Estimation of Glutathione Peroxidase in Hemodialyzed Patients in Baghdad City

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Abstract: Oxidative stress (OS) expressed by oxidants and antioxidants balance disturbance, and it can be indicated through different species that belong to either of its definition terms. OS has been implicated to the worsening of several disorders, and the development of many pathological conditions. Renal failure (RF) is one of the most prevalence disorders in Iraq, affecting both children and adults in high rates. Additionally, hemodialysis, one of the most common therapeutic approaches for RF, has been reported to drive serious consequences on the health of the patients. The current investigation was established to test glutathione peroxidase (GPx) and malondialdehyde (MDA) in the serum of hemodialyzed RF patients. Sixty RF patients were gathered from Baghdad Medical City and controlled with 30 healthy people of comparable ages and body mass indexes (BMIs). The results have shown a rough reduction of GPx level in the serum of hemodialyzed RF patients, encountered by a significant elevation in the level of MDA when compared to healthy people. Other biomarkers were shown significant increase in RF patients including; urea, creatinine, sodium, and potassium. Moreover, GPx was correlated inversely with MDA level and both of which were shown excellent sensitivity for the prognosis of hemodialyzed RF patients. At last, it is safe to presume that both GPx and MDA are key regulators of health in hemodialysis RF patients and can be used as indicators for the patient's prognosis. **Keywords:** Renal failure, oxidative stress, hemodialysis, glutathione peroxidase, malondialdehyde.

1. Introduction

Renal failure (RF) is a health condition in which the kidneys are incapable of performing normal function to filtrate the blood and remove the wastes from the body [1]. Chronic renal disease is increasing as a complicated worldwide health concern, imposing a significant cost burden on both patients' families and the medical delivery system [2]. Unlike acute kidney damage, which results in normal functional kidney repair, chronic and persistent injuries from long-term and progressively nephropathies lead to gradual kidney fibrosis and loss of the normal renal morphology. This influences the kidney's three compartments, which include the glomeruli, tubules, interstitium, and arteries [3]. In many developed nations throughout the world, RF is the ninth biggest cause of mortality [4]. Hemodialysis is one of the major technologies that have been used to treat RF over decades [5]. This approach of treatment faced many problems by raising the risks for developing pathological conditions including cardiovascular diseases [6-9]. Therefore, it is important to understand the effect of hemodialysis on the health of RF patients to maintain their health at stable condition. Free radicals and reactive oxygen species (ROS) are involved strongly in the progression of many disorders, by enhancing the oxidative destruction of the cellular components [10, 11]. Oxidative stress (OS) is the term that used to describe these events and can be controlled by synergistic defense system called antioxidants [12]. The latter composed of wide spectrum of materials with the ability of detoxifying ROS or their harmful effects [13, 14]. Malondialdehyde (MDA) is used extensively as a biomarker for OS, specifically, lipid peroxidation [15]. Therefore, the increase of MDA level indicates a condition of OS.

Glutathione peroxidase (GPx) is one of the most important first line defense antioxidants [16]. This enzyme has the activity of detoxifying both hydrogen peroxide and lipid peroxides, and therefore, preventing their harmful effects on the cellular function and integrity [17]. Many studies have shown the strong effect of OS on the development of cardiovascular diseases [18, 19]. In this study, I have attempted to study the effect of MDA and GPx in the serum of hemodialyzed renal failure (HRF) patients and investigate their role in the consequences of hemodialysis operation.

2. Materials and Methods

The blood specimens of the participants were collected and the serum was stored by freezing (-20 ºC) before analysis. These samples were taken from 30 healthy people and 60 hemodialyzed RF (HRF) patients, whom age was ranged from 19 to 65 years old. The participants were gathered from Baghdad Medical City.

The serum was analyzed for sodium, potassium, urea and creatinine at the time of analysis using BioLabo commercial kits (France) based on spectrophotometric methods, where the absorbance was read by apel PD-303 spectrophotometer (Japan) at λmax of each kit. The level of GPx was estimated by using an enzyme linked immune sorbent assay (ELISA) kit (Bioassay Technology Laboratory) where the absorbance was read on ELX800 ELISA reader (BioTech). Furthermore, MDA was estimated according to the spectrophotometric method that developed by Benge and Aust [20], which depends on the reactivity between thiobarbituric acid and MDA to yield a bink colored solution where it's intensity can indicate the amount of MDA in the serum samples. Finally, the results were processed statistically for comparisons, correlations, and diagnostic sensitivities on SPSS software version 26.0.

3. Results

The results in Table 1 indicate significant disturbance of OS in HRF patients. The level of MDA was elevated while the level of GPx was reduced in HRF patients. Moreover, HRF patients have shown significant increase of urea, creatinine, and potassium levels.

Table 1: The info of participants.

Parameter	Healthy people	HRF patients	*p*-value
N	30	60	--
Gender	15M/15F	30M/30F	--
Age (year)	38.00±12.40	40.02±12.24	0.468
BMI (kg/m^2)	24.233±2.72	25.14±3.12	0.161
MDA (µmol/L)	0.094±0.016*	0.518±0.233	<0.001
GPx (ng/mL)	9.69±2.48*	3.37±2.42	<0.001
Urea (mg/dL)	26.52±7.03*	115.08±22.07	<0.001
Creatinine (mg/dL)	0.839±0.109*	8.339±1.615	<0.001
Potassium (mmol/L)	4.17±0.43*	6.12±0.59	<0.001
Sodium (mmol/L)	139.32±1.65	139.53±4.30	0.729

N: number; M: male, F: female: *significant.

Table 2 shows the correlation of MDA, and GPx with the rest of the studied parameters. MDA was correlated negatively with GPx, urea, creatinine, and potassium, while GPx was correlated positively with urea, creatinine, and potassium in HRF patients.

Table 3: Correlation of MDA and GPx in HRF patients.

Parameter	MDA		GPx	
	r	*p*-value	r	*p*-value
MDA (µmol/L)	-	-	-0.485*	<0.001
GPx (ng/mL)	-0.485	<0.001	-	-
Age (year)	-0.084	0.522	-0.081	0.538
BMI (kg/m^2)	0.068	0.603	-0.025	0.852
Urea (mg/dL)	-0.298*	0.021	0.333*	0.009
Creatinine (mg/dL)	-0.316*	0.014	0.260*	0.045
Potassium (mmol/L)	-0.353*	0.006	0.337*	0.009
Sodium (mmol/L)	-0.023	0.861	-0.127	0.335

Receiver operating characteristic (ROC) analysis has shown that MDA can be used as excellent sensitive biomarker for the prognosis of HRF (Fig. 1). Moreover, ROC analysis was indicated similar observations for GPx (Fig. 2).

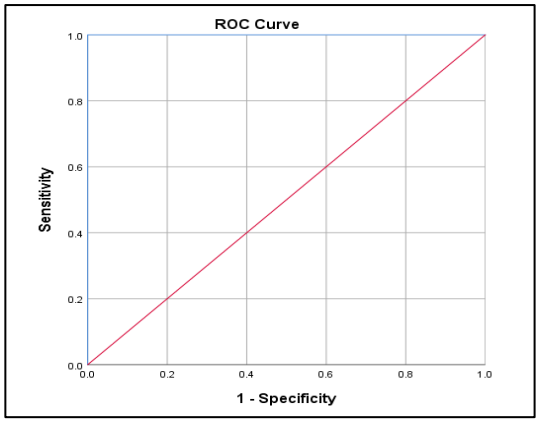

Figure 1: ROC curve of MDA in the prognosis of HRF.

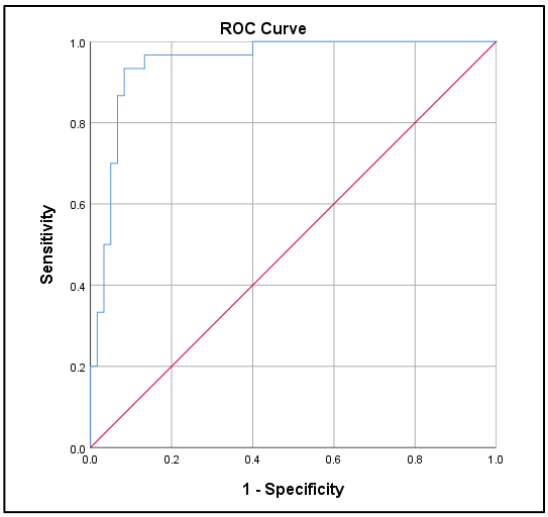

Figure 2: ROC curve of GPx in the prognosis of HRF.

4. Discussion

Hemodialysis operation has been reported to give serious consequences on systemic health [21]. In this study, OS was investigated in these patients to predict their health quality on regular hemodialysis. Serum MDA was significantly higher in HRF patients compared to healthy people, which reflects a condition of increased lipid peroxidation. Roob *et al.* have reported a higher than the normal of the level of MDA in HRF patients [22]. Boaz et al. indicated a high level of MDA in HRF patients and linked this increment to the development of cardiovascular diseases in these patients [23]. Several studies have reported similar observations regarding MDA in HRF patients [24-27]. The increase of MDA level reflects a condition of raise in OS in HRF patients [28]. OS has been observed in HRF patients throughout other biomarkers. Abod *et al.* have observed an increase in the total oxidant status, with low antioxidant capacity in the serum of chronic RF patients [1]. Tepel et al. reported an overproduction of ROS in HRF patients, which essentially responsible for the raise of OS in these patients [29].

The HRF patients have shown low level of GPx, and since it represents an important portion of the antioxidant defense system, its effect on the raise of OS would be significant. Dursun *et al.* reported significant reduction of GPx in HRF patients, whereas the hemodialysis operation did not change the level of GPx significantly and the main reason of reduction was attributed to the dysfunction of the kidneys [30]. A more recent study has shown a non-significant change of GPx activity in HRF patients [31], which disagrees with the current results. Maraj *et al.* have reported significant decrease of GPx in HRF patients compared to the healthy people. The authors have linked the status of OS in HRF patients to the nutritional habits [32]. The reason for GPx reduction may link to the overproduction of ROS, since it can cause oxidative damage to the proteins, therefore it can produce oxidative damage to the GPx at very high levels causing a rapid degradation of the GPx and reducing its amount.

Urea and creatinine are small metabolites that are used to evaluate the function of the kidneys [33]. In accordance, urea and creatinine levels are increased rapidly and massively in RF patients [4]. Morena *et al.* have indicated a lose of antioxidants and raise in OS associated with hemodialysis in HRF patients. They have proposed that very high levels of urea lead to an imbalance between ROS and antioxidants. This imbalance triggered by a lose of the antioxidants in the system which make the detoxification of ROS less efficient [34]. Additionally, MDA was correlated negatively with GPx in HRF patients. This negative correlation prove the relationship between the increase of oxidants and the decrease of antioxidants in HRF patients. Surprisingly, MDA was correlated negatively with urea, creatinine and potassium, while GPx was correlated positively with them. This may result from the trigger of the biological system for the production of more GPx to neutralize the very high amounts of ROS. In conclusion, MDA and GPx can be used to predict the harmful effects of hemodialysis operation on the health of RF patients.

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