Evaluation of total oxidant status and antioxidant capacity in sera of acute- and chronic-renal failure patients

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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 depending on the period of the disease. The study was designed to investigate the level of oxidative stress in RF patients. Seventy-five subjects had enrolled in the study, who divided into three groups equally, in which they are healthy control, ARF patients, and CRF patients. The results had shown a significant (P<0.01) increase in the level of TOS for RF patients when compared with control. Also, a significant difference in the level of TOS has been observed between ARF and CRF patients, where the CRF patients have shown to have the highest level of TOS. The level of TAC was significantly decreased (P<0.01) in RF patients when compared with control. In conclusion, oxidative stress develops and raise with time during renal failure situations, and thus could cause further toxic problems for RF patients.

Keywords: Renal failure, oxidative stress, antioxidant, ROS.

1. 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. 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. [1]. ARF is the syndrome in which glomerular filtration declines abruptly in which the kidneys abruptly stop working entirely or almost entirely (hours to days) and is usually reversible in which the kidneys stop working entirely or almost entirely [2]. CRF, also known as chronic renal insufficiency, in which the kidneys are incapable of handling wastes for long time period and the damage could result in both shutting down both kidneys from work. The CRF leads to kidney impairment and end-stage renal disease, which is becoming a global public health problem affecting in all regions of the world and is linked to poor health outcomes, higher risk of cardiovascular and mortality [3].

Free radical (FR) in the terms of biology and medicine, is used to describe any chemical species that has the ability of independent existence is a chemical species such as an atom, a molecule, or an ion which that contains an uneven number or, unpaired electrons [4]. FRs instability is a result of its containing of odd electron(s), which is the reason for their highly reactivity and short half-life. As a consequence of FRs reactivity they seek and abstract electrons from neighboring compounds to stabilize themselves, but this attack would create other FRs which create chain reactions that affect a living cells [5]. Both ROS and RNS constitute the free radicals and other non-radical reactive species [6]. In living system,
among the radical species, oxygen-derived radicals are most important and it is called reactive oxygen species (ROS) is a term used to define oxygen-containing reactive species [7]. ROS includes superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), as well as hydroxyl radical (·OH), singlet oxygen (O$_2^*$), peroxyl radical (LOO$^*$), alkoxyl radical (LO$^*$), lipid hydroperoxide (LOOH), peroxynitrite (ONOO$^-$), hypochlorous acid (HOCl), and ozone (O$_3$) [4]. Likewise, the term reactive nitrogen species (RNS) has been used to include nitric oxide (NO), peroxynitrite, nitrogen dioxide (NO$_2^*$), as well as other oxides of nitrogen or nitrogen-containing reactive species [8]. The generation of ROS and RNS can occur as a product of biochemical reactions, in mitochondria, peroxisomes, cytochrome P450, and other. The major process of ROS production in aerobic cellular components by the mitochondrial electron respiratory chain (ETC). ROS, and RNS produce tissue injury by lipid peroxidation, enzyme inactivation, damage to DNA and structural proteins. The human body has developed defense mechanisms against these radicals by Antioxidants [9]. Antioxidant materials are chemical substances act to neutralize the free radicals or interfere with their action, doing so prevent the occurrence, breakdown, or cellular damage that results from oxidation of other molecules [10]. Antioxidants will interfere with those free radicals and thus end this continuous reaction by getting rid of the free radical intermediary as well as prevent other oxidation reactions from occurring by oxidizing themselves. Antioxidants can work through one or two methods: chain-breaking or prophylactic. This method is summarized for the chain-breaking process, that when the free radicals release an electron or work to steal it, another second free radical is formed and one another where this free traction performs the same actions on another molecule and continues until the free radicals are installed by the antioxidants that work to break the chain from examples of these antioxidants (Vitamin E, Ascorbic acid, carotenoids, etc.), or simply decomposes into a stable product. The classic example of such a chain reaction is lipid peroxidation. While the second method, which is known as the preventive method, it is this method Explains that the antioxidant enzyme such as (SOD, Lactase, and GPX) can work by preventing oxidative stress by reducing the rate at which the chain initiation occurs, and this process occurs, either by cleaning or reducing free radicals or by stabilizing the transition metal roots, such as iron and copper [11].

2. Materials and Methods

2.1. Subjects

The study included three group control (A).Acute renal (B) .and chronic renal (C). Twenty-five healthy were collected as controls for the study at Mustansiriyah University. As well as twenty-five of each of the remaining groups were collected from Kidney Diseases and Transplantation Center and Baghdad Teaching Hospital in Medical City. The ages of the samples were between (18-70) 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

Blood samples were collected from the individuals in a plastic disposable 5mL syringe was used for venipunctures and blood were drawn slowly. Then the blood was translocated into a gel tube and left for 10 min at room temperature to clot. Blood samples then centrifuged at 4000rpm for 10 min and the obtained serum was stored in three Eppendorf tubes at -20 ºC until analysis. Anthropometric measurements was obtained also from the participants including height, weight.

2.3. Methods
2.3.1 Total Oxidant Status

Serum total oxidant status (TOS) concentration was determined by using an automated colorimetric method for measuring total oxidant status by Erel [12].

Reagent 1 contains 150 μM xylene orange, 140 mM sodium chloride, and 1.35 M glycerol in 25 mM sulfuric acid, pH=1.75. Reagent 2 contains 5mM Fe$^{3+}$, and 5 mM 4-(4-amino-3-methoxyphenyl)-2-methoxyaniline in 25mM sulfuric acid. The procedure is demonstrated in Table 1.

Table 1: The procedure of TOS.

| Reagent             | Sample     | Standard | Blank |
|---------------------|------------|----------|-------|
| Serum               | 105 μL     | -        | -     |
| Hydrogen peroxide   | -          | 105 μL   | -     |
| Reagent 1           | 625 μL     | 625 μL   | 625 μL|
| Reagent 2           | 33 μL      | 33 μL    | 33 μL |

The absorbance were read at 650nm against blank after 3 min.

To calculate TOS, standard curve was constructed first, then the regression equation $y = mx \pm b$ was used.

2.3.2 Total Antioxidant Capacity

Total antioxidant capacity (TAC) of serum samples was determined by using a spectrophotometric method, developed by Erel [13].

Reagent 1 contains 5mM Fe$^{3+}$, and 10 mM 4-(4-amino-3-methoxyphenyl)-2-methoxyaniline in (75 mM, pH 1.8) Clark and Lubs solution. Reagent 2 contains 7.5 mM hydrogen peroxide in Clark and Lubs solution. The procedure is demonstrated in Table 2.

Table 2: The procedure of TAC.

| Reagent | Sample     | Standard | Blank |
|---------|------------|----------|-------|
| Serum   | 35 μL      | -        | -     |
| Vit C   | -          | 35 μL    | -     |
| Reagent 1 | 1000 μL   | 1000 μL  | 1000 μL|
| Reagent 2 | 50 μL     | 50 μL    | 50 μL |

The absorbance were read at 444nm against blank after 3min.

To calculate TAC, standard curve was constructed first, then the regression equation $y = mx \pm b$ was used.

3. Results and Discussion

The results are expressed in the form of mean ± SD, and considered significant at $P \leq 0.05$. There was a non-significant ($P>0.05$) differences in age among control, and patients with acute and chronic renal
failure. The results have shown that the weight of patients was significantly lower \((P<0.05)\) than that of control, and also their body mass index (BMI), Table 3.

**Table 3**: Information about subjects.

| Parameters          | Control, N=25 | Acute renal failure, N=25 | Chronic renal failure, N=25 | \(p\)-value |
|---------------------|---------------|---------------------------|-----------------------------|-------------|
| Age (year)          | 35 ± 10.8     | 40.8 ± 15.69              | 40 ± 14.99                  | 0.355       |
| Weight (kg)         | 72.7 ± 8.2    | 65.5 ± 7.2                | 65.6 ± 10.6                 | 0.018       |
| BMI (kg/m\(^2\))    | 24.61 ± 3.46  | 23.72 ± 4.91              | 20.52 ± 4.29                | 0.011       |
| Sex                 | Male 56%      | Male 60%                  | Male 50%                    |             |
|                     | Female 44%    | Female 40%                | Female 50%                  |             |

The level of TOS was significantly increased \((P<0.01)\) in RF patients \((3.9 ± 0.54 \text{ for acute, and 6.98 ± 0.9 for chronic patients})\) when compared with healthy control \((1.3 ± 0.05)\). The highest level was for CRF patients, and it was significantly higher \((P<0.01)\) than that of patients with ARF. There were non-significant observations between males and females in all groups, Fig. 1.

![Figure 1](image1.png)

**Figure 1**: The level of TOS in control, ARF, and CRF.

The level of TAC was significantly decreased \((P<0.01)\) in RF patients \((0.821 ± 0.14 \text{ for acute, and 0.722 ± 0.01 for chronic patients})\) when compared with healthy control \((1.5 ± 0.07)\). The lowest level was for CRF patients, and it has non-significant difference with the level of TAC in ARF patients. There were a non-significant observations between males and females in all groups, Fig. 2.
Figure 2. The level of TAC in control, ARF, and CRF.

The ratio of TOS over TAC (IOS) has significantly increased ($P<0.01$) from control ($0.87 \pm 0.07$) toward RF patients ($4.76 \pm 0.63$ for ARF, and $9.66 \pm 1.23$). The difference in the IOS ratio between ARF and CRF patients was highly significant ($P<0.01$), where the CRF patients have the highest ratio.

Till this moment, there are several studies have investigated the oxidative stress status in renal failure disease. In the present work, it was found that a serious elevation in the status of total oxidative stress occurs in the sera of RF patients. The great increase in the level of TOS in the sera of CRF patients compared to ARF patients demonstrate the crucial role of kidney dysfunction in the development of oxidative stress.

The results agreed with the finding of Himmelfarb et al. who reported a significant increase of plasma protein-thiol oxidation, a marker of oxidative stress, in patients with ARF [14]. In another work has done by Ushanthika et al. a significant increase of plasma malondialdehyde level had observed in CRF patients (MDA is an end product of lipid peroxidation as a result of ROS effect), they had also reported significant decrease of SOD activity, ultimately the prolonged effect of ROS and the decrease of antioxidants capacity, resulting in oxidative stress development [15].

ROS affects the cells of the body, especially at sites of inflammation, which are abundant in acute and chronic renal failure [16]. pro-and anti-inflammatory cytokine gene polymorphisms might play a pivotal role in the regulation of host inflammatory responses and contribute to greater morbidity and mortality in patients with ARF [17]. ROS causes upregulation of cytokine production [18]. Thus, oxidative stress link with the exacerbation of inflammation in ARF may be a major path for chronic renal failure occurrences.

4. Conclusion

The present study emphasizes the relationship between oxidative stress and renal failure disease. The great increase of oxidative stress status found in patients with chronic renal failure compared to patients with acute renal failure may indicate to a major path in the progression of renal failure disease.
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5. References

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