presence of cytokines such as; tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6, in the blood contribute to chronic inflammation [13]. Tumor necrosis factor (TNF-α) is one of an important proinflammatory cytokine and essential factor of inflammatory tissue injury. Which release by dendritic cells (DCs) in the renal interstitium . Most researchers reported a role of TNF in acute and chronic renal disease pathogenesis. Thus, after renal injury the early proinflammatory mediator is TNF-α. [14]. Interleukin-6 (IL-6) is a proinflammatory cytokine that was first identified as a B-cell stimulatory factor, IL-6 has many functions in the regulation and coordination of the immune system, metabolism, and nervous system. It plays a role in the body's defense against infection, in many regenerative processes [15].

The elevated plasma IL-6 level is commonly observed in CKD patients [16].it has been suggested that Interleukin-6 accelerates the progression of CKD not only by aggravating kidney injury but also by initiating its complications, especially the chronic vascular disease (CVD). It is demonstrated that IL-6 initiates the endothelial injury mainly *via* reducing endothelial nitric oxide synthase (eNOS) and adiponectin (an anti-atherogenic adipokine) expression. [17].

Materials and Methods

The current study included 30 patients with acute renal failure, these patients with age group (15-65) years who were admitted to the AL- Kadimyiah Teaching Hospital and Baghdad Teaching Hospital during the period from December 2020 to March 2021. All patients were diagnosed as having chronic renal failure based on previous medical reports, laboratory tests and clinical examination by consultant nephrologists. The results were compared with (30) healthy subjects with age group (15-65) years as a control group.

P-ISSN: 2664-0562
E-ISSN:2664-0554

The control group subjects were selected as healthy individuals without a history of kidney disease, current or previous kidney stones and not suffering from diabetes mellitus or hypertension depending on previous medical reports and laboratory investigation. blood sample (5ml) were taken from each patient and healthy individuals. We collected serum according to the technique low speed centrifugation at 3000 × *g* at 4 °C, for 10 min. The serum was removed, aliquoted and stored at -20 °C until time of assay. Then serum were used for detection of biochemical parameters include (urea, creatinine, sodium, potassium and calcium) in addition for cytokines analysis which include IL-6, TNFα. Laboratory diagnosis was depended on using auto-analyzer known as Cobas c 311, Germany for estimation serum urea and creatinine while serum calcium, potassium, sodium were measured by using auto-analyzer known as Beckman coulter au480, USA. in addition IL-6, TNFα were identified by ELISA test, Human reader, Germany. Methods was conducted according to the instructions of manufacturing companies leaflet.

Data analysis

In this study Chi-square test was used to detect the significances between variables. All the statistical analysis was done by SPSS program (version-20). Data was presented as Mean ± Stander error. P-value was considered significant less than < 0.05.

Result

The current study involved 60 samples divided into two groups (30 chronic renal failure and 30 healthy apparent controls). Age of the study population ranged from 15-90 years**.** Table (1) shows Chronic patients aged (40-60) had the highest prevalence of 18 (60%) when compared with other groups while only 6 (20%) of patients within age group (61-90) and 6 (20%) within (15-39). There are highly significant differences between the incidences of the different age groups among chronic renal patients (P<0.001).

In same table the study group were categorized according to gender in chronic patients group 22 (73.3%) female whereas 8 (26.7%) were male. According to gender there was non-significance difference at (p=0.107).

Also the table demonstrated other diseases associated with chronic patients group which included that 7 (23.33%) of chronic was among diabetic mellitus (DM) followed by 3 (10%) hypertension (HT) while 20 (66.6%) without any disease.

P-ISSN: 2664-0562
E-ISSN:2664-0554

Table (1): Demographic of study groups according to clinical characteristics (Age, gender and other diseases).

Characteristics		Chronic group NO (%)
Age	15-39	6 (20%)
	40-60	18 (60%)
	61-90	6 (20%)
	Total (%)	30 (100%)
	P-value	0.0007**
Gender	Male	8 (26.7%)
	Female	22 (73.3%)
	Total (%)	30 (100%)
Other Diseases	P-value	0.0001**
	Diabetic mellitus (DM)	7 (23.33%)
	Hypertension (HT)	3 (10%)
	NO DM and NO HT	20 (66.6%)
	Total (%)	30 (100%)

Results of biochemical tests between chronic renal patients group and control group listed in Table (2) .The results revealed that mean urea for chronic group was (121.78 ± 11.94) whereas mean urea for control (28.35 ± 1.42) while mean creatinine for chronic was (3.97 ± 0.43) compared to mean creatinine for control (0.668, ± 0.03) the mean serum potassium for chronic was (4.71 ± 0.13) while mean potassium for control (4.21 ± 0.08) the mean serum calcium for chronic was (8.62 ± 0.16) while mean serum calcium for control (9.22 ± 0.10) the mean serum sodium for chronic was (136.34±0.91) while serum sodium for control (139.12 ± 0.89). Statistically, highly significant difference of urea at (p = 0.001), creatinine at (p = 0.001), S. k at (p = 0.0061) and S. Ca at (p =0.0043) while significant difference of serum sodium was found at (p=0.035) between two group.

P-ISSN: 2664-0562
E-ISSN:2664-0554

Table (2): level of Biochemical tests in chronic patients and control group.

Studied Groups	Mean ± SE				
	Urea	**Creatinine**	**S. K**	**S. Ca**	**S. Na**
Chronic group	121.78 ±11.94	3.97 ±0.43	4.71 ±0.13	8.62 ±0.16	136.34 ±091
Control group	28.35 ±1.42	0.668 ±0.03	4.21 ±0.08	9.22 ±0.10	139.12 ±0.89
P-value	0.0001**	0.0001**	0.0061**	0.0043**	0.0355*
*** (P≤0.05) Significant, ** (P≤0.01) Highly Significant.S.k=serum potaasium,S.ca= serum calcium, S.Na= serum sodium**					

As shown in Table (3), the Result of IL-6 between chronic renal patients and control group which indicated that mean for chronic was (26.26± 1.80) and mean of control (13.02± 0.34) the results revealed highly significance difference at (p=0.0001). Regarding to the TNF-α level mean in chronic group was (92.96± 1.80) while mean for control (66.55± 0.45) Statistically, Highly significance at (p=0.0001).

Table (3): levels of cytokines between chronic renal patients and control group.

Studied Groups	Mean ± SE	
	IL-6	**TNF-α**
Chronic group	26.26 ±1.48	92.96 ±1.80
Control group	13.02 ±0.34	66.55 ±0.45
P-value	0.0001**	0.0001**
**** (P≤0.01) Highly Significant, IL-6= interleukin 6, TNF-α= Tumor necrosis factor, SE=Standard Error**		

The Correlation between Cytokines (IL-6 and TNF-α) and biochemical parameters among chronic renal failure were shown in Table (4). The results clarified that there was non significance between urea, creatinine, S. K, S. Ca and S. Na and cytokines parameters.

P-ISSN: 2664-0562
E-ISSN:2664-0554

Table (4): Relationship between Cytokine parameters and biochemical parameters among chronic renal patients.

Pearson Correlation		Biochemical parameter				
		Urea	**Creatinin**	**S. Na**	**S.ca**	**S. K**
IL-6	R	0.21	-0.23	0.09	-0.15	**0.17**
	P- value	0.251	0.180	0.502	0.331	**0.294**
	C/S	NS	NS	NS	NS	**NS**
TNF-α	R	-0.02	0.31	-0.02	0.32	**-0.28**
	P- value	0.973	0.089	0.973	0.083	**0.102**
	C/S	NS	NS	NS	NS	**NS**

R*=Pearson correlation coefficient, C/S= correlation significant, NS= Non significant

Discussion

Chronic kidney disease (CKD) is a major social health problem. In the current study demonstrated that highest prevalence (60%) of chronic renal failure within age group (40-60) years similar results were found by other Study [18] which confirmed that a high prevalence of CKD in the elderly less than (65 years). Also in this study, the CKD prevalence in our elderly population was 20% within age group more than 60 years in the same table. this results shown some compatibility to other studies. [19, 2] which reported that age group more than 60 years constituted (25.8%) from all cases of CKD patients. Regarding data of the National Institutes of Health in the United State [21] CKD is more common in people aged 65 years or older (38%) than in people aged 45–64 years (12%) or 18–44 years (6%) and those results were unlike with the current study. this variation due to the sample size, geographic distribution, life style different from country to country.

It was proposed that growing prevalence of decreased renal function in older persons can be due to an increase in age-related risk factors for progression to the CKD such as diabetes, hypertension and cardiovascular disease [2] however, aging undergoes several changes in body that impact kidney function, so GFR declines with age [2].

In concordance to other studies [20 ,22,24] our study showed a higher prevalence of CKD in women (73.3%) compared with men (26.7%). So the female gender was the strongest risk factor for CKD in the current study. It may be a result of the difference between women and men in glomerular structure, glomerular haemodynamics, muscle mass, and the hormone metabolism , Additionally, these days the higher CKD prevalence in women might be caused by lower physical activity and high prevalence of cardiometabolic risk factor [25].

P-ISSN: 2664-0562
E-ISSN:2664-0554

Moreover, other Study [26] by Population-based studies indicate that CKD epidemiology differs by sex, affecting more women than men. The result of this study in line with present study. In Iraq study in 2020 [27] which observed that high percentage of CKD in male (60.8%) more than female (39.20 %). this results disagreement with our data. the reason for this variation may be to the difference in sample size**.** The present study indicated that in CKD patients the level of urea and serum creatinine were highly significantly increased ($p \leq 0.01$) in comparison to healthy group. These results were agreed to the other studies [28, 29] which explained that essential solute were eliminated by the kidneys include serum creatinine and blood urea, also exposed that urea was the first organic solvent identified in CKD patients' blood. In chronic kidney disease if the kidney had lost its ability to remove nitrogenous wastes from blood, these substances will accumulate leading to raise in urea and creatinine levels. Blood urea was elevated when renal functions falls to around 25-50% therefore it considered a sensitive indicator of renal disease [30] but creatinine level slightly increase due to damage to the kidney therefore decline GFR due to inflammation of the kidney [31]. It has been noticed in present study that the potassium increase in CKD group in compared with healthy group with statistically highly significant differences ($p \leq 0.01$). This results resembling with other observations [29,32].

In CKD hyperkalemia is very common and life threatening electrolyte disorder [33]. It becomes highly prevalent as CKD advances [6]. As well as our observation shown that significant decrease in serum sodium in CKD patients group as paralleled with their associated healthy group. These findings like with other trends [28,34] were demonstrated that Hyponatremia is predominantly increase in dialysis patients, because of hypotonic fluid intake or excess water. Moreover, other study [35] which explained that prevalence of hypernatremia, but not hyponatremia, increased in patients with more progressive kidney disease. These results unlike with our data. Potential explanations for heterogeneity included the severity of the disease, sample size, study design, cut off value that used for estimation level of serum sodium in addition the measurement of serum sodium levels at a single time point could have resulted in the misclassification of dysnatremia due to the fluctuation of sodium values between dialysis sessions [26].

In addition, the current observation appeared that mean of S. Ca was significant low among CKD in comparing with control group. This results homogenous with other studies [36,37]. In Iraqi, in 2018 [29] which identified there was decrease in the concentration of total calcium in serum of CKD patients group in comparing with control group. These results agreement with our results**.** In contrast to the present study, previous studies [38,39] which demonstrated that significant increases of S.Ca in CKD.

P-ISSN: 2664-0562
E-ISSN:2664-0554

This variation in results may be due to the lack of a standardized reference range for serum calcium, which is instead established by individual laboratories or commercial suppliers of the assays or analyzers. While guidelines exist for determining normal ranges including subject selection, sample size, and biologic variables in addition the normal ranges may vary with age, gender, or ethnicity and are often dynamic in relationship to meals or time of day. Serum calcium is particularly challenging since it is the ionized component, rather than the total [40].

Studies have shown that patients with CKD will suffer from various inflammations, of which the pathogenic factors are mainly renal disease and infection immunodeficiency. Inflammatory infection is not caused by bacteria or viruses, but by higher concentration of IL-6 released by T lymphocytes and increased expression levels of CRP and TNF-α after the antigen stimulation in CKD patients. [41, 42]. The present study revealed highly significant increase of IL-6 level in CKD patients when comparing with control group. these results in keeping with other study [43]. The current investigation appeared highly significant increase of TNFα levels among chronic renal failure patients in comparing with control group in the same table. These outcomes consistent with previous studies [44,4].

The present study concluded that chronic renal patients were prevalent among those Iraqi females with age group (40-60) years in addition elevated Pro- inflammatory cytokines (IL-6, TNFα) in chronic renal diseases indicated these cytokines participate in the pathophysiology of reduced renal function and role of these cytokines as principle mediators of inflammatory reaction in renal damage and these cytokines could be potential therapeutic targets. so it need more future studies with large Iraqi samples to shed light and confirm the role of cytokines (IL-6, TNFα) in chronic renal patients.

P-ISSN: 2664-0562
E-ISSN:2664-0554

References

1. Astor B.C. Matsushita K. Gansevoort R.T,et al. (2011). Kidney International Supplement Chapter 1: Definition and classification of CKD. Jan;3(1):19-62.

2. Inker LA, Astor BC, Fox CH.et al. (2014). KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. Am J Kidney Dis. 63(5):713-35.

3. Webster AC, Nagler EV, Morton RL, Masson P (2017). Chronic Kidney Disease. Lancet;389(10075):1238-1252.

4. Zhang H, Ho YF, Che CT, et al (2012). Topical herbal application as an adjuvant treatment for chronic kidney disease–a systematic review of randomized Health led clinical trials. Journal of advanced nursing.68:1679- 1691.

5. Sandiya Bindroo , Bryan S Q , Hima J.et al. (2020).Renal Failure.Stat Pearls. Treasure Island (FL): StatPearls Publishing; 2021 Jan.

6. Chang A, Kramer H (2012). CKD progression: a risky business.Nephrol Dial Transplant; 27(7):2607-9.

7. Rumeyza K. (2013). Risk factors for chronic kidney disease: an update. 3(4): 368–371.

8. American Diabetes Association (2017). Comprehensive medical evaluation and assessment of comorbidities published correction appears in Diabetes Care. 40(7):985. Diabetes Care.40(suppl 1): S25–S32.

9. Shindler R. (2004). Causes and therapy of microinflammation in renal failure. Nephrol Dial Transplant*;* 19:34–40.

10. Chertow GM, Soroko SH, Paganini EP, Cho KC, Himmelfarb J, Ikizler TA, Mehta RL. (2006).Mortality after acute renal failure: models for prognostic stratification and risk adjustment. *Kidney Int* ; 70:1120–1126.

11. Amore A, Coppo R. (2002). Immunological basis of inflammation in dialysis. *Nephrol Dial Transplant.;* 17:16 –24.

12. Girndt M, Kaul H, Leitnaker CK, Sester M, Sester U, Köhler H. (2001). Selective sequestration of cytokine-producing monocytes during hemodialysis treatment. *Am J Kidney Dis* ;37:954–963.

13. Simmons EM, Himmelfarb J, Sezer MT, Chertow GM, Mehta RL, Paganini EP, Soroko S, Freedman S, Becker K, Spratt D, et al. (2004). Plasma cytokine levels predict mortality in patients with acute renal failure. *Kidney Int.;* 65:1357–1365.

14. Singbart K, Formec, C L and Kellum, J. A. (2019). Kidney-immune system crosstalk in AKI. In: Seminars in Nephrology 39(1): 96-106.

P-ISSN: 2664-0562

E-ISSN:2664-0554

15. Rothaug, Becker-Pauly M, Rose-John C.(2016).The role of interleukin-6 signaling in nervous tissue. Biochim Biophys Acta .18636 Pt A 6 pt A1218–1227.

16. Pecoits-Filho R, Heimburger O, Barany P, Suliman M, Fehrman-Ekholm I, Lindholm B, et al. (2003). Associations between circulating inflammatory markers and residual renal function in CRF patients. *Am J Kidney Dis* ; 41:1212–8.10.1016/S0272-6386(03)00353-6.

17. JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, et al. (2003) . Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. *Am J Physiol Endocrinol Metab*285:E527–33.10.1152.

18. Mary Mallappallil, Eli A Friedman, Barbara G Delano,et al. (2014).Chronic kidney disease in the elderly: evaluation and management. 11(5): 525–535.

19. Alfonso O G , de Francisco A. , Gayoso P , García F. (2010). Prevalence of chronic renal disease in Spain: Results of the EPIRCE study.nefrologia.Vol.30. Issue.1.Janauary. pages 1-142.

20. Leila M , Parviz K, Maryam P , Alireza M .(2013) . Prevalence of Chronic Kidney Disease and Its Related Risk Factors in Elderly of Southern Iran: A Population-Based Study.

21. National Institutes of Health. (2020). USRDS Annual Data Report: Epidemiology of Kidney Disease in the United States. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2020.

22. Hosseinpanah F , Kasraei F , Nassiri A. A , Azizi F. (2009) .High prevalence of chronic kidney disease in Iran: a large population-based study .BMC Public Health, vol. 9, article44.

23. Hansberry M. R , Whittier W. L , Krause M. W . (2005). The elderly patient with chronic kidney disease," Advances in Chronic Kidney Disease, vol. 12, no. 1, pp. 71–77.

24. Rothenbacher D., Klenk J., Denkinger M. et al., (2012). Prevalence and determinants of chronic kidney disease in community-dwelling elderly by various estimating equations, BMC Public Health, vol. 12, article 343.

25. Silbiger S. R. and Neugarten J , (2003) . The role of gender in the progression of renal disease. Advances in Renal Replacement Therapy, vol. 10, no. 1, pp. 3–14.

26. Juan Jesus Carrero , Manfred Hecking ,Nicholas C Chesnaye. et al. (2017). Sex and gender disparities in the epidemiology and outcomes of chronic kidney disease. Mar;14(3):151-164.

27. Ali Manal Kamil ,Shrouk Abdulrazak Hass an , Rajaa A. Mahmoud. (2021). Prevalence of chronic kidney disease and hypertension as a risk factor in Basrah province- Iraq. Annals of Tropical Medicine and Public Health. March 24(4):498-502.

P-ISSN: 2664-0562

E-ISSN:2664-0554

28. Hussein Mahdi Kadhim , Hussein Hazim Al-Ghanimi, Rusul Malik Al-Dedah. (2020). Haematological Parameters and Biochemical Indices in Patients with Chronic Kidney Disease Before Haemodialysis Al-Furat Al-Awsat Governorates / Iraq. The 8th International Conference on Applied Science and Technology (ICAST).

29. Ekhlas Abdallah Hassan, (2018). Biochemical Study in Iraqi Patients with Chronic Renal Failure Therapy by Regular Hemodialysis. Diyala jounral for pure science s. Vol: 14 No: 4, October.

30. Sharma A, Hirulkar N.B, Wadel P, Das P. (2011). Influence of Hyperglycemia on Renal Function Parameters in Patients with Diabetes Mellitus. International Journal of Pharmaceutical & Biological Archives;2(2):734-739.

31. Moses O and Johnkennedy N. (2013). The Alteration of Serum Glucose Urea and Creatinine Level of Malaria Patients in Obowo Local Government Area of Imo State Nigeria. International Journal of Advanced Medicin; 1: 1-6.

32. Tsering Dhondup and Qi Qian (2017).Acid-Base and Electrolyte Disorders in Patients with and without chronic Kidney Disease: An Update. 2017 Dec; 3(4): 136–148.

33. Sarafidis P. A, Blacklock R, Wood E, Rumjon A , et al . (2012). Prevalence and factors associated with hyperkalemia in predialysis patients followed in a low-clearance clinic. Clinical Journal of the American Society of Nephrology;7(8), 1234-1241.

34. Berl T. (2008). Impact of solute intake on urine flow and water excretion. J Am Soc Nephrol; 19:1076–1078.

35. Kovesdy CP. (2012). Significance of hypo- and hypernatremia in chronic kidney disease. Nephrol Dial Transplant.;27(3):891–898.

36. Charles W. O'Neill (2015). Targeting serum calcium in chronic kidney disease and end-stage renal disease: is normal too high.

37. David E. Leaf and Marta Christov.(2018).Dysregulated Mineral Metabolism in AKI. 39:41−56.

38. Kovesdy CP, Kuchmak O, Lu JL, et al. (2010). Outcomes associated with serum calcium level in men with non-dialysis-dependent chronic kidney disease. Clinical journal of the American Society of Nephrology: CJASN; 5:468-476.

39. Mitchell R. Lunn, Jair Muñoz Mendoza, Lezlee J. Pasche , et al. (2010).Hyperparathyroidism with hypercalcaemia in chronic kidney disease: primary or tertiary? NDT Plus. Aug; 3(4): 366–371.

40. Horowitz G.L , Altaie S, Boyd J.C , et al. (2010). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. 3rd ed. Clinical and Laboratory Standards Institute, Wayne, PA: 28.

P-ISSN: 2664-0562

E-ISSN:2664-0554

41. Li ZY, Zheng Y, Chen Y, Pan M, et al. (2017). Brazilin ameliorates diabetic nephropathy and inflammation in db/db mice. Inflammation. 40:1365–1374.

42. Batchu SN, Hughson A, Gerloff J, Fowell DJ , et al. (2013). Role of Axl in early kidney inflammation and progression of salt-dependent hypertension. Hypertension. 62:302–309.

43. Ishigami, J , Taliercio, J , Feldman, H. I , Srivastava, A , et al. (2021). Inflammatory Markers and Incidence of Hospitalization with Infection in Chronic Kidney Disease the Chronic Renal Insufficiency Cohort Study. American journal of epidemiology, 189(5), 433-444.

44. Rofyda H. Aly, Amr E. Ahmed, Walaa G. Hozayen, Alaa Mohamed Rabea, et al. (2020). Patterns of Toll-Like Receptor Expressions and Inflammatory Cytokine Levels and Their Implications in the Progress of Insulin Resistance and Diabetic Nephropathy in Type 2 Diabetic Patients. Front Physiol.; 11: 609223.

45. Cewen Liu , Hui Li. (2018) .Correlation of the severity of chronic kidney disease with serum inflammation, osteoporosis and vitamin D deficiency. Experimental and therapeutic medicine . November 1,Volume 17 Issue 1. Pages 368-372.

P-ISSN: 2664-0562

E-ISSN:2664-0554