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Evaluation of biochemical and hematological parameters and Receiver Operating Characteristic Curves analysis of some oxidative stress markers in Hemodialysis Patients

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Abstract

The objective of this study is to evaluate the variation and importance of certain hematological, biochemical, and oxidative stress markers in hemodialysis patients. Sensitivity and specificity of oxidative stress biomarkers in serum, erythrocytes and leucocytes were estimated using receiver operating characteristics (ROC) curve design. The results showed that Red blood cell, hemoglobin, hematocrit and glomerular filtration rate levels were significantly decreased ($p < 0.05$) while white blood cell, Granulocyte, urea, creatinine, sodium and potassium levels were significantly increased ($p < 0.05$) in hemodialysis patients as compared to control. Our results also showed a significant decrease ($p < 0.001$) of leukocytic catalase activity and erythrocytic GSH level and a significant increase ($p < 0.001$) of erythrocytic MDA and serum ORAC levels in hemodialysis patient group compared to control. ROC analysis indicated that GSH, and ORAC levels are diagnostic tools better than other oxidative markers. In conclusion Our results indicated that erythrocytes GSH level and serum ORAC activity are shown to be a high specificity and sensitivity. It is suggested to insert it into the analytical diagnostic list for prediction of CKD.

Keywords: Chronic Kidney Disease, Hemodialysis, Oxidative stress, ROC analysis

Full length article **Corresponding Author*, e-mail: dersamebio@gmail.com

1. Introduction

Chronic kidney disease (CKD) is a physiological change that produces loss of kidney function, leading to end-stage kidney disease [1]. It is increasingly recognized as a global public health problem [2]. During the past three decades, the incidence and prevalence of end stage renal disease (ESRD) have risen progressively. The global CKD prevalence was reported to be 13.4 %, in USA and 13.6 % in Europe. The lowest prevalence was 4 % in northern Africa macro area, and the highest 16.5 % in west and central west Africa and the average prevalence in the entire Africa continent was 10.1 % [3]., CKD prevalence found to be 650 pmp in Egypt, 323 pmp in Libya, 734 pmp in Tunisia, 300 in morocco and 475 in Algeria [4]. In addition, the total number of ESRD patients receiving renal replacement therapy in Algeria with population of 37 100 000 (in 2001) reached 17000 in 2011. The prevalence and incidence of ESRD was 100 pmp and 109 pmp respectively [5]. Chronic kidney disease (CKD) has emerged as a global public health burden for its increasing number of patients, high risk of progression to end-stage renal disease (ESRD), and poor prognosis of morbidity and mortality [6]. It attracts worldwide attention to its epidemiology, risk factors, treatment plans and preventive. Oxidative stress is an important factor causing metabolic and physiological alterations and various diseases in the body [7]. The oxidative stress have a central role in the pathophysiological process of uremia and its complications. However, there is little evidence to suggest how early oxidative stress starts developing during the progression of CKD [8]. Faced with these problems, this study aims to evaluate some biological parameters and evaluate the sensitivity and specificity of oxidative stress markers in HD patients of Djamaa (Algeria) region.

2. Methods

2.1. Study subjects

Ethical approval was requested and approved by the Ethics Committee of the Department of Cellular and Molecular Biology, Faculty of Natural Sciences and Life, University of El Oued. Our study was carried out on 41 men volunteers, mean age 45.05 ± 2.65 years, were divided into

two groups; first group is control (21 healthy), the second group of 20 hemodialysis patients of hospital Saad Dahleb–Djamaa. Therefore, their social and demographic information including age, weight, social case, and blood group were collected by completing the questionnaires from their medical records or through a direct discussion with patients.

2.2. Inclusion and exclusion criteria

Inclusion criteria for voluntary persons (patients and controls) living in the Djamaa region was as follows: hemodialysis patients on clinical diagnosis showed chronic kidney disease of which they were suffering from two years. It was confirmed by nephrography diagnosis and specialist doctors. Patients were also undergoing hemodialysis but no other type of chronic kidney disease treatment from past 3 months. These experimental suspects were recruited by hemodialysis service of hospital SAAD DAHLEB Djamaa. The controls were healthy peoples and were not suffering from any chronic or acute diseases and had not consumed any drug for last 3 months. Exclusion criteria was to eliminate the factors that might affect oxidative stress parameters We excluded all patients having diabetics, arterial hypertension, anemia or endocrine disorders in their medical history from patient groups and healthy controls.

2.3. Laboratory investigations

2.3.1. Blood sampling

Blood sampling was done in the morning for both groups of control and hemodialysis, but for the last group their sampling is done before and after dialysis, after this operation we collected the samples blood in two types of tubes**:** anticoagulant (EDTA) tubes for hematological (FNS) and oxidative stress (MDA, GSH, Catalase) markers assay. Blood EDTA tubes contents were centrifuged at 2000 rpm for 10 min and removed the plasma. The cap of EDTA tube was lysis with 50 ml of TBS buffer (EDTA 2.92 M; tris 1.21 M; pH = 7) and incubated 30 min in Freezer. After incubation, it was centrifuged at 2500 rpm for 10 min, and the obtained supernatant (erythrocyte homogenate) was used for the determination of antioxidant activity. After separation of erythrocyte, the rest of EDTA tube contents were centrifuged at 2000 rpm for 10 min and removed the plasma. Wash pellet with lysis buffer and shake incubate in Freezer for 30 min. After incubation centrifuged at 2500 rpm for 10 min. followed this step by washing with lysis buffer until the Leukocyte pairing and then recovered to make the dosage of oxidative stress tests [9]. And in dry tubes, samples are centrifuged at 3000 rpm for 10 minutes to obtain the serum and utilized for urea, creatinine, calcium, ionogram analysis and ORAC and FRAP activity assay, Serum samples were stored at -20°C until analysis.

2.3.2. Biochemical and oxidative stress measurements

Serum urea, creatinine, calcium parameters levels were determined by autoanalysis (BIOLIS24j) use commercial kit from spinreact, Spain (urea-20141, creatinine-20151, calcium-20051). Hematological analysis (FNS) was performed by the hematology autoanalyzer (Sysmex) and determination of the ionogram parameter (sodium, potassium and chlorine) by Automatic electrolyte analyzer (Easylute). Malondialdehyde (MDA) and reduced glutathione (GSH) were determined in white blood cells (leukocytes), and red blood cells (erythrocytes) by following previously described methods in literature [10] [11]. Catalase enzyme activity in white blood cells (leukocytes) was determined by the Aebi method [12]. Ferric reducing antioxidant power (FRAP) was determined by method of Oyaizu (1986) [13]. The total antioxidant power of the serum (ORAC: Oxygen Radical Absorbance Capacity) was determined according to the method of Blache and Prost (1992) [14].

2.4 Statistical analysis

The values of the results were expressed as mean ± SEM. Student's t-test of independent samples was applied to results by using Minitab 14 statistical program. To analyze, the relationships between the different parameters we have used coefficient of Pearson's correlation test. Diagnostic model of CKD with several factors was based on OR risk factored regression analysis using SPSS statistics 25 software and the Excel 2007 (Microsoft). The statistical parametersincluding the area under curves (AUC), the receiver operating characteristics (ROC), were used to show the potency of a biomarker in the diagnosis of CKD. Specificity, sensitivity, AUC and 95 % confidence interval (CI) values were calculated. The value of $P < 0.05$ indicated statistically significant difference.

3. Results and discussions

3.1. Socioeconomic characteristics of patients

In our study, experimental population was characterized by many different characters age, weight, sex, social case, and blood group. In our work, we selected 18 hemodialysis patients and 20 individuals in control group. The statistical reliable results are presented in the table 1.

Table 1: Description of study population

¤		Control¤	Hemodialysis·Patients¤
Age¤		46.61±2.84¤	46.03±2.95¤
Body·weight¤		61.20±2.09¤	59.96±2.62¤
Social·Case¤	Married·%¤	29.870¤	29.870¤
	Single·%¤	23.376¤	15.584¤
Blood·Group¤	A·%¤	18.181¤	7.791¤
	B·%¤	10.389¤	10.388¤
	AB·%¤	7.791¤	3.896¤
	O·%¤	16.882¤	18.181¤

3.2. Biochemical markers

The biochemical markers are presented in table 2. The results revealed a significant increase ($p < 0.01$) in urea and creatinine concentration and a significant decrease ($p < 0.001$) in Glomerular filtration rate (GRF) level in hemodialysis patient group compared to control. The electrolytes levels significantly decreased ($p < 0.001$) serum sodium and potassium concentrations and a significant increase ($p<0.01$) in sodium and potassium concentrations in hemodialysis patient group was observed as compared to control. However, there was no significant change in chlorine and calcium concentrations in hemodialysis patient group as compared to control.

Table 2: Biochemical markers and serum electrolytes level in control and patients groups

Parameters	Controls N=21	Hemodialysis patients N=18	P-*value*
Serum Urea (g/l)	0.17±0.01	0.90±0.06	0.000
Serum Creatinine (mg/l)	7.96±0.27	87.49±5.46	0.000
GFR (ml/min/1.73m^2)	92.96±3.31	6.240±0.417	0.000
Serum Sodium (mmol/l)	138.53±0.66	135.37±0.40	0.000
Serum Potassium (mmol/l)	4.69±0.19	5.59±0.19	0.000
Serum Chloride (mmol/l)	108.6±1.26	103.05±1.31	0.545
Serum Calcium (mg/l)	78.59±2.23	82.93±6.00	0.479

Values are mean± SEM. GFR: Glomerular filtration rate

3.3. Hematological markers

The hematological markers are presented in table 3. The results obtained show that the levels of red blood cells, hemoglobin and hematocrit were significantly decreased while white blood cells and granulocyte were significantly increased ($p < 0.001$) in hemodialysis patients as compared to control group. The results illustrated also show that there was no significant change ($p > 0.05$) in the levels of lymphocytes and platelet linein hemodialysis patients with CKD compared to the control group.

Table 3: Hematological parameters of control and Hemodialysis patient groups

Values are mean± SEM

Parameters	Control N=21	Hemodialysis Patient N=18	P-*value*
Red blood cell (10^6/l)	4.75 ± 0.18	3.48 ± 0.22	0.000
Hemoglobin (g/dl)	13.07 ± 0.405	9.83 ± 0.46	0.000
Hematocrit (%)	37.73 ± 1.26	29.73 ± 1.51	0.000
Platelet (10^3/l)	249.1 ± 23.3	238.2 ± 19.4	0.581
White blood cell (10^3/l)	4.86 ± 0.48	6.34± 0.31	0.000
Lymphocytes (10^3/l)	1.33 ± 0.16	1.28± 0.19	0.782
Granulocytes (10^3/l)	3.28 ± 0.44	4.57 ± 0.33	0.001

3.4. Oxidative stress markers

According to the results shown in table 4, analysis of oxidative stress status revealed a significant decrease ($p < 0.001$) of leukocytic catalase activity and erythrocytic GSH level whereas a significant increase ($p < 0.001$) of erythrocytic MDA, serum ORAC and FRAP levels was observed in hemodialysis patient group compared to control. However, no significant change in leukocytic GSH and leukocytic MDA concentration was recorded.

Table 4: Oxidative stress biomarkers in leukocyte, erythrocyte and serum of control and hemodialysis patients' groups

Parameter		Control N=21	Hemodialysis Patient N=18	P-*value*
Leukocyte	GSH (nmol/mg Hb)	0.43±0.04	0.49 ± 0.04	0.211
	Catalase (UI/g Hb)	11.19±1.01	10.06 ± 0.24	0.000
	MDA (nmol/mg Hb)	5.12±0.58	5.12 ± 0.36	0.946
Erythrocyte	GSH (nmol/mg Hb)	0.42±0.06	0.24± 0.06	0.005
	MDA (nmol/mg Hb)	11.00±0.37	12.89 ± 0.64	0.006
Serum	ORAC (UI)	0.41±0.10	0.53 ± 0.09	0.000
	FRAP (%)	96.12±0.48	98.18 ± 0.78	0.119

Values are mean ± SEM

3.5 Receiver operating characteristic analysis of oxidative stress parameters

The results of ROC curve analysis presented (table 5 and figure 1) demonstrate a significant ($p < 0.05$) sensitivity in serum ORAC level and higher percentage of specificity (AUC = 77 %). Moreover, there was a low significant sensitivity and specificity in erythrocytes GSH level (AUC = 31 %). And no significant sensitivity and specificity for erythrocytic MDA level, leucocytic catalase activity and serum FRAP activity was observed.

Table 5: AUC, Sensitivity and Specificity of oxidative stress parameters

Parameters	Sensitivity %	Specificity %	AUC	CI_{95}	P-*value*
Erythrocytic MDA	71	33	0.600	0.387-0.813	0.359
Serum ORAC	43	87	0.771	0.596-0.947	0.013
Serum FRAP	79	73	0.848	0.692-1.000	0.061
Erythrocytic GSH	16	32	0.314	0.129-0.499	0.050
Leucocytic Catalase	58	58	0.528	0.326-0.729	0.770

AUC = Area under the ROC curve, CI=Confidence Interval, P=Significance level

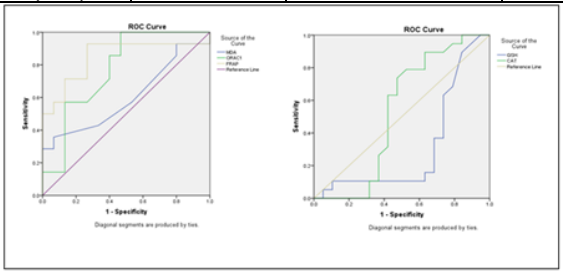

Figure 1: Receiver Operating Characteristic (ROC) curve for oxidative stress markers

3.6. Correlation between oxidative stress markers and biochemical parameters

Our correlation results between oxidative stress markers and biochemical parameters are represented in table 6 which clarify a significant positive correlation ($p < 0.05$) between (catalase/creatinine) and between (erythrocytic GSH/calcium) in control group. However, a negative significant correlation ($p<0.01$) between (Leucocytic MDA/sodium) and between (serum ORAC/sodium) in the same group was observed. Concerning patients group, there was a significant positive correlation ($p<0.05$) between (catalase/Na), (Leucocytic MDA/Na), (serum ORAC/Na), (Erythrocytic MDA/creatinine), (Erythrocytic MDA/GFR), and between (Erythrocytic GSH/GFR). Furthermore, a significant negative correlation ($p < 0.05$) between (catalase/creatinine), (catalase/potassium), (leucocytic MDA/cratinine), (leucocytic MDA/K), (erythrocytic MDA/K) and (erythrocytic GSH/calcium) in the same group. While that the level B in both groups i.

Table 6: Correlation results between oxidative stress and biochemical markers

Correlation		Control		Hemodialysis Patients	
		R	P	R	P
Leucocytic Catalase	Creatinine	0.398	0.001	-0.300	0.027
	Sodium	-0.018	0.940	0.485	0.000
	Potassium	-0.149	0.543	-0.272	0.047
Leucocytic MDA	Creatinine	-0.160	0.489	-0.404	0.002
	Sodium	-0.901	0.000	0.351	0.009
	Potassium	-0.404	0.002	-0.273	0.045
Erythrocytic MDA	Creatinine	-0.125	0.589	0.280	0.041
	GFR	-0.023	0.885	0.269	0.022
	Potassium	-0.189	0.438	-0.392	0.003
Erythrocytic GSH	GFR	-0.305	0.050	0.485	0.003
	Calcium	0.357	0.006	-0.329	0.015
Serum ORAC	Sodium	-0.718	0.000	0.331	0.014

Hemodialysis is one of the renal replacement therapy where body waste product like urea, creatinine and free water are removed from the blood, when the kidneys are impaired. By the diffusion of solutes through a semi permeable membrane [15]. Our results showed a significant increase of serum urea and creatinine levels and a large decrease in GFR before dialysis and observed a clear reduction of serum creatinine after dialysis. but for the serum urea no significant decrease was noted. During dialysis urea and creatinine being small molecules flow through membrane into the sterile solution and was removed due to the counter-current flow of blood and dialysate and removed more urea and creatinine from the blood [16]. According to literature the serum urea level cannot be used to monitor the renal function in CKD patients but may indicate non renal influence [17,18]. When several conditions studied which lead to protein breakdown and resultant increased urea excretion, whether it be feeding a protein-rich diet was associated with increases in all five urea cycle enzymes proportional to the increase in urea synthesis [19]. Routinely used tests for diagnosis of CKD are serum creatinine and urea levels but they are considered as late indicators as they can be observed on at least a 33% decrease in Glomerular filtration rate (GFR) [20]. Our study obtained a significant increase in serum Na and Ca levels and serum K level increasing in patients group compared to controls. The kidney plays an important role in the regulation electrolytes. When kidney is impaired electrolytes imbalance appears [21]. According to our results which showed that a significative decrease in RBC, HB and HT and a significant increase of WBC and Granulocytes number. The explanation of hemoglobin result is based on erythropoietin which is a natural glycopeptide hormone developed by the kidneys also by the liver which stimulates erythropoiesis, the process of production of erythrocytes (red blood cells) [22]. Anemia in ESRD is almost universal can be caused by erythropoietin, iron and vitamin deficiency or blood loss and shortened red cell life span [23]. Moreover, it is a common and often early complication in CKD [24] mostly due to diminished production of erythropoietin (EPO) which is a primary regulator of RBC and mostly produced by renal epithelial cells [25]. Our study obtained a significant variation of oxidative stress parameters when GSH level and catalase activity was found decreasing with significant specificity of GSH (32 %, AUC = 31 %). Also noted an increase of MDA in erythrocytes of CKD patients against to controls. Oxidative stress contributes to the development and the progression of CKD and the associated complications including atherosclerosis, cardiovascular disease, erythropoietin-resistant anaemia, immune deficiency [26]. Glutathione (GSH) is a non-enzymatic antioxidant that contributes to the defense system in the body against oxidative stress induced by reactive oxygen species [27]. The study of Mehryar and Omid [28] proposed that a loss or inactivation of antioxidant factors as GSH is coupled with increased of lipid peroxidation in erythrocytes of HD patients where the MDA was increased significantly in CKD so it is a good indicator for oxidative stress evaluation [29], especially in erythrocytes which are well known markers of ROS overproduction and increased lipid peroxidation [30]. On another side, several assays that can measure total antioxidant capacity of serum such as FRAP and ORAC [31], FRAP can measure the antioxidant capacity of foods and water soluble antioxidant compounds that can interact with different ROS sources [32].FRAP assay is a powerful test for determination of total serum antioxidant capacity in the body [33]. Our results represented a significant ORAC and FRAP increasing in patients groups with high significant specificity (87%, AUC=77% and 73%, AUC=85%), respectively compared to control which demonstrated the presence of oxidant stress status. Antolini et al., (2004) [34] study showed that total antioxidant power measured with FRAP and ORAC paradoxically increased in CKD patients. So, the chronic renal failure (CRF) was characterized by a peroxidant state and deferent value of antioxidant compounds such as vitamin C (which increased in plasma of

HD patients) [35]. The result obtained from this study provides correlation between oxidative stress markers and biochemical parameters [36]. In addition, serum creatinine level was significantly elevated in hypertensive patients when the marker of oxidative stress was increased in the same group [37].

4. Conclusions

Our results indicated an oxidative stress associated with the CKD which contributed to imbalance of antioxidants defense system and overexpression of free radicals and leads to cells membrane alteration and disease progression. Therefore, the erythrocytes GSH level and serum ORAC activity are shown to be a predictive and a new reliable marker for CKD. It is suggested to insert this marker into the analytical diagnostic list for prediction of CKD.

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