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INDOLEAMINE 2,3-DIOXYGENASE LEVEL AND OXIDATIVE STRESS PARAMETERS IN THE SERUM OF PATIENTS WITH CHRONIC RENAL FAILURE

F. M. Y. SAEED, R. F. JASIM✉

College of Education for Girls, Department of Chemistry, University of Mosul, Iraq;
✉*e-mail: ra.fadhel@uomosul.edu.iq*

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Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-degrading enzyme belonging to the kynurenine pathway. IDO activity has been suggested as a biomarker for diagnosis of chronic kidney disease. The aim of the study was to estimate the level of IDO, urea, creatinine, uric acid, phosphate, calcium, albumin, MDA, GSH, and activity of peroxidase, catalase, arylesterase in the serum of chronic renal failure (CRF) patients treated with dialysis compared to the healthy control group. The results showed a significant increment in IDO level in patients compared with the control. Linear regression analysis using the Pearson correlation coefficient showed that increased IDO level correlates positively with urea, creatinine, uric acid, phosphate, MDA level and peroxidase activity whereas negatively with albumin, calcium, glutathione level, catalase activity and glomerular filtration rate. We concluded that IDO level might be a possible marker of oxidative stress and inflammation in patients with CRF.

K e y w o r d s: indoleamine 2,3-dioxygenase, renal failure, serum, biochemical parameters, correlation analysis.

Chronic renal failure disease (CRF) is defined as damage of the kidney or a decrease in glomerular filtration rate (GFR) to be under 60 ml/min/1.73 m^2 for more than 3 months. CRF disease usually becomes worse with age, high blood pressure, diabetes, high levels of elements and glomerulonephritis [1, 2]. The kidney has a key role in regulating fluids in the body, waste products and the acid-base balance. Electrolytes and imbalances of acid-base are the main causes related to bone demineralization, catabolism of muscle, and elevated risk of chronic kidney disease (CKD) and mortality [2]. Indoleamine-2,3-dioxygenase (IDO) contribute to many processes including processes of immune like infection, metabolism, inflammation and autoimmunity [2, 3]. IDO is a heme enzyme responsible for stimulating and maintaining immunosuppression since it binds to nearly all the cells in the immune system such as dendritic and monophyte cells, but mainly, IDO affects lymphocytes [4]. IDO catalyzes the breakdown of tryptophan (TRP) into kynurenic acid, kynurenine (KYN) and quinolinic acid [5], this immune enzyme has a key role in protecting the fetus from rejection by suppressing maternal T cells [6]. Also, the enzyme is associated with renal fibrosis and diabetic kidney failure, and the activity of the enzyme is dangerous for CKD, because it is associated negatively with the kidney glomeruli and glomerular filtration rate, and affects the severity of the disease and inflammation of the kidneys, IDO level is suggested as a new marker for chronic kidney disease [6, 7].

Oxidative stress is related to many diseases, such as atherosclerosis, hypertension, vascular disease and anaemia, as well as the development and complications of CKD, oxidative stress leads to increasing the speed of progression of CKD [8].

Aim of research. Due to the increase in the incidence of chronic renal failure in Iraq for the last few years and since there are a few previous studies on indolamine 2,3-dioxygenase enzyme and its relationship with oxidative stress, we evaluated its role in CRF patients and its relationship with the oxidative stress.

Material and Methods

The study included (88) healthy people (44 females, 44 males) aged (15-70 years) and (74) patients (29 females, 45 males) suffering from chronic kidney failure (CRF) disease and undergoing hemodialysis

with age (15-70 years) from Ibn Sina Teaching Hospital-Nephrology Unit in Mosul/Iraq. This research was approved by local Ethics Committee (Protocol No 22438 dated 06.07.2022).

The blood serum samples obtained from the participants were analyzed to assess the following biochemical parameters. IDO level was estimated using Wuhan Fine Biotech Kit (China) using Enzyme-Linked Immunosorbent Assay (ELISA) Technology. The concentration of urea, creatinine, albumin, uric acid, phosphate and calcium were estimated by using BIOLABO kit (France), glutathione (GSH) concentration was determined using Ellman reagent by a modified method [9], malondialdehyde (MDA) concentration was estimated using thiobarbituric acid by a modified method [10]. Also, peroxidase activity was determined by enzymatic oxidation of hydrogen peroxide [11], catalase activity was determined by colourimetric assay using hydrogen peroxide [12] and arylesterase activity estimated using a modified method by hydrolysis of phenyl acetate enzymatically to produce acetic acid and phenol [13].

Glomerular filtration rate (GFR) is a measure of blood filtration rate by the kidneys. To calculate GFR, we used chronic kidney disease epidemiology collaboration (CKD-EPI) equation. For female (with creatinine < 62 μmol/l): GFR (ml/min/1.73 m^2) = $144\times(Cr/61.6)^{-0.329}\times(0.993)^{Age}$; for female (with creatinine > 62 μmol/l): GFR (ml/min/1.73 m^2) = $144\times(Cr/61.6)^{-1.209}\times(0.993)^{Age}$; for female (with creatinine < 80 μmol/l): GFR (ml/min/1.73 m^2) = $141\times(Cr/79.2)^{-0.411}\times(0.993)^{Age}$; for female (with creatinine > 80 μmol/l): GFR (ml/min/1.73 m^2) = $141\times(Cr/79.2)^{-1.209}\times(0.993)^{Age}$ [14].

Analysis of data. The data of our study were analyzed by Statistical Package for Social Sciences (SPSS), mean and standard division calculated by methods of Standard statistics. Anova one way was used for comparing more than two variants.

To compare between two variants, T-test was used and to find the relation between different parameters, the Pearson correlation coefficient (r) was used. $P \leq 0.05$ was considered statistically significant.

Results and Discussion

IDO level in the control group and CRF patients. As shown in Table 1, the level of IDO is (4.74 ± 1.53 ng/ml) in healthy control, it was comparable with the concentration (4.78 ± 0.15 ng/ml) found by [15] and close to the concentration found by [16] which was (2.60 ± 1.29 ng/ml).

Table 1. IDO level in CRF patients and control group

IDO concentration, ng/ml (mean ± SD)	
Control (n = 88)	Patients (n = 74)
4.74 ± 1.53	13.02 ± 4.88***

Note. ***Significant variance if $P \leq 0.001$, SD – stander division

Also, our results found a significant increase in IDO levels in CRF patients compared with control, as shown in Fig. This result was consistent with [17, 18], CRF is a feature of immune imbalance and insufficiency immune response.

IDO exert a key role in immune regulation and promote an inflammatory state, and an increased level of IDO indicate inflammation and an impaired ability to immunoregulation in CRF patients, also, IDO level correlate with renal ischemia and hypoxia [18].

IDO level in CRF patients and control group according to age. The results illustrated a significant increase in IDO level with age in each group as in Table 2, this result was in agreement with the study conducted by Hong et al. [18].

Aging is associated with elevated levels of pro-inflammatory cytokines which are associated with age-related diseases such as CRF, and the number of immunosuppressive cells increases during aging so, IDO levels increase in inflammation and autoimmune disorders [19].

IDO level in CRF patients and control group according to sex. Our results showed a non-significantly variance in IDO level between the male and the female in each group and these results were compatible with [7] that IDO level is not affected by sex as shown in Table 3.

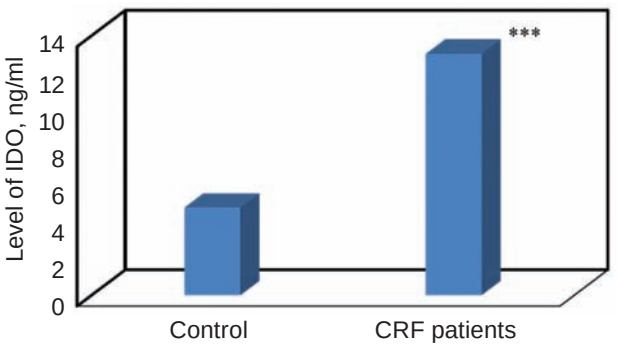

*Fig. IDO level in CRF patients and control. ***Significant variance if $P \leq 0.001$*

Table 2. IDO level in CRF patients and control group according to the age

Age, year	IDO level, ng/ml, (mean ± SD)			
	Control	*n*	Patients	*n*
15-35	2.65 ± 1.15	27	10.03 ± 3.27	21
36-56	6.26 ± 1.86*	30	14.95 ± 2.34**	28
57-77	7.20 ± 2.06**	31	18.09 ± 5.43***	25

Note. *Significant variance vertically if $P \leq 0.05$, if **$P \leq 0.01$, if ***$P \leq 0.001$

Table 3. IDO level in CRF patients and control group according to the sex

Sex	IDO level, ng/ml, (mean ± SD)			
	Control	*n*	Patients	*n*
Male	4.63 ± 1.28	44	12.79 ± 4.96	45
Female	5.12 ± 1.82	44	13.36 ± 3.93	29

Table 4. IDO level in CRF patients according to dialyses

IDO concentration, ng/ml (mean ± SD, *n* = 66)	
pre-dialyses	post-dialyses
9.89 ± 3.62	16.12 ± 4.21***

Note. ***Significant variance if $P \leq 0.001$

IDO level in CRF patients pre- and post-dialyses. A significant increase was observed in IDO level in post-dialyses patients when compared with pre-dialyses patients as shown in Table 4, the results from this study are consistent with one previous study [20]. This increment in IDO level may be due to the hemodialysis session which induces inflammation and activation of the immune at a large scale by the contact of the blood with strange surfaces such as dialyzer tubing or membrane, and this may induce IDO in CKD [21].

Biochemical parameters determined in CRF patients and control. There was a significant increase in urea, creatinine, and uric acid concentration in patients by comparison with control as shown in Table 5. This might be due to the kidney failure to remove these metabolic products from the blood which leads to an increase in its concentration in the blood [22].

Also, there was a significant decrease in albumin concentration in patients compared with the control as shown in Table 5, hypoalbuminemia is a common phenomenon in CRF, albumin decreased in CRF patients because of reduced synthesis and increased degradation of albumin, serum albumin represents a marker of malnutrition and inflammation; and powerful predictor of mortality in CRF [23].

A significant decrease in GFR was found in the patients compared with the control group, which is consistent with [24], decline in GFR indicates the impairment of the mulfunction of the nephron and this might result from diabetic nephropathy or hypertension or increases in protein concentration which increase glomerular capillary pressure [25].

In patients, phosphate concentration increased significantly compared with the control, hyperphosphatemia may be due to kidney failure to excrete extra phosphorus from the body and also, due to

Table 5. Biochemical parameters in CRF patients and control (mean ± SD)

Parameters	Control (*n* = 88)	Patients (*n* = 74)
Urea, mmol\l	4.68 ± 0.75	24.59 ± 7.99***
Creatine, mmol\l	77.80 ± 11.46	909.5 ± 277.2***
Albumin, g\l	34.67 ± 2.71	28.25 ± 8.06**
GFR, μmol/l	99.13 ± 19.78	5.23 ± 1.66***
Uric acid, mmol/l	300.60 ± 48.36	357.70 ± 78.75*
Phosphate, mmol/l	1.04 ± 0.14	1.40 ± 0.56*
Calcium, mmol/l	1.19 ± 0.06	0.91 ± 0.23***

Note. **Significant difference if $P \leq 0.01$, *** if $P \leq 0.001$

Table 6. Oxidants and antioxidants level in CRF patients and control (mean ± SD)

Parameters	Control (n = 88)	Patients (n = 74)
MDA, μmol/l	2.13 ± 0.94	5.36 ± 1.39**
Peroxidase, μmol/l	43.03 ± 18.73	112.50 ± 39.87***
Glutathione, μmol/l	17.13 ± 5.84	10.63 ± 3.01***
Catalase, μmol/l	6.10 ± 1.95	3.89 ± 1.08**
Arylesterase, U/ml	98.56 ± 24.91	61.34 ± 20.52**

Note.**Significant difference if $P \leq 0.01$, *** if $P \leq 0.001$

hyperparathyroidism which results from CRF and affects bone morphology in patients with chronic kidney disease, which includes fibrous osteitis and mixed renal osteonecrosis [26], also the calcium concentration decreases significantly in patients compared with the control, in CRF patients, production of active vitamin D decrease by the kidney which results in decrease absorption of calcium and causes hypocalcemia [27].

Oxidants and antioxidants concentration in CRF patients and control. The results in Table 6 showed a significant increase in malondialdehyde (MDA) and peroxidase in patients compared with the control. Increased peroxidase and MDA are caused by inflammation and malnutrition, which leads to decreased oxidative defence and promote oxidative stress in renal failure patients [28].

Glutathione (GSH) concentration showed a significantly decreasing in patients than in control, glutathione as an antioxidant act to scavenge free radicals and decline lipid peroxidation, also glutathione decreases oxidization of LDL, so its level lower in comparison with the other antioxidants, which make it a more stable marker of antioxidant status [29]. Also, catalase and arylesterase activity showed a significant decreasing in patients when compared with control, this may be because of the enzyme inhibition by free radicals which increase in patients with CRF and react with free sulfhydryl group of arylesterase [30, 31].

Correlation between IDO level and biochemical parameters in CRF patients and control group. Our results showed a positive correlation between IDO level and urea, uric acid, phosphate, and MDA concentration in patients and control, and with creatinine, and peroxidase activity in patients, while there was a negative correlation as in Table 7 between IDO level and concentration of albumin, cal-

Table 7. Correlation of IDO level with biochemical parameters

Parameters	Control group (n = 88)		Patients (n = 74)	
	R	P	r	P
Urea	0.56*	0.029	0.715**	0.002
Creatinine	0.318	0.247	0.675**	0.008
Albumin	-0.656**	0.007	-0.670**	0.006
GFR	-0.535*	0.048	-0.515*	0.049
Uric acid	0.568*	0.026	0.546 *	0.035
Phosphate	0.540*	0.037	0.678**	0.005
Calcium	-0.533*	0.049	-0.523*	0.045
MDA	0.59*	0.012	0.631**	0.006
Peroxidase	0.484	0.056	0.509*	0.043
GSH	-0.29	0.423	-0.33*	0.012
Catalase	-0.566*	0.011	-0.647**	0.004
Arylesterase	-0.29	0.275	-0.33	0.248

Note. *Significant variance if $P \leq 0.05$, ** if $P \leq 0.01$

cium, catalase activity, GFR in each group and GSH in patients.

IDO limits the catabolism rate of tryptophan, which is an essential amino acid and important to survival and immune cell proliferation. The disorder in the metabolism pathway of tryptophan can lead to many diseases, such as CRF. Also, CRF is strongly caused by chronic inflammation, which increases the level of IDO and can cause kidney failure to remove wastes from the blood, such as urea, creatinine, and uric acid which result in increasing their concentration in blood and loss of albumin from the body, with decline in GRF [32].

IDO levels correlate positively with phosphate and negatively with calcium. Many studies show that the elevated level of kynurenine might reduce stiffness and strength of the bone, and change the metabolism of bone, which is common in CRF patients and known as "chronic kidney disease (CKD)-mineral and bone disorder" [33].

The production of antioxidant impaired in CRF patients on hemodialysis and uremic toxins accumulation increase oxidative stress and production of ROS, which results in provoking inflammatory responses [34].

Also, inflammation induces oxidative stress so, oxidative stress and inflammation are related to poor clinical results in CRF patients on hemodialysis, infections and oxidative stress are the first mechanisms that activate IDO so the production of kynurenine mighty be induced by oxidative stress, and tryptophan to kynurenine ratio can indicate to oxidative stress state in CRF patients [35].

Also, 3,3-hydroxy anthranilic acid, one of tryptophan metabolite, is physiologically unstable and produces ROS and increases lipid peroxidation which results in increasing MDA concentration and free radicals, which in turn increase peroxidase activity to remove these free radicals and can cause depletion in antioxidants such as GSH and catalase [36].

Conclusion. IDO level could be a marker of inflammation in CRF undergoing hemodialysis, and there was a strong relation between IDO level and oxidative stress, thus tryptophan to kynurenine ratio may indicate the oxidative stress state in CRF patients.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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РІВЕНЬ ІНДОЛАМІН 2,3-ДІОКСИГЕНАЗИ І ПАРАМЕТРИ ОКСИДАТИВНОГО СТРЕСУ ПАЦІЄНТІВ ІЗ ХРОНІЧНОЮ НИРКОВОЮ НЕДОСТАТНІСТЮ

F. M. Y. Saeed, R. F. Jasim✉

Collage of Education for Girls, Department of Chemistry, University of Mosul, Iraq;
✉e-mail: ra.fadhel@uomosul.edu.iq

Індолеамін-2,3-діоксигеназа (IDO) – це ензим, що розкладає триптофан і належить до кінуренінового шляху. Активність IDO була запропонована як біомаркер для діагностики хронічної хвороби нирок. Метою дослідження було оцінити рівень ІДО, сечовини, креатиніну, сечової кислоти, фосфату, кальцію, альбуміну, МДА, ГСГ, активності пероксидази, каталази, арилестерази в сироватці крові пацієнтів з хронічною нирковою недостатністю (ХНН), які проходили лікування діалізом у порівнянні зі здоровою контрольною групою. Результати показали значне збільшення рівня IDO у пацієнтів порівняно з контролем. Аналіз лінійної регресії з використанням коефіцієнта кореляції Пірсона показав, що підвищений рівень IDO позитивно корелює з сечовиною, креатиніном, сечовою кислотою, фосфатом, рівнем MDA та активністю пероксидази, тоді як негативно корелює з рівнем альбуміну, кальцію, глутатіону, активністю каталази та швидкістю клубочкової фільтрації. Зроблено висновок, що рівень IDO може бути можливим маркером окислювального стресу та запалення у пацієнтів із ХНН.

Ключові слова: індоламін-2,3-діоксигеназа, ниркова недостатність, сироватка крові, біохімічні показники, кореляційний аналіз.

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