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Evaluation of Biochemical and Hematological Parameters in Patients with Chronic Renal Failure from Coastal Maharashtra, India.

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Abstract:
Background*: Chronic Renal Failure (CRF) is a global public health problem, where slow progressive deterioration in kidney function leads to numerous hematological and biochemical dysfunction. These patients become vulnerable to cardiovascular morbidity and mortality if appropriate measures are not taken for their control. The aim of present study was to find out changes, in the common hematological function and levels of some biochemical constituents that may occur in patients of chronic renal failure (CRF); undergoing dialysis; and suggest appropriate measures for their management.*
Materials and Methods:*One hundred and Fifty patients with CRF and on regular maintenance dialysis and 150 healthy adults were recruited for the study. Haemoglobin concentration, total red cell count, total white blood cell count and platelet count along with Biochemical parameters including Serum Creatinine, Serum Total Proteins, Serum Albumin, Serum Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, SGOT, SGPT and Serum Alkaline Phosphatasewere analysed in patients and controls.*
Results: *Results were analysed using SPSS 22.0 version. RBC count, haemoglobin levels, PCV and platelets counts were significantly reduced in the patients of chronic renal failure and the process of haemodialysis further decreased the level of all the above mentioned haematological parameters, with the exception of platelet levels. There was no significant change observed in total leucocyte count , neutrophils, eosinophils. monocytes and lymphocytes . Increase in serum Creatinine and fall in serum Sodium levels, total proteins and albumin was observed in CRF patients prior to dialysis, and further, no significant improvement in the altered levels of these parameters was evident after dialysis.*
Conclusion: *Chronic renal failure is associated with increased serum creatinine, reduced haemoglobin concentration and significant fall in levels of total protein and albumin. This requires proper evaluation for its usefulness in effective management of CRF patients.*
Key Words: *Chronic Renal Failure, Biochemical Parameters, Hematological Parameters, Hemodialysis*

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I. Introduction

Diseases of the kidneys are amongst the most important causes of morbidity and mortality in many countries worldwide. Renal failure is a systemic disease and characterized by a slow and progressive decline of kidney function brought about by various factors including infections, auto immune diseases, diabetes and other endocrine disorders, cancer, and toxic chemicals.[1-3]Chronic kidney disease is a real and growing problem with significant healthcare burden and very high cost of care, especially in developing countries like India.[2]

According to the National Kidney Foundation, kidney disease ranks third amongst life threatening illnesses afflicting Indians, after cancer and heart disease. Over200,000 persons have terminal kidney failure every year. Millions more suffer from lesser forms of kidney disease including CRF.[4]The incidence rate of CRF is 1out of every 5000 population and it affects mostly middle-aged and older people.[5]Patients of CRF require renal Hemodialysis, which works on the principle of diffusion of solutes through a semi permeable membrane[1]. This involves the extracorporeal removal of waste products such as creatinine, urea, and free water from the blood, when the kidneys are impaired.

Kidney diseases are associated with changes in various biochemical and hematological parameters.[2] Anemia is the most common, consistent, and severe of the various hematological abnormalities. In addition to anemia, patients with chronic renal failure are prone to develop infections and hemorrhagic diathesis. Abnormal haemostasias in chronic renal failure is characterized by a tendency to abnormal bleeding and bruising. Decreased Factor III activity, abnormal platelet aggregation and adhesiveness; and impaired prothrombin consumption, contribute to the clotting defect in uremia. Although hemostatic defects in uremia are often

complex, it is probable that platelet dysfunction is the most consistent and clinically the most important feature.[6]In the present study, biochemical and hematological parameters, were evaluated in patients with chronic renal failure to find out the usefulness in management of these patients.

II. Material And Methods

This comparative cross-sectional observational study was carried out in the Department of Biochemistry, B.K.L. Walawalkar Rural Medical College, Sawarde, Dist. – Ratnagiri, Maharashtra State, India. One Hundred and Fifty known Chronic Renal Failure cases in the age group of 20 to 80 years of both genders attending Dialysis Unit were recruited for the study.

Study Design: Comparative cross-sectional observational study.

Study Location: This is a rural 500-bed capacity teaching hospital-based study done in Department of Biochemistry, at B.K.L. Walawalkar Rural Medical College, Sawarde, Dist.– Ratnagiri, Maharashtra State, India.

Study Duration: January 2016 to November 2017.

Sample size: 150 patients.

Sample size calculation: The sample size was estimated based on a single proportion design. We assumed that the confidence interval of 10% and confidence level of 95%.

Subjects & selection method: The study population was drawn from consecutive Chronic Renal Failure patients who presented to B.K.L. Walawalkar Rural Medical College, and Hospital Sawarde., from January 2016 to November 2017.One Fifty apparently healthy age and sex matched adults serving as control group, were also recruited in the study

Inclusion criteria:

Patients with only conformed diagnosis of CRF by the attending nephrologist in the Outpatient Department were included in the study. The diagnosis was confirmed after standard clinical and laboratory investigations.

Exclusion criteria:

Patients with other unrelated comorbid conditions like anemia, diabetes, hypertension, infection, inflammation, dehydration or recent hemorrhagic episodes and any other metabolic disease were excluded from the study.

Procedure methodology

After written informed consent was obtained, five ml of venous blood sample was collected into Ethylene Diamine Tetra Acetic Acid (EDTA) and plain tubes once from each control subject and twice from every patient before and after dialysis for hematological and biochemical analysis respectively.

Hematological parameters like Red Blood Cell count (RBC), Haemoglobin concentration (Hb%), Packed Cell Volume (PCV), Platelet count and Total Leucocyte Count (TLC) were estimated by using Sysmex TransAsia automatic hematology analyzer. Biochemical parameters including Serum Creatinine, Serum Total Protein~~s~~**,** Serum Albumin, Serum Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, SGOT, SGPT and Serum Alkaline Phosphatase were estimated using TransAsia Erba EM-200 fully automated random access chemistry analyzer. Serum Electrolytes (Sodium, Potassium and Chloride) levels were analyzed using Medica Easylyte Plus Na/K/Cl analyzer driven by Ion-Selective Electrode (ISE) method. Statistical analysis was accomplished using SPSS version 22.0 statistical software and findings were compared between normal healthy controls and patients using Student's t-test.

Statistical analysis

Data was analyzed using SPSS version 22.0 statistical software and findings were compared between normal healthy controls and patients using Student's t-test.

III. Result

Age wise and gender wise distribution is presented in Table No 1. The gender wise distribution of CRF patients was skewed predominantly towards males (42 Nos., 84%) as compared to females (8 Nos.,6%). The gender wise distribution of the control group was skewed predominantly towards the males (39 Nos.,78%), while 11 Nos, 22%were females. When segregated by Age, the patient group and control group comprised maximum no. of cases in the 4th decade of life and older.

Table no 1: Age wise and Gender wise distribution of Patients and Control.

	Control			CRF Patients		
Age (Years)	**Male**	**Female**	**No. of Patients (%)**	**Male**	**Female**	**No. of Patients (%)**
20-30	1	0	1 (2)	1	0	2 (4)
30-40	10	1	16 (32)	2	2	4 (8)
40-50	15	4	12 (24)	17	2	18 (36)
50-60	9	4	12 (24)	9	1	10 (20)
60-70	5	2	7 (14)	9	2	11 (22)
70-80	2	0	2 (4)	4	1	5 (10)
Total	39	11	50 (100)	42	8	50 (100)

Results of haematological parameters are presented in Table No 2 and Fig. No 1.In the group of CRF Predialysis patients, the mean values of Haemoglobin, Red Blood Cell count (RBC),Packed Cell Volume(PCV)and Mean Corpuscular Haemoglobin concentration(MCHC) were 9.86± 2.11**(**gm/dl), 3.49 ± 0.85(million/mm^3), 29.90± 6.46 (%) and33.90±4.06(gm/dL**)** respectively. These parameters were significantly reduced ($p<0.001$) as compared to control group. In the group of Post dialysis CRF patients the levels of Hb(gm/dl), RBC count (million/mm^3), PCV (%), MCHC (gm/dl) were also significantly ($p <0.001$) reduced when compared to control group. Except for MCH, MCV and MCHS all hematocrit values were significantly decreased in post hemodialysis group as compared to prehemodialysis group. ($p<0.05$). Changes observed in WBC, Neutrophils, Lymphocytes, Eosinophils, Monocytes were nonsignificant. Platelet levels significantly declined in group I and group II patients as compared to control. However, in post hemodialysis patients the platelet levels increased significantly as compared to prehemodialysis group.

Table No.2: HematologicalParameters as Mean ± SD in Patients and Control.

Hematological Parameters	**Control (n=150) Group I**	**Chronic Renal Failure Patients (n=150)**		**P Value**		
		Pre-Hemodialysis Group II	**Post-Hemodialysis Group III**	**II v/s I**	**III v/s I**	**III v/s II**
Hemoglobin (gm/dl)	14.04± 1.19	9.86± 2.11	8.30± 1.85	0.00007	0.0049	0.2722
RBC (million/mm^3)	4.84± 0.47	3.49± 0.85	2.90± 0.70	0.00018	0.00023	0.0650
PCV (%)	40.72± 7.22	29.90± 6.46	24.77± 6.59	0.00076	0.0001	0.00076
MCV (fL)	81.09± 10.92	86.60± 6.42	84.71± 19.27	0.00319	0.30454	0.3234
MCH (pg)	27.91± 4.57	28.42± 2.13	29.01± 2.21	0.8754	0.6546	0.6700
MCHC(g/dL)	44.45± 5.39	33.90± 4.06	34.76± 13.74	0.002706	0.03275	0.8943
WBC (K/µL)	9238 ± 3152	10053 ±7217	8190 ± 4159	0.22673	0.12864	0.05134
Neutrophils (%)	66.22± 13.99	70.44±14.77	73.20± 12.27	0.98444	0.35114	0.20242
Lymphocytes (%)	25.26± 13.30	20.38±12.84	18.74± 10.19	0.4328	0.54837	0.6404
Eosinophils (%)	5.22± 2.08	5.1± 2.47	4.98± 3.36	0.6273	0.1359	0.3302
Monocytes (%)	3.56± 1.37	4.0± 1.83	3.51± 1.77	0.4116	0.8627	0.8272
Platelets (Lakhs/mm^3)	3.18± 1.11	0.22± 0.99	2.8± 0.44	0.000014	0.00032	0.01293

Results of Biochemical parameters are presented in Table No 3 and Fig. No. 2. Serum Creatinine levels in CRF predialysis patients and CRF post dialysis patients were significantly increased ($p<0.001$) as compared to controls. In both the patient groups Creatinine Clearance and Glomerular Filtration rate values were significantly decreased ($p<0.001$) as compared to control group. A significant fall ($p < 0.01$) in Serum Total Protein, Serum Albumin and Sodium levels was observed in both the patient groups when compared with controls. Changes observed in the levels of Serum Globulin, A:G ratio, Serum Total Bilirubin, Serum Direct Bilirubin, SGOT, SGPT, Serum Alkaline Phosphatase, Potassium and chlorides were marginal and nonsignificant**.**

Table No.3: Biochemical Parameters as Mean ± SD in Patients and Control

Biochemical Parameters	Control (n=150)	Chronic Renal Failure Patients (n=150)		P Value		
	Group I	Pre- Hemodialysis Group II	Post- Hemodialysis Group III	II v/s I	III v/s I	III v/s II
Serum Creatinine (mg/dl)	0.71 ± 0.28	9.97±4.0	5.10±2.18	0.0001	0.0001	0.0001
Creatinine Clearance (ml/min)	93.02 ± 11.4	7.18±2.65	15.40±4.80	0.0001	0.0001	0.0001
GlomerularFiltrationRate(ml/min/ 1.73m^2)	120.08 ± 3.5	6.71±2.69	16.69±6.70	0.0002	0.0001	0.0032
Serum Total Proteins (gm/dl)	7.038 ± 2.5	5.90±1.1	5.5 ± 1.41	0.0107	0.41036	0.05432
Serum Albumin (gm/dl)	4.03 ± 0.76	3.47±0.63	3.38 ± 0.73	0.0627	0.02883	0.0458
Serum Globulin (gm/dl)	2.74 ± 0.67	2.43±0.69	2.33 ± 0.51	0.6723	0.44861	0.7923
A/G ratio	1.63 ± 0.59	1.50±0.52	1.49 ± 0.42	0.8167	0.78203	0.83061
Serum Total Bilirubin (mg/dl)	0.64 ± 0.33	0.68±1.55	0.66 ± 1.85	0.5438	0.23648	0.4906
Serum Direct Bilirubin (mg/dl)	0.30 ± 0.14	0.43±1.02	0.42 ± 2.30	0.7390	0.78342	0.5692
Serum Indirect Bilirubin (mg/dl)	0.34 ± 0.20	0.25±0.5	0.23 ± 10.6	0.2064	0.541	0.489
SGOT (U/I)	27.90 ± 8.57	26.43±27.82	26.47 ± 67.12	0.669	0.8792	0.727
SGPT (U/I)	25.62 ± 14.18	20.88±14.81	21.18 ± 16.66	0.6426	0.8254	0.3734
Alkaline Phosphatase (IU/L	74.99 ± 16.98	71.75±26.50	71.51 ± 3.60	0.5384	0.3285	0.6582
Serum Sodium (mmol/L**)**	138.74 ± 5.67	130.91±20.07	135.6 ± 2.03	0.0123	0.0195	0.296
Serum Potassium (mmol/L**)**	4.23 ± 0.67	4.20±1.1	4.21 ± 1.31	0.7905	0.5742	0.6306
Serum Chlorides (mmol/L**)**	100.68 ± 4.79	100.70±2.13	100.72 ± 14.15	0.397	0.6901	0.2683

Fig.1

Mean value of Hemoglobin, RBC, MCV and Platelets in Pre-Hemodialysis, Post Hemodialysis and Control.

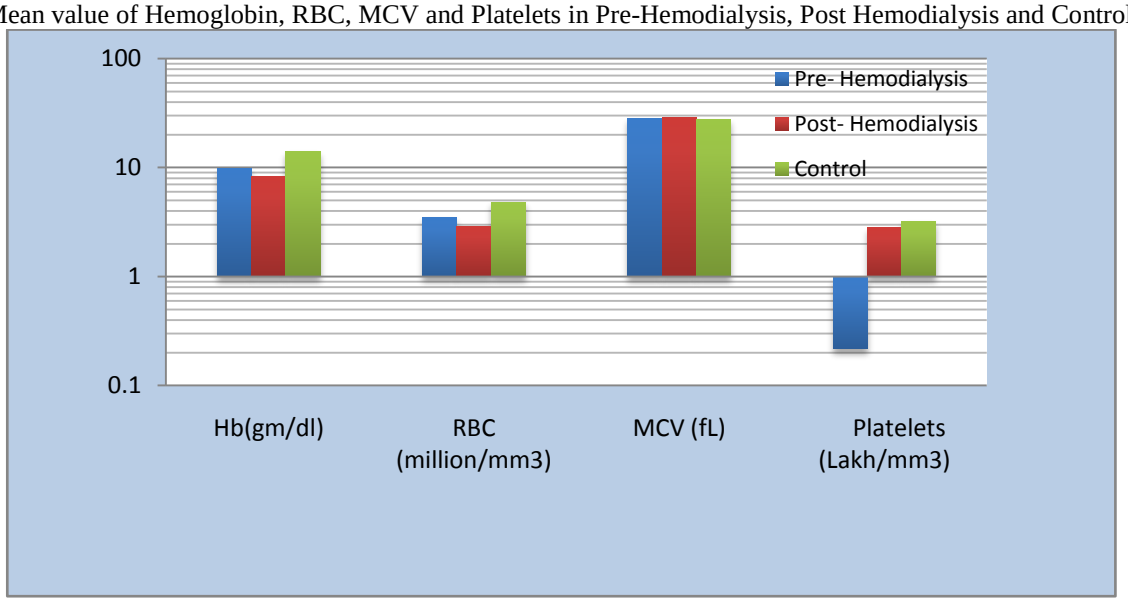
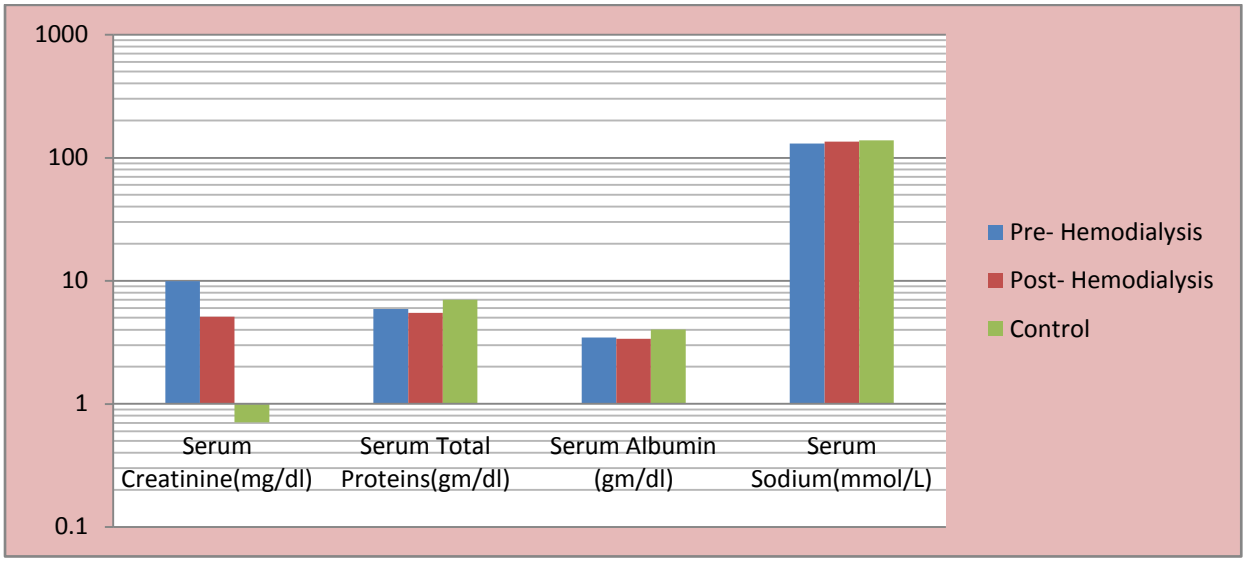


Fig.2
Mean value of Creatinine, Total Protein, Albumin and Sodium in Pre-Hemodialysis, Post Hemodialysis and Control.



IV. Discussion

The present study is a comparative cross-sectional study conducted in the Department of Biochemistry, B.K.L. Walawalkar Rural Medical College, Sawarde, Dist. Ratnagiri, Maharashtra State, India. One Hundred and Fifty Chronic Renal Failure (CRF) patients undergoing dialysis were recruited for the study. These patients (n=150) were divided into two groups: viz. predialysis and post dialysis patients in the ages ranging from 20years to 80 years for both genders. Only those patients were recruited who routinely attended the dialysis Unit of the hospital. One Hundred and Fifty healthy, age and sex matched adults were also recruited into the study as controls. In this study most of the patients with CRF were found in the age groups of 40-50 years,50-60 years,60-70 years, comprising 18(36%),10(20%),11(22%) respectively.

A significant ($p< 0.0001$) decrease in Hb%, PCV & RBC levels (Table No. 2) as compared to the control is observed. Similar decreasing trend for these parameters has also been reported.[9]The essential cause of decreased RBC count and consequent decrease in the Hb concentration and packed cell volume in chronic renal failure can be attributed to impaired erythropoietin production and other factors which suppress marrow erythropoiesis and shortened red cell survival.[2]The hemoglobin concentration and hematocrit generally provide an accurate reflection of the extent to which the circulating red cell mass is reduced. In chronic renal disease, impaired erythropoietin secretion and increased destruction of red blood cells, leads to a fall in red blood cell count, which reduces the hemoglobin concentration and hematocrit. A decrease in hematocrit is apparent even among patients with mild to moderate renal insufficiency.[11]An inverse relationship normally exists between serum/plasma erythropoietin levels and hemoglobin (Hb) concentration. As the hemoglobin and hematocrit decreases the erythropoietin level rises.

RBC survival is decreased in uremic patients in proportion to the blood urea nitrogen concentration, and it improves significantly after intensive hemodialysis.[2]Most studies in literature state that uremic patients are almost invariably anemic.[6] Anemia of the chronic renal failure is multifactorial. The pathogenesis of this type of anemia has been attributed to decreased plasma erythropoietin due to renal damage, inhibitors of erythropoiesis in uremic plasma and decreased hemoglobin oxygen affinity.[6]

In the present study platelet count was notably reduced in CRF patients(predialysis), as compared to control group ($P< 0.0001$). This is similar to study reported by Suresh M et al,[9] Yassein, R.B et al.[13], Alghythan, AK et.al[14],Dorgalaleh A et.al[15].Platelet count increased in post dialysis group as compared to predialysis patients. Thrombocytopenia is a common side effect of hemodialysis. The membrane of dialyzer is responsible for platelet adhesion, aggregation, and activation hemodialysis resulting into platelet activation which has been manifested by raised levels of platelet, factor 4 and thromboxane. In CRF patients, there is diminished production of erythropoietin resulting into reduction in total platelet count. Erythropoietin potentiates the effect of megakaryocyte colony stimulating factors, acetyl hydrolase (PAF-21 AH) and paraoxonase (PON1). Some studies have also concluded that occurrence of thrombocytopenia following hemodialysis were interconnected with complement activation, precisely C3a, along with the platelet activation.[12]

It is well known that hematological parameters are reduced in CRF. The most affected ones are erythrocyte indices. This is because majority of erythropoietin is synthesized in the juxta glomerular apparatus except 10% in liver and other organs. Apart from decreased erythropoietin, changes in red blood cells (RBCs) indices may be caused by vitamin B_{12}, iron and folic acid deficiencies, which are consequences of dietary insufficiency or blood loss or by decreased erythrocytes life span. Other causes of anemia in CKF may include gastrointestinal bleeding; severe hyperparathyroidism and systemic inflammation.[2]

We noticed that in the present analysis pre-dialysis group showed an increase in the levels of the creatinine which were statistically significant (p-value < 0.001). Our result is similar to Noor Ul Amin et al.[16], Islam MN et al.[17]Dialysis has positive impact on serum creatinine level resulting in reduced level towards normal value. Results showed that most of the patients had serum creatinine below 7 mg/dl after dialysis (Table no. 4). The increased serum creatinine level is due to the fall in the GFR in CKD patients. As the GFR falls, plasma levels of creatinine rise as they are eliminated by glomerular filtration and tubular secretion. Serum creatinine levels in the post-dialysis group showed a significant fall in comparison with the pre-dialysis group.[18]

In our study, levels of serum total protein and albumin were significantly decreased in predialysis patients as compared to controls. Nevertheless, a significant fall in these parameters were noticedin post-dialysis patients as compared to the pre-dialysis patients. This may be attributed to either changes in the structure of basement membrane of glomeruli which consequently leads to the leakage of albumin and some low molecular weight proteins or restriction protein intake protein malnutrition [19]and inflammation. Hypoalbuminemia due to decreased rate of its synthesis is the powerful predictor of mortality of chronic kidney disease patients. Significant decrease in total protein and albumin level in CKD patients compared to the control group has been documented[20].

Serum sodium levels significantly declined (P<0.01) in patients with renal failure.The change in serum sodium can be attributed to lowering of renin secretion from the kidney that is important in sodium control. Due to moderate to severe failure in kidney function[21]there is no significant change in serum potassium levels in both before and after hemodialysis groups.

V. Conclusion

In the present study it can be concluded that patients with chronic renal failure with continuous dialysis show abnormal hematological and biochemical parameters. Impaired erythropoietin secretion leads to destruction of red blood cells and thereby a fall in red blood cell count, and reduced hemoglobin concentration. Reduction in the levels of total protein and albumin level in CKD patients, is probably due to malnutrition and/or inflammation. These findings can assist clinicians in identifying and determining the course of the disease and providing direction for appropriate management of patients.

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