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Assessment of Some Biochemical Markers in Chronic Kidney Disease Patients in Al-Najaf Al-Ashraf Governorate

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Abstract : Chronic Kidney Disease was considered of the public health problems, which was loss in kidney function and structure with decrease of GFR for three month or more. In the present study was used biomarker (Neutrophil Gelatinase-Associated Lipocalin (NGAL), Adiponectin (ADPN), Erythropoietin(EPO)) and other parameter to diagnosis of chronic kidney disease (CKD). The study was conducted by taking 68 patients with CKD (34 men, 34 women) attending the Artificial Kidney Unit in Al-Sadder Teaching city and in Al-Hakeem hospital in the province of Al-Najaf Al-Ashraf and 20 healthy group. The concentration of NGAL, ADPN and EPO were measure, also urea, creatinine and other physiological were estimated in patients and healthy group. The result show significant increase ($p< 0.05$) in the concentration of NGAL, ADPN, Urea and Creatinine but showed significant decrease ($p< 0.05$) in the EPO, Hb, RBC, WBC and Lymphocytes in patients with CKD compared with healthy group. The result showed positive significant ($p< 0.05$) positive correlation of NGAL with creatinine and urea. the study also shown significant ($p< 0.05$) positive correlation of ADPN with NGAL and Creatinine. The study was revealed significant ($p< 0.05$) positive correlation of EPO with hemoglobin, Red blood cells and platelets, but significant ($p< 0.05$) negative correlation of EPO with NGAL and ADPN. The present study was concluded that NGAL, ADPN and EPO were marker to diagnosis and detection of chronic kidney disease.
Key words : chronic kidney disease, NGAL, ADPN, EPO.

Introduction

Chronic kidney disease was definition as an irreversible deterioration of renal function over 3 months or more, in the CKD the glomerular filtration rate was decrease leading to the increase in the creatinine concentration and other organic substance in the blood. CKD in the End Stage required renal replacement therapy[1].

Chronic kidney disease was considered the most widespread disease in the world. In US about 35,000 deaths recorded yearly due to the chronic kidney disease. The proportion of renal disease death in the US could be rise in the past sixteen years. CKD in west Malaysia was 9.0% that occurrence in adult population (above 18 years old) [2]. In 2010 about 300,000 patients have kidney disease in United States therefore rise in the morbidity and mortality with renal failure[3].

Adiponectin was 30-kDa polypeptide exudation by adipocytes. It consists from 244 amino acids in monomer structure including collagen-like fibrous stalk and a globular domain. it is structure resemble to complement factors C1q[4]. Adiponectin circulates in three forms: low-molecular-weight adiponectin (LMW)was term trimer ,middle-molecular weight adiponectin (MMW) called hexamer was produced from twolow-molecular weight adiponectin by disulphide bonds within the collagen stalk and high-molecular-weight

(HMW)isoform collected from middle-molecular-weight oligomers [5]. In normal kidney function ADPN was identified in the urine in small amount [6] but in the kidney disease the amount of ADPN was increase therefore usage to detect the development of CKD[7].

Neutrophil gelatinase-associated lipocalin was 25-kDa secretory glycoprotein covalently bound to neutrophil gelatinase. Also NGAL was 178 amino acids that belonging to lipocalins family, that was specialized in attaching and transferring small hydrophobic molecules. The expression gene of neutrophil gelatinase-associate lipocalin was normally found in several adult human tissues, including salivary gland, prostate, gastric, trachea, colon, lung, liver, and kidney[8].

The lipocalins structure share a molecular organization including eight β -strands arranged in a complex β -barrel structure which define a calyx shape, This represents their binding site [9]. NGAL have important role in detection of kidney damage and progressive of CKD[10].

Erythropoietin was characterization as a hormone secretion from the peritubular cells in the adult kidney [11]. EPO have important role in the erythropoiesis[12]. In kidney disease the secretion of the EPO was decline leading to the anemia[13].

Aim of study is to determine the biomarker parameter and relation with progressive of disease in patients with chronic kidney disease undergoes hemodialysis and study the correlation of gender, age and Body Mass Index with parameter in patients of CKD undergoes hemodialysis.

Experimental

The study was conducted by taking 68 patients with CKD attending Artificial Kidney Unit in Al-Sadder Teaching city and in Al-Hakeem hospital in the province of Al-Najaf Al-Ashraf. It was carried out from .the age of the patients group was range from the 15-75 years.

The information of patients were obtained through questionnaire consisted name, gender, age, weight, height. Patients with Hepatitis were excluding from the study.

A group of 20 was considered healthy subjects (10 men and 10 women). The age of healthy group was range from the 20 -60 years.

Collection of the sample

Blood sample were drawn from vein by sterilized synergies with 5 milliliters. The sample put in the two labeled tubes, first group of tubes contain EDTA as anti-coagulants to prevent clotting of blood to be used for physiological studies. The second group of tubes was without anti-coagulant as plain tubes, for blood to be used for preparing serum for following biochemical and biomarker parameter. Blood was left at room temperature for 10 minutes for clotting, centrifuged 6000 rpm for 10 minutes, and then serum was separated and freezing at -20 ºC until time for performed the laboratory analysis for study.

Biomarker measurement

Determination of Neutrophil Gelatinase associated lipocalin (NGAL)

The level of NGAL determining by using Enzyme-linked immunosorbent assay (ELISA) method, according procedure provide by the manufacture instructions (Elabscience, China Cat-No. E-EL-H0096).

Determination of Erythropoietin (EPO)

Erythropoietin (EPO) concentrations in the serum examination by using enzyme- linked immunosorbent assay (ELISA) were conducted by preparing processed from (Elabscience, China-Cat-No. E-EL-H0066) .

Determination of Adiponectin

This examination was conducted by preparing processed from (Elabscience, China Cat-No.E-EL- 0004) using enzyme- linked immunosorbent assay method to determine the level in the serum of patients with chronic kidney disease.

Biochemical measurement

Determination of serum Urea concentration

Colorimetric method used to determination of urea concentration in the serum (bioMerieux, France). Read absorption at 500 nm sample and standard.

Determination serum creatinine concentration (BIOLABO)

Colorimetric reaction to determine the concentration of creatinine in the serum (BIOLABOSA, France) read adsorption at 510 nm was absorption 1 and after 2 minutes read adsorption 2.

Physiological Parameter

Measurement of Hemoglobin Estimation

The use of Hemoglobin Meter and Drabkin Solution as a dilution solution to estimating the concentration of hemoglobin [14] .

Estimation of Leucocytes Count

1- Total Leucocytes Count

Utilizing blood cells counter and Turks fluid to estimate the total leucocytes count by microscope according this question [15].

Total Leucocyte count /mm^3=the cells count ×50

2-Differential Leucocytes count

Preparation of blood smear and staining dye with Leishman stain then diagnosis under oil immersion lens to measure differential leucocytes count[14]**.**

Measurement Total platelets Count

Used method of blood cells count and Ammonium oxalate solution as a diluted solution to count of total platelets then calculate the number of platelets from this equation[15].

Platelet number/mm^3=platelet count ×1000

Estimation of Red Blood cells

Utilizing the counting chamber and formal citrate solution to diluted the blood to estimating the RBC count according this question [16].

RBC count = No. counted cells/ cubic mm x10, 000

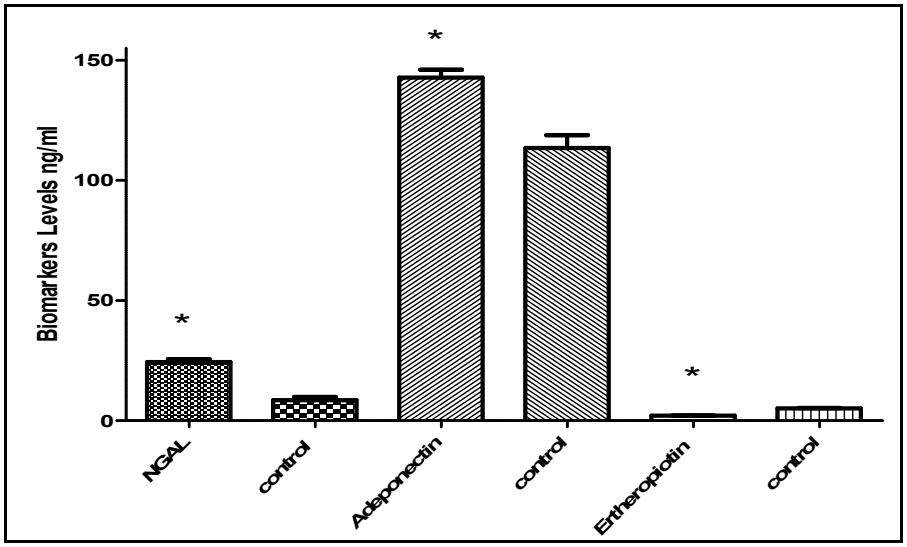
Statically analysis

The data were analyzed by using windows software packages Graph pad prism v5, data were offered as the mean± standard error (SE). Statistical analysis of comparison between the patients and healthy groups were tested by one way ANOVA test, while the comparison between subgroups was analysed by t-test. It carried out the correlation between the parameters correlation coefficient of Pearson. A level of statistically significant determination by P-value < 0.05.

Results

Change in the Concentration of biomarker in patients with CKD

The result in Figure (1) indicated significant ($p< 0.05$) increase in the concentration of NGAL24.33 ±1.279ng/ml and ADPN 142.8±3.278ng/ml in patients with CKD comparing with healthy group. Also in the same Figure showed significant($p< 0.05$) decrease in the concentration of EPO2.051±0.083ng/mlin patient with CKD comparing with healthy group.



***Statistically significant differences (P < 0.05) between patient and Healthy**
Figure 1: Change in the Concentration of biomarker in patients with CKD

Effect of some chemical parameter on patients of the Chronic kidney disease

In the table (1) showed significant increase($p< 0.05$) in the concentration of urea and creatinine in patients with chronic kidney disease , also showed significant ($p< 0.05$) decrease in glomerular Filtration Rate(GFR) in patients with chronic kidney disease.

Table 1: Effect of some chemical parameter on patients of the Chronic kidney disease

Parameter	Chronic kidney disease patients (Mean±SE) N=68	Healthy group (Mean ±SE) N=20
Urea (mg/dl)	158.7± 6.328*	35.85±1.510
Creatinine(mg/dl)	8.059 ± 0.3334*	1.850± o.1138
GFR mL/min per 1.73 m^2	7.897±0.4430*	99.60±3.826

***Statically significant difference ($p< 0.05$).**

Effect of some physiological parameter for blood on patients of the Chronic Kidney Disease

Table (2) revealed significant($p< 0.05$) decrease in the levels of Hemoglobin, Red Blood Cell, Platelet, Total White Blood Cell and Lymphocytes, but no significant ($p< 0.05$) in the Neutrophils, Eosinophils, Basophils, Monocytes in patients have chronic kidney disease

Table 2:Effect of some physiological parameter for blood on patients of the Chronic Kidney Disease.

Parameter	Chronic kidney disease patients (Mean± SE)N=68	Healthy group (Mean ±SE)N=20
Hb(g/dl)	**8.006±0.1801***	**13.10±0.4184**
RBC×10^6cell/ml	**3.299±0.07711×10^6***	**5.243±0.1341×10^6**
Platelet×10^3cell/ml	**150.1±4.775***	**253.2±13.45**
WBC (cell/ml)	**7.460± 0.5032×10^3**	**8.098 ±0.2714 ×10^3**
Lymphocytes(cell/ml)	**1.693 ±0.1129×10^3 ***	**2.688 ± 0.1560×10^3**
Neutrophils(cell/ml)	**4.390±0.3890×10^3**	**4.657 ± 0.2643×10^3**
Eosinophils(cell/ml)	**0.2426± 0.03906×10^3**	**0.2011 ± 0.03528×10^3**
Basophils(cell/ml)	**0.08892± 0.01447×10^3**	**0.07265±0.005837×10^3**
Monocytes(cell/ml)	**0.6297± 0.07817×10^3**	**0.6794±0.04256×10^3**

***Statically significant difference ($p< 0.05$).**

In the present study result showed significant ($p< 0.05$) Negative correlation between NGAL and EPO, but showed significant($p<0.05$) positive correlation between NGAL and ADPN,also in the study showed significant($p< 0.05$) positive correlation between Hemoglobin and EPO.As well showed significant($p< 0.05$) positive correlation between NGAL and creatinine and positive ($p< 0.05$) correlation between ADPN and creatinine(figure 2).

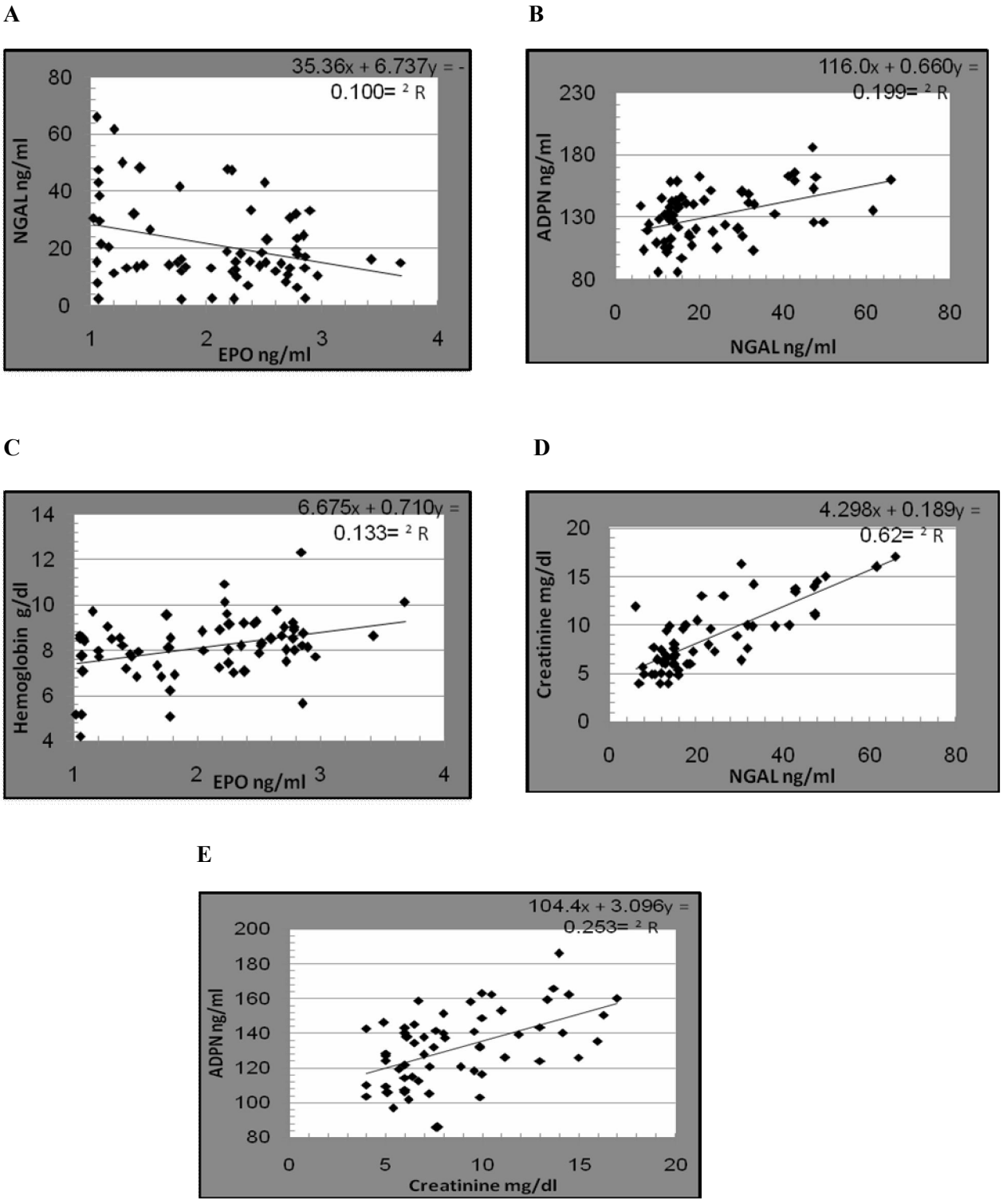


Figure 2: Correlation between some biomarkers (A- NGAL & EPO ,B- ADPA &NGAL, C- Hemoglobin & EPO, Creatinine & NGAL, and ADPN & Creatinine) in patients with Chronic Kidney.

Discussion

In this study was showed significant increase in the NGAL in patients of CKD relative to the control group .This result agreement with previous studies[10,17,18]. NGAL expressed from the tubular epithelial cell and tubular epithelium of the distal nephron after damage, therefore increase NGAL in the urine and serum reflect to the kidney disease[19]. Another study was found increase in the expression of NGAL in the present inflammation and injured epithelia for this considered the NGAL is one of the first proteins produced in the kidney after nephrotoxic insult or ischemic[20]. They were present correlation between NGAL with the progression of renal function deterioration and severity of renal damage[21]. In study revealed the positive correlation between the NGAL and serum creatinine in CKD.The concentration of NGAL was increased in serum and urine with ongoing renal damage[22]. Neutrophil gelatinase associated lipocalin was used to identified renal damage, detection primary site of renal damage, able to distinguish AKI from chronic kidney disease [23]. Numerous current studies were showed role of NGAL in chronic kidney disease and detection the severity of the CKD, serum level of NGAL correlated rather better than cystatin C with lower value of GFR[24 ,17] but some studies suggested that used NGAL as biomarker in acute kidney injury[25].

From the result there is significant increase of adiponectin concentration in the patients of chronic kidney disease relative to the control group. This result agreement with previous studies[26,27,28,29]. Several studies showed the causes to increase concentration of ADPN in unclear, but suggested decrease of renal clearance, adiponectin resistance, with an abnormal receptor-ligand interaction and increase production of the ADPN causes high level of adiponectin in the plasma[30,31]. Hemodialysis patients have high level of ADPN this leads to the decrease in mortality; therefore the correlation between adiponectin and the cardiovascular events was inversely[32]. In current study indicated present positive correlation between creatinine and ADPN because level of ADPN decrease after successful kidney transplant.Therefore using adiponectin as a marker of CKD[33]. NGAL was evaluated in patients of CKD[17] and also Adiponectin evaluated in patients of CKD[27], therefore in the study showed present significant positive correlation between NGAL and ADPN that suggested using this biomarker to diagnosis the CKD. This result agreement with another study[34].

In the study result was showed significant decrease in the EPO concentration in patients of CKD, this agreement with other studies[35] EPO production impaired in patients of kidney disease therefore cause erythropoietin deficiency[36,37,38]. Cells responsible for secretion of EPO in the kidney is called renal Epo-producing (REP) cells, this cells present in the interstitial space between renal tubules transform into myo-fibroblastic cells, because this ability to transformed, the REP cells associated with renal fibrosis in the inflammatory condition and miss to secretion EPO in hypoxic[39]. Inflammatory cytokines was produced from the activation T-lymphocytes such as IFN-gamma, TNF-α, this cause inhibit secretion of EPO from the kidney, therefore impair growth of erythroblasts and promote death by damage erythroblasts[40,41]. Other studies explanation the decrease in the blood flow to the renal cause reduced in the activity of tubular transport system, consequently the level of oxygen in renal was remain constant to allow stable of the kidney. Therefore EPO secretion dependent on alteration the level of oxygen and independent on the difference in renal blood flow ,this lead to decrease in secretion of EPO[42,43]. In the study revealed positive correlation between hemoglobin and EPO in patients of CKD, this agreement with another study was showed normal inverse correlation between EPO and hemoglobin level therefore when hemoglobin level decrease the EPO level rise ,but in patients with CKD was positive correlation between hemoglobin level and EPO[44]. EPO releasing from the REP cells in tubular[45] and NGAL releasing from the damage tubules[19]. Therefore in the study shown negative correlation between EPO and NGAL, when decrease in EPO produced increase in the NGAL from this result shown can be detected the progressive of anemia from these markers.

From the result there is significant rise in the urea and creatinine concentration in the serum patients of CKD relative to the control in. this result agreed to the other studies[46,47]. Some studies were explanation the essential solute eliminated by renal considered blood urea and serum creatinine, also revealed the urea was initial organic solute identified in the blood of patients with CKD[48,49]. Increase in the urea and creatinine level occurs in CKD patients because the kidney loses ability to eliminate nitrogenous wastes from the blood results in accumulation of these substances in the blood. Other reasons of increase urea and creatinine in the blood from the excessive protein intake, shock, gastrointestinal hemorrhage etc. could also contribute to this[50]. In this study was showed a significant decrease in hemoglobin concentration in patients relative to the control group. It is signs to the anemia in the CKD. many of studies showed that anemia was the most common complication of advanced chronic renal disease .This result was agreed with previous studies[51,52,53]. Reason of anemia in

chronic kidney disease is deficiency of the kidney to produce erythropoietin hormone, it is responsible to the erythropoiesis in the bone marrow[12]. Uremia toxic accumulation in the blood is considered another cause of aneamia in the CKD[54]. Iron deficiency may consider secondary reason of anemia in chronic kidney disease[55]. Also deficiency in the folic acid and B12 vitamin and Hyperparathyroidism causes anemia[56].

This study showed a significant decrease in Red blood cell count in patients relative to the control group, this result agreed to the other study[57]. The major causes of the RBC count decrease are deficiency to produced erythropoietin from the kidney which causes suppression in the erythropoiesis[58]. Also shorten RBC life spin lead to the lower RBC count, shorted in the life spin of the RBC result from the uremia lead to the rises the expression of phosphatidylserine in red blood cells on the outer cell surface ,this enhance RBC damage by macrophage therefore decrease survival of cell[59].

In this study was indicated significant decrease in platelets in patients comparative to the control group leading to the bleeding also result from the platelet dysfunction, this result agreed to the other studies[58,60,61]. The decrease of the platelets due to the acidosis in the blood in patients of chronic kidney disease, the increase in the acidosis in the blood lead to the decrease in the protein synthesis and increase in the protein catabolism in the body, some studies was showed present positive correlation between bicarbonates and platelets[62,63]. Thrombopoietin dysfunction was one causes of decrease platelets count that it responsible to regulated thrombopoiesis and megakaryocyte in the bone marrow[64,] while other studies suggested the megakaryocyte number in bone marrow is normal, thrombopoiesis is reduced, but elevated thrombopoietin levels[65,66].

In this study has been show slight decrease in the total White blood cell in patient of chronic kidney disease comparative to the control group and also showed decrease in the lymphocytes in the patients comparative to the control group, this agreed to the previous studies[58,67]. Another study was showed the decrease of White blood cell due to the membrane of dialysis, after exposure the blood to the membranes leading to the activation of the complement. The complement is typically C3a or C5a, when complement activation was lead to neutrophil accumulation and adherence to endothelial surface after that reduction in WBC count[68]. Several immune abnormalities may be contributed to impaired immunity in CKD patients have been low number of T-lymphocytes, B-lymphocytes and natural killer cell (NK) in CKD patients, abnormal T-lymphocytes function in dialyzed patients[69].

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