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A comparative study of antioxidant status in patients on haemodialysis and healthy control subjects

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Abstract

Chronic renal failure is a progressive loss of renal function over a period of months or years. Chronic Kidney Disease (CKD) encompasses a spectrum of different patho-physiological processes associated with abnormal Kidney functions and a progressive decline in glomerular filtration rate (GFR). This study aims to estimate the levels of serum Malondialdehyde (MDA), Urea and Albumin in healthy control subjects and in patients with chronic renal disease undergoing haemodialysis. This was a prospective hospital based, case-control study conducted on 100 individuals aged between 25-75 years of both sexes, 50 cases were taken from Nephrology Department (Dialysis Unit) as study group and 50 controls were healthy individuals in PBM hospital during 2019. Blood samples of control as well as study group were withdrawn and analyzed. The serum MDA, were estimated by UV-VIS spectrophotometer. The serum Albumin, and Serum Urea were estimated by fully autoanalyser. The results of present study showed that in CKD patients under-going haemodialysis serum MDA was significantly increased (P-value <0.0001). and Serum urea also increased whereas serum albumin level decreased in haemodialysis patients. The results may be significant in understanding the possible contribution of above mentioned parameters in pathophysiological process of CKD, and role of antioxidants in patients on haemodialysis. This study may help us to optimize antioxidant supplementation as adjuvant therapy in treatment of renal disorder patients who are on haemodialysis, which will help in preventing the impending complications caused by progression of disease and subsequent Haemodialysis.

Keywords: chronic renal failure (CRF), hemodialysis (HD), malondialdehyde (MDA)

Introduction

Chronic kidney disease encompasses a spectrum of different pathophysiological processes associated with abnormal kidney function and a progressive decline in GFR. Chronic renal failure is a progressive loss of renal function over a period of months or years. Haemodialysis is the mainstay of treatment for patient with end stage renal disease who are waiting for or who are not suitable to undergo renal transplantation. The CRF patients who undergo dialysis are subjected to an oxidative stress. The Global Burden of Disease (GBD) 2015 study estimated that in 2015, 1.2 million people died from kidney failure, an increase of 32% since 2005 [1]. In 2010, the National kidney and Urologic Disease information Clearinghouse reported that more than 20 million Americans aged 20 and older had CKD. In 2009, the Clearinghouse reported that more than 871,000 people were being treated for ESRD, more than 90,000 of whom died. The survival rates for patients with ESRD at one, five and ten years were 95.5%, 81.4%, and 10.5% respectively [2]. A study conducted in north India reported an approximate prevalence of CKD of about 800 per million populations and the incidence of ESRD of about 150-200 per million populations [3]. In India, Screening and Early evaluation of kidney disease (SEEK), a community based voluntary health screening program which was started in 2006, showed the overall prevalence of CKD in the SEEK-India cohort to be 17.2% with nearly 6% of patients having CKD stage 3-5 [4].

Oxidative Stress in CKD

Oxidative stress arises when there is an imbalance between free radical production and antioxidant defense. As a result, certain biomolecules are oxidized leading to structural and functional modifications of these molecules [5]. This oxidant production process occurs mainly in the mitochondria, with the help of mitochondrial cytochrome oxidase enzymes such as cytochrome P_{450}. The ROS products from these processes likely contribute to the progression of renal injury and the pathogenesis of atherosclerotic diseases in CKD [6].

In uremic patients, the prooxidant state arises from reduced antioxidant system (catalase, glutathione peroxidase, glutathione) activity [7], decreased intracellular vitamin E and vitamin C levels, and decreased levels of high-density lipoproteins, thiols, and apolipoprotein A-I [8]. Increased activity of prooxidants is associated with CKD risk factors such as old age, diabetes, chronic inflammatory state, uremic toxins, and dialysis membranes and solutions [9]. Oxidative stress damage is further compounded in patients requiring dialysis therapy due to the bio-incompatibility of dialysis membranes in HD or solutions in PD [10].

Material and Method

Design of study a Case-control analytic study setting

The present study was carried out in Department of Biochemistry in collaboration with Department of Nephrology, Sardar Patel Medical College and Associated Group of PBM Hospital, Bikaner, Rajasthan. The study plan was approved by the Ethical Committee of the Institute. Valid consent was taken from all the patients before each sample collection.

Study Population
It was a prospective hospital based, case control study, conducted on 100 individuals of similar age group (25years to 75years). 50 CKD patients undergoing dialysis in Nephrology Department (Dialysis Unit) as study group. 50 healthy individuals registered in hospital and known relatives represented as the control group. All 100 subjects were randomly selected irrespective to their caste and creed.

Study Protocol
Following criterias were considered for selection of subjects in the study:

Inclusion Criteria
The diagnosed patients of CKD by nephrologists undergoing dialysis. Patients aged between 25- 75 years of both sexes. Subjects with normal nutritional habits without supplementing with any vitamins during last three months are included in the study.

Exclusion Criteria
The patients with associated Coronary heart disease, liver disease, lung disease. Patients with history of recent infection, malaria, dengue fever. Patients having some autoimmune disease, inflammatory disease. Subjects who are taking vitamin supplementation. Patients who are taking drugs like Methotrexate, Carbamazepine or Phenytoin that could alter the required parameter.

Procedural Steps
Informed consent was obtained from all subjects for participating in the study. 5 ml blood sample was collected by venupuncture by aseptic technique. The serum separated from the samples was analyzed for the following biochemical parameters. Blood sample was allowed to clot at room temperature for 30 minutes and then transferred to a centrifuge tube. The serum was separated by centrifugation at 3000 revolutions per minute (rpm) for 10 minutes. Then serum was transferred to cuvette.
Analytic grade chemical, kits, standard and auto analysar used and following estimations were done:

1. Malondiadehyde was estimated by thiobarbituric Acid (TBA) assay method described by Buege and Aust (1978) [11]
2. The determination of Serum Albumin using Bromocresol Green - Manual method of Doumas *et al*. (1971), modified Spencer and Price(1977) [14]
3. Estimation of serum Urea: The Berthelot reaction has been adapted for use with the autoanailyzer by Wilcox *et al*. (1966) and Wilson (1966) [15].

Statistical Method
Statistical analysis was performed using Microsoft excel. Unpaired‘t’ test with unequal variance was used to test the significance of differences between the groups. A p value less than 0.05 was considered as significant.

Results
Mean MDA concentration was found to be 1.3630 nmol/ml in healthy subjects (control group) and 3.6498nmol/ml in haemo dialysis patient (study group). The increase in MDA level in HD patients was highly significant with P-value <0.0001. Mean urea concentration is compared with healthy control. Mean urea levels in control group were 26.980 mg/dl and in study subjects were 121.75 mg/dl (P-value <0.0001). Mean albumin concentration is compared with healthy control. Mean albumin levels in control group were 3.994 g/dl and in study subjects were 2.764 g/dl (P- value <0.0001).

Table 1: Comparison of Mda, Urea, and Albumin Concentration In Haemodialysis Patients (Study Group) And Healthy Subjects (Control Group)

Blood Parameters	Control Group (n=50)	Haemo dialysis Patients (n=50)	Significance		
	MEAN±SD	MEAN±SD	DF	t-value	p-value
MDA	1.3630±0.4593	3.6498±1.1465	98	13.09	<0.0001**
UREA	26.980±7.190	121.754±43.064	98	15.3492	<0.0001**
ALBMIN	3.994±0.401	2.764±0.430	98	14.7907	<0.0001**

Highly Significant **

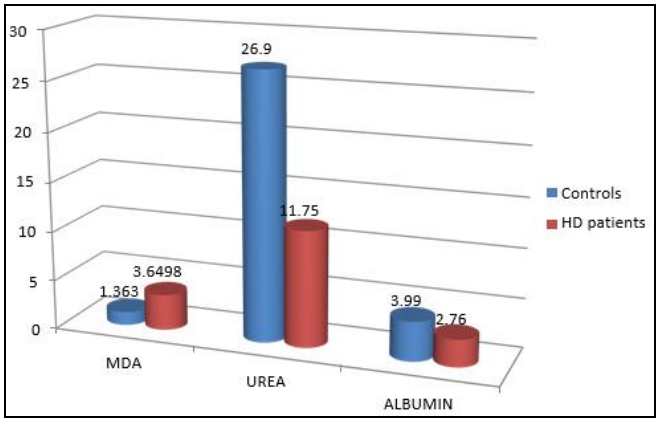

Fig 1: Comparison of Mda, Urea, and Albumin Concentration In Haemodialysis Patients (Study Group) And Healthy Subjects (Control Group)

Discussion
The increase in MDA level in HD patients was highly significant with P-value <0.0001 in our study. MDA is thus a reliable marker for lipid peroxidation and is significantly elevated in HD patients as compared to healthy subjects. These results are similar to various studies and research. Study by Loughrey CN. *et al* [16] in 1994, evaluated MDA levels in dialysis patients and control group, MDA levels were significantly higher in dialysis patients(1.16 ± 0.08 umol/l) than control group(0.66 ± 0.10). Priya R. *et al* [17] in 2009 studied extent of free radical damage on lipid (measured as MDA). The level of MDA was significantly increased in CRF patients as compared to normal subjects. Guo *et al* [18] in 2010, found that patients on HD as compared to controls have significant higher plasma concentration of MDA. Urea levels were found to be higher in patients undergoing HD as compared to healthy subjects. Similar results were observed in study conducted by Kadham. A.J. *et al* [19] in 2008, that serum urea levels were significantly increase in HD subjects as compared to control group (P<0.05). Kadhum A.J *et al* [19] in 2008 conducted study to evaluate both the lipid peroxidation and some antioxidant levels in patients with chronic renal failure. Plasma total protein, albumin were significantly low in patients when compared with control group (P<0.05). There was a significant negative correlation of MDA with albumin (r=- 0.66, P<0.05).
In the present study there is statistically significant increase in serum MDA level might be due to increased oxidative stress during haemodialysis.

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