DETERMINATION OF REACTIVE OXYGEN SPECIES, GLUTATHIONE, URIC ACID IN SERA OF RENAL FAILURE PATIENTS BEFORE - AND AFTER DIALYSIS

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ABSTRACT
Renal failure is a disease of the kidney, in which the renal excretory function is failed to process due to depression of the GFR. Renal failure is divided into acute and chronic by depending on the period of disease. The study designed to investigate the level of oxidative stress in RF patients. One hundred and twenty subjects had enrolled in the study, who divided onto three groups equally, in which they are healthy control, before dialysis patients, and after dialysis patients. The results had shown significant (P<0.01) increase in the level of ROS for before and after dialysis patients when compared with control. Also, significant (P<0.01) increase has observed at patients after dialysis compared to their situation before the dialysis. Also, the results had shown significant (P<0.01) decrease of reduced glutathione level for before and after dialysis patients when compared with control. The differences was also significant between the same patients before dialysis and after dialysis, in which hemodialysis decrease the level of GSH significantly (P<0.01). The concentration of uric acid has increased significantly (P<0.01) in patients before dialysis. Uric acid concentration in patients after hemodialysis was significantly (P<0.01) reduced compared to same patients before dialysis, and it was non-significantly differ with that of control’s. In conclusion, oxidative stress develops and raise up with time during renal failure situation, and thus could cause further toxic problems for RF patients.

Keywords: Renal Failure, Oxidative Stress, Antioxidant, ROS, GSH.

I. INTRODUCTION
Renal failure (RF) is a kidney disease in which the renal excretory suffers from a failure in its function due to defect in glomerular filtration rate leading to retention of nitrogenous waste products from the blood. RF is classified, according the time of disease, into acute renal failure (ARF) which refers for short period of incidence, and chronic renal failure (CRF) which refers for long period of incidence (Rahman, 2018). The CRF leads Thus leading to kidney weakness, as well as kidney disease in its final stages, and thus it has become at the present time one of the global health problems that have become affected in all regions of the world and that is associated with poor health outcomes, and that leads to a high risk of heart disease and also blood vessels And that leads to deaths (Centers for Disease Control and Prevention, 2017). Individuals with stage terminal chronic kidney disease, which means those with a glomerular filtration rate <15 mL/min/1.73 m², will need some the rapty to replace renal function. This therapy can include kidney transplantation or one of the available dialysis There are two primary types of dialysis, peritoneal dialysis and hemodialysis (Hayat & Baglin, 1973). Peritoneal dialysis (PD) is a renal replacement therapy based on infusing a sterile solution into the peritoneal cavity through a catheter and provides for the removal of solutes and water using the peritoneal membrane as the exchange surface (Viviana Teixeira Henriques et al., 2012), is one of several renal replacement therapies (RRTs) used in the treatment of renal failure to remove excess water and salt, balance the other electrolytes in the body, and remove waste products of metabolism and restore chemical Hemodialysis uses a machine filter called a dialyzer or artificial kidney (Bny Uoda, 2019).

In the various fields of biology and medicine, free radicals are more generally known as ROS or reactive nitrogen species (RNS), free radicals are molecules/molecular fragments containing one or more unpaired electrons, the
presence of which usually makes them highly reactive FRs instability is a result of its containing of odd electron(s), which is the reason for their highly reactivity and short half-life (Poprac et al., 2017), as a consequence of FRs reactivity they seek and abstract electrons from neighboring compounds to stabilize themselves, but this attack would create another FRs which create chain reactions that affects living cells. Among the most important ROS are the hydroxyl radical (OH), the superoxide radical anion (O2·), nitric oxide (NO), and peroxyl radicals (ROO•) [4], as well as non-radical species such as hydrogen peroxide (H2O2), singlet oxygen (1O2), hypochlorous acid (HOCl), and peroxynitrite (ONOO⁻) (Mohammed et al., 2020). ROS can cause various damages to biologic macromolecules. macromolecules as oxidation targets for ROS, membrane fatty acids are so sensitive due to lipid peroxidation (LPO) process, because once LPO is initiated, a harmful chain reaction begins, nucleic acids such as DNA and RNA are also very sensitive to ROS attack, oxidation of these nucleic acids causes mutations in both mitochondrial and nuclear genomes, nearly, all amino acids in a protein or in an enzyme can be oxidized by ROS, and these oxidation can cause modifications which lead to losses of function ultimately resulting in cell death (BA, 2018). Antioxidant materials are chemical substances act to neutralize the free radicals or interfere their action that prevent cellular damage caused by oxidation of other molecules, antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves (Ahmed & Mohammed, 2020). The living system contain substances with antioxidant activity such as uric acid and reduced glutathione etc. (Sarangarajan et al., 2017). Defined, Oxidative stress occurs when the not balance between antioxidants and ROS are disrupted because of either depletion of antioxidants or accumulation of ROS is probably mediated by increased production of chemically reactive oxygen species (ROS) in the mitochondria (Birben et al., 2012). Cellular protection against ROS is provided by a variety of antioxidant molecules and enzymes, including the glutathione (GSH)-dependent antioxidant system. The GSH-dependent antioxidant enzyme system provides vital cellular protection against ROS, particularly hydrogen peroxide and certain organic hydroperoxides, under pathological and toxicological conditions, by using selenium-dependent and -independent peroxidases to reduce hydrogen peroxide or lipid peroxides to water or the respective alcohols, with the concurrent oxidation of GSH to glutathione disulfide (GSSG). In the mitochondria, limitations of GSH synthesis and transmembrane transport suggest that optimal functioning of the mitochondrial GSH system, and maintenance of adequate thiol–disulfide redox tone is essential to protect against the injurious effects of ROS (Donovan & Fernandes, 2000).

II. MATERIALS AND METHODS

2.1. Subjects
The study included three group control (A) Pre-dialysis (B).and Post-dialysis (C). Forty healthy were collected as controls for the study at Mustansiriyah University there ages ranged from 18 to 60 years old. As well as Forty of each of the remaining groups were collected from Kidney Diseases and Transplantation Center and Baghdad Teaching Hospital in Medical City, there ages ranged from 18 to 65 years old. The laboratory side of the study was performed at the laboratory of biochemistry research at the department of chemistry science, Mustansiriyah University.

2.2. Sample Collection
The blood was transferred to gel tube, and then the serum was separated by the centrifuge instrument, centrifugation instruction were 2500 g for 10 minutes, and the process was done at room temperature. Then 33 the serum was divided on three Eppendorf tubes, and stored in a deep freezer (~20 °C) until time of analysis. Anthropometric measurements was obtained also from the participants including height, weight.

2.3. Methods
2.3.1 Determination of Serum GSH Concentration
The serum thiol concentration was measured according the Ellman method &as follows:

(a) H2NaPO4 (0.2M) was prepared by dissolving (0.2) in (100ml) D.W.

(b) HNa2PO4 (0.2M) was prepared by dissolving (0.2) in (100ml) D.W.

(1) Reagent A:(Phosphate buffer (0.2M), pH=7) was prepared by mixing 41ml of (b) with 9ml of (a). Volume was completed to 100ml by D.W. and pH was adjusted.
(2) Reagent B: (Phosphate buffer (0.2M), pH=8) was prepared by mixing 5ml of (a) with 45ml of (b) and Volume was completed to 100ml by D.W. and pH was adjusted before and after add D.W.

(3) Reagent C (DTNB reagent): This solution prepared by dissolving (39.6 mg) of DTNB in 10 ml of reagent A with added tiny amount of Na₂CO₃.

The Procedure of GSH

1. 20μl of serum was added to 1000μl of D.W. in test tube.
2. Then 1000μl of reagent B was added and mixed well.
3. 1500μl was draw from above mixture and 20μl of reagent C was add. The solution mix well and incubated at 37 °C for 60 min
4. Blank was prepared as the same steps in (1,2 and 3) with the exception that the same volume of D.W. was added instead of serum in step 1.
5. The absorbance was read at λ=420nm. In order to calculate GSH use this equation (GSH con.in serum μmol/L=(T-B)×d.f/Ɛ ×10⁶)

2.3.2 Determination of Reactive Oxygen Species (ROS)
The ROS were measured by ELISA test for the quantitative determination of ROS in human serum.

2.3.3 Determination of Uric Acid
Uric acid was measured by spectrophotometric analysis by using commercial kit.

III. RESULTS AND DISCUSSION

The results are expressed in the form of mean ± SD, and considered significant at P ≤ 0.05. There are non-significant (P>0.05) difference in age between control and chronic renal failure patients. Chronic renal failure patients have shown highly significant (P<0.01) higher values of BMI compared to the study control. Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control, N=40 Mean ± SD</th>
<th>Renal Failure Patients, N=40 Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>33.43 ± 10.63</td>
<td>38.30 ± 13.35</td>
<td>0.124</td>
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<tr>
<td>BMI (kg.m⁻²)</td>
<td>21.72 ± 2.08</td>
<td>28.76 ± 4.49</td>
<td>&lt; 0.0001</td>
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<td>Gender</td>
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</tr>
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<td>Male</td>
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<td>50%</td>
<td>1.0</td>
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<tr>
<td>Female</td>
<td>50%</td>
<td>50%</td>
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</tbody>
</table>

The level of ROS has increased significantly (P<0.01) in chronic renal failure patients before dialysis (191.57 ± 42.41 IU/mL) and after dialysis (216.37 ± 30.35 IU/mL) compared to healthy control (85.51 ± 31.8 IU/mL). Also, significant (P<0.01) increase has observed at patients after dialysis compared to their situation before the dialysis. Fig. 1.
Himmelfarb et al. (1993), have reported significant increase of ROS level in the blood of renal patients after 15 min of dialysis, and this increasing remains significant for 30 min after the operation (Ault & Hakim, 1993). Mimić-Oka et al. (1999), have reported significant increase of hydrogen peroxide level in plasma of renal patients after hemodialysis compared to their study control, yet the increase was non-significant between control and patients before dialysis, they have assumed that the imbalance in the activity of extracellular antioxidant enzymes in chronic renal failure may result in accumulation of free radical species, and in unscheduled oxidation of susceptible molecules (Mimić-Oka et al., 1999). The results of ROS at the present study is agreed with all of the researches that previously mentioned above. ROS play an important role in the physiological regulation of kidney function which consequently makes the kidney especially vulnerable to redox imbalances and oxidative stress. Formation of ROS or changes in ROS production can occur both in the renal cortex and medulla, with a broad range in effects, going from alteration in renal blood flow over sodium/fluid retention to inflammation and fibrotic changes and onset of proteinuria (Nistala et al., 2008). In chronic kidney disease, increased mitochondrial ROS generation and mitochondrial dysfunction are frequently reported. Especially in diabetic nephropathy, mitochondrial dysfunction has been well explored with findings on both morphological as well as functional disturbances in the renal mitochondria (Galvan et al., 2017). Recent evidence also found a role for mitochondrial dysfunction in nondiabetic chronic kidney disease (Ishimoto et al., 2017) (Andries et al., 2019). Furthermore, NADPH oxidase, the important source of ROS production, was observed with elevated activity in chronic kidney disease which leads to the overproduction of ROS (Ling & Kuo, 2018). Overall, dialysis patients are vulnerable to oxidative stress with a marked increase in ROS production and antioxidant depletion. ROS induces activation of nuclear factor kappa B (NF-κB), which is translocated to the cell nucleus stimulating cytokine production, in turn causing inflammation (Subrata Kumar Biswas Department, 2016). Another impact of the hemodialysis treatment is the activation of polymorphonuclear white blood cells, which trigger production of ROS and other pro-oxidants. Indeed, increased indices of oxidative damage along with decreased indices of antioxidant defense have been observed in hemodialysis patients post-dialysis. Additionally, involuntary removal of vitamins also occurs with every hemodialysis session,(Sahathevan et al., 2020). this removal leads to a decrease of ROS detoxification system, and increase in ROS level.

The level glutathione shows significant (P<0.01) decrease of reduced glutathione level in sera of chronic renal failure before dialysis (1056.3 ± 20.51) and after dialysis (767.87 ± 71.72) compared to healthy control (1295.05 ± 98.4). The differences was also significant between the same patients before dialysis and after dialysis, in which hemodialysis decrease the level of GSH significantly (P<0.01), for graphic demonstration see. Fig. 2.
Ceballos-Picot et al. (1996), have reported significant decrease of reduced glutathione level in severe chronic renal failure patients compared to control, yet non-significant changes has observed with hemodialysis patients. They have declared that such disturbances in antioxidant systems that occur from the early stage of chronic uremia and are exacerbated by dialysis provide additional evidence for a resulting oxidative stress that could contribute to the development of accelerated atherosclerosis and other long-term complications in uremic patients (Ceballos-picot et al., 1996). Chugh et al. (2000), have reported significant lower level of reduced glutathione in chronic renal failure patients compared to healthy control. The GSH level has significantly reduced after hemodialysis. There was an evidence of oxidative stress in patients of CRF before hemodialysis which increased further after hemodialysis (Chugh et al., 2000). Annuk et al. (2001), have studied the oxidative stress in renal failure patients. They have reported significant decrease of reduced glutathione in renal failure patients compared to healthy control. Also, they have concluded that an impaired endothelium vasodilation function and oxidative stress are related to each other in patients with CRF (Annuk et al., 2001). The decrease of reduced glutathione is supports the results of TAC, which considered as part of the total antioxidant within the system. At CRF there is an overproduction of ROS; on the other hand, the activity of antioxidant enzymes is reduced and the level of antioxidants with low molecular weight is lowered. The decrease in the activity of SOD, decrease in the level of GSH, and higher GSSG/GSH ratio were described in RBC from hemodialyzed patients (Gwozdzinski et al., 2021).

The concentration of uric acid has increased significantly (P<0.01) in the sera of chronic renal failure patients before dialysis (8.81 ± 1.01 mg/dL) compared to healthy control (4.7 ± 1.16 mg/dL). Uric acid concentration in CRF patients after hemodialysis (4.38 ± 0.77 mg/dL) was significantly (P<0.01) reduced compared to same patients before dialysis, and it was non-significantly differ with that of control’s. see. Fig. 3.

Figure 2. Mean, Range, and Median of GSH in Patients and Control
Bergesio et al. (1998), reported significant increase of uric acid level in CRF patients compared to heathy control, but it decreased after hemodialysis. Also, they have reported significant positive correlation with total antioxidant capacity (Bergesio et al., 1998). Erdog’an et al. (2002), have reported significant increase of uric acid level in CRF patients on hemodialysis compared to healthy control (Erdog & Kuru, 2002). Mok et al. (2012), have reported significant linear association between serum uric acid level and chronic kidney disease, in which higher serum uric acid levels increased the risk of CKD, suggesting that at least part of the reported association between serum uric acid and cardiovascular disease may be connected to CKD (Mok et al., 2012). Experimental studies demonstrated that hyperuricemia caused the low development of kidney disease, with the development of albuminuria, microvascular disease, glomerulosclerosis, and tubulointerstitial fibrosis. Hyperuricemia was also found to accelerate renal disease of other etiologies, particularly the remnant kidney model. A variety of mechanisms were identified, including the stimulation of intrarenal renin expression with renal hypertrophy, glomerular hypertrophy, acceleration of intrarenal microvascular disease, and the development of glomerular hypertension and renal vasoconstriction. Recent clinical studies have also confirmed that uric acid is a major independent risk factor for the development of renal disease both in the normal population and in subjects with kidney disease due to immunoglobulin A nephropathy (Nakagawa et al., 2006). Nakatani et al. (2017), have reported significant association between serum uric acid and the activity of xanthine oxidase in CKD patients (Nakatani et al., 2017). This could explain the increase of uric acid since it is the enzyme responsible for uric acid synthesis.

IV. CONCLUSION

The level of oxidative stress was significantly elevated in chronic renal failure patients through both, increase of oxidants and decrease of antioxidant parameters. Furthermore, the increase of oxidative stress has observed at greater level after the hemodialysis process, this would increase the health problems beyond the renal dysfunction to systemic level in which the rest of the body’s cells would affected and oxidatively damaged; hence a development of further pathological conditions would appear.

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