Effects of Uremia on White Blood Cells, Platelets and Coagulation Parameters: A Single-Centre Ambispective Study

Indira K Shastry¹, Sushma Belurkar²

¹Resident, Department of Pathology, Kasturba Medical College, Manipal University, India
MD., DNB, Assistant Professor, Department of Pathology, American university of Antigua College of Medicine, Manipal University, West Indies

²Associate Professor and Clinical Lab In-Charge, Department of Pathology, Kasturba medical college, Manipal University, Indira

Abstract: Our study aimed at analyzing the total number of chronic kidney disease (CKD) patients with abnormalities in white blood cells and coagulation parameters. A total of 300 patients were analysed in an ambispective, correlation study. Neutrophilia was observed in 45% of cases and 77% of them were in stage 5 CKD without infections, and toxic change were observed in 55%. Prolongation of PT was seen in 16.1% of cases and in this group, 33% of them were being treated for sepsis. Similarly, a prolonged aPTT was observed in 14.7% of cases and among them 34% had sepsis. Conclusion: Alteration in the coagulation profile and leucocytes number and morphology can be due to infections because of defects in immune system or interference by uremic toxins.

Keywords: Chronic kidney disease, White blood cells, Platelets, Bleeding time, Prothrombin time and Activated partial thromboplastin time

1. Introduction and Literature Review

Bacterial Infections remains the second most common cause of death in patients with end stage renal disease (ESRD), this is largely due to decreased immune response because of depletion of memory and regulatory T cells, and a reduction in the number of B-cells in uraemia. Chronic kidney disease (CKD) is also associated with systemic inflammation and oxidative stress due to activation and release of reactive oxygen species by the cells of innate immune system directed by monocytes / macrophages, granulocytes and other non-immune cells. Systemic inflammation includes atherosclerosis, cardiovascular disease, cachexia and anaemia[1].

The extent of naive and central memory T-cell depletion is directly proportional to severity of azotemia, oxidative stress, secondary hyperparathyroidism, iron overload and inflammation. Hendriks et al demonstrated an increased apoptosis and a marked reduction in the T regulatory cells (CD4+, CD25+), and their anti-inflammatory response in dialysis independent CKD patients and in ESRD patients maintained on peritoneal and haemodialysis[2]. This theory was supported by incubation of isolated T-reg cells from normal individuals in the uremic serum. Upon observation it was noted that uremic milieu lowered the number of T-reg cells and reduced their immune suppression capacity, thus pointing to its harmful effect on these cells. This effect was reproduced by addition of oxidised LDL, which is consistently elevated in CKD patients. These researches illustrate the interconnection between oxidative stress, lipid disorders, immunological abnormalities and atherogenic abnormalities in CKD[2][3][4]. Other causes for a defect in host defence mechanism are related to nutritional deficiency and therapy, such as Vit D3, zinc, Selenium, Pyridoxine deficiency, protein malnutrition and fibronectin deficiency. Iron overload causes a decrease in phagocytic activity of innate immune cells. Mobilisation of iron stores by deferoxamine is associated with a marked increase in incidence of mucormycosis, yersinia enterocolitis and other infections[5].

Platelet dysfunction in uremia: Multiple factors are responsible for platelet dysfunction in uremia, leading to bleeding. According to a study by Zachee Pet al, three factors that contribute to platelet dysfunction are[5].
1. Uremic Toxins.
2. Anaemia.
3. Nitric Oxide.

In the same study it was found that a reduction in megakaryocytic ploidy can produce platelets of lower functional abilities and defective hemostasis[5]. Furthermore, an interference in hemostasis as a result of competitive binding of circulating fibrinogen to Glycoprotein (GP) IIb/IIIa receptors on the surface of the platelets can cause an interference in clot formation[6].

Nitric Oxide is produced by endothelial cells and platelets, and it is a potent inhibitor for platelet aggregation. Elevated levels of guanidinosuccinic acid, a uremic toxin has been hypothesised as a precursor of increased nitric oxide production[7].

 Patients in advanced renal disease can also have procoagulant abnormalities such as impaired release of tissue plasminogen, increased PAI-1, elevated fibrinogen, D-dimer, and increased TF/FVIIa, along with a platelet function defect with an increased risk for cutaneous and mucosal bleeding[8].

So, our study aimed at identifying the total number of CKD patients with;
1) Abnormality in total leukocyte counts, absolute counts of each type of leukocytes and their morphology. In addition, we also tried to identify the cause for these abnormalities.

2) Altered platelet counts, bleeding time (BT), prothrombin time (PT) and activated partial thromboplastin time (aPTT). And the cause for their abnormal values.

2. Materials and Method

Total of 300 chronic kidney disease patients with available data on white blood count, differential counts, platelet count, BT, PT and aPTT were selected. However, few of the patient’s records did not show information on PT and aPTT. The patients were randomly chosen irrespective of treatment status and the study was of ambispective type. Institutional ethical clearance was taken.

Study population was divided into five groups depending on the stage of renal failure.

Stage of renal failure was determined by measuring eGFR, by using the formula =175 × standardized Scr −1.154 × age−0.203

Statistical analysis was done by using SPSS 20 and the data interpretation was done by using Spearman’s rho correlation and a P-value of less than 0.05 was considered significant.

3. Results

In three hundred patients with CKD, 206 (68.7%) were found to be in stage 5, followed by stage 4 (22.3%), stage 3 (8.7%) and stage 2 (0.3%).

Diabetes (38.3%) was the leading cause of CKD followed by obstructive uropathy (10.3%), hypertension (7.3%) and glomerular diseases (11%). Rare causes for the CKD in the study population were; drug induced renal failure due to non-steroidal anti-inflammatory drug abuse and ayurvedic medicines (2.3%), chronic tubulointerstitial nephritis due to various aetiologies (2.3%), autoimmune diseases like SLE (1.7%), renal amyloidosis (1.3%), multiple myeloma (1%), renal artery stenosis (0.7%) and inherited causes like Autosomal dominant polycystic kidney disease (1.7%) and mitochondrial cytopathy (0.3%).

3.1 White blood cells

The total WBC count ranged from 1,200 to 40,000/μl. Among these patients, 201 (67.1%) had normal WBC counts, 94 (31.3%) had increased WBC counts and 5 (1.6%) had decreased WBC counts. Furthermore, increased WBC counts were seen mostly in patients with high blood urea levels (>100mg/dl), whereas in other cases (with urea levels < 100 mg/dl) it was associated with infections. Common types infectious agents observed in patients with neutrophilia were; staphylococcus aureus, pseudomonas, klebsiella and E coli.

A Spearman’s rho correlation coefficient between high WBC count and stage of kidney failure was 0.124 and the P value was 0.032. Thus, it was deemed to be a statistically significant positive correlation.

<table>
<thead>
<tr>
<th>Stage of renal failure</th>
<th>Total WBC count (cells/μl) for n=300</th>
<th>Neutrophil toxic change for n=300</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;4,000</td>
<td>4,000-11,000</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>133</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>201</td>
</tr>
</tbody>
</table>

A total of 135 (45%) patients showed neutrophilia and only 4 (1.3%) cases showed neutropenia. Major number of patients with neutrophilia (77%) were observed to be in stage 5 CKD (Table-2). The patients with neutrophilia and toxic changes were further scrutinized for the presence of infection/s and it was found that only 19% of the patients were positive for culture with bacterial agents like staphylococcus aureus, pseudomonas, klebsiella and E coli. Therefore, it was concluded that the patients in stage 5 CKD with very high blood urea and creatinine levels can demonstrate neutrophilia and toxic change even in the absence of an infection. When the absolute neutrophil count and the presence of toxic changes were analysed in these patients, a P value of 0.012 was obtained, it was considered statistically significant.

A statistical analysis of absolute counts of Lymphocytes, monocytes and eosinophils with the stage of renal failure was statistically insignificant.

<table>
<thead>
<tr>
<th>Stage of renal failure</th>
<th>Absolute Neutrophil count (cells/μl) for n=300</th>
<th>Neutrophil toxic change for n=300</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;2000</td>
<td>&gt;2000-7000</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>98</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>161</td>
</tr>
</tbody>
</table>

3.2 Platelets, Bleeding time, PT and aPTT

Platelet count ranged from 12,000-696,000 /μl, with a mean value of 235,000/μl. Thrombocytopenia was seen in 42 cases (14%) and in majority of them it was associated with systemic infection (sepsis). In addition to sepsis, peripheral smear of these patients showed features of hemolysis, especially in patients with stage 5CKD. One patient in stage 3 renal failure had steroid responsive Idiopathic Thrombocytopenic Purpura (ITP). An increased platelet count was seen in 13 cases (4.3%). (Table-3).

The infectious agents detected in the patients with low platelet counts were, staphylococcus aureus, pseudomonas, klebsiella and E coli. Eight cases were associated with tuberculosis and 10 were positive for Hepatitis B and C.
A correlation of platelet count with stage of renal failure was significant with a P value of 0.021 and it showed positive relationship on Spearman rho analysis.

**Table 3:** Correlation of platelet count with stages of renal failure  

<table>
<thead>
<tr>
<th>Stages of renal failure</th>
<th>Platelet count (μl) for n=300</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;150,000</td>
<td>&gt;150,000-400,000</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>24</td>
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<tr>
<td>4</td>
<td>6</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>164</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>245</td>
</tr>
</tbody>
</table>

Bleeding time (BT) in CKD patients ranged from 1.30-6.30 min with a mean of 3.4±/-.79 min. 2 cases (0.66%) showed prolonged bleeding time, and they were found to be in sepsis. A spearman’s rho correlation coefficient was 0.194 and a P value of 0.001 was noticed.

Prothrombin time (PT) ranged from 12.7-37.3 sec with a mean value of 15.8±/-.2.1 sec. An increased PT was seen in 48 cases (16.1%), among which 16 cases (33%) had sepsis and infection. The correlation coefficient was found to be 0.156 with a P value of 0.007.

Activated Partial thromboplastin time (aPTT) ranged from 18.9-120 sec, with a mean value of 34.25+/-. 7.9 sec. Prolonged aPTT was seen in 44 cases (14.7%) and 15 of them (34%) were associated with sepsis and infection. The correlation coefficient when compared with stages of renal failure was found to be 0.13 and a P value of 0.024 was noted.

### 4. Discussion

#### 4.1 White blood cells

Chronic kidney disease (CKD) in late stages can affect multiple systems and multiple causes can lead to chronic kidney disease. Uremia is common in stage 5 CKD and this can alter the function of immune cells, thus predisposing an individual to infections. In addition to this, patient can have dysfunctions in adaptive immune system leading to systemic inflammatory state. So, the blood cells in these patients can show leucocytosis, neutrophilia and toxic changes in the absence of infections. In a study done by Wastiet al[16], 29.8% cases had leucocytosis and, in a study done by Agarwal et al[12], leucocytosis was identified to be an independent risk factor for the mortality in End Stage Renal Disease (ESRD). In our study, after analysing the patients with leucocytosis it was interpreted to be due to infections and continuous inflammatory process in patients with altered kidney function, since, 31.3% cases had increased WBC counts. The most common leukocyte anomaly found in CKD patients is in neutrophils, due to oxidative stress. A 45% of patients in our study showed absolute neutrophilia, so as a study by Talwar et al[13](46% of the cohort). Moreover, a 77% of the cases with neutrophilia had increased urea level, only 15% had infection. The data in a study by Agarwal et al[12] revealed neutrophilia 6.03% of cases. In the same study the patients with CKD, neutrophilia, monocytes and eosinophilia had increased risk of progression to ESRD.

#### 4.2 Platelets, Bleeding time, PT and aPTT

A decreased platelet count in CKD can be due to use of heparin, infections or hypersplenism, and a decrease in platelet count is rarely severe[13]. Mean platelet count in our study population was normal in 81.6% cases, so as the studies by Singh et al[6], Wastiet al[11]. However, reduction in the platelet count in our study population was majorly attributed to sepsis, it can also be due to impaired erythropoietin secretion in CKD causing a decrease in platelet count, because of considerablesimilarities between erythropoietin and thrombopoietin. Another possible factor for a reduction in platelet mass is possibly due to a decreased in thrombopoietin activity[15].

In the study by Din SU et al[16], 1% patients in stage 4 had prolonged PT, 1% and 2.5% of patients in stage 3 and 4 renal failure respectively had prolonged aPTT. In our study an increased PT and aPTT was seen in 16.1% and 14.7% cases respectively and among them 34% cases were suffering from infection. It was also observed that a higher incidence of prolonged PT and aPTT were more prevalent in stage 4 and stage 5 CKD patients. The changes in coagulation were hypothesized to be due to sepsis and toxic effects of urea.

### 5. Conclusion

We concluded that leucocytosis and neutrophilia were commonly associated with advanced stage of kidney failure and infection. We could explain the changes in the coagulation profile as due to infections (sepsis), this could probably due to immune system dysregulation seen in stage 5 CKD patients. A conclusive correlation between the effects of uremic toxins on the coagulation parameters could not be established, because the study was not funded to facilitate lab investigations and comprehensive clinical correlation.

### 6. Acknowledgements

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### 7. Financial support and sponsorship

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### 8. Conflict of interest

None

### References


