A Comparative Study of Biochemical and Hematological Parameter in Non Dialysis Dependent Chronic Kidney Disease and Dialysis Dependent Chronic Kidney Disease Patients

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\section*{ABSTRACT}

\textbf{Introduction:} CKD patients are at higher risk for development of anemia and decline in liver function associated with increased mortality rate. This study was conducted to access the hematological and liver parameters in DD-CKD and NDD-CKD patients.

\textbf{Materials and Methods:} This cross sectional study was conducted among 419 CKD patients attending Sumeru Hospital, Nepal. Among them 147 were dialysis dependent and 272 were non dialysis dependent CKD and its stages were diagnosed based on eGFR (CKD-EPI). Hematological, biochemical profile and viral infection were determined. ALT and Aspartate-Platelet ratio index were used respectively for the identification of hepatotoxicity and liver fibrosis.

\textbf{Result:} The Hb concentration, Platelets count, ALT and AST in DD-CKD patients (9.06 g/dL, 191 x 10^9 Cells/L, 23 U/L and 15 U/L) were lower as compared to NDD-CKD patients (12.41 g/dL, 206 x 10^9 Cells/L, 29 U/L and 25.5 U/L). ALP was significantly increased in DD-CKD (141 U/L) as compared to ND-CKD (99 U/L). In addition, the occurrence of HCV and HBV infection was 3.1% each and HIV was 2.6%. Furthermore, the frequency of hepatotoxicity and liver fibrosis were found to be 12.9% and 8.1% respectively. The Pearson correlation of eGFR with Hb, Platelets, ALT, MCV, MCH were positively correlated whereas, it was negatively correlated with total WBC count and ALP.

\textbf{Conclusion:} The severity of anemia, leukocytosis, thrombocytopenia and liver deterioration increases along with the increasing severity of CKD and the severity are at highest in DD-CKD as compared to NDD-CKD patients.

\textit{Key words:} DD-CKD, NDD-CKD, Hematology profile, Liver profile

\section*{INTRODUCTION}

CKD is a global health issue with an alarming mortality rate which is predominant in Asian countries\textsuperscript{(1)} mostly in developing region with high health care cost. The prevalence of CKD in urban Nepal is found to be 10.6\%\textsuperscript{(2)} According to WHO the death rate due to CKD in Nepal was 2.74\% in 2018. ESRD is the final stage of CKD where there is only less than 10\% of nephron functioning with markedly reduction in eGFR resulting in imbalance in body fluid and electrolyte concentration followed by uremia\textsuperscript{(3)}. Three major causes of ESRD are believed to be Diabetes Mellitus, Hypertension and Glomerulonephritis. Hemodialysis and renal replacement therapy is the most widely used therapy for people with ESRD\textsuperscript{(2)}. 
Biochemical and hematological profile dysfunction is associated with kidney disease and the effect rises more as the disease progress. Due to decrease in erythropoietin production, chronic blood loss and hemolysis there is the emergence of anemia in CKD. Similarly, severe hyperparathyroidism, gastrointestinal bleeding and systemic inflammation might be the case of anemia. Moreover, there is decreased platelet count in CKD which is regarded as a consequences of hemodialysis (3,4).

To access and monitor the hepatic function, liver enzymes; AST, ALT and ALP are commonly used. But as the disease progress, serum aminotransferase tends to decrease and this effect is predominant in patient with hemodialysis. Similarly studies suggested that HCV infection patient with CKD undergoing hemodialysis have lower serum aminotransferase level as compared to patient with HCV infection only (5). The cause of lower aminotransferase level is unclear but possible reason may be due to pyridoxine deficiency or hemodilution or the presence of an inhibitory substance (6). Similarly ALP is higher in CKD patient mostly in stage 4 and 5, this might be due to renal osteodystrophy which may contribute to cardiovascular burden and increases the risk for mortality (7). Thus, diagnosis, treatment and management of liver damage in CKD is being challenge to the health care (8).

In CKD patient, infection with HCV is higher with increased mortality rate due to liver cirrhosis, hepatocellular carcinoma and development of glomerulonephritis followed by CKD. HCV infection leads to the development of IgM autoantibody with rheumatoid factor activity which causes mixed cryoglobulinemia and deposition of these compound in kidney tubules and glomerular capillaries (8, 9). Furthermore, antiviral agents such as tenofovir and/or lamivudine risen the risk of nephrotoxicity development in these individual (10).

MATERIALS AND METHOD
This was a cross-sectional study conducted at Department of Biochemistry at Sumeru Hospital, Nepal for the period of 11 months. 419 patients at different stages of CKD were recruited consecutively for the study among which 147 were undergoing hemodialysis. Selections of patient were based on previous diagnosis of CKD and patient presenting with predisposing factors like diabetes mellitus and microalbuminuria. The blood sample withdrawn from patients were analyzed for complete blood cell count, hepatic and renal function tests and serological examination for screening viral infection. eGFR was calculated by CKD-EPI formula which include serum creatinine concentration, age, gender and race of an individual (10). Categorization of CKD in different stage was done according to the classification by National Kidney Foundation (11). Assessment of hepatotoxicity was based on the activity of ALT (>50U/L). Grading of hepatotoxicity into 1, 2, 3 and 4 grade was done when ALT activity was 50.0–99.9 U/L, 100.0–199.9 U/L, 200.0–399.9 U/L and >400.0 U/L respectively (12). Similarly, we used Aspartate-platelets-ratio index (APRI) for determining liver fibrosis APRI = (AST/ULN) × 100)/platelet count (10^9/L) where ULN denotes for upper limit of reference range (5-45 U/L) of AST and APRI index >1.5 indicates liver fibrosis (9).

Data were analyzed in IBM SPSS version 23. Normally distributed data were expressed as mean and standard deviation and compared by independent sample t-test and for more than two groups ANOVA test was used. Non-normally distributed data were presented as median and Interquartile Range and compared by Mann Whitney test and for more than two group Kruskal Wallis test was used. Categorical variables were compared by Pearson's Chi-square test. Pearson's correlation was used to determine the correlation between eGFR and different variables.
RESULT
A total of 419 CKD patients were included in the study out of which 147 were undergoing hemodialysis. Among the study population, 62.05% (n=260) were male and 37.94% (n=159) were female, the maximum number of patient were from stage 5. Majority of participants were male in each stage of CKD (Fig.1). The mean age group of the population is 51.25 ±16.94 years (Fig. 2).

The comparison of hematological and hepatic parameters in different stages of CKD shows that Hb level, Platelet count, MCV, MCH, AST, ALT, Urea, Creatinine, and eGFR were significantly ($p<0.05$) reduced as the disease in higher order of chronicity whereas ALP activity is significantly ($p<0.01$) increased along with increasing severity of CKD. The incidence of hepatotoxicity and liver fibrosis were 12.88% (n=54) and 7.78% (n=26) respectively with Grade 3 hepatotoxicity seen in stage 4 and 5. Moreover, the incidence of viral infections were also seen significantly ($p<0.05$) higher in stage 5 CKD (Table 1).

The comparison of AST, ALT, Hb, Platelet count, MCV and MCH level shows significantly ($p<0.01$) lower in DD-CKD as compared to NDD-CKD whereas ALP is found to be significantly ($p<0.01$) increased in DD-CKD in contrast as compared to NDD-CKD (Table: 2).
The incidence of hepatotoxicity, liver fibrosis and viral infections (HIV, HCV and HBV) were found to be higher in DD-CKD as compared to NDD-CKD patients (Table: 3).

In addition, the Pearson’s correlation of eGFR shows a significant positive relation with Hb, Platelet count, MCV, MCH and ALT (p<0.05) whereas, significant negative relation with ALP (p<0.01) (Table 4).

### Table 1: Comparison of Hematological parameter, Liver status and viral infection between different stages of CKD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>13.4 ± 2.1</td>
<td>13.4 ± 2.3</td>
<td>11.6 ± 2.5</td>
<td>10.3 ± 2.2</td>
<td>9.1 ± 2.1</td>
<td>&lt;0.000**</td>
</tr>
<tr>
<td>T. WBC (10^3/L)</td>
<td>7.4±9.9</td>
<td>7.4±9.3</td>
<td>8.7±12.1</td>
<td>7.9±13.8</td>
<td>8.2±11.4</td>
<td>0.106*</td>
</tr>
<tr>
<td>Platelets (10^3/L)</td>
<td>217 (153-281)</td>
<td>219 (150-289)</td>
<td>193 (101-285)</td>
<td>177 (91-263)</td>
<td>190 (88-292)</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>93.0 ± 6.5</td>
<td>91.8 ± 7.6</td>
<td>92.6 ± 8.6</td>
<td>89.9±8.7</td>
<td>88.1±8.4</td>
<td>&lt;0.05**</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>29.4 ± 2.8</td>
<td>29.6 ± 2.7</td>
<td>29.5 ± 2.7</td>
<td>29.1±3.7</td>
<td>28.6±2.6</td>
<td>0.05**</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>31.5 (24.0-46.0)</td>
<td>26.5 (17.7-37.2)</td>
<td>24 (17.5-35)</td>
<td>16 (10-33)</td>
<td>15 (10-26)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>31.5 (25.0-39.0)</td>
<td>30 (24-37)</td>
<td>29.22±49.5</td>
<td>23±16.8</td>
<td>23±15.4</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>94.3±75.2-123.7</td>
<td>72±75.1-113.5</td>
<td>110±83.5-155.5</td>
<td>129±91-208</td>
<td>141±95-204</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>T. Bilirubin (mg/dl)</td>
<td>0.3 (0.2-0.4)</td>
<td>0.2 (0.2-0.4)</td>
<td>0.3 (0.2-0.6)</td>
<td>0.2 (0.2-0.3)</td>
<td>0.2 (0.2-0.3)</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

### Table 2: Comparison of hematological and biochemical parameters between DD-CKD and NDD-CKD patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DD-CKD Mean±SD</th>
<th>Median(IQR)</th>
<th>NDD-CKD Mean±SD</th>
<th>Median(IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>7.8 ± 2.6</td>
<td>15 (11-21)</td>
<td>29.25±23.21</td>
<td>29.5±27</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>8.4 ± 3.8</td>
<td>1.4 (1.0-1.8)</td>
<td>7.7 ± 3.04</td>
<td>6.4 ± 3.1</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>0.9 ± 3.07</td>
<td>15 (10-26)</td>
<td>12.4 ± 2.9</td>
<td>25.5±17.38</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

### Table 3: Incidence of Liver disease and viral infection between DD-CKD and NDD-CKD patients.

<table>
<thead>
<tr>
<th>Liver status and Viral infection</th>
<th>DD-CKD (n)</th>
<th>NDD-CKD (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatotoxicity</td>
<td>18 (12.2%)</td>
<td>36 (13.2%)</td>
<td>0.852</td>
</tr>
<tr>
<td>Hepatotoxicity Grades</td>
<td>Grade 1</td>
<td>11 (6.1%)</td>
<td>23 (13.5%)</td>
</tr>
<tr>
<td></td>
<td>Grade 2</td>
<td>5 (27.8%)</td>
<td>12 (33.3%)</td>
</tr>
<tr>
<td></td>
<td>Grade 3</td>
<td>2 (11.1%)</td>
<td>2 (11.1%)</td>
</tr>
<tr>
<td>Liver fibrosis</td>
<td>12 (8.3%)</td>
<td>22 (15.8%)</td>
<td>0.979</td>
</tr>
<tr>
<td>HIV</td>
<td>9 (6.1%)</td>
<td>20 (7.0%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HCV</td>
<td>11 (7.5%)</td>
<td>20 (7.0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HBV</td>
<td>10 (6.8%)</td>
<td>3 (1.1%)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

The incidence of hepatotoxicity, liver fibrosis and viral infections (HIV, HCV and HBV) were found to be higher in DD-CKD as compared to NDD-CKD patients (Table: 3).
DISCUSSION

The increasing incidence of CKD mainly in the developing regions is creating economic and global health burden (14). The increasing grade of anemia and liver impairment has also been well established in association with increasing severity of CKD and increases the mortality risk. This study was intended to determine the incidence of different stage of CKD and was emphasized to measure the hematological and biochemical parameter with viral disease among NDD-CKD and DD-CKD patients.

On the basis of WHO criteria for anemia (Male: <13g/dl and Female: <12g/dl) (15), almost all CKD patients in the study were anemic and grade of anemia was proportionally higher with increasing severity of CKD. The study also revealed that the grade of anemia was higher in DD-CKD patients as compared to NDD-CKD patients, which is in support of numerous studies (4, 15). The probable causes for development of anemia in CKD patients are erythropoietin deficiency due to kidney destruction, uremia-induced inhibitors of erythropoiesis, shortened erythrocyte survival, disordered iron homeostasis by excess hepcidin, impairing dietary iron absorption and mobilization from body stores (16). It may be linked with other abnormalities like diminished cardiac dysfunction, fatigue and mental acuity. So continuous monitoring of the patient for cause, severity of anemia and its treatment is important (4).

The study has revealed the decrease in platelet count with decrease in eGFR as well as significant decrease in platelet count in DD-CKD patients as compared to NDD-CKD patients which is in agreement with the studies of Islam MN et al. (15) and Gafter et al. (17). Decreased thrombopoetin level and interaction of platelet with dialysis membrane leads to the platelets adhesion, aggregation and activation may cause thrombocytopenia (18).

Total WBC counts were slightly higher in DD-CKD patients as compared to NDD-CKD patients as well as negatively correlated with eGFR at a significant level which is in support with substantial publications supporting the hypothesis that traditional systemic inflammation induced leukocytosis may serve as a risk factor for CKD development (3, 19, 20).

The interrelationship between the kidneys and the liver is complex. Functional renal failure is a common feature in patients with liver disease, due to underlying hepatorenal pathophysiology, marked by splanchnic vasodilation, arterial underfilling and renal vasoconstriction that progresses in patients with decompensated cirrhosis. Liver enzymes, liver fibrosis and viral infection were assessed in DD-CKD and NDD-CKD patients. Serum concentrations of transaminases are routinely measured to assess liver function in patients with and without CKD. The aminotransferases are normally present in the circulation in low concentrations, usually <40 U/L. Substantial publications have established that the level of transaminases in CKD patients commonly fall within the lower end of normal range. Our study has also revealed that the level of AST and ALT in DD-CKD patients were comparatively lower in NDD-CKD patients which was found to be similar with the study of Ray et al. (6), Allawi et al (21) and Sette et al. (22). The low level of these enzymes in CKD may be due to consumption of NADPH by increased serum lactate; the presence of uremic factors that would inhibit the activity of these enzymes; and the deficiency of pyridoxal phosphate (6).

The activity of ALP enzyme is found to be increase in CKD patients due to high bone turnover (6). Finding from the study has revealed significantly increasing ALP activity as the disease progress with higher activity among DD-CKD patients. Furthermore, there was a significant negative correlation between ALP and eGFR which is in support with the study of Freethi et al. (23). Increased ALP activity in CKD patient increases the risk of mortality as ALP leads to the vascular calcification.
and promotes metabolic bone disease related bone fracture (24).

In the study the incidence of liver fibrosis in CKD patients using APRI score was found 8.1% which is similar with the study of Chien et al. and Kim et al. (25, 26). Even though NAFLD is not the independent risk factor for CKD, patients with advanced liver fibrosis are facing CKD. Common factors underlying the pathogenesis of liver fibrosis and CKD may be insulin resistance, oxidative stress, activation of rennin-angiotensin system, and inappropriate secretion of inflammatory cytokines by steatotic and inflamed liver (27).

HCV infected patients are at higher risk for the development of CKD. In HCV immune complexes are deposited in the glomerulus leading to glomerulonephritis (9). In our study, the incidence of HIV, HCV and HBV were 2.6%, 3.1% and 3.1% respectively among the total CKD patients whereas the incidence of viral infection in DD-CKD patients was significantly higher as compared to NDD-CKD patient. The incidence of HIV, HCV and HBV in DD-CKD patient was 6.1%, 7.5% and 6.8% respectively where as in NDD-CKD patients the incidence of HIV, HCV and HBV were 0.7%, 0.7% and 1.1% respectively. This finding is in agreement with the studies of Ibrahim et al. (28), Kansay et al. (29), and Chang et al. (30) where the incidence of viral infection is higher in DD-CKD patients as compared to NDD-CKD patients. The risk factor for viral infection may be age, history of blood transfusion, treatment of patient at various centers and use of intravenous drugs (30).

CONCLUSION

The study concluded that the progression of anemia, leukocytosis and thrombocytopenia is associated with increasing severity of CKD. Moreover serum transaminases were decreased with increasing severity of CKD which suggest the need of establishing the separate reference ranges in different stages of CKD. ALP levels and incidence of liver fibrosis and viral hepatitis increases with increasing severity of CKD. The related complications were more severe in DD-CKD patients as compared to NDD-CKD patients. Anemia and liver diseases are recommended to be treated in CKD patients to alleviate related complications and minimize the mortality rate and economic burden.

ACKNOWLEDGMENTS

The authors are deeply thankful to all the staffs of laboratory department, management and officials of Sumeru Hospital, Lalitpur for providing the opportunity to carry out this research work.

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