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Estimate the Correlation Between Some Antioxidant Biomarkers and Pseudo Peroxidase Activity of Hemoglobin in Patients With Chronic Kidney Disease

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Abstract **Background:** Hemoglobin, being a hemoprotein, may operate as a peroxidase due to the hemolysis process and the release of heme into blood stream. **Objective:** Evaluation of the oxidative biomarkers and peroxidase activity of hemoglobin in patients with final stage of renal failure. **Materials and Methods:** The study encompassed a total of 200 blood samples, with 140 samples collected from patients diagnosed with Chronic Kidney Disease (CKD) and 60 samples obtained from individuals who were considered healthy control subjects. **Results:** Levels of various serum parameters, including total protein, urea, creatinine, albumin, free amino, disulfide, ischemia-modified albumin (IMA), peroxidase activity, and specific activity, exhibited a significant increase in patients with Chronic Kidney Disease (CKD) when compared to the control group consisting of healthy individuals. Conversely, CKD patients demonstrated a notable decrease in levels of glomerular filtration rate (GFR), hemoglobin, native thiols, and total thiols compared to the control group. **Conclusion:** Oxidative biomarkers may present useful information about the oxidative stress and help to diagnose CKD patients in their advanced stages of the disease.

Key Words chronic kidney disease (CKD), oxidative biomarkers, peroxidase activity, renal failure, glomerular filtration rate (GFR)

1. Introduction

Human peroxidases can be divided into 2 groups: true peroxidases are enzymes whose main function is to generate free radicals in the peroxidase cycle and (pseudo) hypohalous acids in the halogenation cycle. The major true peroxidases are myeloperoxidase, eosinophil peroxidase and lactoperoxidase and pseudo peroxidases (are not real enzymes, but rather compounds with peroxidase-like activity because of their molecular arrangement or interaction with different substances [1]), perform various important functions in the body, but under the influence of external conditions they can display peroxidase-like activity. As oxidative intermediates, these peroxidases produce not only active heme compounds, but also protein-based tyrosyl radicals. Hemoglobin, myoglobin, cytochrome c/cardiolipin complexes and cytoglobin are considered as pseudo-peroxidases [2]. Hemoglobin (Hb) is a hemoprotein found in the red blood cells (RBCs) that transports O_2 from lungs to the body organs [3]. It is made up of heme and globin and has a binding capacity of 1.34 ml O_2 / gram [4]. Hemoglobin is produced by cells in the bone marrow that mature into the RBCs, thus having it in the circulation is required for appropriate tissue oxygenation [5], [6]. In the presence of oxidative equivalents, such as H_2O_2, Hb can act as peroxidase activity with a very high oxidative potential [7]. Oxidation of Hb is accompanied by generation of highly oxidized forms of Fe and globin radicals that have high oxidative activity and are toxic to cells, also the activity of peroxidase may indicate structural changes that occur in the hemoglobin molecule because of chemical modification [7].

Extracellular Hb is known to have substantial pro-oxidative and cytotoxic properties [8], as well as the ability to oxidize lipids, proteins, and nucleic acids [9].

When Hb is liberated from RBCs due to hemolysis, it can function in vivo as a pseudo peroxidase enzyme [10]. The heme group in Hb, like genuine peroxidases, can facilitate the substrate oxidation by H_2O_2 [11]. The leakage of Hb into the circulatory system caused by hemolysis can also cause pathophysiology linked to poor clinical outcomes in hemolysis patients [12], including chronic and acute vascular disorders, inflammation [13], thrombosis [14], and kidney failure [15].

The hemoglobin and its importance as a blood protein, along with its direct connection with the heme group has made it possible to study different biomarkers that are closely connected to the chronic kidney disease, although these biomarkers could be related to other pathophysiological issues [16]. The aim of this study was to examine the hemoglobin as a pseudoperoxidase enzyme and estimate the levels of some antioxidant biomarkers in patients with chronic kidney disease (CKD), then studying the correlation coefficient between them.

2. Patients and Methods

This study was conducted at the Department of Chemistry/University of Kirkuk in Kirkuk/Iraq between October 2022 to April 2023. Blood samples were obtained exclusively from Kirkuk General Hospital, Renal Dialysis Unit.

A. Blood Samples collection

A number of (60) samples (35 males and 25 females) apparent healthy subjects as control and (140) samples of patients with CKD (60 males, 80 females) were collected, the total number of samples was (200) samples. Blood was collected directly from the individuals' vein using single-use disposable syringes and the blood was poured into gel tubes, centrifuged, then the blood serum was collected. Heparin tubes were also used to maintain the whole blood non-clotted for the assessment of the peroxidase enzyme activity and specific activity. A special form has been made to collect the data of each patient which included the following: the age of the patient, whether he/she is a smoker or not, alcoholic or not, does he have/has any chronic illness, and how many times does the patient is having the renal dialysis during the week.

B. Methods

Total protein estimated by Zaia et al. method [17]; bromo cresol green method is employed to ascertain the level of albumin [18]; total thiol and native thiol estimated by the modified Ellman's method, [19]; The developed method by Levine et al. method used to estimate protein carbonyls [20]; Ischemia Modified Albumin (IMA): estimated by modified procedure of Dervisoglu et al. [1]; Free amino: by Zaia et al. [17]; Glomerular Filtration Rate (G.F.R): by Florkowski and Chew-Harris [21]; Urea and creatinine: by Kamal et al. using the Drabkin's method [22]; Hemoglobin: procedure by Srinivasan et al. [23]; Globulin: by the subtraction of albumin from total protein; Di-sulfide level by Ates et al. [24]; Peroxidase, pseudoperoxidase enzyme activity/specific activity: A modified procedure by Minai-Tehrani et al. [25] was followed.

C. Statistical Analysis

Statistical analysis was performed using GraphPad Prism Version 8.0.2 (263), the results were shown as (mean ± SD) utilizing the t-test function for the comparison between the obtained results in the mentioned program. In addition to the parameters correlation that has been applied using P value ($P \leq 0.05$).

D. Ethical Approve

The study was carried out in accordance with the ethical norms outlined in the Kirkuk Health Directorate's statement. Before taking the sample, the patients verbal, and analytical consent were obtained. To get this permission, the study protocol details, and consent form were evaluated and authorized by a local ethics committee in accordance with document numbered (245) and dated (4/4/2022).

3. Results

(Table 1) shows the levels of the studied parameters as (mean ± SD) in patients group compared to control.

The results in (Table 1) have shown a significant correlation between the control and patients' group for albumin, total protein (T.P.), glomerular filtration rate (G.F.R.), urea, creatinine, free amino, carbonyl, peroxidase activity, and specific activity, where ($P \leq 0.001$). whereas non-significant correlation has been seen for other parameters including hemoglobin (H.b.), globulin, Ischemia modified albumin (IMA), total thiol, native thiol and disulphide when comparing the levels in both patients vs. control groups, with P values (≥ 0.05). However, a notable increase was seen in the levels of urea, creatinine, free amino, IMA, disulphide, pseudoperoxidase activity, and pseudoperoxidase specific activity in the blood of patients compared to the control group. Whereas the other biomarkers' level in patients' blood including albumin, T.P., and carbonyl have shown a slight increase when compared to the control group.

G.F.R, H.b., total thiol and native thiol have shown a great decrease in their level in the blood of patients compared to healthy subjects. Moreover, only slight decrease in globulin level has been noticed.

Table 3 presents correlation coefficients between peroxidase enzyme activity, specific activity, and different parameters in male and female patients with chronic kidney disease.

4. Discussions

Proteins serve as significant targets for oxidation reactions, which are defined as a mismatch between the way free radicals generated and their antioxidant function inside the cells, due to their abundance in tissues, outside of the cells, and fluids from physiological processes, in addition to their quick rate of reaction with oxidants, which can cause oxidative stress. Lipids and carbohydrates degrade create highly reactive chemicals that assault proteins in several functional locations. As a result, a diverse set of proteins that alter irreversible changes contribute elements that hasten the appearance of many diseases [11]. Some oxidized protein products are appealing possibilities as oxidative damage markers due to their chemical stability and large production volume. The current study explores the different biomarkers that are strongly connected to the chronic kidney disease CKD,

Parameter	Control (n=60) Mean ± SD	Patients (n=140) Mean ± SD	P value
T.P. (g/dL)	7.356 ± 1.482	8.838 ± 2.114	<0.05
Albumin (g/dL)	4.373 ± 1.350	5.722 ± 1.714	<0.05
Globulin (g/dL)	3.029 ± 0.023	3.242 ± 0.537	0.3694
Albumin/globulin ratio	1.443 ± 0.443	1.765 ± 0.526	<0.05
IMA (ABSU)	0.485 ± 0.011	0.896 ± 0.263	0.0008
G.F.R (mL/min)	123.275 ± 12.169	45.654 ± 4.593	<0.05
Urea (mg/dL)	25.700 ± 7.733	163.769 ± 14.441	<0.05
Creatinine (mg/dL)	0.843 ± 0.053	10.050 ± 1.768	<0.05
H.b. (g/dL)	12.963 ± 0.610	8.955 ± 1.339	0.4562
Free Amino (mmole/L)	8.301 ± 1.355	30.750 ± 4.679	<0.05
Free amino/T.P.	1.128 ± 0.617	3.479 ± 1.284	<0.05
Carbonyl (nmole/mL)	69.984 ± 3.009	73.672 ± 12.645	<0.05
Carbonyl/T.P.	9.513 ± 2.169	8.335 ± 2.838	<0.05
Total Thiol (µmol/L)	45.117 ± 8.481	23.325 ± 24.974	0.954
Total Thiol/T.P.	61.334 ± 5.389	26.391 ± 10.030	<0.05
Native thiol (µmol/L)	44.127 ± 6.661	21.148 ± 13.514	0.665
Disulphide (µmol/L)	16.205 ± 1.467	22.361 ± 4.473	<0.05
Peroxidase Activity (U/mL)	8.102 ± 1.952	37.380 ± 8.050	<0.05
Pseudoperoxidase Activity (U/mL)	1.836 ± 0.323	39.610 ± 5.992	<0.05
Peroxidase Activity/ Pseudoperoxidase Activity (U/mL)	4.413 ± 0.974	0.943 ± 0.0352	<0.05
Peroxidase Specific Activity	1.030 ± 0.346	4.277 ± 0.979	<0.05
Pseudoperoxidase Specific Activity	0.251 ± 0.065	4.481 ± 1.737	<0.05
Where n=number of samples taken. SD = Standard Deviation.			

Table 1: The studied parameters levels in the patients and healthy subjects

Parameter	Males		P value	Females		P value
	Control (n=25) Mean ± SD	Patients (n=60) Mean ± SD		Control (n=25) Mean ± SD	Patients (n=60) Mean ± SD	
T.P. (g/dL)	7.274 ± 1.363	8.634 ± 1.604	<0.05	7.436 ± 1.673	9.054 ± 1.501	<0.05
Albumin (g/dL)	4.282 ± 1.407	5.688 ± 1.018	<0.05	4.418 ± 0.994	5.772 ± 0.971	<0.05
Globulin (g/dL)	2.324 ± 0.976	3.523 ± 0.696	<0.05	3.276 ± 0.737	3.091 ± 0.459	<0.05
Albumin/globulin	1.842 ± 0.5957	1.614 ± 0.748	<0.05	1.348 ± 0.463	1.867 ± 0.685	<0.05
IMA (ABSU)	0.485 ± 0.146	0.896 ± 0.107	<0.05	0.537 ± 0.121	0.912 ± 0.194	<0.05
G.F.R (mL/min)	121.217 ± 8.730	37.783 ± 7.197	<0.05	123.85 ± 27.866	47.108 ± 7.948	<0.05
Urea (mg/dL)	25.478 ± 4.824	165.087 ± 31.376	<0.05	25.667 ± 5.775	165.189 ± 25.146	<0.05
Creatinine (mg/dL)	0.817 ± 0.137	9.752 ± 1.835	<0.05	0.843 ± 0.190	10.224 ± 1.783	<0.05
H.b. (g/dL)	10.987 ± 2.635	9.935 ± 1.899	0.0880	12.763 ± 2.872	7.864 ± 1.377	<0.05
Free Amino (mmole/L)	7.950 ± 1.593	25.485 ± 4.824	<0.05	8.265 ± 1.860	37.466 ± 5.348	<0.05
Free Amino/T.P.	1.092 ± 0.489	2.951 ± 3.514	<0.05	1.111 ± 0.491	4.138 ± 3.113	<0.05
Carbonyl (nmole/mL)	65.401 ± 18.020	69.536 ± 13.221	<0.05	75.312 ± 16.945	79.461 ± 12.508	<0.05
Carbonyl/T.P.	8.991 ± 3.866	8.053 ± 3.691	0.0059	10.128 ± 4.783	8.776 ± 4.2027	<0.05
Total Thiol (µmol/L)	48.561 ± 45.118	25.248 ± 47.927	<0.05	46.671 ± 85.011	23.923 ± 39.609	<0.05
Total Thiol/T.P.	66.760 ± 19.204	29.242 ± 15.849	<0.05	62.764 ± 31.376	26.422 ± 13.703	<0.05
Native thiol (µmol/L)	45.984 ± 23.62	22.5.395 ± 42.852	<0.05	44.912 ± 79.053	21.987 ± 33.309	<0.05
Disulphide (µmol/L)	18.305 ± 3.327	20.731 ± 3.931	0.0149	16.205 ± 3.646	22.361 ± 3.810	<0.05
Peroxidase Activity (U/mL)	7.532 ± 2.193	39.611 ± 7.562	<0.05	8.075 ± 1.817	34.397 ± 5.944	<0.05
Pseudoperoxidase Activity (U/mL)	1.768 ± 0.235	46.761 ± 8.857	<0.05	1.921 ± 0.372	36.852 ± 4.213	<0.05
Peroxidase Activity/ Pseudoperoxidase Activity (U/mL)	4.260 ± 1.263	0.847 ± 0.186	<0.05	4.203 ± 1.087	0.933 ± 0.115	<0.05
Peroxidase Specific Activity	0.943 ± 0.197	4.630 ± 0.808	<0.05	1.083 ± 0.244	3.802 ± 0.643	<0.05
Pseudoperoxidase Specific Activity	0.943 ± 0.197	4.914 ± 0.934	<0.05	1.083 ± 0.244	3.802 ± 0.643	<0.05

Table 2: The studied parameters in sub-divided healthy and CKD patients (males and females)

Parameter	Patients	Males with CKD	Females with CKD
	r/p	r/p	r/p
PA-T.P.	0.2091/0.6945	0.09901/0.7513	0.03203/0.7204
PA-Albumin	-0.2922/0.3306	-0.3830/0.4406	-0.4308/0.3933
PA-Globulin	0.01600/0.6554	-0.2924/0.8961	-0.6477/0.3057
PA-Albumin/globulin	-0.4919/0.1304	-0.3887/0.1350	0.0985/0.6184
PA-IMA	-0.002440/0.2561	-0.6613/0.08127	-0.5181/0.1529
PA-G.F.R	-0.5278/0.05722	-0.6218/0.1475	-0.04980/0.3184
PA-Urea	-0.1824/0.4300	-0.1290/0.6332	0.0537/0.7915
PA-Creatinine	-0.1988/0.4161	-0.2479/0.5534	-0.5855/0.2029
PA-H.b.	0.1328/0.7323	-0.3943/0.6136	-0.7404/0.06076
PA-Free Amino	0.9238/0.9826	0.7010/0.9710	0.8314/0.9776
PA-Free Amino/T.P.	-0.1192/0.0532	0.0970/0.6162	-0.1331/0.4893
PA-Carbonyl	0.4790/0.8050	0.1762/0.4844	-0.5614/0.2123
PA-Carbonyl/T.P.	0.1831/0.8055	0.0774/0.5966	-0.1591/0.3607
PA-Total Thiol	0.1767/0.2995	-0.08137/0.6612	0.1851/0.3979
PA-Total Thiol/T.P.	-0.1823/0.4401	-0.0454/0.0643	-0.2378/0.9160
PA-Native thiol	-0.05349/0.2944	0.3359/0.1126	-0.2459/0.5549
PA-Disulphide	-0.4884/0.3669	-0.6612/0.08137	-0.5637/0.2339
SA-T.P.	-0.1192/1.0525	0.0970/0.9915	0.5716/1.3864
SA-Albumin	0.06046/0.7554	-0.4437/0.4413	-0.5020/0.6379
SA-Globulin	-0.4192/0.3114	-0.8331/0.5042	-0.5286/0.6160
SA-Albumin/Globulin	0.1831/0.8055	0.0774/0.5966	-0.1594/1.2776
SA-IMA	-0.4192/0.3114	-0.8331/0.5042	-0.5286/0.6160
SA-G.F.R	-0.41958/0.06922	-0.1518/0.1725	0.2915/0.6597
SA-Urea	-0.1434/0.4420	0.341/0.6582	0.395/1.1328
SA-Creatinine	-0.1598/0.4281	0.2221/0.5784	-0.2442/0.5442
SA-H.b.	0.1718/0.1946	0.0757/0.1960	-0.3991/1.3189
SA-Free Amino	0.9628/0.8170	0.692/0.5094	0.679/0.5442
SA-Free Amino/T.P.	0.2558/0.8782	-0.0518/0.4674	-0.01704/0.5209
SA-Carbonyl	0.518/0.3115	0.6462/0.6862	-0.2442/0.7392
SA-Carbonyl/T.P.	0.4334/1.0558	0.4348/0.9540	0.7577/1.2776
SA-Total Thiol	0.2157/0.3064	0.38863/0.1376	0.5264/0.8962
SA-Total Thiol/T.P.	-0.02584/0.9169	-0.4236/0.4397	0.3350/0.8284
SA-Native thiol	-0.4494/0.3789	-0.1912/0.1063	-0.2224/0.5752
SA-Disulphide	0.09946/0.3243	-0.3631/0.5292	-0.1873/0.9573
PsA-T.P.	0.02834/ 0.7796	0.1019/0.7464	0.1101/0.3983
PsA-Albumin	-0.07134/ 0.4806	-0.0164/0.8718	-0.4205/0.4192
PsA-Globulin	-0.06015/0.5522	-0.3050/0.7952	-0.06676/0.5093
PsA-Albumin/globulin	0.1968/0.0496	-0.0942/ 0.9258	0.1110/ 0.2716
PsA-IMA	-0.0258/0.2561	-0.6714/0.0926	-0.4981/0.1631
PsA-G.F.R	0.04818/0.634	-0.1596/0.0917	-0.0512/0.4005
PsA-Urea	-0.1794/0.3930	-0.1370/0.7121	0.09669/0.3386
PsA-Creatinine	-0.1698/0.3916	-0.2815/0.6438	-0.6194/0.2387
PsA-H.b.	0.3475/0.0004	-0.1101/0.3983	-0.758/0.959
PsA-Free Amino	0.7261/0.8592	0.6953/0.9301	0.7150/0.9646
PsA-Free Amino/T.P.	-0.0189/0.0954	0.1746/0.9325	-0.0163/0.8187
PsA-Carbonyl	0.09272/ 0.5175	0.0919/0.4553	-0.4827/0.2218
PsA-Carbonyl/T.P.	0.1746/0.7982	0.0635/0.5815	-0.0494/ 0.6254
PsA-Total Thiol	0.05464/0.5892	-0.0531/0.5561	0.1706/0.4382
PsA-Total Thiol/T.P.	-0.1538/0.4979	-0.06946/0.5123	-0.05464/0.5892
PsA-Native thiol	-0.1366/ 0.1753	0.1677/0.2639	-0.1476/0.5947
PsA-Disulphide	0.07953/ 0.4316	-0.6612/0.8137	-0.596/0.736
PsSA-T.P.	-0.1531/0.8509	0.0753/0.4185	0.4997/1.2580
PsSA-Albumin	0.05464/0.5892	-0.0197/0.7791	-0.1660/0.0987
PsSA-Globulin	-0.1196/0.3817	-0.3128/0.5709	-0.3921/0.5984
PsSA-Albumin/Globulin	0.04818/0.634	-0.0992/0.6237	-0.1614/0.9691
PsSA-IMA	-0.0391/0.3526	-0.04941/ 0.6254	0.1011/0.3168
PsSA-G.F.R	-0.4195/0.0967	-0.0926/0.2418	0.3225/0.7186
PsSA-Urea	0.0795/ 0.4316	0.3559/0.6867	0.3426/ 0.9258
PsSA-Creatinine	-0.1647/0.4782	-0.05664/0.5757	-0.1553/0.5691
PsSA-H.b.	0.1827/0.2736	0.0918/0.3642	0.1526/0.7358
PsSA-Free Amino	0.1110/ 0.2716	0.6834/0.5725	0.0629/0.6153
PsSA-Free Amino/T.P.	-0.0915/0.8167	-0.0534/0.3864	-0.0170/0.5824
PsSA-Carbonyl	0.1101/0.3983	0.1827/0.7918	-0.1251/ 0.2892
PsSA-Carbonyl/T.P.	0.3596/0.9495	0.1641/0.4352	0.2568/0.9891
PsSA-Total Thiol	0.4268/ 0.8284	0.3886/0.1376	0.4391/0.7791
PsSA-Total Thiol/T.P.	0.1353/ 0.5921	-0.0521/ 0.7382	0.0753/0.6187
PsSA-Native thiol	-0.3382/0.2716	-0.2734/0.1784	-0.2672/0.6826
PsSA-Disulphide	0.1682/0.4927	-0.2160/0.0917	-0.1846/ 0.9258

Table 3: Correlation coefficient between the Peroxidase Activity (PA), Specific Activity (SA), Pseudoperoxidase activity (PsA) and Pseudoperoxidase Specific Activity (PsSA)) the studied parameters

especially at the last stage where patients are already reached the hemodialysis advanced level of the disease.

Oxidative stress in CKD patients creates a proinflammatory environment that is at least largely responsible for the higher mortality and morbidity in this group of patients [26]. A study by Russa et al. [27] revealed that the oxidative stress and the inflammation in CKD patients is not only connected to cardiovascular disease but also represent a strong risk factor. It has been hypothesized that the use of antioxidant could help to reduce the oxidative stress significantly in terms of improving the many biomarkers in CKD patients [28].

The results in (Table 1) show the general trend of the studied biomarkers in both males/females' healthy subjects as well as CKD patients. It can be seen that the level in the control group of albumin, T.P., urea, creatinine, free amino, globulin, IMA, carbonyl, disulphide, peroxidase activity, and specific activity were (4.373, 7.356, 25.700, 0.843, 8.301, 3.029, 0.485, 69.984, 16.205, 8.102, and 1.030) respectively. These parameters have been clearly increased in the patients' group to be (5.722, 8.838, 163.769, 10.050, 30.750, 3.242, 0.896, 73.672, 22.361, 37.380, and 4.277).

The comparison of the different parameters between male and female individuals, as well as between control subjects and patients with chronic kidney disease (CKD), is shown in (Table 2) and discussed as follows:

Albumin levels are higher in both male and female CKD patients compared to the respective control groups (Male Control: 4.282 g/dL, Male Patients: 5.6886 g/dL, Female Control: 4.418 g/dL, Female Patients: 5.772 g/dL). This indicates an increase in albumin levels in CKD patients [29].

Total protein (T.P.) levels are also higher in CKD patients compared to the control groups in both males and females (Male Control: 7.274 g/dL, Male Patients: 8.634 g/dL, Female Control: 7.436 g/dL, Female Patients: 9.054 g/dL). This suggests an overall increase in protein levels in CKD patients [30].

The glomerular filtration rate (G.F.R.) values are lower in CKD patients compared to the control groups in both males and females (Male Control: 121.217 mL/min, Male Patients: 37.783 mL/min, Female Control: 123.85 mL/min, Female Patients: 47.108 mL/min). This indicates a decrease in kidney function in CKD patients [31].

Urea levels are significantly higher in CKD patients compared to the control groups in both males and females (Male Control: 25.478 mg/dL, Male Patients: 165.087 mg/dL, Female Control: 25.667 mg/dL, Female Patients: 165.189 mg/dL). This is expected as impaired kidney function leads to a buildup of urea in the blood [32].

Creatinine levels are higher in CKD patients compared to the control groups in both males and females (Male Control: 0.817 mg/dL, Male Patients: 9.752 mg/dL, Female Control: 0.843 mg/dL, Female Patients: 10.224 mg/dL). Elevated creatinine levels indicate impaired kidney function [33].

Hemoglobin (H.b.) levels are lower in CKD patients compared to the control groups in both males and females (Male Control: 10.987 g/dL, Male Patients: 9.935 g/dL, Female Control: 12.763 g/dL, Female Patients: 7.864 g/dL). This suggests that CKD can lead to anemia, which is characterized by a decrease in hemoglobin levels [34].

Free amino acid levels are significantly higher in CKD patients compared to the control groups in both males and females (Male Control: 7.950 µmol/L, Male Patients: 25.485 µmol/L, Female Control: 8.265 µmol/L, Female Patients: 37.466 µmol/L). This may be due to altered protein metabolism in CKD, leading to increased levels of free amino acids [35].

Globulin levels are higher in CKD patients compared to the control groups in both males and females (Male Control: 2.324 g/dL, Male Patients: 3.523 g/dL, Female Control: 3.276 g/dL, Female Patients: 3.091 g/dL). This indicates an increase in globulin proteins in CKD patients, which may be a result of the immune response and inflammation associated with CKD [36].

Ischemia-Modified Albumin (IMA) levels are higher in CKD patients compared to the control groups in both males and females (male control: 0.485 ABSU, male patients: 0.896 ABSU, female Control: 0.537 ABSU, female patients: 0.912 ABSU). This suggests increased oxidative stress in CKD, as IMA is a marker of oxidative stress and ischemia [37].

Carbonyl levels are higher in CKD patients compared to the control groups in both males and females (male control: 65.401 nmol/mL, male patients: 69.536 nmol/mL, female control: 75.312 nmol/mL, female patients: 79.461 nmol/mL). Elevated carbonyl levels indicate increased protein oxidation in CKD, which is associated with oxidative stress and damage to cellular proteins [38].

Total thiol levels are lower in CKD patients compared to the control groups in both males and females (male control: 485.613 µmol/L, male patients: 252.482 µmol/L, female control: 466.714 µmol/L, female patients: 239.231 µmol/L). Thiol groups play a crucial role in antioxidant defense mechanisms, and a decrease in total thiol levels suggests impaired antioxidant capacity in CKD patients [39].

Native thiol levels are also lower in CKD patients compared to the control groups in both males and females (Male Control: 459.846 µmol/L, male patients: 225.395 µmol/L, female control: 449.125 µmol/L, female patients: 219.874 µmol/L). Native thiol represents the reduced form of thiol groups, and a decrease in its levels further supports the impaired antioxidant status in CKD patients [40].

Disulphide levels, which represent the oxidized form of thiol groups, are slightly higher in CKD patients compared to the control groups in both males and females (male control: 18.305 µmol/L, male patients: 20.731 µmol/L, female control: 16.205 µmol/L, female patients: 22.361 µmol/L). This suggests an imbalance between the reduced and oxidized forms of thiol groups in CKD patients [40].

Peroxidase activity, an indicator of oxidative stress, is significantly higher in CKD patients compared to the control groups in both males and females (Male Control: 7.532 U/mL, male patients: 34.397 U/mL, female control: 8.075 U/mL, female patients: 39.611 U/mL). This suggests in-

creased oxidative stress in CKD patients, which is characterized by an imbalance between the production of reactive oxygen species and the antioxidant defense mechanisms [41].

Specific activity, which represents the ratio of peroxidase activity to total thiol levels, is also higher in CKD patients compared to the control groups in both males and females (Male Control: 0.943, male patients: 3.802, female control: 1.083, female patients: 4.630). this indicates an increased propensity for oxidative stress in CKD patients, as the specific activity reflects the efficiency of the antioxidant defense system in counteracting oxidative damage [42].

In Table 1, it can be noticed that the peroxidase activity in CKD patients is 37.380 U/mL. However, the specific activity of pseudoperoxidase for CKD patients was 39.610 U/mL. The level of pseudoperoxidase and pseudoperoxidase activity is important because it reflects the oxidative stress status in the body. Pseudoperoxidase is an enzyme that can catalyze the oxidation of various substrates, leading to the production of reactive oxygen species (ROS). ROS are highly reactive molecules that can cause damage to cells and tissues if their levels are not properly regulated. In the case of CKD patients, the pseudoperoxidase activity is significantly higher compared to healthy individuals. This suggests that CKD patients may have increased oxidative stress and higher levels of ROS production. It is important to note that the specific activity of pseudoperoxidase is a measure of the enzyme activity per unit of protein concentration. In the case of CKD patients, the specific activity of pseudoperoxidase is significantly higher compared to healthy individuals. This indicates that the enzyme is more active in CKD patients, potentially leading to increased ROS production and oxidative stress [43].

In Table 2, the levels of peroxidase activity in both males and females were higher in CKD patients compared to healthy controls. Specifically, in males, the peroxidase activity was 39.761 U/mL in patients compared to 7.532 U/mL in controls. In females, the peroxidase activity was 36.852 U/mL in patients compared to 8.075 U/mL in controls. Similarly, the peroxidase specific activity was higher in CKD patients compared to healthy controls in both males and females. In males, the peroxidase specific activity was 4.914 in patients compared to 0.943 in controls. In females, the peroxidase specific activity was 4.165 in patients compared to 1.083 in controls [43].

The correlation coefficients in (Table 3) and (Figures 1 to 6), generated by GraphPad Prism, demonstrate a combination of positive and negative relationships between peroxidase enzyme activity, specific activity, and the different parameters studied in male and female patients with CKD. Certain associations exhibit statistical significance, others do not.

For the above parameters, Hemoglobin can act as a (pseudo)-peroxidase in vivo, and heme has a pro-oxidative potential that can contribute to intravascular hemolysis. Free hemoglobin with its pseudo-peroxidase activity can interfere in the detection of hemolysis in CKD patients [44].

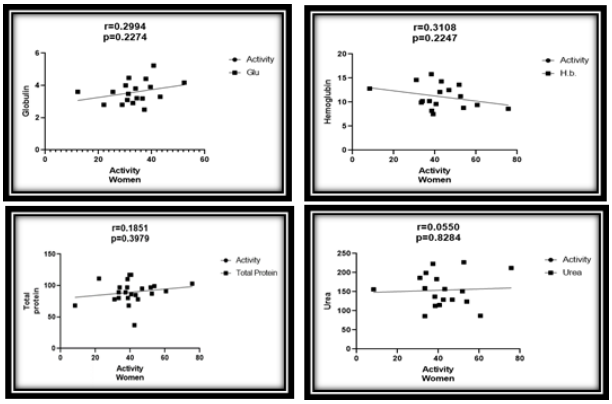

Figure 1: A comparison between globulin, hemoglobin, total protein and urea vs. peroxidase enzyme activity in females

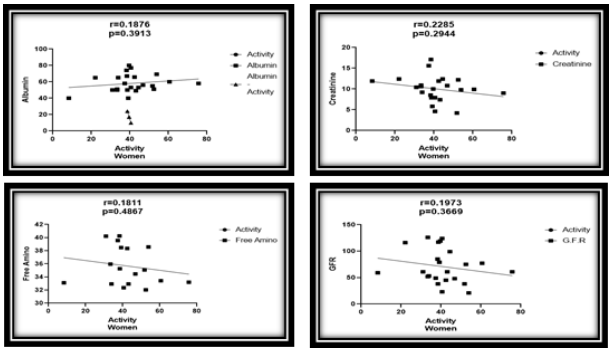

Figure 2: A comparison between albumin, creatinine, free amino and GFR vs. peroxidase enzyme activity in females

5. Conclusions

In summary, the comparison of different parameters between healthy individuals and those with chronic kidney disease (CKD) revealed significant differences. CKD patients have generally exhibited higher levels of albumin, total protein, urea, creatinine, globulin, IMA, carbonyl, peroxidase activity, and specific activity indicating the impaired kidney function and increased oxidative stress. On the other hand, CKD patients tend to have lower levels of glomerular filtration rate (G.F.R.), hemoglobin (H.b.), total thiol, native thiol, and disulphide, suggesting decreased kidney function, anemia, and compromised antioxidant capacity. the level of pseudoperoxidase and pseudoperoxidase activity in CKD patients suggests an imbalance in the antioxidant defense system, leading to increased oxidative stress. This information can be valuable in understanding the pathophysiology of CKD and developing potential therapeutic interventions to mitigate oxidative damage. The different parameters highlight the impact of CKD on various physiological processes and emphasize the need for appropriate management and treatment strategies for CKD patients.

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

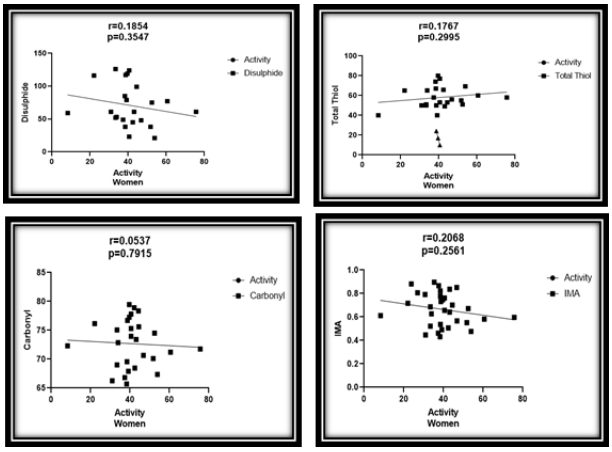

Figure 3: A comparison between disulphides, total thiol, carbonyl and IMA vs. peroxidase enzyme activity in females

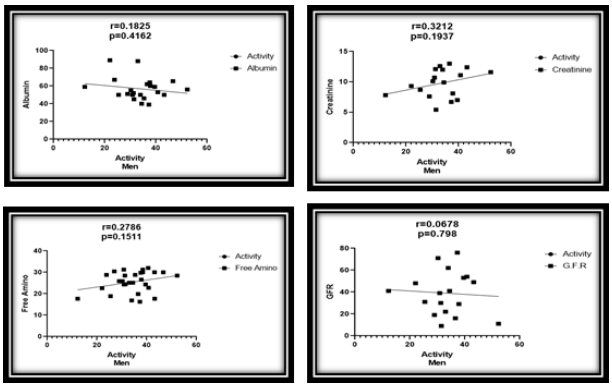

Figure 4: A comparison between albumin, creatinine, free amino and GFR vs. peroxidase enzyme activity in males

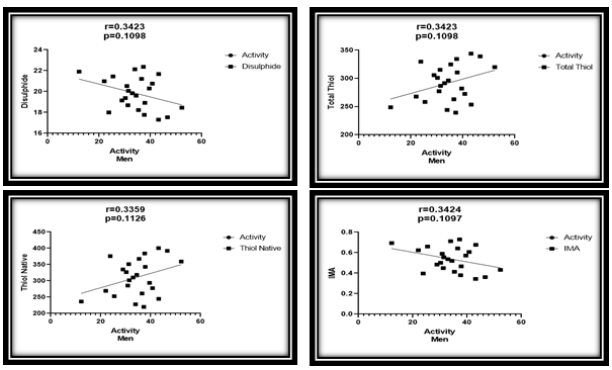

Figure 5: A comparison between dislphides, total thiol, native thiol and IMA vs. peroxidase enzyme activity in males

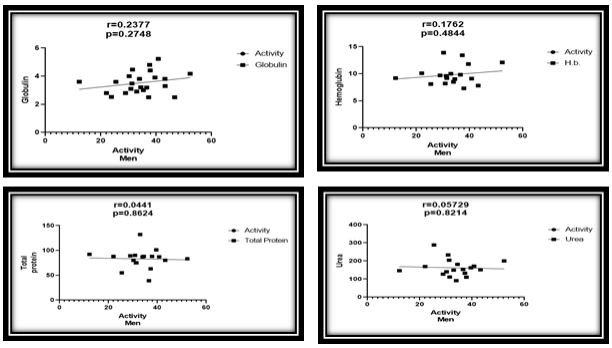

Figure 6: A comparison between globulin, hemoglobin, total protein and urea vs. peroxidase enzyme activity in males

Authors Contribution

All authors contributed equally in this paper.

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