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Estimating the Level of Interleukin-2 in Blood Serum and its Role in Distinguishing Between Early and Late Stages of Kidney Disease

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ABSTRACT

Background: *Interleukin 2 (IL-2) is a type of cytokine. It have essential role in proliferation and regulation of T cell. In kidney disease, IL-2 play a role in regulation of immune response. This study aims to evaluate the level of IL-2 and its relationship to the five stages of the disease.* ***Subject and method****: Ninety blood samples are taken from participant in this study and it divided into two groups, control group (30) samples and kidney disease group (60) samples. The parameters were measured include; (IL- 2, urea, creatinine), and GFR was estimated. Statistical analysis was performed for all parameters under the study.* ***Results****: The low level of IL-2 have a high significance difference in disease group as compared to control group (P=0.001), also there was a positive correlation between IL-2 and kidney disease stages. Furthermore, there were significant different in the mean serum levels of IL-2 among GFR stages. ROC curve gives a good result in differentiation patients from control* ***Conclusion****; it is possible to rely on the level of IL-2 in the blood to distinguish between the early and late stages of the disease, as it plays an important role in tracking the disease and its development.*
Key words: Kidney disease, Interleukin2, eGFR.

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INTRODUCTION

Interleukins are elements of the cytokines network affect the entire human organism. They are protein in nature synthesis from many cell in the body spatially by immune cell. Interleukin 2 (IL-2) one type of cytokines have essential role in proliferation and regulation of T cell [1]. In exposure to foreign body, T cell secreted IL-2 that bind with specific receptor on regulatory T cell (Tregs) promote its differentiation to activate Treg cell [2]. Human IL-2 is a protein that contains 153 amino acid residues with a total molecular weight of 15.5 kDa that includes a hydrophobic signaling sequence of 20 amino acids [3]. It encoded by the gene IL2 that is located on locus 4q27 of chromosome 4 [4]. After synthesis IL2, its bind with specific receptor called IL-2R exposed in many immune cell like T cell, B cell, natural killer cell that regulated proliferation, survival, and immune responses of these cells [5].

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There are many diseases that pose a threat to human health, such as kidney disease. There are primary or secondary causes of the disease, primary causes change in structure and function of kidney like Polycystic Kidney Disease while secondary causes by effect other organ such as cardiovascular disease and autoimmune kidney disease [6] [7]. During this condition, immune system disrupt and result in inflammation and tissue damage of glomeruli and tubuli [8]. IL2 have essential role in regulating the immune response by regulated activity of Treg to prevent hyperactive of Treg and reduced effect of inflammation process in renal structure [9]. Inflammation is closely relate to kidney disease and involves a complex network of interactions between kidney parenchymal cells and the immune cells found in the kidneys such as macrophages and dendritic cells. IL2 act as growth factor for Treg by enhanced the cell cycle, some of molecular study find that high dose of IL2 can used for therapy of autoimmune disease and cancer in those effect kidney due to the important function of IL2 as regulated to proliferation of Treg cell [10] [11]. One of these applications used to reduce chronic effect of autoimmune kidney disease such as lupus nephritis, IgA nephropathy, and vasculitis by used high dose inject of IL2 [12]. Other therapy application include used IL2 to stimulate of anti-tumor immune response in patients with melanoma, kidney cancer and other diseases caused by abnormal cells, in addition be approved for the treatment of renal cell carcinomas and metastatic melanomas [13] [14]. Overall, IL-2 is a key player in immune regulation and can influence kidney function in various ways, particularly in the context of immune-mediated kidney diseases and transplantation [15] [16]. Understanding the roles of IL-2 and related immune pathways in kidney health and disease is important for developing targeted therapies and improving patient outcomes [17]. This study aims to measure the level of IL-2 in patients' blood serum who suffering from kidney disease and evaluate the extent of its relationship to glomerular filtration rate and disease stages**.**

SUBJECT AND METHOD

Study design: It is Case-control study, made in AL-NAJAF AL-ASHRAF. The sample collected from participants in AL-SADER medical city and GIT hospital between December 2023 and January 2024, and then divided into two groups, 60 samples collected from kidney disease participants (35 males' samples and 25 female's with age between 45-70 years), and apparently healthy people that taken as control were 30 samples. The laboratory test measured for both disease and control include IL-2, serum urea, serum creatinine, estimated GFR.

Sample collected: four milliliters of blood collected from all participants and placed in gel tube. Then blood separate to serum by centrifuge in 5000 rpm for 5 min, serum result from separation process collected in khan tube and stored in –20 °C for used later to measure serum (IL2, urea, creatinine).

METHOD

The samples of kidney disease and control participants collected as ordinary blood samples and examinated for measured of kidney function test (urea, creatinine) by spectrophotometric procedure and ELIZA technique for measured IL-2 level.

Human IL-2 (Interleukin 2) ELISA Kit, Elabscience Company/ USA,

Catalog No: E-EL-H0099.

Colorimetric methods for measuring serum urea, creatinine, Abbott Company/ U.S.A

While the GFR was estimated using the following equation:

$$CrCl\left(mL/min\right) = \frac{\left(140 - age\ in\ years\right) \times \left(body\ weight\ in\ kg\right)}{SCr\ mg/dl \times 72}$$

Ethical Approval

Initial approvals were taken from the College of Medicine / Tikrit University, Al-Sader medical city and GIT hospital / Al-Najaf. The research was conducted by taking blood samples from volunteers. Ethical conditions were applied in every step of this study.

RESULTS

Interleukin-2 biomarker

The mean level of IL- 2 in kidney disease group (2.12 ng/ml) with SD (1.06) and for control group (1.24 ng/ml) with SD (0.46). IL-2 give high significance result in kidney disease group as compare with control group, (P=0.001) table 1.

Urea and Creatinine

According to table 2: The mean serum level of (urea, creatinine) for disease group were [(109.34mg/dl) with SD (59.16), (4.99mg/dl) with SD (4.50)] respectively, and the mean serum level of (urea, creatinine) for control group were [(23.55mg/dl) with SD (6.22). (0.76mg/dl) with SD (0.12)].There are high significant level of serum urea and creatinine in disease group comparison with control group, p-value (0.001).

Estimated GFR

According to table 2: The mean serum level of eGFR for kidney disease (41.37 ml/min/1.73m^2) with SD (33.73) and for control group (126.23 ml/min/1.73m^2) with SE (18.77). There was high significant difference of eGFR in kidney disease group as compare with control group, p-value (0.001).

Table 1: Comparison of IL-2 between patients and control groups

Variable	Groups	N	Mean	SE	SD	Median	IQR	p-value
IL-2 (ng/ml)	**Kidney disease**	60	2.12	0.14	1.06	1.96	1.17-2.70	0.001* #
	Control	30	1.24	0.08	0.46			

**** Significant differences at p value <0.05. SE: Standard Error of Mean. SD: standard division. IQR: inter quartal range. #: Mann Whitney U test.***

Table 2: Comparison of kidney function parameters between patients and control groups

Variables	Groups	N	Mean	SE	SD	IQR	p-value
Urea (mg/dl)	**Kidney disease**	60	109.34	7.57	59.16	56.94-152.50	0.001* #
	control	30	23.55	1.14	6.22		
Creatinine (mg/dl)	**Kidney disease**	60	4.99	0.58	4.50	1.29-8.50	0.001*
	control	30	0.76	0.02	0.12		
eGFR (ml/min/1.73m^2)	**Kidney disease**	60	41.37	4.32	33.73	10.40-65.50	0.001* #
	control	30	126.23	3.43	18.77		

**** Significant differences at p value <0.05. SE: Standard Error of Mean. SD: standard division. IQR: inter quartal range. #: Mann Whitney U test.***

The relationship between IL-2 and kidney function parameters

The data in table 3 showed that, there were highly significant positive correlation between serum IL-2 level and (urea, Creatinine) in patients group [(r=0.652, P<0.001), (r =0.610, P<0.001)] respectively. In addition table 3 and figure 1 showed there was highly significant negative correlation between serum IL-2 level and eGFR (r=-0.664, P<0.001). While the data did not show any significant correlation in control group.

Interleukin-2 and kidney disease stages

The mean levels of IL-2 in G1and G2 were (0.93, 1.5) ng/ml respectively, and the mean level in control group was (1.24 ng / ml); there were no significant difference in their means. While there were significantly elevated in the mean serum levels of IL-2 for G3 and G4 kidney disease (1.98, 2.39) ng / ml respectively. In addition, there was highly elevated of IL-2 level in G5 of kidney disease (dialysis and end stage kidney disease), the mean level was (2.81 ng / ml) table 4, figure 2.

The diagnostic and validity criteria of IL-2 by used receiver operating characteristic curve (ROC curve):

Table 5 and figure 3, show the area under curve (AUC) of the biochemical marker IL-2 in differentiating of kidney disease patients from control; equal to (0.761), The results revealed that serum level of IL-2 at cut-off (< 1.7900) ng/ml, (Sensitivity = 0.6, Specificity = 0.87, with P=0.0001). Accordingly, the biochemical marker IL-2 considered good in performing this differentiation.

Table 3: The correlations between IL-2 and variables in groups under the study.

Groups / Variables	IL-2(ng/ml)	
	Control (n=30)	kidney disease (n=60)
Urea (mg/dl)	0.012	0.652**
	0.951	< 0.001
Creatinine (mg/dl)	0.023	0.610**
	0.811	< 0.001
eGFR (ml/min/1.73m^2)	0.185	-0.664**
	0.329	< 0.001

**Correlation is significant at the 0.01 level
* Correlation is significant at the 0.05 level

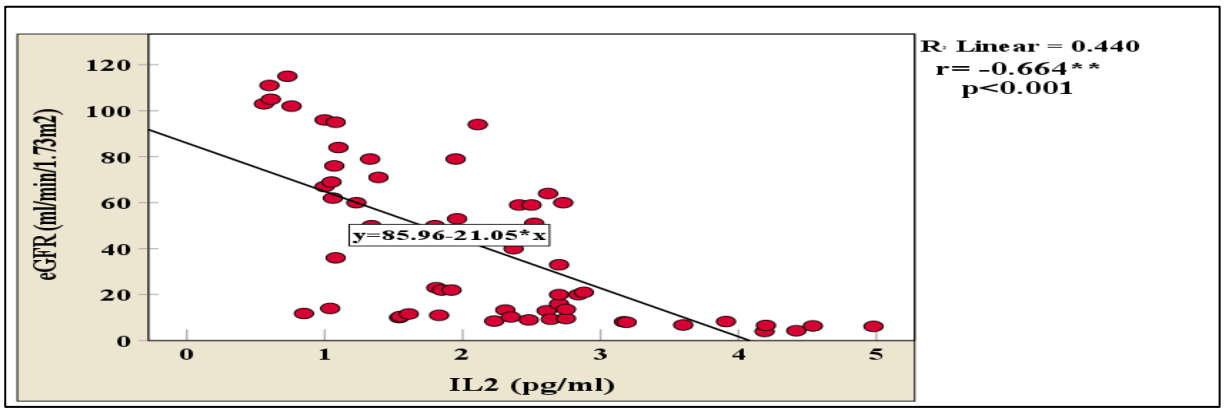

Figure1: Scatter diagram with fitted regression line showing the correlation between

IL-2 and eGFR in kidney disease group.

Table 4: The mean of IL-2 serum levels among disease stages .

Variable	G1 > 90	G2 60 - 89	G3 30 - 59	G4 15- 29	G5 < 15	p-value
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	
IL-2 (ng/ml)	0.93(0.18)	1.5(0.19)	1.98(0.16)	2.39(0.19)	2.81(0.24)	0.001*

**** Significant differences at p value <0.05. SE: Standard Error of Mean. Ns: non-significant.***

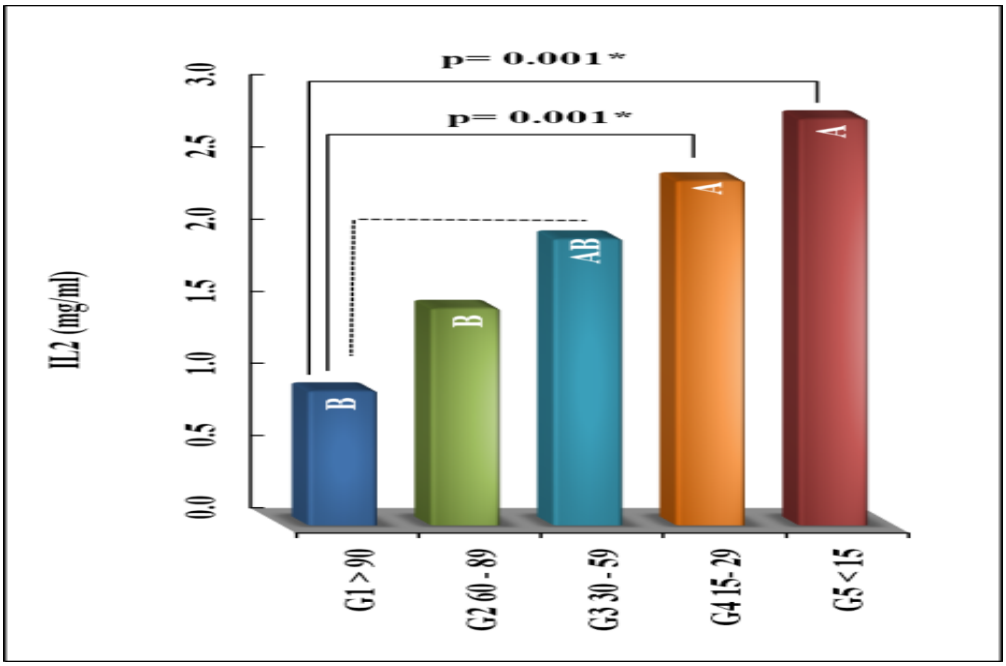

Figure 2: Bar chart showing the difference in mean of serum IL-2 among kidney disease stages.

The diagnostic criteria and validity of IL-2 markers as.

Table 5: Differentiator of (kidney disease) group from (Control) group.

Test Result	Area	Std. Error[a]	p-value	95%CI	cut point	Sensitivity	1 - Specificity
IL-2 ng/ml	0.761	0.049	0.0001	0.665-0.856	1.7900	0.607	0.133

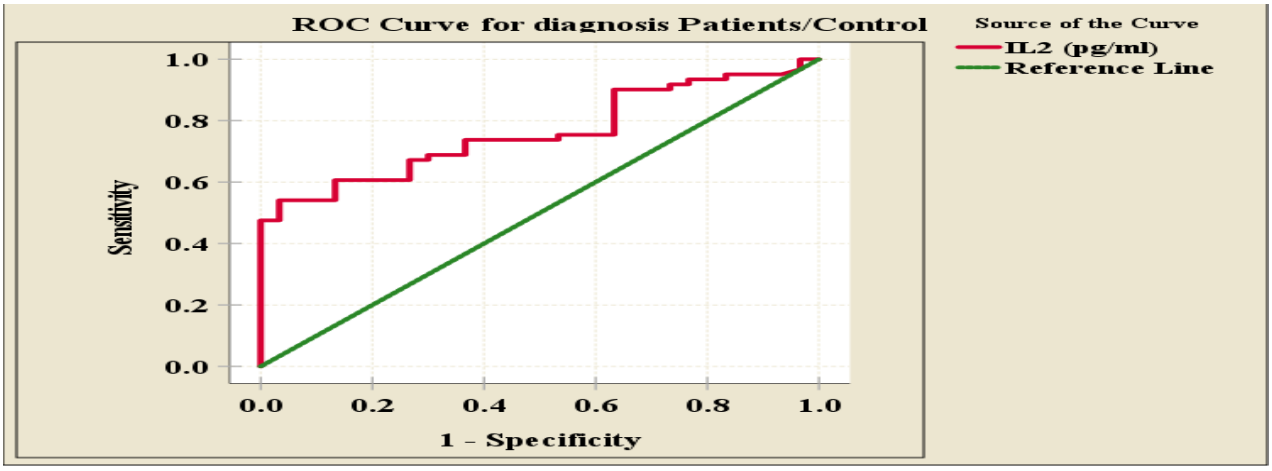

Figure 3: ROC curve of IL-2 in kidney disease patient and control group.

DISCUSSION

According to the result in table 1, patient with kidney disease had elevated in the level of IL-2 in blood due to disturbance in immune cell activity that result from secondary effect of kidney disease [18]. In this condition, the stimulation of lymphocyte T cell during immune cell activation causes increase secretion of IL2 and other inflammatory mediator cytokine. This result agreement with study made by (Costa E., et al). Showed that, chronic kidney disease patient had immunophenotype with chronic T cell stimulation causes higher production of IL-2, interferon gamma (INF-Y), tumor necrosis factor alpha (TNF-α). As well as T cell from CKD patient release higher amount of this cytokines in vitro stimulation[19].

Other study agreement with the current study made by (Al-rawi K.F., et al). That found, IL-2 and IL-17 significant increase in-patient with CKD compare with control. They detect relationship between IL-2 and IL-17 with serum creatinine, serum albumin in kidney disease patient. As well as, the significant increase of IL-2 associated with significantly high concentrations of creatinine, serum urea and urine albumin[20]. According to the current study, IL-2 elevated in disease that effect kidney because the disturbance occur in immune cell include lymphocyte T cell causes increase secretion of cytokine that IL-2 on of them, Study made by (Stenvinkel P., et al) is agreement with this current study. They detected elevated in proinflammatory mediator cytokine include IL-1b, TNF, and IL-2 in polycystic kidney disease. This study used animal to detected significant role of this inflammatory cytokines in development and proliferation of Polycystic Kidney Disease[21].

Dependent on the result in table 3 and figure 1, the mean levels of IL-2 classified according to stages of kidney disease. The level of IL-2 in G1 and G2 about (0.93 ng/ml, 1.5 ng/ml) near to the normal level of this marker in control samples (1.24 ng/ml), in G1 and G2 the filtration function of kidney are normal or slight decrease than normal state. IL-2 start to elevated above normal result in G3 (1.98 ng/ml) when the function of kidney clearly reduced but the kidney damage not diagnosis because its asymptomatic and the routine kidney function test not elevated yet. Finally, in chronic kidney disease and end stage kidney disease (G4 and G5), IL-2 level is significant increase (2.39 ng/ml, 2.81 ng/ml). This result agreement with study made by (Mertowska P., et al) and other study made by (Kalavrizioti D., et al) they found elevated of multiple cytokine include IL-1β, IL-2 and IL-4 in patient with focal segmental Glomerulosclerosis (common type of acute kidney injury) .

They detect significant higher excretion of IL-2 and other cytokines in urine of patient compare with control. Also they found, the

Glomerulonephritides; a common type of chronic kidney disease induced by furious inflammatory factor include IL-2, INF-γ, TNF-α, IL-6, IL-17 [22] [23]. Finally; serum urea, creatinine and eGFR give significant difference in compared between kidney disease patient and control. This result suspected because these tests used in hospitals to estimated kidney function and detect damage in renal tissue (glomeruli, tubuli) [24]. In other hand, these tests not detect asymptomatic kidney damage or acute case of kidney disease when the damage not widely spread through renal tissue because these tests not changed until 50% of kidney function was lost [25]. Therefore, assessing the GFR, in addition to evaluating the interleukin-2 level in blood, is important in such cases and gives good results in distinguishing between patients and healthy people, in addition to determining the stage of the disease (this is shown in the tables 4, 5and figure 2,3).

CONCLUSION

The level of IL-2 in the blood serum increases significantly for kidney disease patients, and this increase is directly proportional to the deterioration of the patient's condition and his arrival to the late stages of the disease (IL-2 in G1<G2<G3<G4<G5). In addition IL-2 gives good indicator in differentiation among stages of the disease.

REFERENCE

[1] "Mertowska, P., Mertowski, S., Smarz-Widelska, I., & Grywalska 'Biological role, mechanism of action and the importance of interleukins in kidney diseases.' International journal of molecular sciences 23.2 (2022): 647.".

[2] "Abbas, A. K., Trotta, E., R. Simeonov, D., Marson, A., &Bluestone, J. A 'Revisiting IL-2: Biology and therapeutic prospects.' Science immunology 3.25 (2018): eaat1482.".

[3] "Bendickova, Kamila, and Jan Fric. 'Roles of IL-2 in bridging adaptive and innate immunity, and as a tool for cellular immunotherapy.' Journal of Leucocyte Biology 108.1 (2020): 427-437.".

[4] "Dimberg, J., Shamoun, L., Landerholm, K., Andersson, R. E., Kolodziej, B., & Wågsäter, D 'Genetic variants of the IL2 gene related to risk and survival in patients with colorectal cancer.' Anticancer research 39.9 (2019): 4933-4940.".

[5] "Mitra, Suman, and Warren J. Leonard. 'Biology of IL-2 and its therapeutic modulation: Mechanisms and strategies.' Journal of leukocyte biology 103.4 (2018): 643-655.".

[6] "Kalantar-Zadeh, K., Jafar, T. H., Nitsch, D., Neuen, B. L., & Perkovic, V. 'Chronic kidney disease.' The lancet 398.10302 (2021): 786-802.".

[7] "Connaughton, D. M., Kennedy, C., Shril, S., Mann, N., Murray, S. L., Williams, P. A., ... & Hildebrandt, F. 'Monogenic causes of chronic kidney disease in adults.' Kidney international 95.4 (2019): 914-928.".

[8] "Tecklenborg, J., Clayton, D., Siebert, S., & Coley, S. M. 'The role of the immune system in kidney disease.' Clinical & Experimental Immunology 192.2 (2018): 142-150.".

[9] "Knauf, Felix, J. Richard Brewer, and Richard A. Flavell. 'Immunity, microbiota and kidney disease.' Nature Reviews Nephrology 15.5 (2019): 263-274.".

[10] "Stenvinkel, P., Chertow, G. M., Devarajan, P., Levin, A., Andreoli, S. P., Bangalore, S., & Warady, B. A 'Chronic inflammation in chronic kidney disease progression: role of Nrf2.' Kidney international reports 6.7 (2021): 1775-1787.".

[11] "Thaha, Mochammad, and I. Gde Raka Widiana. 'The role of inflammation in chronic kidney disease.' Indonesian Journal of Kidney and Hypertension 2.3 (2019): 4-13.".

[12] "Mitra, Suman, and Warren J. Leonard. 'Biology of IL-2 and its therapeutic modulation: Mechanisms and strategies.' Journal of leukocyte biology 103.4 (2018): 643-655.".

[13] "Mortara, L., Balza, E., Bruno, A., Poggi, A., Orecchia, P., & Carnemolla, B. 'Anti-cancer therapies employing IL-2 cytokine tumor targeting: contribution of innate, adaptive and immunosuppressive cells in the anti-tumor efficacy.' Frontiers in Immunology 9 (2018): 424754.".

[14] "Dhupkar, Pooja, and Nancy Gordon. 'Interleukin-2: old and new approaches to enhance immune-therapeutic efficacy.' Immunotherapy (2017): 33-51.".

[15] "Li, Y., Liu, X., Wang, W., Wang, S., Zhang, J., Jiang, S., ... & Huang, H 'Low-dose IL-2 expands CD4+ regulatory T cells with a suppressive function in vitro via the STAT5-dependent pathway in patients with chronic kidney diseases.' Renal Failure 40.1 (2018): 280-288.".

[16] "Sadasivam, Mohanraj, et al. 'Activation and proliferation of PD-1+ kidney double-negative T cells is dependent on nonclassical MHC proteins and IL-2.' Journal of the American Society of Nephrology 30.2 (2019): 277-292.".

[17] "Hellemans, R., J-L. Bosmans, and D. Abramowicz. 'Induction therapy for kidney transplant recipients: do we still need anti-IL2 receptor monoclonal antibodies?.' American Journal of Transplantation 17.1 (2017): 22-27.".

[18] "Hartzell, S., Bin, S., Cantarelli, C., Haverly, M., Manrique, J., Angeletti, A., ... & Cravedi, P 'Kidney failure associates with T cell exhaustion and imbalanced follicular helper T cells.' Frontiers in immunology 11 (2020): 583702.".

[19] "Costa, E., Lima, M., Alves, J. M., Rocha, S., Rocha-Pereira, P., Castro, E., ... & Santos-Silva, A. 'Inflammation, T-cell phenotype, and inflammatory cytokines in chronic kidney disease patients under hemodialysis and its relationship to resistance to recombinant human erythropoietin therapy.' Journal of clinical immunology 28 (2008): 268-275.".

[20] "Al-Rawi, K. F., Ali, H. H., Guma, M. A., Aldahham, B. J. M.,Alaaraji, S. F. T., Al-Ani, O., & Ali, A. T. 'Relationship Between IL-2, IL-17 Concentrations, and Serum Creatinine Levels in Men with Chronic Kidney Diseases.' Reports of Biochemistry & Molecular Biology 10.4 (2022): 664.".

[21] "Stenvinkel, P., Chertow, G. M., Devarajan, P., Levin, A., Andreoli, S. P., Bangalore, S., & Warady, B. A. 'Chronic inflammation in chronic kidney disease progression: role of Nrf2.' Kidney international reports 6.7 (2021): 1775-1787.".

[22] "Kalavrizioti, D., Gerolymos, M., Rodi, M., Kalliakmani, P., Provatopoulou, S., Eleftheriadis, T., ... & Goumenos, D. S. 'T helper (Th)-cytokines in the urine of patients with primary glomerulonephritis treated with immunosuppressive drugs: can they predict outcome?.' Cytokine 76.2 (2015): 260-269.".

[23] "Mertowska, Paulina, et al. 'Biological role, mechanism of action and the importance of interleukins in kidney diseases.' International journal of molecular sciences 23.2 (2022): 647.".

[24] "Ferguson, Michael A., and Sushrut S. Waikar. 'Established and emerging markers of kidney function.' Clinical chemistry 58.4 (2012): 680-689.".

[25] "Manoeuvrier, G., Bach-Ngohou, K., Batard, E., Masson, D., & Trewick, D 'Diagnostic performance of serum blood urea nitrogen to creatinine ratio for distinguishing prerenal from intrinsic acute kidney injury in the emergency department.' BMC nephrology 18 (2017): 1-7.".

