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Correlation between Oxidative Stress and Electrolytes in Diabetic End Stage Renal Disease.

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ABSTRACT

Long-term uncontrolled diabetes mellitus enhances the production of free radicals. In development of various cardiovascular diseases particularly in diabetic end stage renal disease, dysregulation of sodium, potassium and other ions has an important role. To analyze the impact of oxidative stress parameters on serum electrolytes in diabetic end stage renal disease and to further investigate its probable use as a marker to assess the progression of the disease. This prospective study involvesGroup-1 (control), Group-2 (diabetics without complication) and Group-3 (diabetics with diabetic end stage renal disease) of 50 patients each. Oxidative stresswas measured using erythrocyte glutathione&malondialdehyde and plasma thiols. Serum electrolytes, renal profile and blood glucose levels were measured using standard methods. One-Way Anovaand Correlation test were used to analyze the result. A significant increase ($p<0.001$) in oxidative stress was observed in all the three groups. The study further suggests that there is a significant correlation ($p<0.001$) between serum electrolytes and oxidative stress. Thisstudyprovides confounding evidence that the electrolyte imbalance is as a result of oxidative stress damaging the erythrocytes and reducing the activity of Na^+-K^+ ATPase.

Keywords: End-stage renal disease, GSH, MDA, oxidative stress, Na^+-K^+ ATPase

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INTRODUCTION

Diabetes mellitus is a metabolic disorder with characteristics of hyperglycemia and insufficiency of secretion or action of endogenous insulin. As the disease progresses, patients are at increased risk for the development of specific complications including retinopathy leading to blindness, nephropathy leading to renal failure, neuropathy and atherosclerosis [1,2]. Patients with uncontrolled hyperglycemia may develop complications leading to acute life-threatening state, such as ketoacidosis or hyperosmolar coma.

Diabetic patients are susceptible to a series of complications that cause morbidity and premature mortality. Among these complications, renal disease is a leading cause of death and disability in diabetes [3]. About half of the cases of end stage renal disease (ESRD) in the United States are now due to diabetic nephropathy. Diabetic nephropathy may be functionally silent for long periods (10-15yrs).

Cardiovascular and renal diseases are the major health problems and are the main causes of morbidity and mortality in India. These issues are no longer unban-centric but an ever increasing prevalence among the rural population in India contributes to the burden. Ischemic heart disease, hypertension and chronic cardiac failure are the most common cardiovascular problems amongst commonest cardiac complications in diabetics. In development of various cardiovascular diseases, as indicated in various studies, dysregulation of sodium, potassium and other ions has an important role, especially in diabetics with complications [4].

Recent studies have also suggested that, diabetes mellitus and its complications along with free radicals have a common pathway. The majority of reviews focus on the role of oxidative stress in development of diabetic complications. Overwhelming evidences are available highlighting the role of free radicals and oxidative stress in diabetes and its complications. There is also considerable evidence suggesting that reactive oxygen species (ROS) are implicated in the pathogenesis of ischemic, toxic, and immunological mediated renal injury.

The red cell's Na^+-K^+ ATPase plays a central role in the regulation of intra and extra cellular cationic homeostasis. Alterations of this transport enzyme are thought to be linked to several complications of diabetes mellitus [5]. ROS causes lipid peroxidation of cells and organelle membranes and hence, disruption of structural integrity and capacity for cell transport and energy production, especially in the red blood cell and proximal tubule system. This eventually disrupts the membrane stability which in turn affects the activity of Na^+-K^+ ATPase. Moreover there are possibilities that the free radical production in diabetics can in fact target the enzyme itself.

It has been reported that there is an inverse relationship between serum sodium and potassium levels in diabetic coma. This association may be based on the movement of electrolytes between intra and extra cellular spaces dependent on impaired insulin action [6].

The purpose of this study was to analyze the impact of oxidative stress parameter onserum electrolytes in diabetic end stage renal disease (ESRD) and to further investigate its probable use as a marker to assess the progression of the pathology.

The study aims

- To assess the correlation between electrolytes and oxidative stress in diabetics without complications and diabetic ESRD patients.
- To assess the predictability of oxidative stress parameter for analyzing the electrolyte imbalance in diabetics and diabetic ESRD.

MATERIALS AND METHODS

Study population

The study was conducted in accordance with the regulation of the ethical committee of Sri Devaraj Urs Medical College. An informed consent was obtained from the entire participant in this study.

The control group consisted of 50 normal, healthy individuals between the age group of 30 to 60 years from Kolar district. The second group consists of 50 patients presenting with diabetes without any complications, aged between 30 to 60 years visiting to RL Jalappa Hospital and research center. The third group consisted of 50 clinically proven diabetic ESRD patients posted for hemodialysis at RL Jalappa hospital and research center. The patient's chosen were aged between 30 to 60 years.

Sample collection

This prospective study was conducted at Sri RL Jalappa Hospital and Research Center, Kolar, India, a rural tertiary care center.

The control group (Group 1) consisted of 50 normal, healthy, age and sex matched individuals, the second group (Group 2) consists of 50 patients presenting with diabetes mellitus (diagnosed as per the criteria proposed by American Diabetes Association.[4]) without any complications and no other concurrent disease or illness, aged between 30 to 60 years. The third group (Group 3) consisted of 50 patients, clinically and biochemically diagnosed diabetic ESRD patients as per the literature criteria[4] posted for hemodialysis, with no other concurrent disease or illness & on no other medication apart for the disease under study at RL Jalappa hospital and research center.

Exclusion criteria

Pregnant and lactating women, smokers, alcoholics and obese individuals were excluded from the study groups. Individuals with any history of recent surgery, or any other illness not related to diabetes were also excluded.

Method of sample collection

A detailed personal history was taken from subjects in group 1 and for group 2. a complete case history was taken group 3 and the blood was collected from this group before dialysis to ensure that the process of dialysis dose not interfere with the dialysis as shown in many studies.

- 5ml of venous sample was taken after overnight fast form groups 1 and 2. Form group 3, 5ml of overnight fasting sample, just before hemodialysis.
- Of the 5ml of blood sample, 2.5ml was collected in vacutainers containing EDTA as anticoagulant for analysis of oxidative stress parameters and the remaining 2.5ml in plain vacutainers for analyzing electrolytes, renal profile and glucose levels.

Preparation of sample for analysis

Blood sample from the anticoagulant containing vacutainer was centrifuged at 3000rpm for 10 min, supernatant plasma was aspirated and stored in aliquot tubes to assay thiols groups. The buffy coat was discarded. The packed cells were suspended in equal volume of cold phosphate buffer saline and re-centrifuged. The supernatant was discarded. The washing of packed cells was repeated twice, the packed cells were used for analysis of GSH and MDA [6] and the thiols levels were estimated in plasma.

The blood sample from plain vacutainers was centrifuged at 3000 rpm for 10 mins, the serum was aspirated and was further used for analysis of blood glucose, electrolytes and renal profile. All of these parameters were analyzed by auto analyzer using standard methods adopted in the clinical laboratory.

Estimation of oxidative stress parameters

All chemicals used in the experiments were of analytical reagent grade (AR)

Estimation of Glutathione (GSH) in erythrocytes membrane

The role of GSH, especially in RBC's has been of immense interest in the study of oxidative stress status, the reason being that most of the enzymes for which GSH is an important factor are present in the erythrocytes. The levels of GSH present in the blood are proportional to those present in various other tissue, like hepatic, brain, renal etc., and a high

proportion of these are present in erythrocytes. Hence an estimate of GSH levels in Erythrocytes was considered a good measure of oxidative stress [5].

The major non-protein sulfhydryl groups of RBC's are in the form of reduced GSH. This RBC membrane glutathione is estimated using 5, 5' dithiobis 2-nitrobenzoic acid (DTNB) as a disulfide chromogen that is readily reduced by sulfhydryl compounds to an intensely yellow compound[7]. The absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to GSH concentration.

Estimation of malondialdehyde (MDA) in erythrocytes membrane

Free radical activity is determined indirectly by measuring lipid peroxidation products on the erythrocyte membrane. MDA is formed from the breakdown of polyunsaturated fatty acids serves as an index for determining the extent of peroxidation. MDA reacts with Thiobarbituric acid (TBA) to give a pink chromogen, which can be read at 532 nm[6] and is estimated as TBAR's.

Estimation of serum protein thiols

Total serum protein Thiols, included all the free SH groups containing protein in the serum, it was measured colorimetrically using dithionitrobenzoic acid (DTNB) [7].

Estimation of hemoglobin, electrolytes and renal profile parameters

Sodium, potassium and chloride, creatinine, urea, glucose and hemoglobin were estimatedin serum. All of these parameters were estimated by standard methods used in the clinical laboratory.

All the results were analyzed using SPSS 18.

RESULTS

A one-way ANOVAanalysis (Table 2) done comparing the means of serum electrolytes and oxidative stress parameters with group1, group2 and group 3 showed that they were highly significant with a p value of < 0.005 for all the parameters.

A one-way ANOVA analysis (Table 2) done comparing the means of serum electrolytes and oxidative stress parameters between male and female showed no significance.

A Pearson's bivariate correlate analysis (Table 3) was done using SPSS software which suggeststhe existence of a correlation between Na^+, K^+, Cl^- and erythrocyte GSH. The correlation between erythrocyte GSH and K^+ was highly significant with a p value of <0.005 whereas Na^+ and Cl^- had a p value of < 0.05. It is also observed that there exist a strong correlation between erythrocyte MDA and K^+ having a p value of <0.005 and Na^+ with p value <0.05. Further, the

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results also shows a strong correlation of serum Thiol and K^+ with a p value of <0.005 and Cl^- with a p value of <0.05.

A simple linear regression model shows that among the oxidative stress parameters, erythrocyte MDA and GSH influenced the serum K^+ and Na^+ values. However serum thiols were not significantly involved in influencing the serum electrolytes values.

Table 1: Descriptive Statistics

	Mean	Std. Deviation
Na+	138.25	4.02
K+	4.56	.75
Cl-	104.53	5.100
GSH (mg/gm of Hb)	7.67	3.89
MDA (nmol/gm of Hb)	12.37	4.30
Thiol (micro mol/ lit)	261.91	59.54

Table 2: ANOVA

ANOVA					
		df	Mean Square	F	Sig.
Na^+	Between Groups	2	134.9	9.2	.000
	Within Groups	147	14.6		
	Total	149			
K^+	Between Groups	2	13.3	33.6	.000
	Within Groups	147	.4		
	Total	149			
Cl^-	Between Groups	2	307.5	13.9	.000
	Within Groups	147	22.2		
	Total	149			
GSH (mg/gm of Hb)	Between Groups	2	953.1	400.9	.000
	Within Groups	147	2.4		
	Total	149			
MDA (nmol/gm of Hb)	Between Groups	2	698.3	74.9	.000
	Within Groups	147	9.3		
	Total	149			
Thiol (micro mol/ lit)	Between Groups	2	102231.5	46.4	.000
	Within Groups	147	2202.4		
	Total	149			

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Table 3: Pearson Correlation

		Na^+	Cl^-	K^+	GSH (mg/gm of Hb)	MDA (nmol/gm of Hb)	Thiol (micro mol/ lit)
Na^+	Pearson Correlation	1	0.037	-.197*	.195*	0.058	-0.007
	Sig. (2-tailed)		0.652	0.016	0.017	0.481	0.937
	N	150	150	150	150	150	150
Cl^-	Pearson Correlation	0.037	1	0.014	-.179*	0.116	-.209*
	Sig. (2-tailed)	0.652		0.869	0.028	0.156	0.01
	N	150	150	150	150	150	150
K^+	Pearson Correlation	-.197*	0.014	1	-.461**	.453**	-.371**
	Sig. (2-tailed)	0.016	0.869		0	0	0
	N	150	150	150	150	150	150
GSH (mg/gm of Hb)	Pearson Correlation	.195*	-.179*	-.461**	1	-.585**	.602**
	Sig. (2-tailed)	0.017	0.028	0		0	0
	N	150	150	150	150	150	150
MDA (nmol/gm of Hb)	Pearson Correlation	0.058	0.116	.453**	-.585**	1	-.494**
	Sig. (2-tailed)	0.481	0.156	0	0		0
	N	150	150	150	150	150	150
Thiol (micro mol/ lit)	Pearson Correlation	-0.007	-.209*	-.371**	.602**	-.494**	1
	Sig. (2-tailed)	0.937	0.01	0	0	0	
	N	150	150	150	150	150	150
*. Correlation is significant at the 0.05 level (2-tailed).							
**. Correlation is significant at the 0.01 level (2-tailed).							

DISCUSSION

The increase in intra-erythrocytes sodium and serum potassium levels with decreased intra-erythrocyte potassium and serum magnesium in diabetic subjects is a consequence of decreased Na^+-K^+-ATPase activity [10]. Na^+-K^+-ATPase is a ubiquitous enzyme that ensures that trans-membrane gradients of sodium and potassium concentration are maintained. Alterations of this transport enzyme arethought to be linked to several complications of diabetes mellitus [5]. In humans, this enzyme activity is mainly studied in the erythrocyte membrane because these take the brunt of the effect of free radicals and the advance glycated end product (AGE). Significant decrease has been previously reported in uncontrolled diabetic type 1 patients [11].

In another study disturbances of the membrane lipid organization can also explain the decrease in Na^+-K^+-ATPase activity [12]. A dysfunction of Na^+-K^+-ATPase is implicated in the pathophysiology of diabetic nephropathy.

Hypertension that frequently accompanies diabetes mellitus is characterized by abnormalities of sodium metabolism at all physiologic levels, whole body renal and cellular. The most consistently described abnormalities is an expansion of exchangeable sodium, which seems to be closely associated with increased proximal renal tubular sodium re-absorption and suppression of membrane Na^+-K^+- ATPase activity together with inadequate stimulation of the pump leads to a transitionary sodium cell retention as also observed by Syed M Shahid et al [10].

Diabetics differ markedly in their erythrocyte reactions regarding potassium permeability whereas patients with Diabetic ESRD show an efflux of potassium during investigation as there is a decrease of potassium concentration in plasma in diabetics [13].

The statistical analysis done with our results suggests that damage to the erythrocyte membrane due to oxidative stress in diabetics with and without complications, to a large extent, influenced the changes in the serum electrolytes. It's also implies that the raise in the oxidative stress in the serum did not directly influence the electrolyte value, rather the OS damaging the RBC membrane was responsible for the change in electrolytes among diabetics and diabetic ESRD cases.

Further extrapolating from our results it is conclusive that the main enzyme Na^+-K^+ ATPase regulating the movement of electrolytes between serum and RBC's are affected due to a raise in the OS damaging the erythrocytic membrane.

Our results also reveal that erythrocyte sodium, serum potassium, and magnesium and Na^+-K^+-ATPase activity were more deranged male when compared to female diabetic patients suggesting that male patients are at higher risk of diabetic complication.

CONCLUSION

The results from studypresents a confounding evidence implying that the electrolyte imbalance is as a result of oxidative stress damaging the erythrocytes which in turn reduces the activity of Na^+-K^+ ATPase. It also concurs earlier studies suggesting the raise is oxidative stress as a consequence of prolong elevated hyperglycemic state. And finally the results from this study proposes the importance of analyzing oxidative stress parameter in providing a significant insight of electrolytes imbalance in diabetic ESRD providing an opportunity for early intervention.

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