Article

Estimated Advanced Glycation end Products Level and Biochemical parameters for Renal Failure Patients in Kirkuk city

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Abstract

Advanced glycation end product (AGE) levels are elevated in patients with decreased renal function, which may contribute to the excess cardiovascular disease in this population. So, the present study assessed the advanced glycation end product level of patients with renal failure. 100 subjects (31 males and 69 females with ages: 15-75 years) reported chronic kidney disease disease who attended Azadi Teaching Hospital, Kirkuk Hospital, and Al-Watan Hospital, Kirkuk, Iraq, from October 2023 to April 2024. The participants in this study were divided into two groups: 100 patients with chronic kidney disease and 40 subjects as control group. The results showed that nearly all of patients were across the age of 60 (39%). Patients had an average body mass index of 18.5-24.9 kg 52%. Eighty-three percent of patients were living in cities. The smoking status of patients was non-smoking 68%. The current outcomes demonstrated a reduction in the concentration of total protein that is significant ($P<0.05$), albumin, and globulin in patients compared to the control group. On the other hand, the results show a significant ($P<0.05$) increase in the concentrations of carbonyl and amino group, while a decrease in thiol levels in patients compared to the control group. Otherwise, the results showed that the AGE levels in the serum of patients (3.82 ± 0.016 AU) are significantly higher ($P<0.05$) than in the control group (3.09 ± 0.028 AU). It is concluded that chronic kidney disease leads to elevated glycation levels and

changes in oxidative status.

Keywords: **Glycation; Chronic kidney disease; amino group; carbonyl group.**

Introduction

The kidneys are a vital organ for the proper functioning of the human body. Its function is to filter blood, remove waste products, and control fluid balance in the body and urine formation [1]. Chronic kidney disease (CKD) refers to all conditions of the kidney, lasting more than three months, where a person has had evidence of kidney damage and/or reduced kidney function. Evidence of kidney damage manifests as either proteinuria or albuminuria, hematuria or scarring detected by imaging tests [2-3]. There are five stages of chronic renal failure (CRF) based on the glomerular filtration rate [4]. End-Stage Renal Disease (ESRD) is the final stage of chronic kidney disease and is defined as a severe, irreversible decline in a person's kidney function that can be fatal with no dialysis or transplantation [5-6]. The kidney is a metabolically active organ, via epithelial cells rich in mitochondria in which redox reactions occur. Furthermore, ROS play an important role in the physiological regulate renal function. This makes the kidney increasingly susceptible to oxidative stress, which may speed up the progression of kidney disease [6-7]. Oxidative stress can be induced and enhanced in the context of the uremic milieu [7-8], and of CKD-associated chronic low-grade inflammation [9– 10]. Other pro-oxidant conditions, which are frequently present in moderate-to-severe stages of CKD and even more in ESRD (e.g., advanced age, diabetes), can exacerbate oxidative stress., dyslipidemia, hypertension, obesity, and infectious complications) [11], and by the lower levels of water-soluble antioxidant vitamins measured in CKD patients due to the dietary restriction of fresh fruits and vegetables required of them in order to avoid hyperkalaemia [12-13]. Furthermore, Hemodialysis (HD) can lead to increased oxidative stress [14-15], which is instead mitigated by restoring renal function [16-17]. Advanced glycation end products (AGEs) are stable posttranslational modifications of proteins that occur during a spontaneous reaction with glucose and related metabolites. Important AGEs consist of MG-H1, Nε-carboxymethyl-lysine (CML), and glucosepane [18]. They contribute to the development of chronic kidney disease (CKD), so the present study conducted to assess the Glycation level and oxidative status of in patients with renal failure.

Materials & methods

Subjects

One hundred subjects (31 males and 69 females with age: 15-75 years) reported with CKD disease who attended at Azadi Teaching Hospital, Kirkuk hospital and Al-Watan hospital, Kirkuk, Iraq, from October 2023 to April 2024. The participants in this study were divided into two groups as follows:

- 100 CKD as a patients group
- And 40 subjects as control group with same age.

Inclusion criteria

Patients with CKD who are between the ages of 15 and 75 who do not receive any therapeutic drugs.

Exclusion criteria

Patients with CKD used therapeutic drugs, as well as those with other types of organ failures such as the heart, etc.

Blood samples

Every patient had a blood sample taken by skilled nurses. Five milliliters of venous blood were donated by each patient, and they were split between a 4.0 ml vacutainer plain tube and a 1.0 ml EDTA tube. After centrifuging for ten minutes at 4000 rpm, the serum was extracted. After being separated, the serum was put into tubes and kept cold until it was time for analysis

Measurements

- **Total protein**: Total protein analysis was performed for patients and control according to the colorimetric method described by the manufacturer (Biolabo/France).

- **Albumin**: Albumin analysis was performed for patients and control according to the colorimetric method describedby the manufacturer (Biolabo/ France).

- **Total thiol**: Total thiol ELISA Kit (Cell Biolabs, USA, NO.:MET-5053) assays total thiol level in human serum using Sandwich-ELISA.

- **Carbonyl group**: Carbonyl group ELISA Kit (Sigma- Aldrich, USA, NO.: MAK094) assays carbonyl group levels in human serum using Sandwich-ELISA.

- **Advanced glycation end products (AGEs)**: AGEs ELISA Kit (Cell Biolabs, USA, NO.: STA-817) assays AGEs levels in human serum using Sandwich-ELISA.

Statistical analysis

The Data were analyzed using a statistical Minitab program. A statistical difference between the means of the experimental groups was analyzed using one-way analysis of variance (ANOVA).

Results and Discussion

Socio-demographic variables

Table 1 displayed a few sociodemographic factors. Around 39% of patients were over 60 years old, making up the largest age group Patients' Body Mass Index (BMI) ranged from 18.5-24.9 kg with a 52% percentile. 83% of the patients' residential areas were urban. Patients had a 68% non-smoking rate for smoking status.

Table (1): socio-demographic variables of studied groups

Characteristics	Classes	Groups Control	Patients
Age	>20-30 years	2 (5%)	7 (7%)
	31-40 years	5 (12.5%)	11 (11%)
	41-50 years	7 (17.5%)	19 (19%)
	51-60 years	11 (27.5%)	24 (24%)
	> 60 years	15 (37.5%)	39 (39%)
	Total	40	100
Body Mass Index (BMI)	< 18.5	14 (35%)	37 (37%)
	18.5-24.9	21 (52.5%)	52 (52%)
	25-29.9	5 (12.5%)	11 (11%)
	Total	40	100
Residential Area	Urban	32 (80%)	83 (83%)
	Rural	8 (20%)	17 (17%)
	Total	40	100
Smoking Status	Yes	6 (15%)	18 (18%)
	Non	29 (72.5%)	68 (68%)
	Passive	5 (12.5%)	14 (14%)
	Total	40	100

Proteins profile

According to table (2), the totalprotein concentration in the blood of male patients (5.751±0.535 mg/dl) with renal failure is lower than control group (7.298±0.357 mg/dl) and the reduction is significant (P<0.05). the albumin level of male patients (2.855±0.437) with renal failure is significantly lower (P<0.05) than that of the control group (4.205±0.443 mg/dl). Otherwise, there are no non- significant (P<0.05) differences between the control group's serum globulin level (2.91±0.461 mg/dl) and male patients (2.749±0.305 mg/dl).

On the other hand, for female subject, the totalprotein level in female patients (5.561±0.427 mg/dl) is lower than in the control group (7.198±0.51 mg/dl) that is significant (P<0.05)., as shown in table (3). Albumin levels in female patients (2.639±0.21 mg/dl) are significantly lower (P<0.05) than in the control group (4.225±0.398 mg/dl). Globulin levels in female patients (2.675±0.415 mg/dl) are lower (P0<0.05) than in the control group (3.406±0.51mg/dl) that is significant (P<0.05), as shown in table (2).

Table (2): protein profile in studied groups

Groups Parameters	Male		Female	
	Control mean±SD	Patients mean±SD	Control mean±SD	Patients mean±SD
TP mg/dl	7.298±0.357	5.751±0.535*	7.198±0.51	5.561±0.427*
Albumin mg/dl	4.205±0.443	2.855±0.437*	4.225±0.398	2.639±0.21*
Globulin mg/dl	2.91±0.461	2.749±0.305	3.406±0.51	2.675±0.415*

* mean there is a significant (P<0.05) differences between groups.

Table (2) shows that females are more susceptible to low levels of total protein and albumin compared to males with chronic kidney failure. The albumin levels in our study showed significant (P<0.05) reduced in CKD patients compared to control, in the table (2), Hypoalbuminemia is one of the most important risk factors for death in CKD patients. These findings were supported by many researchers[19-20] who shared that decreased serum albumin levels have been linked to nutritional inflammation markers in HD patients. Our findings extend the existing literature and back up the idea that decreased albumin the likelihood of CKD progression regardless of whether participants had different baseline eGFR or proteinuria. The findings supplied guidance for clinical ALB level intervention to decrease the risk of CKD development. Hypoalbuminemia possibly reflects several conditions: liver disease, poor nutrition, severe proteinuria, and chronic inflammation. Kadono et al. reported a significantly increased hazard of inflammation in lower serum levels of albumin [21]. As a consequence, the mechanism of hypoalbuminemia on CKD progression may be related to the manifestations stated above. Investigation in the Cardiovascular Health Study found that serum albumin was independently associated with a higher hazard of decreased renal function, while several markers of inflammation were not significantly associated [22]. According to findings of Abdulkader, the total protein level in the serum of patients with CKD reduced and reached (4.9) g/dl with a significant (P<0.001) difference because the renal is able to be retaining formed substances that include RBC, WBC, and proteins at the glomerulus level. The results of the current study revealed that the level of total protein demonstrate a substantial (P<0.05) reduced compare healthy individuals. Protein consequently exits the circulation through the kidney and into the urine [23]. In the current investigation, it was discovered that when compared with control, albumin levels reduced significantly in both males and females (P 0.05). An earlier Japanese study found an independent relationship between blood albumin levels and proteinuria in Japanese DN individuals, and a subsequent study examined the effects of albumin reducing on the progression of renal disease in 343 Caucasian (77%) and black DN patients. They found that a quicker rate of GFR decline was also associated with hypoalbuminemia [24].

Carbonyl group and total thiol

In male, Carbonyl levels in serum of CKD patients (0.779±0.032 mg/dl) are significantly higher (P<0.05) than in the control group (0.415±0.039 mg/dl). Thiol levels in serum male patients (14.11±4.01) are lower (P<0.05) than in healthy individuals group (26.078±2.89l) that is significant (P<0.05). In female, Carbonyl levels in CKD patients (0.71±0.092 nmol/mg) lower than in healthy individuals group (0.408±0.019 nmol /mg) that is significant (P<0.05).

Thiol levels in serum of CKD patients (9.432±2.44) are lower than in healthy individuals group (22.801± 3.475) that issignificant (P<0.05), as shown in table (3).

Table (3): total thiol carbonyl group in studied groups

Groups Parameter	Male		Female	
	Control mean±SD	Patients mean±SD	Control mean±SD	Patients mean±SD
Carbonyl nmol/mg	0.415±0.039	0.779±0.032*	0.408±0.019	0.71±0.092*
Thiol nmol/mg	26.078±2.89	14.11±4.01*	22.801±3.475	9.432±2.44*

* mean there is a significant (P<0.05) differences between groups.

This study demonstrated that the level of serum thiol was decreased in patients group compared to control subjects, and table (3) shows that females are more susceptible to low levels of total thiol compared to males with chronic kidney failure. These results were agreed with KemidiIIaiah et al [25], they found that protein thiols were decreased in patients with uremia ,they are sacrificed to quench ROS which are produced excessively in CKD patients, thus the levels of these proteins decrease in these patients.Totalt hiol status in the body, especially thiol (SH) groups present onprotein are considered as major plasmaantioxidant in vivo are present in a concentration higher than albumin and are major reducing groups present in the body fluids [26]. Protein CO groups as biomarkers of oxidative stress possess certain advantages across other oxidation products as a result of relative early formation and relative stability. This study confirmed increased carbonyl formation in CRF patients. Jasmina et al, [27], found that level of protein sulphydryl groups (P- SH) in plasma, which are important chain   breaking

"sacrificial" antioxidant, were markedly reduced in individuals with CRF compared to healthy subjects and carbonyl level as a marker of oxidative modification of proteins, increased in plasma of all chronic renal failure patients compared to healthy subjects. According to the findings of the current study, renal failure and critically sick patients had blood protein thiol levels that were much lower than those of healthy participants. thiol levels and serum albumin levels were positively correlated in patients with renal failure. Study the causes of low thiol concentrations in critically ill patients and those with kidney failure in furtherdetail. Lower blood albumin levels could be one of the main explanations of this observation [28].

The advanced glycation end products (AGEs) level

Table (4) showed that the advanced glycation end products levels in serum of CKD patients (3.82±0.016 AU) are significantly higher (P<0.05) than inthe control group (3.09±0.028 AU).

Table (4): levels of advanced glycation end products in studied groups

Groups Parameters	Control group	Patients group
AGEs (AU) ($X10^5$)	3.09±0.028	3.82±0.016*

* mean there is a significant (P<0.05) differences between groups.

There have been several studies reporting serum AGE levels in uremic patients. Some authors report no difference in the serum AGE levels in the patients with and without DM on chronic dialysis treatment [29]. On the contrary, other authors [30] described higher AGE levels in dialyzed patients with DM. Additionally, AGE levels in uremic patients were found to be several times higher than in patients with diabetes and normal kidney function. Mechanisms other than hyperglycemia therefore must be involved [31]. In this study, the AGE levels in CKD patients were elevated. Nevertheless, this finding suggests that the decreased AGE disposal in CKD patients is more essential for accumulation of AGEs than long-term glycemia in those CKD patients. The increase in AGE levels was evident from CKD. In CKD patients, the reason for the increase in AGEs is a decreased ability of the kidney to clear AGEs from circulation [32] and also increased carbonyl stress, a major pathogenic mechanism of AGE formation and risk factor for patients with CKD [33].

Conclusions

Based on the results of the current study, it was found that Chronic kidney Disease led to the accumulation of advanced glycation end products in the blood and thus caused an increase in its levels in patients. Thus, it can be considered an important indicator in the diagnosis of CKD.

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