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# Study of Serum Malondialdehyde and Whole Blood Reduced Glutathione in Chronic Kidney Disease

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***Abstract:*** **Chronic Kidney Disease (CKD) is associated with high incidence of morbidity and mortality. The imbalance between oxidants and antioxidants is thought to play an important role in the tissue damage in CRF. The objective is to evaluate oxidant and antioxidant imbalance in CRF patients.**

**Methods: A total number of 80 subjects comprising of 40 healthy controls and 40 Chronic Kidney Disease patients attending dialysis unit before dialysis were studied. In all the subjects, serum levels of malondialdehyde (MDA) as a biomarker of lipid peroxidation and antioxidant whole blood reduced glutathione (GSH) were estimated.**

**Results: The level of whole blood reduced glutathione was significantly decreased in CKD Patients when compared to healthy controls. Serum MDA was significantly increased in CKD patients when compared to controls.**

**Conclusion: The presence of increased systemic oxidative stress in chronic kidney disease patients seems to be associated with accumulation of uremic toxins, increased gycation end products and increased homocysteine level. The decrease in antioxidants levels appears to be mainly a consequence of increased oxidative stress. This suggests that oxidative stress is likely to be involved in the tissue damage in CKD patients.**

***Keywords:*** **Oxidative stress, Antioxidants, CKD, Whole blood reduced glutathione, MDA.**

## I. INTRODUCTION

Chronic kidney disease (CKD) or Chronic Renal Failure (CRF) is associated with high morbidity and mortality and is a growing health problem globally with rising incidence and prevalence. Chronic kidney disease is a progressive loss in renal function over a period of months or years. The symptoms of worsening kidney function are not specific and might include feeling generally unwell and experiencing a reduced appetite. CKD often diagnosed as a result of screening of the people known to be at risk of renal problems, such as those with diabetes mellitus or hypertension and those with chronic kidney disease in a blood relative. The CKD also identified when it leads to complications such as cardiovascular disease, anaemia or pericardiatis.[1]

Oxidative stress has been defined as an imbalance between formation of reactive oxygen species and antioxidant defence mechanisms in body.[2] ROS are highly reactive molecules generated by biochemical redox mechanisms that occurs during normal cellular metabolism and also during free radical mediated diseases such as diabetes mellitus, cancer, cardiovascular diseases and renal disease. In view of the profound biological effects of reactive oxygen species, in recent years numerous experimental and clinical studies have been focused on detection of signs of oxidative stress in CKD patients.[3]

The Tripeptide glutathione (GSH) represents major low molecular weight thiol compound present in red blood cells. The reduced form of Glutathione (GSH) functions as an important scavenger of hydrogen peroxide and plays an important role in the prevention of peroxidative damage in tissues. The reduced form of glutathione is an efficient antioxidant of both intracellular and extracellular medium. Extracellular GSH protect cells from oxidants produced and released by

inflammatory cells, and intracellular GSH protect cells from oxidants produced in normal biochemical reactions and from xenobiotic compounds.[4]

Malondialdehyde is an organic compound with the formula $CH_2(CHO)_2$. The product of this aldehyde occurs naturally and used as a biomarker for the measurement of oxidative stress in an organism. Reactive oxygen species or free radicals degrade polyunsaturated lipids present in cell membrane producing Malondialdehyde. This compound is a reactive aldehyde and is one of the many reactive electrophilic species that cause oxidative stress in cells and also form the covalent protein adducts which are known as advanced lipoxidation end products (ALE).[5]

Present study is undertaken to evaluate antioxidant whole blood reduced glutathione and serum Malondialdehyde as a marker of oxidative stress in controls and in Chronic kidney disease patients

## II. MATERIALS AND METHODS

The sample size includes 80 subjects among them 40 normal healthy persons as controls and 40 CKD patients attending for dialysis unit before dialysis were selected. Study is carried out over a period of one year at KLE's Hospital and Medical Research centre, Belgaum, attached to Jawaharlal Nehru Medical College, Belgaum. All study subjects included were in the age group ranging from 40-60 years of both sexes.

**Exclusion criteria:**

1) Subjects with history of medical disorders such as hypertension, diabetes, hepatic, neoplasia and cerebrovascular disorders
2) Smokers, alcoholics, obese( BMI>30), family history of dyslipidemia
3) Subjects on medications steroids, vitamin supplementation and herbal medications

**Collection of blood sample:**

About 5ml of blood was collected from large peripheral vein under aseptic precaution before undergoing dialysis. Out of which 2ml was taken in an anticoagulant (EDTA) bulb for estimation of whole blood reduced glutathione (GSH) and 3ml in a plain bulb for estimation of serum malondialdehyde (MDA).

**Estimation of serum Malondialdehyde (MDA)[6]**

Serum malondialdehyde estimated by thiobarbituric acid method. The reaction depends on the formation of pink coloured complex between malondialdehyde and thiobarbituric acid (TBA), having an absorption of maximum at 532 nm.

**Estimation of Whole Blood Reduced Glutathione[7]**

Whole blood reduced glutathione was estimated by Ernest Beutler et al., method. It is based on the principle that all of the non-protein sulphydryl groups of red blood cells are in the form of reduced glutathione (GSH). 5,5[1] - dithiobis-2-nitrobenzoic acid (DTNB) is a disulphide compound, which is readily reduced by sulphydryl compounds, forming a highly colored yellow compound. Optical density of which is measured at 412nm and is directly proportional to the GSH concentration.

**Statistical Analysis:**

Results are expressed as mean ±SD and range values. Unpaired't' test is used for comparing different biochemical parameters between cases and controls. p value of < 0.05 was considered as statistical significance

**Table 1: Comparison of Serum MDA and Whole Blood Reduced Glutathione in Controls and CKD cases**

| Groups | MDA(nmol/ml) | GSH(mg/dl) |
|---|---|---|
| Controls | 3.78 ± 0.52 | 33.45 ± □□□□ |
| CKD Cases | 6.52 ± 0.24 | 27.53 ± □□□□ |
| Mean Difference | 2.74 | 5.92 |
| t* | 23.44 | 15.45 |
| p | < 0.001 | < 0.001 |

* Unpaired t-test

## III. DISCUSSION

Oxidative stress defined as the tissue damage resulting from an imbalance between an excessive generation of oxidant compounds and insufficient antioxidant defense mechanisms. Oxidant compounds include reactive oxygen species and reactive nitrogen species. They are called reactive because of their unstable nature and the way they interact with their surrounding molecules. The release of one reactive oxygen species may lead to the production of several other free radicals through chain reaction.[8]

The results in this study indicate that there is increase in oxidative stress and decrease in antioxidant level in CKD patients when compared to controls. When compared to controls chronic kidney disease patients have significantly (p value < 0.001) increased level of MDA. This is in accordance with the study of Kayode Solomon et al.[9], Durak I et al.[10], and Yawata Yet al.[11] MDA is a reactive aldehyde and is a lipid peroxidation product which is produced during peroxidative process of PUFA by reactive free radicals. All of the major classes of biomolecules present in the body can be attacked by reactive oxygen species but lipids among them are the most susceptible to theses reactive oxygen radicals. Cell membranes are abundant sources of polyunsaturated fatty acids which can be readily attacked by these reactive species. The oxidative destruction of polyunsaturated fatty acids by deleterious free radical reactions is known as lipid peroxidation. Lipid peroxidation has been implicated in a wide range of tissue damages and diseases.[4]

The increased serum MDA level in chronic kidney disease patients is associated with increased production of reactive oxygen species. The numerous possible sources of ROS in CKD patients include accumulation of uremic toxins, increased advanced glycation end products which are potent mediators of inflammation leads to activation of macrophages. Reactive oxygen species in CKD patients may also be produced due to increased homocysteine level in these cases and also treatment of anemia with high doses of iron may also induce oxidative stress.[12]

When compared to controls CKD patients have significantly decreased (p value < 0.001) level of GSH. This is in accordance with the study of Francesco et al.[13], Sangeeta salagar et al.[14] Glutathione exists in reduced (GSH) and oxidized (GSSH) states. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent to other unstable molecules, such as reactive oxygen and nitrogen species. In donating electron, glutathione itself becomes reactive, but readily reacts with another reactive glutathione to form glutathione disulfide (GSSG). In normal healthy cells and tissues, more than 90% of the total glutathione pool is present in the reduced form and less than 10% present in disulfide form (GSSG). An increased GSSG to GSH ratio is indicative of oxidative stress in cell1[4].

Glutathione other than antioxidant activity has many physiological functions including detoxification of xenobiotics, regulation of cell proliferation and modulation of redox regulated signal transduction in cells.[13] Reduced whole blood glutathione in chronic kidney disease patients may be due to inhibition of glucose-6-phosphate dehydrogenase activity by accumulated uremic toxins in blood.[14]

## IV. CONCLUSION

In conclusion there is an oxidant and antioxidant imbalance in chronic kidney disease patients when compared to healthy controls and this imbalance play an important role in tissue damage in CKD patients. Hence further studies are required on beneficial effect of consumption of diet rich in antioxidants and their effect on prevention of oxidative damage in chronic kidney disease.

I/we believe the manuscript represents valid work. Neither this manuscript nor one with substantially similar content under my/our authorship has been published or is being considered for publication elsewhere, except as described in the covering letter.

I/we certify that all the data collected during the study is presented in this manuscript and no data from the study has been or will be published separately.

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