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Effects of haemodialysis on Hemoglobin and red cell indices in chronic kidney disease patients at a tertiary health care institute

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Abstract
Background: Chronic kidney disease (CKD) is a global health problem, with very high cost of care and greater burden particularly in developing countries like India.
Material and Method: This prospective observational study of effects on hemoglobin and red cell indices in CKD before and after hemodialysis conducted on 150 CKD patients in SRM Medical College Hospital & Research Centre.
Results: The most frequent morphologic features were normochromic-normocytic (78.7%), microcytic hypochromic (14.7%), macrocytic (3.3%) and dimorphic (3.3.%). Haemoglobin (Hb) levels and platelets counts are significantly reduced in post dialysis with a mean level of Hb 8.7 when compared to pre dialysis mean value of 7.78g/dL. There was a significant increase in MCV, with decrease in MCH and MCHC in the post dialysis values compared to pre dialysis.
Conclusion: These hematological abnormalities expose CKD patients to higher risk of anemia-related complications. The present investigation might help clinicians to initiate precautions before and after dialysis procedures.

Keywords: Chronic kidney disease, dialysis, Hemoglobin, anemia, indices

Introduction
Chronic kidney disease (CKD) is a global health problem, with a very high cost of care and a great burden particularly in developing countries like India. The Kidney Disease Outcomes Quality Initiative describes 5 stages of CKD, with the Stage 5 being End Stage Renal Disease (ESRD) which is characterized by progressive, irreversible deterioration in the renal function and the body failing to maintain fluid and electrolyte balance resulting in uremia [1]. A high prevalence of CKD in India is contributed by several factors, the most common being diabetes and hypertension [2]. More than 1.1 million patients are estimated to have renal failure worldwide with an annual increase at a rate of 7% [3]. HD is the most common modality of treatment in CKD followed by, transplantation and peritoneal dialysis (PD) is a distant third [4]. Dialysis involves the elimination of urea and other toxic substances from the plasma as well as the correction of electrolyte imbalance [5]. There are over 120,000 patients receiving routine hemodialysis (HD) in India, and the number is increasing by about 232 per million population, a reflection of increasing longevity in general.
In patients with CKD, anemia is a clinically substantial burden and it becomes more prevalent with deteriorating glomerular filtration rate (GFR). Anemia is associated with reduced quality of life and an increased risk of cardiovascular mortality and morbidity [6]. Hematological derangements are common in CKD due to relative deficiency/reduction in erythropoietin (EPO) production which remains as one of the major cause of anemia in CKD patients. Other factors such as increased hemolysis, suppression of bone marrow erythropoiesis, hematuria and gastrointestinal blood loss also contributes to anemia [7]. A normocytic normochromic red cell morphology is common in CKD, whereas a microcytic and hypochromic blood picture suggests either iron deficiency or aluminium intoxication. Macrocytic anemia is usually due to Vitamin B12 and folate deficiency. Both these types of anemia can occur in CKD patients [8].
Therefore the present study has been designed to observe the effects on hemoglobin and red cell indices in chronic kidney disease patients before and after dialysis.

Materials & Methods

This prospective observational study of effects on hemoglobin and red cell indices in chronic kidney disease patients before and after dialysis was conducted in the Department of Pathology in collaboration with the Department of Nephrology, SRM Medical College Hospital & Research Centre between May 2017 to July 2018 and approved by our Institutional Ethical Committee. A total of 150 patients diagnosed as CKD and subjected for hemodialysis were included in this study. An informed consent was obtained from all the subjects. Hematological changes were assessed before and after dialysis, by taking into account the parameters such as Hemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC). Red Blood Cell (RBC) morphology were recorded for all the cases. Patients of all age group and gender diagnosed as CKD in the Department of Nephrology and initiated for renal replacement therapy in the form of hemodialysis were included in this study.

Patients suffering from muscular atrophy**,** malignancy, inherited or acquired blood diseases, hepatitis or other liver diseases, infection, acute or chronic inflammation, connective tissues diseases, dehydration, or recent haemorrhagic episode were excluded from this study.

Clinical history of Age, Gender, Cause of the disease, Duration of hemodialysis and associated co-morbid illness (diabetes, hypertension) were recorded.

The parameters assessed for each case included:

Hb and Red cell indices

Under aseptic precautions, two millilitres of venous blood were obtained by routine phlebotomy procedure from renal failure patients before and after hemodialysis within 2hours. The samples was collected in an Ethylene Diamine Tetra Acetic Acid (EDTA) vacutainer and analysed within 2 hours for Hb, MCV, MCH and MCHC using XT 1800i (automated hematology analyser). A peripheral smear was prepared from the same sample.

Statistical analysis

Statistical analysis was done using SPSS software 17.0. Chi square test was used for the comparison between two proportions. p value <0.05 was considered to be statistically significant.

Results

In present study, out of 150 CKD patients, 104 (69.3%) were males and 46 (30.7%) were females. The total number of cases were compared between males and females and was not statistically not significant (Table 1).

Table 1: Age and gender distribution of CKD patients

Age group	Female (n=46)		Male (n=104)		Chi Square & p value
	N	%	N	%	
21 – 30	3	6.5%	4	3.8%	x^2= 3.382 p= 0.496
31 – 40	7	15.3%	12	11.5%	
41 – 50	11	23.9%	17	16.4%	
51 – 60	14	30.4%	33	31.8%	
>60	11	23.9%	38	36.5%	

a) Hemoglobin

For 150 CKD patients, haemoglobin concentration were compared before and after the hemodialysis session. Hemoglobin ranges from 2.9 – 12.8g% with mean of 8.17g% in pre dialysis and hemoglobin ranges from 3.1–11.6g% with mean of 7.78g% in post dialysis. A significant decrease in the mean hemoglobin was observed in post dialysis when compared with pre dialysis values, with p value <0.05 (Table 2).

Table 2: Hemoglobin in pre and post dialysis values of CKD patients (n=150)

Period	Hemoglobin		Paired T test	p value
	Mean	SEM		
Pre dialysis	8.17	0.16	12.865	0.0001
Post dialysis	7.78	0.15		

Severity of anemia

Among 150 patients undergoing dialysis in this study, patients presented predominantly with moderate degree of anemia, which was found in 79 patients (56.7%) in pre dialysis and in 74 patients (49.3%) of post dialysis (Figure 1). Severe degree of anemia was found in 42 pre dialysis patients (28%) and 56 post dialysis patients (37.4%). The severity of anemia was found to be increased after dialysis session but was not statistically significant (Table 3).

Table 3: Grading of anemia in pre and post dialysis values of CKD patients

Grading of anemia	Pre dialysis		Post dialysis		Chi Square & p value
	Cases	%	Cases	%	
Mild anemia	12	8%	3	2%	x^2=6.73 p=0.035
Moderate anemia	75	50%	71	47.3%	
Severe anemia	63	42%	76	50.7%	

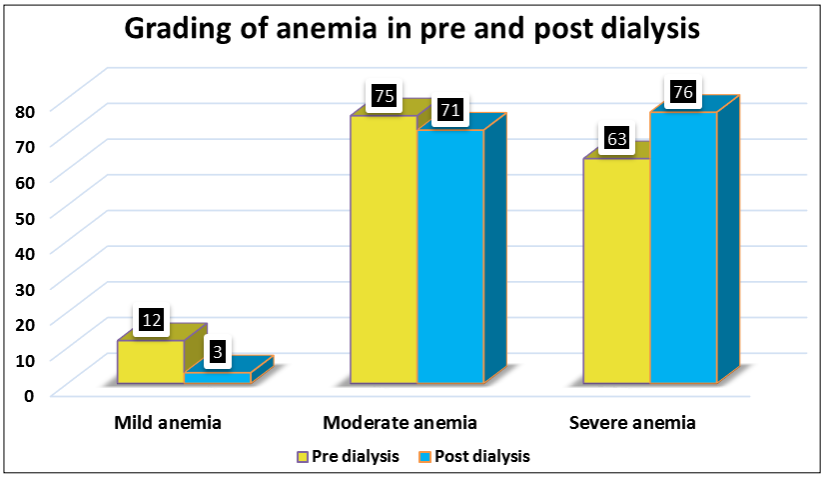

Fig 1: Grading of anemia in pre and post dialysis values of CKD patients

b) RBC Morphology

The most frequent morphologic features in both pre and post dialysis were normochromic-normocytic (78.7%), followed by microcytic hypochromic (14.7%), macrocytic (3.3%) and dimorphic (3.3%) (Figure 2). The other associated findings included varying degree of Anisopoikilocytosis such as tear drop cells, fragmented cells, pencil cells and macro-ovalocytes. RBC morphology was not altered after dialysis in this study.

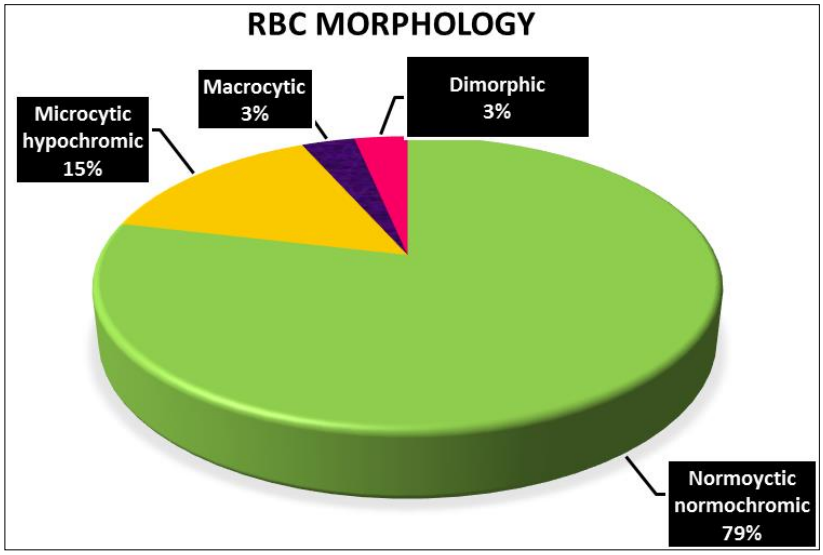

Fig 2: RBC morphology in patients with CKD

c) Red Cell Indices

Pre dialysis values of red cell indices such as MCV, MCH and MCHC of 150 CKD patients were compared with post dialysis values. A statistically significant increase in MCV with a mean value of 84.95 in pre dialysis to a mean value of 85.61 in post dialysis was observed. A mild reduction in MCH with mean value of 27.79pg in pre dialysis to 27.17pg in post dialysis was noted which was statistically significant. There was slight decrease in MCHC with mean from 32.59g/dl in pre dialysis to 32.05g/dl in post dialysis which was statistically significant (Table 4).

Table 4: RBC indices in pre and post dialysis values of CKD patients

RBC Indices	Pre dialysis	Post dialysis	Paired T test	p value
MCV	84.95 ± 0.63	85.61 ± 0.63	-13.688	0.0001
MCH	27.79 ± 0.22	27.17 ± 0.22	14.169	0.0001
MCHC	32.59 ± 0.16	32.05 ± 0.16	12.858	0.0001

Discussion

The prevalence of anaemia in patients with CKD has been widely studied. As renal function declines, the frequency of anaemia increases [9]. In this present study, hemoglobin values of 150 CKD patients were compared before and after dialysis. Hemoglobin value ranges from 2.9 – 12.8g/dl with a mean 8.17g/dl in pre dialysis and ranges from 3.1 – 11.6g/dl with a mean 7.78g/dl in post dialysis. There was a significant decrease in the mean haemoglobin in post dialysis. The cause of decrease in the hemoglobin concentration in chronic kidney disease is mainly due to impaired erythropoietin production and other factors which

suppress marrow erythropoiesis and shortened red cell survival[1]. Thus, anemia is a common complication encountered in both pre dialytic and post dialytic group. The mean GFR in our study was 7.3ml/min/m^2. Lower the EPO or GFR production, greater the loss of hematopoietic nutrient elements and inflammation due to dialytic membrane which then causes a lower mean hemoglobin in hemodialysis patients. RBC Indices are also affected after dialysis. In our study, there was an increase in MCV after dialysis with a mean value of 85.61fl compared to mean 84.95fl in pre dialysis. A slight reduction in MCH was noted with mean value of 27.17pg after dialysis when compared to pre dialysis mean value which was 27.79pg. There was slight decrease in MCHC in post dialysis with mean 32.05g/dl compared to pre dialysis mean value of 32.59g/dl. Haemodialysis influences the transport of water through the erythrocytic membrane and induces morphologic and functional modifications [5].

Similar findings were reported by *Pandian et al.* [8] showed a significant decrease in mean hemoglobin from 8.38g/dl to 8.02g/dl in pre and post dialysis respectively. MCV ranges from 66 to 97fl in pre-dialysis patients and ranges from 66 to 96fl in post dialysis patients. There was no significant difference in mean MCV of pre-dialysis patients in comparison with post dialysis patients. There was significant reduction in mean MCH from 28.02pg in pre-dialysis to 27.81pg in post dialysis patients. There was also a slight decrease in mean MCHC from 32.47 g/dl in pre dialysis patients to 32.27 g/dl in post dialysis patients. *Gautam et al.* [10] found that the mean haemoglobin in pre dialysis was 7.69 ± 1.95 whereas in post dialysis patients, the mean value was 8.46 ± 2.87 with a significant increase. Mean value of MCV showed only a mild increase post dialysis. Mean value of MCH in pre dialysis was 27.01 ± 2.82 and increased following dialysis to 27.67 ± 2.51. Mean value of MCHC in pre dialysis was 30.48 ± 2.77 whereas after dialysis it got increased to 31.09 ± 2.33 (Table 5).

Alghythan et al. [11] in their study observed that the hemoglobin concentration showed a statistically significant decrease in pre-HD patients (11.70 ± 1.29) as compared to the post dialysis group (13.16±1.57). *Chowdhury et al.* [5] observed in their study, that the mean MCV(fl) was decreased from 96.20(±11.57)fl to 92.80(±10.75)fl in pre and post dialysis respectively. In pre-dialysis and post-dialysis sample the mean MCH(pg) was 29.10(±3.62)pg and decreased to 28.79pg respectively. In pre-dialysis and post-dialysis sample the mean MCHC(g/dL) was 29.25g/dL and increased to 30.25gm/dL respectively.

Table 5: Hemoglobin and RBC Indices in post dialysis compared to pre dialysis with previous studies

Study	Year	Cases	Hb	MCV	MCH	MCHC
Alghythan *et al*. [5, 9]	2012	100	Increased	Increased	Increased	Increased
Chowdhury *et al*. [6]	2017	40	-	Decreased	Decreased	Increased
Gautam *et al*. [12]	2018	80	Increased	Increased	Increased	Increased
Pandian *et al*. [9]	2017	120	Decreased	No change	Decreased	Decreased
Present study	2018	150	Decreased	Increased	Decreased	Decreased

The severity of anemia was also compared among pre and post dialysis values of hemoglobin. Grading of anemia was done according to WHO classification. Moderate anemia was predominant 50% followed by severe anemia noted in 42% among pre dialytic patients. The severity of anemia increased following dialysis which showed 50.7% with severe anemia and 47.3% with moderate anemia. Mild anemia was present only in 8% and 2% in pre and post dialytic patients respectively. These results were compared with the study done on 100 CKD patients by *Afshar et al.*, where the severity of anemia among pre dialysis group was mild in 45% and moderate in 55% whereas in post dialysis, mild was 5%, moderate in 70% and severe anemia in 25% [12].

Considering the RBC morphology, our study showed highest number of cases with normocytic normochromic picture (78.7%) followed by microcytic hypochromic (14.7%), macrocytic (3.3%) and dimorphic (3.3%). The normocytic normochromic anemia is due to the fact that CKD is a state of uremic environment which causes a shortened life span of RBCs. Iron deficiency and folic acid deficiency causes microcytic and macrocytic anemia respectively. Aluminum in the dialysis water could interfere with iron incorporation in erythroid cells and cause microcytic anemia, occasionally osteomalacia and encephalopathy. Folic acid should be repeated in patients who are undergoing intensive dialysis because folic acid is dialyzable and may be lost in the dialysis bath. *Chakravarti et al*. conducted a study on 114 patients who had been undergoing hemodialysis for ESRD and found that 77.19% had normocytic normochromic anemia followed by 14.9% diagnosed as dimorphic anemia, 6.14% with microcytic hypochromic and 1.75% with macrocytic anemia [13].

Limitations of the study

Hemodialysis patients are more anemic compared to pre dialysis patients. Further evaluation of iron and water soluble vitamins in CKD patients might help in establishing the true cause of anemia. However this can be included in further study.

Conclusion

The results of this study indicate that Hb and Red cell indices in pre or post HD were either elevated or lowered. The most frequent finding is the moderate degree of anemia in both pre and post dialysis patients, and also, there is increase in severity of anemia after hemodialysis in many patients. However, in both pre and post dialysis, the anemia is normocytic normochromic. These findings expose CKD patients to higher risk of anemia related complications, which may have a role in increasing the rate of patient mortality and morbidity.

In the light of this study, there is a need for nephrologist to monitor the hematological profile of CKD patients on dialysis, and treat any derangements in the same, so as to improve outcome for these patients.

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