“Impact of Hemodialysis Frequency and Duration on Changes in Haematological Markers and Electrolytes in Patients with End-Stage Renal Disease: A Prospective, Multi-Center Study”

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Abstract

Background: Chronic kidney disease affects 200 million people globally. Global prevalence was 8–16%, rising 7% yearly. India's National Renal Foundation ranks kidney illnesses third among life-threatening diseases. GFR, proteinuria, azotemia, and uremia indicate kidney impairment. Anemia, fluid and electrolyte imbalances and metabolic bone disease resulting from kidney dysfunction.

Material and Methods: We conducted a prospective and retrospective observational study on 150 End Stage Renal Disease (ESRD) patients who had been on hemodialysis for at least a year. The impact of dialysis duration on various haematological parameters and electrolytes was observed and interpreted. The change in the percentage mean of each parameter was calculated with respect to duration and frequency.

Results: Out of 150 patients maximum were of 41 to 60 years. Prevalence of ESRD was higher in males, also hypertension was major the cause. Levels of haemoglobin, PCV level, RBCs count, s. creatinine, s. urea and electrolytes were statistically significant in the higher duration of dialysis as compared to the lower duration. The majority of patients were observed with moderate anemia. All ESRD patients showed hyperparathyroidism.

Conclusion: Higher duration of dialysis was proven better than a lower duration of dialysis. No significant difference was observed with respect to a different frequency.

Keywords: End stage renal disease, Hemodialysis, Anemia, Electrolyte imbalance, erythropoietin

INTRODUCTION:

Chronic kidney disease (CKD) also recognized as chronic renal insufficiency, progressive kidney disease, or nephropathy, is a major public health problem worldwide that affects 200 million people from all racial and ethnic groups (1-3). The worldwide prevalence was estimated to be 8–16% which increases annually at a rate of 7% (4). The adverse outcomes of CKD are renal failure, cardiovascular disorders, and early death. The National Kidney Foundation states that in India, kidney diseases rank third amongst life-threatening disease, after cancer and heart disease (5). The prevalence of CKD in a semi-urban Indian population was apparently higher (6) (7). In 2011, CKD registry of India declared a cumulative annual report which shows 63538 CKD patients in which 44854 male and 18684 female with mean age 50.0 ± 14.6 years. Major
causes of CKD include diabetes, congenital anomalies, vasculitis, secondary glomerular nephritis, glomerulonephritis, neoplasms or tumors, and iatrogenic (8-10). Conferring to the Kidney Disease Improving Global Outcomes (KDIGO) declaration, GFR of less than 60 mL/minute/1.73 m² is an indication of CKD. The decline in GFR category (≥ 90 [G1], 60–89 [GII], 45–59 [GIIIa], 30–44 [GIIIb], 15–29 [GIV], < 15 [GV] ml/min/1.73 m²). Stage V is also called end-stage renal disease (ESRD) (5). The decrease in GFR occurs due to the gradual replacement of normal kidney architecture with interstitial fibrosis leading to irreversible structural damage to existing nephrons and progressive deterioration in kidney function over several months to years and leading to a decline in GFR (11). Declining renal function disturbs the kidney’s homeostasis, leading to hypervolemia, hyperkalemia, hyperphosphatemia, hypocalemcaemia, hypoponatremia/ hypernatremia and bicarbonate deficiency (metabolic acidosis), anemia and metabolic bone disease (11-14). The development of anemia is quite common during the early stages of CKD and in patients with ESRD (15). Renal anemia pathogenesis includes chronic inflammation, iron deficiency, shortened half-life of erythrocytes, and deficiency of erythropoietin (16,17). Renal transplantation and dialysis are the only options to sustain a life of ESRD patients (1). In India, 29% of the ESRD patients are poor, and kidney transplantation is a costly option to treat ESRD (18) and when pre-emptive kidney transplantation doesn’t seem to be possible, dialysis is being used (19). Hemodialysis was associated with a suboptimal response to erythropoietin therapy in ESRD patients, and all haematological parameters differed between the control group, CKD stages, and age groups (2, 20-22). It may cause bleeding and coagulation abnormalities due to thrombocytopenia (23-26). One study found that metabolic acidosis in well-dialyzed renal failure patients can be completely corrected (27). Furthermore, after HD, QTc and QT intervals decreased and electrolytes changed significantly (28). Also, hemodialysis lowers serum urea and creatinine in CKD patients (29). No reports discussed how dialysis frequency and duration affected haematological parameters and electrolytes. This study examines how end-stage renal disease patients on maintenance hemodialysis respond to haematological parameters and electrolyte imbalances.

MATERIAL AND METHODS

The study protocol was drafted and submitted to the K.B. Institutional Ethics Committee (KBIEC) of the K.B. Institute of Pharmaceutical Education and Research in Gandhinagar. The project was granted approval with protocol number (KBIEC/2015/60).

Site Approval

The study was conducted in three different sites and each hospital’s medical superintendent gave his or her approval in advance for the research to be conducted, as detailed below:

Study Sites

1. Civil Hospital, Palanpur, Dist: Banaskantha with Dr. Shivram Patel having designation M.B.B.S, M.S.
2. Gandhi-Lincon Hospital, Deesa, Dist: Banaskantha with Dr. Jagdish Soni having designation M.B.B.S, M.S.
3. M.B.Patel Sarvajanik Hospital, Isanpur Mota, Dist: Gandhinagar with Dr. Hetal Chaudhary having designation M.B.B.S, M.S.

STUDY DESIGN AND DURATION

It was an observational, prospective and retrospective, multicentre study in ESRD. Study population ESRD patients undergoing regular dialysis for the last 1 year. The study duration was 6 months (November 2015 – April 2016)

SAMPLE SIZE AND ELIGIBILITY CRITERIA FOR SAMPLE SELECTION

Based on inclusion criteria and exclusion criteria 150 patients were enrolled in this study

a) Inclusion criteria:

• Patients undergoing regular hemodialysis, laboratory investigation & data available of since last 1 year
• Patients of either sex having age ≥20 years
• Serum creatinine > 1.5 mg/dl
• Patients on erythropoietin therapy

b) Exclusion Criteria:

• HIV positive patients
• Kidney transplanted patients
PREPARATION OF DOCUMENTS

Documents were designed according to variables; importance and need of the research work.

CASE REPORT FORM (CRF)

Case Report Form (CRF) was designed as per the variables needed and influenced the study. CRF was prepared in the English language. CRF has been divided into three parts based on the types of data collection.

A. Demographic data

B. Clinical investigation included the presence of comorbidity, the duration that they have been receiving RRT, the frequency of hemodialysis per week, etc.

C. Pharmacotherapy

Type and Description of Variables

1. Age: Continuous (type) and it was divided as:
   - below 20
   - between 20-40
   - between 41-60 and above 60.
2. Parameter: Continuous (type) and S.creatinine, S.urea, CBC, Serum Na⁺, K⁺, Ca²⁺, PO₄²⁻, Urine albumin, etc are taken.
3. Gender: Categorical (type) and classified as Male and Female.
4. Diet: Categorical (type) and classified as Vegetarian, Non-Vegetarian, Eggetarian.
5. Source of drinking water: Categorical (type) and classified as drinking mineral (RO) water and drinking tap water.

DETAILS OF DIALYSIS UNIT BEING USED IN HOSPITAL

Clinical and laboratory investigations

Demographic data

<table>
<thead>
<tr>
<th>Demographic characteristic</th>
<th>Number of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.2133±11.0857</td>
<td>150</td>
</tr>
<tr>
<td>21 – 40</td>
<td>46±6.2283</td>
<td>30.66</td>
</tr>
<tr>
<td>41 – 60</td>
<td>86±5.4147</td>
<td>57.33</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>18±3.4127</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 1: Distribution of demographic characteristic and medical history

BIOCHEMICAL PARAMETERS

Blood Parameters

It includes the following tests like HIV I & HIV II test, Hepatitis C virus (HCV) test, Anti HCV, Serum uric acid, Serum creatinine, Serum urea, CBC test, Lactate dehydrogenase (LDH), Blood urea nitrogen (BUN), Serum parathyroid hormone (PTH), Serum electrolytes, Serum random blood sugar level.

Urine Parameters

It includes the following tests like Urine albumin, Urine protein, Urine pH, Pus cells, Epithelial cells, Red blood cells, Cast, Crystals, Bacteria, Macrophage, Total protein, Albumin, Globulin

Statistical Analysis

All data was expressed in terms of Mean ± SD. Mean % change in creatinine, urea, hemoglobin, RBCs, PCV, sodium, potassium, calcium, phosphorus was analyzed by unpaired t-test. While the effect of causes of ESRD on serum phosphorus, calcium, uric acid, LDH and WBCs count were analyzed by one-way ANOVA followed by post hoc Tukey’s test using graph prism pad. The effect of age and gender was analyzed by two-way ANOVA followed by the Bonferroni post-test.

RESULTS:

Demographic and Clinical Characteristics

The demographic characteristics of the study sample are detailed in Table 1. The mean number of study subjects are 150. The majority fell between 41–60 years (57.33%). In addition, the prevalence of males was higher (76.66 %). Hypertension-induced ESRD was (32%) more prevalent compare to diabetes (Table1).
BIOCHEMICAL PARAMETERS

Following tables represent the Mean±SD of the laboratory investigation including S. uric acid, Lactate dehydrogenase, White blood cell, S. phosphorus, S. calcium performed for the study population (Table2-5).

Table 2 Causes wise distribution of laboratory finding in Mean and Standard deviations.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Parameters</th>
<th>Causes</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HTN</td>
<td>DM</td>
</tr>
<tr>
<td>1</td>
<td>S. uric acid</td>
<td>8.47±1.688</td>
<td>10.67±1.819 *</td>
</tr>
<tr>
<td>2</td>
<td>Lactate dehydrogenase</td>
<td>261±61.24</td>
<td>295.4±41.5</td>
</tr>
<tr>
<td>3</td>
<td>White blood cell</td>
<td>6.56±1.514</td>
<td>6.02±1.592</td>
</tr>
<tr>
<td>4</td>
<td>S. phosphorus</td>
<td>9.902±2.251</td>
<td>9.725±2.433</td>
</tr>
<tr>
<td>5</td>
<td>S. calcium</td>
<td>6.82±1.506</td>
<td>7.07±1.711</td>
</tr>
</tbody>
</table>

HTN: Hypertension, DM: Diabetes, HCV: Hepatitis C Virus.
* indicates significant difference from all other group of ESRD. (P < 0.001)

Table 3 Duration wise distribution of Biochemical parameters in Mean and Standard deviations.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Parameters</th>
<th>Duration (Hr)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. urea</td>
<td>51.8±85.17</td>
<td>19.9±168.1 *</td>
</tr>
<tr>
<td>2</td>
<td>S. creatinine</td>
<td>16.0±22.14</td>
<td>46.9±22.19 *</td>
</tr>
<tr>
<td>3</td>
<td>Haemoglobin</td>
<td>21.3±21.43</td>
<td>35.9±21.19 *</td>
</tr>
<tr>
<td>4</td>
<td>Red blood cell</td>
<td>17.1±15.02</td>
<td>25.5±14.37 *</td>
</tr>
<tr>
<td>5</td>
<td>Packed cell volume</td>
<td>33.5±13.69</td>
<td>44.1±11.36 *</td>
</tr>
<tr>
<td>6</td>
<td>S. sodium</td>
<td>0.7±12.82</td>
<td>1.8±23.4 *</td>
</tr>
<tr>
<td>7</td>
<td>S. potassium</td>
<td>10.6±21.85</td>
<td>32.8±26.16 *</td>
</tr>
<tr>
<td>8</td>
<td>S. calcium</td>
<td>5.8±12.11</td>
<td>11.5±17.35 *</td>
</tr>
<tr>
<td>9</td>
<td>S. phosphorus</td>
<td>15.8±12.74</td>
<td>33.4±31.06 *</td>
</tr>
</tbody>
</table>

* indicates significant difference from all other group of ESRD. (P < 0.001)
Table 4: Effect age and gender on parathyroid hormone in mean and standard deviations.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Gender and Age</th>
<th>No of patients</th>
<th>PTH</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male, 50</td>
<td>70±6.7554</td>
<td>637±310.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2</td>
<td>Female, 50</td>
<td>25±6.6725</td>
<td>586.7±208.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Male &gt; 50</td>
<td>45±5.6948</td>
<td>619.3±252.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Female &gt; 50</td>
<td>10±7.2694</td>
<td>1343±213.2*</td>
<td></td>
</tr>
</tbody>
</table>

*indicates significant difference from all other group of ESRD. (P < 0.001)

Table 5: Severity of anemia before and after treatments of EPO in ESRD patients

<table>
<thead>
<tr>
<th>Severity of anemia</th>
<th>No of patients before treatment</th>
<th>Percentage</th>
<th>No of patients after treatment</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild anemia (≥ 10 g/dL)</td>
<td>3</td>
<td>2</td>
<td>129</td>
<td>86</td>
</tr>
<tr>
<td>Moderate anemia (7.1-9.9 g/dL)</td>
<td>88</td>
<td>58.66</td>
<td>19</td>
<td>12.66</td>
</tr>
<tr>
<td>Severe anemia ≤ 7 g/dL</td>
<td>59</td>
<td>39.33</td>
<td>2</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Effect of duration of dialysis on mean % change in s. urea, creatinine level, haemoglobin level, RBC count, PCV level, s. sodium level, s. potassium level, s. calcium level, s. phosphorus level.

Mean % change in s. urea was significantly different in higher duration of dialysis (4 Hr) (194.9±168.1) as compared to the lower duration of dialysis (3:30 Hr) (51.84±85.17) with (P value 0.0015) [Figure 1 (a)].

In s. creatinine, the mean % was significantly different in higher duration hemodialysis (4 Hr) (46.69±22.19) as compared to the lower duration of hemodialysis (3:30 Hr) (16.07±27.14) with (P value < 0.001), [Figure 1(b)].

**Figure 1(a)** Effect of duration of dialysis on mean % change in s. urea level. Data expressed as Mean±SD. Student t-test. * indicates significant difference from 3:30 Hr with (P < 0.01)

**Figure 2(b)** Effect of duration of dialysis on mean % change in creatinine level. Data expressed as Mean±SD. Student t-test. * indicates significant difference from 3:30 Hr with (P < 0.01)
In hemoglobin, the mean % was significantly different in the higher duration of hemodialysis (4 Hr) (35.96±21.19) as compared to the lower duration of hemodialysis (3:30 Hr) (21.31±21.43) with (P value 0.0001), [Figure 1].

**Figure 3I** Effect of duration of dialysis on mean % change in haemoglobin level. Data expressed as Mean±SD. Student t-test. * indicates significant difference from 3:30 Hr with (P < 0.01)

In RBCs count, the mean % was significantly different in higher duration of dialysis (4 Hr) (25.57±14.37) as compared to the lower duration of dialysis (3:30 Hr) (17.10±15.02) with (P value 0.0011), [Figure 1(d)].

**Figure 4(d)** Effect of duration of dialysis on mean % change in RBC count. Data expressed as Mean±SD. Student t-test. * indicates significant difference from 3:30 Hr with (P < 0.01)

In PCV, the mean % was significantly different in higher duration of dialysis (4:00 Hr) (44.18±11.36) as compared to the lower duration of dialysis (3:30 Hr) (33.56±13.69) with (P value < 0.0001), [Figure 1].

**Figure 5I** Effect of duration of dialysis on mean % change PCV level. Data expressed as Mean±SD. Student t-test. * indicates significant difference from 3:30 Hr with (P < 0.01)
In s. sodium mean % was significantly different in higher duration of dialysis (4:00 Hr) (1.856±3) as compared to the lower duration of dialysis (3:30 Hr) (0.7814±2.820) with (P value 0.0434), [Figure 1(f)].

**Figure 6(f)** Effect of duration of dialysis on mean % change s. sodium level. Data expressed as Mean±SD. Student t-test. * indicates significant difference from 3:30 Hr with (P < 0.01)

In s. potassium, the mean % was significantly different in higher duration of dialysis (4:00Hr) (32.88±26.16) as compared to the lower duration of dialysis (3:00 Hr) (10.68±21.85) with (P value < 0.0001), [Figure 1(g)].

**Figure 7(g)** Effect of duration of dialysis on mean % change in s. potassium level. Data expressed as Mean±SD. Student t-test. * indicates significant difference from 3:30 Hr with (P < 0.01)

In s. calcium, the mean % was significantly different in higher duration of dialysis (4:00 Hr) (11.55±17.35) as compared to the lower duration of dialysis (3:00 Hr) (5.824±12.11) with (P value 0.0407), [Figure 1(h)].
Effect of duration of dialysis on mean % change in s. calcium level. Data expressed as Mean±SD. Student t-test. * indicates significant difference from 3:30 Hr with (P < 0.01)

In s. phosphorus, the mean % was significantly different in higher duration of dialysis (4:00 Hr) (33.4±31.06) as compared to the lower duration of dialysis (3:30 Hr) (15.84±12.74) with (P value 0.0002), [Figure 1(i)].

Effect of causes of ESRD on s. phosphorus level, s. calcium level, WBC count, s. uric acid level, LDH level, gender and age on PTH level, prevalence of hyperparathyroidism.

The Serum phosphorus level was significantly elevated in stone induced (12.05±3.195) ESRD compared to all other ESRD groups with (P value = 0.0054), [Figure 2(a)].
The serum calcium level was significantly elevated in stone-induced ESRD compared to all other ESRD groups with (P value 0.0002), [Figure 2(b)].
The WBC count was significantly elevated in HCV patients (13.276±4.057) compared to all other groups of ESRD with (P value = < 0.0001), [Figure 2(c)].
The Serum uric acid level was significantly elevated in DM+HTN (11.07±2.671), DM (10.67±1.819) induced ESRD with (P value = < 0.0001), [Figure 2(d)].
The LDH level was significantly elevated in HCV patients (397.5±80.90) compared to all other groups of ESRD. (P= < 0.0001), [Figure 2(e)].
The PTH was significantly elevated in older age (> 50) females compare to all other groups. (P < 0.0001), [Figure 2(f)].
The Prevalence of hyperparathyroidism was higher in the age of 20-50 in males (70±6.7554), [Figure 2(g)].
DISCUSSION:

Chronic kidney disease (CKD) is a complex disease impacting millions of individual over the world (30). Progression of CKD is associated with a number of serious complications, including increased incidence of cardiovascular disease, hyperlipidemia, anemia, electrolyte imbalance, metabolic bone disease, and secondary hyperparathyroidism (SHPT) (31, 32). Hemodialysis and renal transplantation are the only treatment options for ESRD (1). Hemodialysis removes metabolic waste products like urea and creatinine from the blood (29). Conventional hemodialysis remains the most common treatment for ESRD worldwide and is usually performed for 3 to 5 hours, 3 days per week (5).

Various hospitals had the same dialysis setup for hemodialysis but the duration of the dialysis cycle and frequency differs with dialysis centers. In this study, we tried to find out the effect of dialysis duration and frequency on various haematological parameters, electrolytes, and serum biochemistry. We collected all demographic and clinical data viz. age, gender, diet, source of drinking water, socio-economic status, causes of ESRD, co-morbid conditions, and current pharmacotherapy of 150 ESRD patients. We also collected data for various haematological parameters viz. Hb, RBC count, PCV, MCV, MCH, MCHC, ESR, WBC Count, platelets, and Red Cell Distribution Width (RDW). Data for serum biochemical parameters viz. PTH, LDH, total cholesterol, and triglyceride; and urine analysis viz. urine protein, urine albumin, pus cells, epithelial cells, and total protein were collected.

Various studies/authors have reported that ESRD affects either sex and it involves any age group but the majority of the patients in the study were males (115 out of 150), suggesting higher prevalence of the male gender. This was similar to a previous study done by Arun, Prabhu et al. (33). In our study we also recorded the causes of ESRD following this the maximum number of patients ESRD had hypertension (n=48) as a cause of followed by HCV positive patients (n=44), diabetes (n=20), stone (n=16), diabetes with hypertension (n=15) and other (n=7). It is supported by various studies conducted which showed the major cause for the development of ESRD is hypertension (34, 35). A few studies also showed that diabetes mellitus was also a major cause for CKD (20, 36).

Higher hemodialysis duration significantly improved the haematological parameters like haemoglobin, RBC count, and PCV (20, 37). Treatment with EPO and iron supplements significantly elevated basal Hb, PCV, and RBC count. This is supported by previous studies which showed that EPO and iron supplements should be administered/prescribed for the
treatment of anemia (38). Moderate anemia was observed in the majority of patients (88 out of 150). This supported that Normocytic normochromic anemia was observed in our study (33) There was no significant difference in haematological markers across age groups. This is corroborated by a prior study which demonstrated that all age groups of CKD patients had considerably lower haematological values than the control group (25). In CKD patients with positive HCV, WBC count was notably higher than other causes group of CKD. Leucocytosis is more common in patients with hepatitis and infection as per various studies conducted in past (39, 40).

Electrolytes like sodium, potassium, calcium, and phosphorus level were significantly improved in patients undergoing a higher duration of dialysis as compared to lower. Few studies showed that electrolyte levels were significantly changed after dialysis (37, 41, 42). Basal serum calcium and phosphorus levels were significantly higher in kidney stone-induced CKD. It may correlate that these patients had a high prevalence of stones probably because of calcium oxalate and calcium phosphate deposition in the kidney (43).

Serum urea and creatinine level were significantly higher in ESRD patients than in the normal range. High-duration hemodialysis caused a significant decline in mean % change in blood urea and creatinine level. No significant difference was observed in basal serum creatinine & urea and/or in mean % change creatinine & urea with respect to age, gender, and causes of ESRD. Glomerular nephritis, polycystic kidney disease, and uric acid was significantly elevated in DM, DM with HTN as compared to other causes group of ESRD (44-46). Basal serum uric acid level is higher in CKD patients which is supported by several studies conducted previously (47).

Serum LDH level is a novel biomarker for acute renal injury and CKD. LDH level was markedly elevated in HCV patients in the study population. Basal serum LDH level was also elevated in the renal and hepatic injury (48, 49). PTH level at baseline was significantly higher in the older age group female as compared to all other groups. SHPT is a frequently encountered problem in patients with CKD resulting from disturbances in the regulation of PTH, calcium, phosphorus, and vitamin D. An age-related increase in PTH level has been demonstrated in several studies (50).

CONCLUSION:
It was found that longer dialysis sessions were more beneficial than shorter dialysis sessions. Regarding the various frequencies, we could not find any statistically significant differences.

Financial Support And Sponsorship: Nil.

Conflicts Of Interest: There are no conflicts of interest.

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