OSMOTIC FRAGILITY STATUS OF RED BLOOD CELL IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Abstract

Background: Anaemia is an almost invariable consequence of Chronic Kidney Disease. Among other causes, excessive haemolysis is also seen in advanced renal failure. The aim of this study is to observe the range of osmotic fragility of RBC in patients with chronic kidney disease and correlate the fragile states of RBC with different stages of chronic kidney disease. **Materials and methods:** It is a hospital based cross sectional observational study. 100 patients of diagnosed case of chronic kidney disease admitted in the Department of Nephrology, Chittagong Medical College Hospital were selected as cases and 100 apparently healthy persons, age and sex matched were selected as controls. Osmotic fragility of RBC was determined manually by traditional method in the department of Physiology, Chittagong Medical College, Chittagong. Data were analyzed by different statistical methods. **Results:** In our study, among the case group (100 patients), we found that 5.5% were on CKD stage III, 21% on stage IV, 23.5% on stage V. And 50% (100 apparently healthy subjects) were taken as control. Among the case group RBC osmotic fragility was decreased in 69% and remaining 31% was normal. Here the mean (±SD) of strength of NaCl solution were 0.30 (±0.05) and 0.44(±0.04)% in case and control group respectively where RBCs were partially hemolysed. The mean (±SD) of strength of NaCl Solution were 0.11 (±0.10) and 0.21(±0.09)% in case and control group respectively where RBCs were completely hemolysed. Again the mean (±SD) of strength of NaCl Solution were 0.33 (±0.03), 0.29 (±0.06) and 0.30(±0.05)% in CKD patients with stage III, IV and V respectively where RBCs were partially hemolysed. The mean (±SD) of strength of NaCl soluton were 0.14 (±0.09) and 0.08(±0.10)% and 0.12(±0.10)% in CKD patients with stage III, IV and V respectively where RBCs were completely hemolysed. **Conclusion:** The results of this study revealed that osmotic fragility status of RBC of CKD patients was significantly decreased which were inconsistent with other study.

Key words

Chronic kidney disease; Haemoglobin; RBC; Osmotic fragility; Complete haemolysis; Partial haemolysis; NaCl solution; Mean Osmotic Fragility (MOF).

Introduction

Chronic Kidney Disease (CKD) is one of the major public health problems worldwide. Early diagnosis and proper management have important role in prevention of CKD progression to end stage renal disease[1]. Chronic kidney disease, also known as chronic renal failure is characterized by progressive loss in renal function over a period of months or years[2]. Most patients with chronic kidney diseases eventually become anemic. We should review the management of anemia in these patients as a part of the overall management of the many clinically relevant manifestations of chronic kidney diseases. Factors likely contributing to anemia in chronic kidney diseases include blood loss, shortened red cell life span, vitamin C deficiencies, the "uremic milieu," Erythropoietin (EPO) deficiency, iron deficiency, and inflammation. Unfortunately, we know little about the relative contributions of the different factors and conditions in the early stages of chronic kidney disease[3]. Deficiency of erythropoietin is the primary cause of anemia in chronic renal failure, but it is not the only cause.

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Blood loss and red blood cell destruction also frequently contribute to the anemia in patients with renal failure[4]. Anemia is important because it causes many debilitating symptoms (e.g. tiredness and lethargy, muscle fatigue. intolerance to cold, breathlessness on exertion, poor exercise capacity). It is also a major factor in the high prevalence of cardiovascular disease in CKD patients, and it is reported that cardiovascular diseases account for more than 50% of deaths in these patients[4].

Anemia is the universal complication of chronic kidney disease and reflects problem either in abnormally low production of RBC by the bone marrow or excessive hemolysis or blood loss. Excessive hemolysis is also seen in advanced renal failure, the life span of RBC is shortened to average 64 days instead of average 120 days and there is evidence of increased osmotic fragility of RBC probably due to L-carnitine deficiency that destabilizes erythrocyte membrane and its metabolism[5,6]. Fragility, haemolysis or brittleness of RBC means the tendency of the red blood corpuscles to breakdown or disintegrate resulting in discharge of its contents (haemoglobin) into surrounding fluid. It is a test that measures the resistance to hemolysis of Red Blood Cells (RBC) exposed to hypotonic solutions[7]. RBC are exposed to a series of saline (NaCl) solutions with increasing dilution. The sooner haemolysis occurs, the greater is osmotic fragility of RBC. Degree of haemolysis are measured manually at room temperature. Normal Range : hemolysis start at: 0.45-0.5 % NaCl and hemolysis complete at: 0.3-0.33 % NaCl.

This study has been designed to observe the range of osmotic fragility status of RBC in patients with chronic kidney disease and correlate the fragile states of RBC with different stages of chronic kidney disease.

Materials and methods

The present study was a hospital based, cross-sectional case-control study. The study was conducted in the Department of Nephrology, Chittagong Medical College Hospital, Chittagong in collaboration with the Department of Physiology, Chittagong Medical College, Chittagong between January 2011 and December 2011.

The study population consisted of 100 patients of diagnosed case of CKD admitted in the Department of Nephrology, Chittagong Medical College Hospital, Chittagong during study period were selected as cases by the process of purposive sampling and 100 apparently healthy persons, age and sex matched are selected as controls. According to stages of CKD, case group were subdivided into three groups stage III, IV and V. Inclusion criteria include patients with chronic kidney disease (Serum creatinine level ≥2mg/dL) with age 18 years to 70 years, average nutritional status, taking supplementary drugs for CKD (Calcitriol, Iron, Zinc, Folic Acid, etc) with antihypertensive (Calcium channel blocker or ACE or ARB or diuretics) or oral or injectable hypoglycaemic or both antihypertensive and hypoglycaemic agent providing informed written consent. Patients with acute medical conditions like acute MI, Stroke etc, CKD with other preexisting known diseases like tuberculosis and CKD patients with H/O bleeding disorders eg. haemophilia, purpura, haemolytic anaemia etc, CKD patients with H/O gastrointestinal bleeding, CKD with CLD and malignancy were excluded. Ethical clearance was taken properly from the Ethical Committee of Chittagong Medical College, Chittagong.

From all eligible subjects clinical history had been taken and clinical examination performed. Then, for haematological study, about 5-7 ml of venous blood were collected aseptically in a heparinised test tube. Osmotic fragility of RBC was determined manually by traditional method[8,9].

Data were processed and analyzed by computer based soft ware Statistical Package for Social Sciences (SPSS) for windows version 18. Data were expressed as mean ±SD. Confidence level was fixed at 95% level and 'p' value of 0.05 or less was considered significant.

Results

Table I : Distribution of the study groups (n = 200)

Study Groups		Frequency		Percentage (%)	
CASE					
(CKD Patients)	Stage III	11	100	5.5	50.0
	Stage IV	42		21.0	
	Stage V	47		23.5	
CONTROL			100		50.0
Total			200		100.0

Table II : Statistics of Osmotic Fragility Status of RBC among case groups.

Osmotic Fragility Status of RBC	Frequency	Percentage
Decreased	69	69.0
Normal	31	31.0
Total	100	100.0

Table III : Statistics of haemolysis in %NaCl solution among the study groups (With t-test significance)

	Study Groups	N	Mean	± SD	Median	Range	Sign.*
Partial Haemolysis in % NaCl Soluton	Case	100	0.30	0.05	0.30	0.20 – 0.40	t = 22.352 p = 0.000 HS
	Control	100	0.44	0.04	0.45	0.35 – 0.50	
	TOTAL	200	0.37	0.09	0.35	0.20 – 0.50	
Complete Haemolysis in % NaCl Soluton	Case	100	0.11	0.10	0.20	0.00 – 0.30	p = 0.000 t = 7.024 HS
	Control	100	0.21	0.09	0.20	0.00 – 0.30	
	TOTAL	200	0.16	0.11	0.20	0.00 – 0.30	

* Independent samples t-test. HS = Highly Significant (p < 0.001).

Table IV : Statistics of partial haemolysis according to CKD stages among the cases (n = 100) (With ANOVA test significance)

	CKD Stages	N	Mean	± SD	Median	Range	Anova Sign.
Partial Haemolysis in % NaCl Soluton	Stage III	11	0.33	0.03	0.35	0.30 – 0.35	P = 0.082 Not Significant
	Stage IV	42	0.29	0.06	0.30	0.20 – 0.40	
	Stage V	47	0.30	0.05	0.30	0.20 – 0.35	

Table V : Statistics of complete haemolysis according to CKD stages among the cases (n = 100) (With ANOVA test significance)

	CKD Stages	N	Mean	± SD	Median	Range	Anova Sign.
Complete Haemolysis in % NaCl Soluton	Stage III	11	0.14	0.09	0.20	0.00 – 0.20	p = 0.092 Not Significant
	Stage IV	42	0.08	0.10	0.00	0.00 – 0.30	
	Stage V	47	0.12	0.10	0.20	0.00 – 0.30	

Table I showing among the case group patients we found that 5.5% are on CKD stage III, 21% on stage IV, 23.5% on stage V and 50% apparently healthy subjects are considered as control.

Table II showing among the case group RBC osmotic fragility was decreased in 69% and remaining 31% was normal.

Table III showing mean (±SD) of strength of NaCl soluton were 0.30 (±0.05) and 0.44(±0.04)% in case and control group respectively where RBCs were partially hemolysed. The mean (±SD) of strength of NaCl Soluton were 0.11 (±0.10) and 0.21(±0.09)% in case and control group respectively where RBCs were completely hemolysed.

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Discussion

Red Blood Cell Osmotic Resistance (RBCOR) is defined as resistance to osmotic changes in cell integrity after their exposure to hypotonic saline solution. The present study was undertaken to observe red blood cell osmotic fragility status and to evaluate the RBC membrane stability in CKD patients and to find out the correlation between the different stages of CKD and RBC osmotic fragility status.

Here the mean osmotic fragility status of RBC was significantly ($p<0.005$) decreased in 69% CKD patients and was remain normal in 31% of patients. The mean of strength of NaCl solution for partial haemolysis was 0.3(± 0.05) % and 0.11 (± 10) % for complete hemolysis in CKD patients. On the other hand red blood cells were partially hemolysed at 0.44 (±0.04)% and completely hemolysed at 0.21(± 0.09) % of NaCl solution in control group.

The mean (±SD) of strength of NaCl Soluton were 0.33 (±0.03) 0.29 (±0.06) and 0.30(±0.05)% in CKD patients with stage III, IV and V respectively where RBCs were partially hemolysed.

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In this study, osmotic fragility status of RBC of CKD patients were inconsistent with other studies. Gwo dzi ski K et al demonstrated higher osmotic fragility of erythrocytes in the patients with renal failure as compared to normal subjects[10]. Another study demonstrated that erythrocyte deformability and two other related variables such as membrane fluidity and osmotic fragility improve significantly with nifedipine therapy. It is likely that nifedipine inhibiting cytoplasmic calcium accumulation could restore some red blood cell membrane properties[11]. The effect of nifedipine, a calcium-blocking agent, on osmotic fragility of erythrocytes of uremic and essential hypertensive patients was studied. Median Osmotic Fragility (MOF) was found to be higher in uremic patients (0.457 ± 0.016 gm/L) than in healthy controls (0.421 ± 0.0054 gm/L). Mild essential hypertensives also showed higher MOF (0.434 ± 0.013 gm/L) than normal controls (0.421 ± 0.054 gm/L) but lower than the uremic group. Uremic patients with hypertension had the same MOF as uremic patients without hypertension. Treatment with nifedipine resulted in a reduction of MOF in both the uremic and hypertensive patient groups. These results support the suggestion that the shortened red blood cell survival found in uremic patients is caused by enhanced calcium influx into the red cells as a result of the action of parathyroid hormone. Treatment with calcium channel blockers reduces MOF and may improve the anemia of renal failure[12].

Conclusion

In this study, osmotic fragility status of RBC of CKD patients were inconsistent with other studies. 91% hypertensive patients of CKD were receiving antihypertensive drugs like diuretics, beta blocker, calcium channel blocker, Angiotensin Receptor Blocker (ARB) and Angiotensin Converting Enzyme (ACE) inhibitor. All the patients were receiving supportive drugs for CKD like active vitamin D, vitamin B_{12}, multivitamin, micronutrient specially calcium, iron, zinc and folic acid combination. There were evidence of effects of antihypertensive and nutrient supplement that makes RBC more resistant to haemolysis in hypotonic solution that results in decrease osmotic fragility of RBC. There is another important cause of reduced osmotic fragility of RBC is microcytic hypochromic anemia in CKD which frequently occurs due to iron or other nutritional deficiency or defective iron absorption.

Disclosure

All the authors declared no competing interests.

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