



A comparative study of true and pseudo-peroxidase and their relative biomarkers between male and female patients with chronic kidney disease

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ABSTRACT

Introduction: Pseudo-peroxidase enzymes, despite mimicking the catalytic prowess of true peroxidases, lack authentic enzymatic functionality. The critical discernment between pseudo and true peroxidases is imperative when assessing oxidative biomarkers in both sexes, as variations in enzymatic activity may underpin gender-specific disparities in oxidative stress profiles, potentially impacting disease vulnerability and therapeutic modalities.

Objectives: This study aims to ascertain true and pseudo peroxidase activity alongside other oxidative stress biomarkers, comparing these parameters between male and female patients afflicted with chronic kidney disease (CKD).

Patients and Methods: A total of 140 blood samples were analyzed, comprising 80 from CKD patients and 60 from healthy controls. Various parameters, including total protein, albumin, glomerular filtration rate (GFR), urea, creatinine, hemoglobin (Hb), free amino acids, globulins, ischemia-modified albumin (IMA), carbonyls, total thiols, native thiols, disulfides, peroxidase, and pseudo peroxidase activity, as well as specific activity, were assessed in both groups.

Results: Females with CKD exhibited a significant elevation in numerous serum parameters, including total protein, albumin, globulins, IMA, GFR, urea, creatinine, free amino acids, carbonyls, and disulfides compared to their male counterparts with CKD. Conversely, males with CKD demonstrated a notable increase in peroxidase activity, peroxidase specific activity, pseudo peroxidase activity, and pseudo peroxidase specific activity in comparison to females with CKD.

Conclusion: The comparative analysis between male and female CKD patients regarding the assessed parameters revealed notable differences in albumin, total protein, GFR, urea, creatinine, Hb, free amino acids, globulins, IMA, carbonyls, total thiols, native thiols, disulfides, peroxidase, and pseudo peroxidase activity and specific activity.

Implication for health policy/practice/research/medical education:

Pseudo-peroxidase enzymes imitate true peroxidases; however, they are deficient in authentic enzymatic functionality, which is indispensable for accurately evaluating oxidative biomarkers in both male and female subjects. Females with chronic kidney disease showed higher oxidative stress serum parameters than males.

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Introduction

Chronic kidney disease (CKD) represents a substantial global health challenge characterized by the gradual deterioration of renal function. The intricate interplay between oxidative stress and CKD has garnered increasing significance, with peroxidase enzymes, encompassing both authentic and pseudo forms, emerging as pivotal

contributors in this dynamic milieu (1). The enzymatic capabilities of genuine peroxidases, such as catalase and peroxidase, are widely acknowledged for their efficacy in mitigating reactive oxygen species (ROS) and fortifying cellular defenses against oxidative injury). Conversely, pseudo-peroxidases mimic catalytic activities without possessing authentic enzymatic functions, thereby

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prompting inquiries into their potential impact on oxidative stress, particularly within the context of CKD (2).

The pivotal role of oxidative stress in CKD pathogenesis is widely acknowledged, with authentic peroxidases constituting indispensable components of the antioxidant defense system (3). Their enzymatic functions play a critical role in ameliorating oxidative damage to renal tissues by efficiently neutralizing ROS. The implication of oxidative stress in CKD progression is well-established, raising questions about how the non-enzymatic characteristics of peroxidases may disrupt redox dynamics and potentially exacerbate oxidative stress in CKD (4).

An intricate understanding of gender-specific discrepancies in CKD unveils a complex array of oxidative stress profiles between males and females (5). Although the precise mechanisms underlying these differences remain incompletely elucidated, factors such as hormonal fluctuations, genetic predispositions, and lifestyle choices are believed to be contributory. The complex interplay between true and pseudo-peroxidases in the realm of gender-specific oxidative stress in CKD is currently the subject of active investigation. Acknowledging gender-specific intricacies in oxidative stress has the potential to influence treatment responses and guide the development of personalized therapeutic strategies (6).

The identification of differences in peroxidase activity and oxidative stress levels between male and female cohorts with CKD is of paramount importance, given the potential impact of these variations on disease progression and associated complications (7). Researchers are intensively exploring the intricate interaction between peroxidase enzymes and oxidative stress in CKD patients, aiming to enhance our understanding of the disease pathophysiology and establish the groundwork for tailored therapeutic interventions that cater to the distinct needs of male and female CKD populations.

Objectives

This study aims to assess the pseudo peroxidase activity of hemoglobin (Hb) in comparison to peroxidase activity and to estimate a range of oxidative stress biomarkers, particularly in patients with CKD, with a specific focus on gender-based differences between males and females.

Patients and Methods

Study design

The investigation was conducted at the Department of Chemistry, University of Kirkuk, located in Kirkuk, Iraq, spanning the duration from October 2022 to April 2023. Blood specimens were exclusively procured from the Kirkuk General Hospital's renal dialysis unit.

Blood samples collection

A total of 200 samples were procured, comprising 60 samples from apparently healthy individuals (25 males

and 35 females) serving as controls, and 140 samples from patients diagnosed with CKD (60 males and 80 females). Blood was collected via venipuncture using sterile disposable syringes, which were subsequently transferred into gel tubes. The collected blood underwent centrifugation to separate and collect the serum. Heparin tubes were employed to prevent blood clotting for the assessment of peroxidase enzyme activity and specific activity.

A specialized questionnaire was developed to collect patient data, including age, smoking habits, alcohol consumption, presence of any chronic illnesses, and frequency of renal dialysis sessions per week.

Methods

Total protein was assessed using the method described by Zaia et al (8), while the bromocresol green method was employed to determine albumin levels (9). Total thiol and native thiol levels were estimated using a modified version of Ellman's method (10). Protein carbonyls were quantified using the method developed by Levine et al (11). Ischemia-modified albumin (IMA) levels were determined using a modified procedure based on Dervisoglu and Oner (12). Free amino acid concentrations were measured according to the procedure outlined by Zaia et al (8). The glomerular filtration rate (GFR) was estimated using the method described by Florkowski and Chew-Harris (13). Urea and creatinine levels were determined using Drabkin's method, following the procedure by Kamal et al (14). Hemoglobin levels were estimated according to Srinivasan et al (15). Globulin levels were calculated by subtracting the albumin concentration from the total protein concentration. Disulfide levels were determined according to Ateş et al (16). Peroxidase and pseudo-peroxidase enzyme activities and specific activities were estimated using a modified procedure based on the study by Ali et al (17).

Statistical analysis

The statistical analysis was performed using GraphPad Prism version 8.0.2 (263). The results were expressed as the mean \pm standard deviation (SD), and the *t* test tool was utilized to compare the data obtained within the aforementioned software. Additionally, the correlation between the parameters was evaluated using a significance threshold of $P \leq 0.05$.

Results

Table 1 displays the activity of pseudo and true-peroxidases as the mean \pm standard deviation (SD) in the sera of CKD patients, categorized into male and female patient groups, each compared to its respective control group.

Additionally, Table 1 illustrates heightened levels of peroxidase activity and specific activity, as well as pseudo peroxidase activity and specific activity, in CKD patients compared to healthy individuals. Specifically, males with CKD exhibited elevated enzyme levels compared to

Table 1. The activity and specific activity of true and pseudo peroxidases in both male and female CKD patients compared to the control group

Parameter	Males		P value	Females		P value
	Control (n=25) Mean ± SD	Patients (n=60) Mean ± SD		Control (n=35) Mean ± SD	Patients (n=80) Mean ± SD	
Peroxidase activity (U/mL)	7.53 ± 2.19	39.61 ± 7.56	< 0.05	8.08 ± 1.82	34.397 ± 5.94	<0.05
Peroxidase specific activity (U/mL)	0.94 ± 0.197	4.63 ± 0.81	< 0.05	1.08 ± 0.24	3.80 ± 0.64	<0.05
Pseudo-peroxidase activity (U/mL)	1.77 ± 0.24	46.76 ± 8.86	< 0.05	1.92 ± 0.37	36.85 ± 4.21	<0.05
Pseudo-peroxidase specific activity (U/mL)	0.94 ± 0.197	4.91 ± 0.93	< 0.05	1.08 ± 0.24	3.57 ± 0.42	<0.05
Peroxidase activity (U/mL)	0.685 ± 0.461	3.984 ± 0.986	< 0.05	0.633 ± 0.338	4.373 ± 1.479	<0.05

females.

Table 2 presents the levels of the investigated oxidative stress biomarkers as the mean ± standard deviation (SD) in male and female patient groups, each compared to its respective control group.

In general, Table 2 reveals elevated levels of total protein, albumin, IMA, urea, creatinine, free amino acids, carbonyls, disulfides, peroxidase activity, peroxidase-specific activity, pseudo-peroxidase activity, and pseudo-peroxidase specific activity in patients compared to the control groups, with a significant *P* value ($P < 0.05$), observed in both male and female patients. Conversely, GFR, total thiol, and native thiol levels exhibited a decrease in patients compared to the control groups, with a significant *P* value ($P < 0.05$), evident in both male and female populations.

Moreover, globulin levels were elevated in males

compared to the control group, whereas a decrease in globulin levels was observed in females compared to the control group. Hemoglobin levels were decreased in patients compared to the control groups in both male and female subjects, with a non-significant *P* value.

Table 3 illustrates the correlation coefficient analysis between true and pseudo- peroxidase activity and the investigated parameters in male and female CKD patients. Table 3 shows that some of the studied correlations were significant, however, others were not.

Discussion

True peroxidases are enzymes primarily involved in scavenging free radicals and other substances through the peroxidase cycle (17). Pseudo-peroxidases, while serving important roles in physiological processes, can exhibit peroxidase-like behavior under specific conditions. The

Table 2. The levels of oxidative stress biomarkers in both male and female chronic kidney disease patients compared to the control group

Parameter	Males		P value	Females		P value
	Control (n=25) Mean ± SD	Patients (n=60) Mean ± SD		Control (n=35) Mean ± SD	Patients (n=80) Mean ± SD	
Total protein (g/dL)	7.27 ± 1.36	8.63 ± 1.60	<0.05	7.44 ± 1.67	9.054 ± 1.501	<0.05
Albumin (g/dL)	4.28 ± 1.41	5.69 ± 1.02	<0.05	4.42 ± 0.994	5.772 ± 0.971	<0.05
Globulin (g/dL)	2.32 ± 0.98	3.52 ± 0.69	<0.05	3.28 ± 0.737	3.091 ± 0.459	<0.05
AGR	1.84 ± 0.596	1.61 ± 0.75	<0.05	1.35 ± 0.463	1.867 ± 0.685	<0.05
IMA (ABSU)	0.49 ± 0.15	0.896 ± 0.11	<0.05	0.54 ± 0.121	0.912 ± 0.194	<0.05
GFR (mL/min)	121.22 ± 8.73	37.78 ± 7.197	<0.05	123.85 ± 27.87	47.11 ± 7.95	<0.05
Urea (mg/dL)	25.48 ± 4.82	165.09 ± 31.38	<0.05	25.67 ± 5.78	165.19 ± 25.15	<0.05
Creatinine (mg/dL)	0.82 ± 0.14	9.75 ± 1.84	<0.05	0.84 ± 0.19	10.224 ± 1.783	<0.05
Hemoglobin (g/dL)	10.99 ± 2.64	9.94 ± 1.9	0.08	12.76 ± 2.87	7.864 ± 1.377	<0.05
Free amino (mmol/L)	7.95 ± 1.59	25.49 ± 4.82	<0.05	8.27 ± 1.86	37.466 ± 5.348	<0.05
Free amino/total protein	1.09 ± 0.49	2.95 ± 3.51	<0.05	1.11 ± 0.49	4.138 ± 3.113	<0.05
Carbonyl (nmol/mL)	65.4 ± 18.02	69.54 ± 13.22	<0.05	75.31 ± 16.95	79.461 ± 12.508	<0.05
Carbonyl/ total protein	8.99 ± 3.87	8.05 ± 3.69	0.006	10.13 ± 4.79	8.78 ± 4.20	<0.05
Total thiol (μmol/L)	485.61 ± 45.12	252.48 ± 47.93	<0.05	466.71 ± 85.01	239.23 ± 39.61	<0.05
Total thiol/ total protein	66.76 ± 19.20	29.24 ± 15.85	<0.05	62.76 ± 31.38	26.42 ± 13.70	<0.05
Native thiol (μmol/L)	45.98 ± 23.62	22.54 ± 42.85	<0.05	44.912 ± 79.05	21.99 ± 33.31	<0.05
Disulfide (μmol/L)	18.31 ± 3.33	20.73 ± 3.93	0.015	16.21 ± 3.65	22.36 ± 3.81	<0.05

IMA: Ischemia-modified albumin; GFR: Glomerular filtration rate; AGR: Albumin/globulin ratio; ABSU: The absorbance unit of ischemia-modified albumin.

Table 3. The correlation coefficients between true and pseudo-peroxidase activity and the studied parameters in male and female patients with chronic kidney disease

Parameter	Males with CKD	Females with CKD
	<i>r</i> */ <i>P</i> value	<i>r</i> */ <i>P</i> value
PA-total protein (g/dL)	-0.0993/0.7581	-0.0320/0.7204
PA-albumin (g/dL)	0.3803/0.4468	0.4308/0.3933
PA-globulin (g/dL)	-0.2915/0.8927	-0.6477/0.3057
PA-albumin/globulin	0.3984/0.1402	-0.0985/0.6184
PA-IMA (ABSU)	0.6610/0.0836	0.5181/0.1529
PA-GFR (mL/min)	0.6225/0.1503	0.0498/0.3184
PA-urea (mg/dL)	-0.1317/0.6323	-0.0537/0.7915
PA-creatinine (mg/dL)	0.2562/0.5518	0.5855/0.2029
PA-Hb (mg/dL)	-0.3964/0.6290	0.744/0.06076
PA-free amino (mmol/L)	0.7088/0.9717	0.8314/0.9776
PA-free amino/total protein (g/dL)	0.0921/0.6294	0.1331/0.4893
PA-carbonyl (nmol/mL)	-0.1872/0.4837	-0.5614/0.2123
PA-carbonyl/total protein	0.0864/0.5911	0.1591/0.3607
PA-total thiol (μmol/L)	0.0815/0.6608	0.1851/0.3979
PA-total thiol/total protein	-0.0591/0.0615	0.2378/0.9160
PA-native thiol (μmol/L)	0.3402/0.1167	0.2459/0.5549
PA-disulfide (μmol/L)	-0.6636/0.0818	-0.5637/0.2339
SA-total protein (g/dL)	0.0928/0.9914	-0.5716/1.3864
SA-albumin (g/dL)	-0.4414/0.4395	0.5020/0.6379
SA-globulin (g/dL)	-0.7946/0.4990	-0.5286/0.6160
SA-albumin/globulin	-0.0812/0.5934	0.1594/1.2776
SA-IMA (ABSU)	0.8326/0.5009	-0.5286/0.6160
SA-GFR (mL/min)	-0.1517/0.1775	0.2915/0.6597
SA-urea (mg/dL)	-0.3491/0.6632	-0.3954/1.1328
SA-creatinine (mg/dL)	0.2242/0.5854	0.2442/0.5442
SA-Hb (mg/dL)	-0.0866/0.1906	-0.3991/1.3189
SA-free amino (mmol/L)	0.6939/0.5186	0.6792/0.5442
SA-free amino/total protein	0.0518/0.4674	0.0174/0.5209
SA-carbonyl (nmol/mL)	-0.6462/0.6862	-0.2442/0.7392
SA-carbonyl/total protein	0.4348/0.9540	0.7577/1.2776
SA-total thiol (μmol/L)	0.38863/0.1376	0.5264/0.8962
SA-total thiol/total protein	0.4236/0.4397	0.3350/0.8284
SA-native thiol (μmol/L)	0.1912/0.1063	0.2224/0.5752
SA-disulfide (μmol/L)	-0.3631/0.5292	-0.1873/0.9573
PsA-total protein (g/dL)	0.1019/0.7464	0.1101/0.3983
PsA-albumin (g/dL)	0.0164/0.8718	-0.4205/0.4192
PsA-globulin (g/dL)	-0.3050/0.7952	-0.06676/0.5093
PsA-albumin/globulin	0.0942/0.9258	0.1110/0.2716
PsA-IMA (ABSU)	0.6714/0.0926	0.4981/0.1631
PsA-GFR (mL/min)	0.1596/0.0917	-0.0512/0.4005
PsA-urea (mg/dL)	-0.1370/0.1721	0.09669/0.3386
PsA-creatinine (mg/dL)	0.2815/0.6438	0.6194/0.2387
PsA-Hb (mg/dL)	-0.1101/0.3983	-0.7581/0.959
PsA-free amino (mmol/L)	0.6953/0.9301	0.7150/0.9646
PsA-free amino/total protein (g/dL)	0.1746/0.9325	0.0163/0.8187

Table 3. Continued

Parameter	Males with CKD	Females with CKD
	r*/P value	r*/P value
PsA-carbonyl (nmol/mL)	-0.0919/0.4553	-0.4827/0.2218
PsA-carbonyl/total protein	0.0635/0.5815	0.0494/0.6254
PsA-total thiol (μmol/L)	-0.0531/0.5561	0.1706/0.4382
PsA-total thiol/total protein	-0.0694/0.5123	-0.05464/0.5892
PsA-native thiol (μmol/L)	0.1677/0.2639	0.1476/0.5947
PsA-disulfide (μmol/L)	-0.6612/0.8137	-0.5960/0.736
PsSA-total protein	0.0753/0.4185	0.4997/1.2580
PsSA-albumin (g/dL)	-0.0197/0.7791	0.1660/0.0987
PsSA-globulin (g/dL)	-0.3128/0.5709	-0.3921/0.5984
PsSA-albumin/globulin	0.0992/0.6237	0.1614/0.9691
PsSA-IMA (ABSU)	0.0494/0.6254	0.1011/0.3168
PsSA-GFR (mL/min)	0.0926/0.2418	0.3225/0.7186
PsSA-urea (mg/dL)	-0.3559/0.6867	0.3426/0.9258
PsSA-creatinine (mg/dL)	0.0566/0.5757	-0.1553/0.5691
PsSA-Hb (mg/dL)	-0.0918/0.3642	-0.1526/0.7358
PsSA-free amino (mmol/L)	0.6834/0.5725	0.0629/0.6153
PsSA-free amino/total protein	0.0534/0.3864	0.0170/0.5824
PsSA-carbonyl (nmol/mL)	0.1827/0.7918	-0.1251/0.2892
PsSA-carbonyl/total protein	0.1641/0.4352	0.2568/0.9891
PsSA-total thiol (μmol/L)	0.3886/0.1376	0.4391/0.7791
PsSA-total thiol/total protein	0.0521/0.7382	0.0753/0.6187
PsSA-native thiol (μmol/L)	0.2734/0.1784	0.2672/0.6826
PsSA-disulfide (μmol/L)	-0.2160/0.0917	-0.1846/0.9258

IMA: Ischemia-modified albumin; GFR: Glomerular filtration rate; ABSU: The absorbance unit of ischemia-modified albumin; PA: Peroxidase activity; SA: Peroxidase specific activity; PsA: Pseudo peroxidase activity; PsSA: Pseudo peroxidase specific activity.

*r: is Pearson's correlation factor, which measures the strength of the correlation, given by the GraphPad Prism program.

peroxidases under scrutiny have demonstrated the ability to convert both active heme molecules and proteins, generating tyrosyl radicals (18). Hemoglobin, a vital protein found in red blood cells (RBCs), is known to possess pseudo-peroxidase activity in living organisms, engaging in a complex pseudo-peroxidative cycle initiated by its interaction with hydrogen peroxide. This pseudo-peroxidase activity, also termed peroxidase activity, results in the production of various harmful oxidative intermediates. The non-enzymatic peroxidase-like activity of Hb stems from its heme iron moiety, which contains an iron ion (Fe^{2+}) capable of undergoing redox reactions, mimicking certain peroxidase-like functions. Hemoglobin's peroxidase activity is often referred to as pseudo-peroxidase activity due to its lack of specificity and efficiency, distinguishing it from true peroxidase enzymes such as catalase or peroxidase. The heme iron within Hb can undergo redox processes, switching between its ferrous (Fe^{2+}) and ferric (Fe^{3+}) forms (19). This ability allows Hb to participate in electron transfer reactions akin to those observed in peroxidase enzymes. Additionally, Hb has been found to catalyze the decomposition of hydrogen

peroxide (H_2O_2), a reaction commonly associated with peroxidases. The heme iron acts as a catalyst in this decomposition, albeit with lower efficiency compared to true peroxidases. Hemoglobin's pseudo-peroxidase activity may be augmented under specific conditions, such as during oxidative stress, where an abundance of ROS or other oxidative stressors can induce Hb to undergo redox processes, potentially contributing to oxidative damage (20).

The total protein levels were significantly elevated in male (8.634 ± 1.604 g/dL) and female (9.054 ± 1.501 g/dL) patients compared to their respective control groups (7.274 ± 1.363 g/dL for males and 7.436 ± 1.673 g/dL for females), with a significant *P* value ($P < 0.05$). These findings are consistent with those reported by Adnan Khalaf and Ghassan Zainal (21), who conducted a similar study involving patients with end-stage kidney disease in Kirkuk city, where the current study was conducted. Furthermore, these results align with the observations made by Al-Doorii et al (22), indicating an increase in total protein levels in the context of chronic kidney failure.

The albumin levels were significantly elevated in male

(5.688±1.018 g/dL) and female (5.772±0.971 g/dL) patients compared to their respective control groups (4.282±1.407 g/dL for males and 4.418±0.994 g/dL for females), with a significant *P* value (*P*<0.05). These findings are consistent with the results reported by Wyld et al (5), who observed a 30% higher likelihood of females requiring dialysis compared to males, accompanied by elevated serum albumin levels.

The globulin levels were significantly higher in male patients (5.688±1.018 g/dL) compared to their control group (2.324±0.976 g/dL). Conversely, females exhibited lower globulin levels (3.091±0.459 g/dL) compared to their control group (3.276±0.737 g/dL), with a significant *P* value (*P*<0.05). Despite the higher prevalence of CKD in females, males demonstrate a greater propensity to progress to end-stage kidney disease (23). This observation aligns with a study linking the increase in globulin levels in males with CKD to sex hormone-binding globulin (20).

The levels of IMA were significantly elevated in male (0.896±0.107 ABSU) and female (0.912±0.194 ABSU) patients compared to their respective control groups (0.485±0.146 ABSU for males and 0.537±0.121 ABSU for females), with a significant *P* value (*P*<0.05). Literature reports (24) indicate higher IMA levels in female CKD patients, particularly in end-stage renal disease.

The GFR was significantly reduced in male (37.783±7.197 g/dL) and female (47.108±7.948 g/dL) patients compared to their respective control groups (121.217±8.730 g/dL for males and 123.85±27.866 g/dL for females), with a significant *P* value (*P*<0.05). A global comparative study across 195 countries concluded that GFR levels are higher in females compared to males (25).

Serum urea levels were significantly higher in male (165.087±31.376 mg/dL) and female (165.189±25.146 mg/dL) patients compared to their respective control groups (25.478±4.824 mg/dL for males and 25.667±5.775 mg/dL for females), with a significant *P* value (*P*<0.05). These results align with findings by Omar et al (26) and Al-Doorii et al (22).

Serum creatinine levels were significantly elevated in male (9.752±1.835 mg/dL) and female (10.224±1.783 mg/dL) patients compared to their respective control groups (0.817±0.137 mg/dL for males and 0.843±0.190 mg/dL for females), with a significant *P* value (*P*<0.05). These results are consistent with the studies by Omar et al (26) and Al-Doorii et al (22).

Plasma Hb levels did not significantly differ in male patients, with lower Hb levels (9.935±1.899 mg/dL) recorded compared to the control group (10.987±2.635 mg/dL) with a *P* value of 0.0880. Only female patients exhibited a significant increase in Hb levels (7.864±1.377 mg/dL) compared to the control (12.763±2.872 mg/dL; *P*<0.05). These findings are consistent with the study by Okada et al (27), showing reduced Hb levels, especially in females compared to males. The results of lower Hb levels have been confirmed in women with CKD compared

to men, with decreased Hb levels were also noted in the study by Al-Doorii et al (22).

The levels of free amino acids were significantly elevated in patients, males (25.485±4.824 mmol/L), females (37.466±5.348 mmol/L), exceeding the control values (7.950±1.593 and 8.265±1.860 mmol/L) for males and females, respectively, with a significant *P* value (*P*<0.05). Li et al (28) reported similar elevations in free amino acids in patients with end-stage kidney failure undergoing hemodialysis.

The concentrations of carbonyls were significantly higher in patients, males (69.536±13.221 nmol/mL), females (79.461±12.508 nmol/mL), compared to the control values (65.401±18.020 and 75.312±16.945 nmol/mL) for males and females, respectively, with a significant *P* value (*P*<0.05). Colombo et al (29) observed increased carbonyl concentrations in females undergoing maintenance hemodialysis, suggesting a potential biological role of the female gender in hemodialysis-induced plasma protein carbonylation in patients with end-stage renal disease (ESRD).

The concentrations of total thiol were significantly lower in patients, males (252.48±47.927 μmol/L), females (239.23±39.609 μmol/L), compared to the control values (485.61±45.118 and 466.71±85.011 μmol/L) for males and females, respectively, with a *P* value (*P*<0.05). Qian et al (30) also observed reduced thiol concentrations in patients compared to controls.

The concentrations of native thiol were significantly lower in patients, males (225.395±42.852 μmol/L), females (219.987±33.309 μmol/L), compared to the control values (45.984±23.62 and 44.912±79.053 μmol/L) for males and females, respectively, with a significant *P* value (*P*<0.05), in agreement with Ateş et al (16).

The concentrations of disulfide were significantly higher in patients, males (20.731±3.931 μmol/L), females (22.361±3.810 μmol/L), compared to the control values (18.305±3.327 and 16.205±3.646 μmol/L) for males and females, respectively, with a non-significant *P* value in males (*P*<0.01), and a significant *P* value (*P*<0.05) in females, consistent with Kurku et al (31).

The peroxidase activity levels were significantly higher in patients, males (39.611±7.562 U/mL), females (34.397±5.944 U/mL), compared to the control values (7.532±2.193 and 8.075±1.817 U/mL) for males and females, respectively, with a significant *P* value (*P*<0.05), contrasting the results of Rush and Sandiford (32).

The levels of peroxidase specific activity were significantly higher in patients, males (4.630±0.808), females (3.802±0.643), compared to the control values (0.943±0.197 and 1.083±0.244) for males and females, respectively, with a significant *P* value (*P*<0.05). However, Rush and Sandiford (32) found no gender-based differences in peroxidase specific activity levels.

The levels of pseudo-peroxidase activity were significantly higher in patients, males (46.761±8.857 U/

mL), females (36.852 ± 4.213 U/mL), compared to the control values (1.768 ± 0.235 and 1.921 ± 0.372 U/mL) for males and females, respectively, with a significant *P* value ($P < 0.05$), consistent with the literature (33).

The pseudo-peroxidase specific activity levels were significantly higher in patients, males (4.914 ± 0.934), females (1.083 ± 0.244), compared to the control values (0.943 ± 0.197 and 1.083 ± 0.244) for males and females, respectively, with a significant *P* value ($P < 0.05$). Studies suggest that females have higher levels of pseudo-peroxidase activity compared to males, which contrasts with the recent findings (33).

The parameters mentioned above have been extensively investigated in various diseases, both related and unrelated to CKD (22,34). However, the expression and significance of each parameter are highly dependent on the specific pathophysiological conditions of the disease under consideration.

Conclusion

The oxidative stress biomarkers in sera and blood of CKD patients have been studied in this research. The current study also compares the oxidative stress biomarkers between males and females to check the gender disparities, as well as comparing the obtained results with previous studies on ESRD. Females have shown higher levels of total protein, albumin, ischemia modified albumin (IMA), GFR, urea, creatinine, free amino, carbonyl, and disulfide compared to males. Whereas the levels of globulin, Hb, total thiol, native thiol, peroxidase activity, peroxidase specific activity, pseudo-peroxidase activity and pseudo-peroxidase specific activity were lower in females compared to males.

Limitations of the study

This study was conducted on CKD patients undergoing regular hemodialysis and is a single center study. We suggest larger investigation on this subject of ESRD patients.

Authors' contribution

Conceptualization: Israa Ghassan Zainal.

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Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The study was conducted in compliance with the ethical standards specified in the statement of the Kirkuk Health Directorate. Prior to obtaining the sample, the patients' verbal and analytical approval were acquired. In order to obtain this permission, the research procedure details, and consent form underwent evaluation and approval by a local ethics committee, in compliance with document number (ref #245 dated 4/4/2022). Accordingly, written informed consent was taken from all participants before any intervention. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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