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EVALUATION OF TOTAL ANTIOXIDANT STATUS (FRAP) IN CKD AND MHD PATIENTS

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ABSTRACT

Chronic kidney disease (CKD) is a major condition in which oxidative processes are implicated by the amplification of inflammatory responses. Hemodialysis (HD) represents a state of chronic stress for the patient where the oxidative reactions are mainly due to bio incompatibility of components of dialysis apparatus leading to the production of ROS by inflammatory cells. Lipid peroxidation leads to alterations in the biological membranes and is involved in the progression of renal injury. FRAP is said to give more biologically relevant information than that provided by individual antioxidant measurements and which may describe the dynamic equilibrium between pro-oxidants and antioxidants in the plasma. A significant increase in plasma MDA was found in CKD and Pre HD patients and in Hemodialysis with pre HD Increased levels were found when comparing with post HD ($P<0.05$), along with significant decrease in the antioxidants like Uric acid and FRAP levels were found in Pre HD comparing with CKD and Pre HD with Post HD ($P<0.05$). FRAP is being used as a single test to estimate total antioxidant capacity (TAC) of blood. FRAP may be considered as an easy, cost effective method to measure the antioxidant power and it might be incorporated into risk prediction in dialysis and CKD.

Keywords: Chronic kidney disease, Hemodialysis, Oxidative stress; Free radicals, Antioxidants

INTRODUCTION

Chronic kidney disease (CKD) is a worldwide killer. Not only does a poor renal function cause patients to die of uremia, but there is also a strong association between CKD and cardiovascular disease. Chronic kidney disease (CKD) patients have a higher risk to develop cardiovascular disease, atherosclerosis and cancer compared to the general population. Cardiovascular disease is the major cause of death in CKD patients **[1]**. Hemodialysis per se has been suggested to induce oxidative stress, with reactive oxygen species being generated on the surface of dialysis membrane s by activation of polymorphonuclear leukocytes. It has been well documented that even a single session of hemodialysis significantly increases lipid peroxides and decreases antioxidants.

HD is mainly responsible for free radical production as well as non-enzymatic antioxidant losses. This suggests that HD, far from improving oxidative stress, worsens the same. Mechanisms might account for increased oxidative stress, including antioxidant deficiency, neutrophil activation during dialysis, and chronic inflammation. OS is implicated in the pathologic pathways of various conditions, such as diabetes mellitus (DM), atherosclerosis, inflammation, and progression of chronic kidney disease (CKD) to end-stage renal disease (ESRD).

Antioxidants are important in patients with CKD to retard disease progression, and reduce the risk of premature development of cardiovascular disease (CVD) and death. Total antioxidant capacity is a measure of overall body defense against free radicals; it is made up of enzymatic and non-enzymatic components. This study showed a significant reduction in the total antioxidant capacity in patients undergoing HD. Oxidative stress (OS) has been implicated in the pathogenesis of cardiovascular death and CKD patients are at increased risk of both OS and cardiovascular death. Incidence of cardiovascular events and death has been reported to increase soon after commencement of HD, however the mechanism responsible is not fully understood **[2]**. This study further suggests that loss of antioxidants across the semi permeable dialyzer bio-incompatible membrane during the course of HD may play a significant role in increased oxidative stress in patients undergoing HD and may partly contribute to increased mortality. The dialysis treatment itself is also a source of oxidative stress and inflammation. The contact between the blood and the dialysis membrane (that can be more or less biocompatible depending on material) will cause alterations in the constitution of blood cells during hemodialysis **[3-5]**.

In this Paper on CKD patients and HD Patients levels of oxidative stress, and Antioxidant capacity FRAP (TAS) were investigated. Oxidative stress was measured in the blood. The relationships between the study parameters were investigated and the results for CKD, pre dialysis and post dialysis patients were compared. The biochemical markers results will studied and compared with the CKD, dialysis patients and that pre dialysis and post dialysis patients were compared with controls.

MATERIAL AND METHODS

50 Patients with CKD (who never undergone HD) and 50 patients were maintenance hemodialysis for 3 years or more, 100 Controls were recruited after informed consent. Of these, the causes of ESRD were diabetic nephropathy, hypertensive nephropathy, chronic glomerulonephritis, ischemic nephropathy, chronic interstitial nephritis, and unknown. Patients with acute infection were not included in the study group. The dialysis program consists of 4 hrs dialysis sessions three times a week.

Statistical Analysis

Continuous variables were expressed in mean±SEM. The data were transformed into percentage taking the predialytic value as 100% in order to nullify the effect of uremia and reflect change due to the dialysis session. Analysis of variance for repeated measures was performed to assess the overall time course change due to dialysis. A P value of <0.05 was considered significant. Statistical analysis was performed using Microsoft Excel and SPSS version 11.5 for Windows.

RESULTS

A significant increase in plasma MDA was found in CKD and Pre HD patients and in Hemodialysis with pre HD Increased levels were found when comparing with post HD ($P<0.05$), along with significant decrease in the antioxidants like Uric acid and FRAP levels were found in Pre HD comparing with CKD and Pre HD with Post HD. ($P<0.05$). MDA levels were increased in CKD patients when compared with control. Total antioxidant capacity is an indicator of plasma resistance against oxidant agents. The major contributors to the total antioxidant capacity of plasma are urate, ascorbate, vitamin E, and plasma protein. We have measured this status in the form of ferric reducing ability of plasma (FRAP). A significant decrease in Pre (P=0.001) and

Post (P<0.001) in FRAP levels were observed in our study.

DISCUSSION

In this study, the levels of plasma MDA, which serves as evidence of lipid peroxidation were similar before and after HD. Plasma MDA is an intermediate product of lipid peroxidation and a marker of oxidative stress. The result of our study suggests that HD may not be associated with the removal of pro-oxidants molecules such as MDA in CKD patients. Though elevated levels of MDA has been demonstrated in patients with varying degrees of renal functions **[6, 7]**, this study showed that a session of HD did not yield a significant reduction in its plasma levels in CKD patients undergoing first session of HD. This is in agreement with earlier study **[8]** who observed a non-significant difference following HD though it's not stated whether participants were incipient or have been on dialysis. In another study **[9]**, post dialysis sample was collected 30 minutes after completing HD, MDA values were reported to be similar to those pre-dialysis. That study however recruited participants who were on regular HD.

Our study confirmed significant decrease in total antioxidant capacity associated with HD that has been documented in previous studies and further suggests that the possible mechanism may be associated with loss of soluble components during HD. Possible cumulative effects of this coupled with non-clearance of MDA pose dialysis patients at increased risk of oxidative stress. This may enhance disease progression, cardiovascular complications and increase mortality among these patients. However, the use of vitamin E coated dialyzers has been suggested to reduce oxidative stress and endothelial dysfunction associated with HD **[10]**. This study has further shown that HD is associated with significant alteration in antioxidant status of CKD patients. Even a single session of HD may contribute to OS in CKD patients through loss of antioxidants across dialyzer bio incompatible membrane. Clinical trials may be necessary to ascertain the probable beneficial effects of antioxidants supplements and antioxidant-coated dialyzers. Dietary restrictions for CKD patients may be required to diminish the complications of kidney failure and dialysis treatment. The purpose is to decrease uremic symptoms and to keep the patient at a healthy weight by individual restrictions and dietary advice. Reduced intake of proteins, potassium, sodium, phosphorus, calcium and excess fluid are often recommended. Alterations in the diet, in some cases with a reduction of intake of fresh fruits and vegetables to evade hyperkalemia and limit levels of phosphorus and calcium, is a potential cause of antioxidant deficiency.

MDA is the stable end product of lipid peroxidation, and it's produced during the decomposition of polyunsaturated fatty acids. UA is also a physiological free radical scavenger and one of the major contributors of the plasma antioxidant capacity. Thus, UA plays a dual role, both as a pro-oxidant and as an antioxidant **[11]**. Uric acid was shown to be the second highest contributor among the known serum antioxidants, albumin (28.0%), ascorbic acid (3.08%), α-tocopherol (1.74%) and bilirubin (1.0%). In addition, uric acid was found to be elevated with overweight, obesity and visceral fat area **[12]**. Hence, FRAP can be said to provide more biologically and clinically relevant information on antioxidant capacity than that provided by individual antioxidant measurements. Measurement of total antioxidant capacity as FRAP can reflect all these and hence is a better measure of antioxidant status than measurement of the individual antioxidants. FRAP is being used as a single test to estimate total antioxidant capacity (TAC) of blood. In recent years several methods have been developed to assess the TAC of human serum (or) plasma. More biologically relevant information can be obtained by assessing FRAP than that obtained by measuring the concentration of individual antioxidants and may more closely describe the dynamic equilibrium between Pro oxidant and anti-oxidants occurring in the plasma compartment **[13]** FRAP is the global marker of the antioxidant power. FRAP conclude the total activity of antioxidant vitamins and enzymes to difficulty in separate estimation of each antioxidant component of plasma and of the interactions that take place among different components. The strength of the antioxidant system inhibitor trap the free radical produced under normal and pathological condition was evaluated by measuring the level of total antioxidant status. This reflects the status of extracellular antioxidants these antioxidants inhibit delay the oxidative process **[14]**. FRAP may be considered as an easy, cost effective method to measure the antioxidant power and it might be incorporated into risk prediction in dialysis and CKD.

However, we might be able to assume that uric acid did contribute serum total antioxidant status level, when we consider the report 20% total antioxidant capacity (TAC) called as total antioxidant status (TAS) was contributed by uric acid **[15]**. We observed similar tendency in elevated total antioxidant status with increased number of metabolic risk factors. Increased total antioxidant status level may not always represent ones healthier condition, a condition with a low oxidative stress **[16]**. Suggesting that total antioxidant status may be used as a sole indicator of oxidative stress marker. Elevated levels of oxidative stress are frequently reported in CKD patients **[17-20]**. This imbalance can occur as a consequence of both an increased production of ROS and insufficient antioxidant defence. Beyond the generation of ROS during normal cellular metabolism, potential sources of oxidative stress in CKD patients also include systemic inflammation, incidence of diabetes, dialysis treatment, reduced levels of dietary intake of antioxidants and the uremia itself. Inflammation, oxidative stress and malnutrition are closely related in renal failure and contribute to the increased risk for cardiovascular disease and mortality. Raised levels of inflammation markers such as specific cytokines and acute-phase reaction proteins are observed in CKD patients **[21, 22]**.

FRAP is a simple method, speedy, inexpensive. A single test which denotes the antioxidant power of blood was established & estimated as the ferric reducing ability of plasma (FRAP) was found to give more biologically relevant information than the measurement of individual antioxidants. In recent years several methods have been developed to assess the TAC of human serum (or) plasma. More biologically relevant information can be obtained by assessing FRAP than that obtained by measuring the concentration of individual antioxidants and may more closely describe the dynamic equilibrium between Pro-oxidant and anti-oxidants accuring in the plasma compartment **[23, 24]**.

CONCLUSION

Oxidative stress and inflammation are important risk factors that contribute to disease progression and mortality in chronic kidney disease patients. The interrelations between these events are complex and factors including dialysis treatment, medication, diet and oral health are of importance. These results are suggestive of oxidative stress leading to progressive renal injury along with immune suppression. FRAP can be a useful indicator to monitor and optimize antioxidant therapy, which may potentially become an important adjunct in the management of CKD patients. OS is a universal challenge in life and induces a counter response by exposed cell. The enhanced OS status that characterizes HD patients is mainly due to poor dietary intake of exogenous antioxidants, accumulation of oxidative products, and loss of antioxidant molecules during HD and is highly linked with development of atherosclerosis, chronic inflammation, and all-cause and CVD mortality in these patients. It seems that OS is an undisputed component of the uremic environment and since uremia is a well-established nontraditional risk factor for CV events and all-cause/CV mortality, it is tightly linked with early atheromatosis and CV disease. Therefore, OS should be incorporated in a "uremic milieu" abnormality approach and might constitute a novel but quite important therapeutic target in chronic HD patients.

FRAP Summarizes the overall activity of antioxidant vitamins and enzymes. Because of the difficulty in measuring each antioxidant component of plasma separately and of the interactions that take place among different components. FRAP is being used as a single test to estimate total antioxidant capacity (TAC) of blood. FRAP may be considered as an easy, cost effective method to measure the antioxidant power and it might be incorporated into risk prediction in dialysis and CKD.

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