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Research Article

IN VITRO STUDY TO SEE THE EFFECT OF CREATININE AND URIC ACID ON RED BLOOD CELL MEMBRANE PEROXIDATION

Suchanda Sahu[1]., Aju James Ashok[2] and Joseph John[3]

[1]Departments of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar, Odisha
[2]General Surgery, Pondicherry Institute of Medical Sciences, Kalapet, Pondicherry
[3]Pediatrics, All India Institute of Medical Sciences, Raipur, Chhattisgarh

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ABSTRACT

The red blood cell (RBC) membrane of patients suffering from chronic renal failure (CRF) are exposed to increased activity of free radicals by uremic toxins and hemodialysis which leads to decrease in RBC stability and a lower resistance to hemodialysis. Our study was to see the effect of different concentrations of creatinine and uric acid on RBC membrane obtained from healthy individuals. RBC's from 5 healthy individuals were incubated with different concentrations of uric acid and creatinine and the lipid peroxidation levels in RBC membrane was estimated after 0, 5, 10, 15 and 30 minutes. One-way ANOVA showed that with increase in duration of incubation with uric acid, there was significant rise in lipid peroxidation (P=0.000) when compared within and between groups for 5, 15 and 30 min (P=0.000). There was an inconsistent rise and fall with increase in duration of incubation with creatinine. In our study, we observed that there was a rise in RBC membrane lipid peroxidation with increase in duration of exposure and increase in concentration for uric acid and creatinine. The rise in presence of uric acid was more consistent than for creatinine. Uric acid being one of the early markers of renal function impairment needs to be monitored closely and managed aggressively in order to decrease the level of free radical exposure in chronic renal failure patients

INTRODUCTION

The red blood cell (RBC) membrane of patients suffering from chronic renal failure (CRF) are exposed to increased activity of free radicals. These compounds are generated by uremic toxins and hemodialysis [1, 2].They cause an increase in peroxidation of lipids and proteins in RBC membrane. This in turn leads to decrease in RBC stability and a lower resistance to hemodialysis [1]. There is susceptibility to disintegration which results in release of small quantities of hemoglobin and significant reduction in the life of these cells [3].

There have been many reports on the influence of oxidants, antioxidants, trace elements and enzymes on RBC membrane in CRF but there is a paucity of reports on the effect of individual uremic toxins on RBC. Hence, we did this study to see the effect of different concentrations of creatinine and uric acid on RBC membrane obtained from healthy individuals.

AIMS AND OBJECTIVES

To study the levels of RBC membrane lipid peroxidation in vitro

1. At different concentrations of creatinine and uric acid.
2. And with different durations of incubation.

MATERIALS AND METHODS

Materials required

1. RBC's from 5 healthy individuals after obtaining their written consent.
2. Lipid peroxidation levels in RBC membrane [4]

METHODOLOGY

Fresh RBCs were washed with normal saline and were incubated with creatinine whose concentration varied from 1-5 mg% and uric acid concentrations ranging from 3 to 12 mg%. They were then incubated for 5, 10, 15 and 30 minutes and the

Corresponding author:* **Suchanda Sahu
Departments of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar, Odisha

lipid peroxidation products from RBC membrane were estimated.

Procedure for measurement of RBC membrane lipid peroxidation products (4):

The RBC's were washed and packed cell (PC) was collected.500 µl of PC was incubated with 500 µl of different concentrations of uric acid and creatinine solutions. The duration of incubation was noted, thereafter the tubes were centrifuged at 2000g for 5 minutes. The to 200 µl of PC, 800 µl of phosphate buffer, 25 µl of Butylated hydroxy toluene (BHT) and 500 µl of 30% Trichloro acetic acid (TCA) was added in a test tube. Test tube was vortexed and was kept on ice for 2 hours. This was then centrifuged at 2000 rpm for 15 min.1 ml of supernatant was taken in another test tube to which 75 µl of 0.1M EDTA and 250µl of 1% thiobarbituric acid(TBA) in 0.05N NaOH was added. This was incubated at 100^0 C for 15 min. Cooled to room temperature and absorbance was taken at 532 nm and 600 nm in Systronics AU- 2701 spectrophotometer, India.

1,1,3,3-tetramethoxypropane was used as standard for MDA assay.MDA in nM/ml PC = (Absorbance at 532 nm-absorbance at 600 nm) x180. Molar extinction coefficient of MDA-TBA adduct = 1.56 x 10^5 / cm / M (4). Each sample was run in triplicate.

RESULTS

The general characteristics of the five participants in the study showedbiochemical parameters within normal limits. This ruled out any kidney dysfunction (Table 1).

Table 1 General characters of participants

	Mean ± SD	Range
Age (Years)	19.4 ± 1.67	18 - 22
Serum Urea (mg/ dl)	23.2 ± 6.97	15-32
Serum Creatinine (mg/ dl)	0.66 ± 0.11	0.5-0.8
Serum Uric Acid (mg/ dl)	4.72 ± 0.56	3.9-5.4
Serum Sodium (mEq/ l)	136.2 ± 1.3	135-138
Serum Potassium (mEq/ l)	3.94 ± 0.22	3.6-4.2
Serum Chloride (mEq/ l)	102.6 ± 3.05	99-107
SerumBicarbonate (mEq/ l)	26 ± 4.5	22 - 33

The RBC membrane lipid peroxidation at different concentrations of uric acid standards did not show a rise up to physiological levels (6mg%) but increased significantly there after. One way ANOVA showed that with increase in duration of incubation there was significant rise in lipid peroxidation (P=0.000) when compared within and between groups for 5, 15 and 30 min (P=0.000) (Table 2).

Table 2 Mean RBC Lipid peroxidation with different concentration of Uric acid expressed as mmol/ ml of RBC

Uric acid conc.	5 Min	10 Min	15 Min	30 Min
Blank	50.8 ± 3.9	49.9 ± 4.67	51.5 ± 4.3*	61.9 ± 3.46*
3 mg%	50.7 ± 3.88*	56.5 ± 6.9	54.9 ± 6.69*	68.14 ± 2.07*
6 mg%	41.12 ± 2.00*	52.75 ± 4.7	62.04 ± 1.07*	65 ± 3.12*
9 mg%	54.28 ± 1.21*	60.09 ± 6.27	67.88 ± 3.67*	73.06 ± 3.4*
12 mg%	58.92 ± 1.76*	61.13 ± 13.35	73.78 ± 7.44*	79.18 ± 58*

* is p = 0.000.

The lipid peroxidation of RBC membrane at different concentrations of creatinine and with different incubation time showed a rise at 5(P=0.000) and 30 minutes (P=0.001) and the increase at 10 to 15 minutes were not significant. There was an inconsistent rise and fall with increase in duration of incubation (Table 3).

Table 3 Mean RBC Lipid peroxidation with different concentration of Creatinine expressed as mmol/ ml of RBC

Creatinine	5 Min	10 Min	15 Min	30 Min
Blank	42.12 ± 4.27*	46.8 ± 0	39.2 ± 0.44	49.92 ± 6.97**
0.5 mg%	32.52 ± 6.6*	46.64 ± 0.35	40.56 ± 3.48	47.04 ± 0.54**
1.0 mg%	39.4 ± 3.9*	47.24 ± 0.98	45.21 ± 3.48	56.16 ± 6.5**
2.5 mg%	49.4 ± 6.6*	46.84 ± 0.09	40.56 ± 3.48	57.7 ± 4.27**
5 mg%	49.52 ± 3.29*	46.44 ± 0.8	43.68 ± 4.27	56.16 ± 3.48**
10 mg%	42.12 ± 11.8*	46.56 ± 0.53	40.56 ± 0.5	60.84 ± 3.84**

* is p = 0.000 and ** is p = 0.001

With increase in duration of incubation with uric acid there was a significant rise in RBC membrane lipid peroxidation particularly at 30 min when correlated to 5 min (p=0.001), 10 min (P=0.008) and 15 min (P=0.000) (Table 4).

Table 4 Correlation with increase in duration of incubation with uric acid

		Pearson's Correlation ' r'	P
5 min	10min	0.329	NS
	15min	0.328	NS
	30min	0.632	0.001
10 min	15min	0.333	NS
	30min	0.521	0.008
15 min	30min	0.662	0

NS- not significant

Similar observation was seen with incubation with creatinine (Table 5) but the increase was not consistent with time.

Table 5 Correlation with increase in duration of incubation with creatinine

		Pearson's Correlation ' r'	P
5 min	10min		
	15min	-0.373	NS
	30min	0.08	NS
10 min	15min	–	–
	30min		
15 min	30min	0.173	NS

NS- not significant

DISCUSSION

Patients of chronic renal failure are exposed to many uremic toxins like urea, uric acid, creatinine and to disturbances in pH and electrolytes. These disturbances lead to an increased oxidative stress in such patients. The activities of different antioxidant enzymes and levels of several oxidants and / or lipid peroxidation products in serum and RBCs are usually determined to estimate the level of oxidative damage [5, 6, 7]. In the present study, we found that an increase in concentration of uric acid and creatinine and with an increase in duration of incubation, there was a rise in RBC membrane lipid peroxidation. This was similar to a study conducted in Italy by Lucchi L et al. They reported that as opposed to the controls, RBCs from end stage CRF patients exhibited an increased sensitivity to oxidative stress induced in vitro as demonstrated by a significantly higher level of MDA production at all the incubation times (P<0.05) of 0, 5, 10, 15 and 30 minutes [8].

In chronic renal failure per se or due to different treatment modalities adopted that is hemodialysis (HD), continuous ambulatory peritoneal dialysis (CAPD) or conservative

treatment there is an increase in the free radial activity exposure to erythrocytes [1]. They cause peroxidation of lipids and proteins in RBC thereby decreasing stability and lowering resistance to hemolysis. The intensity of changes is according to the stage of the disease. These processes in turn deepen anemia in CRF and make treatment more difficult [1, 2].

Though physiological levels of uric acid are known to have antioxidant properties [9] and at higher levels it leads to many diseases due to it pro-oxidant actions [10], our study showed that there was increase in lipid peroxidation with increase uric acid levels. This may have been probably due to the oxidant property of uric acid in the intracellular compartment rather than in serum [11].

CONCLUSION

In our study, we observed that there was a rise in RBC membrane lipid peroxidation with increase in duration of exposure and increase in concentration for uric acid and creatinine. The rise in presence of uric acid was more consistent than for creatinine. Uric acid being one of the early markers of renal function impairment needs to be monitored closely and managed aggressively in order to decrease the level of free radical exposure in chronic renal failure patients.

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