Relationship between C-Reactive Protein, Differential Leukocyte Count and Uric Acid in Kidney Disease in Erbil Teaching Hospital.

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ABSTRACT

The study purpose was to evaluate the relationship between CRP, differential leukocyte count and uric acid in people with and without chronic kidney disease (CKD). A case-control study carried out at kidney disease unite, Erbil teaching hospital, from June 2013 till November 2013. Patient with diagnosed CKD being included with regular follow-up with no evidence of infection at time of interview by history, clinical examination and investigations including renal function testing, serum uric acid, CRP, general urine examination and other relevant investigations. The control group in general population were apparently healthy evaluated by the same way. There was significant reduction (p<0.001) in lymphocyte level (12.52±0.94) compared to control group (26.0±0.84) and significant increase was observed for CRP (10.56±0.61 ng/mL) in CKD patients compared with the control group (3.36±0.69 ng/mL) at the level (p<0.001). Serum uric acid was significantly high in patients with CKD (7.05±0.205 mg/mL) when compared to controls (4.3±0.27 mg/mL) at the level (p<0.001). Lymphocyte count showed significant reduction (p<0.001) (12.93±0.86) and (13.46±0.84) compared to control group (25.2±1.45) and (26.35±0.7) respectively, while other differential leukocyte count show no significant difference when compared with control group. CRP negatively and significantly correlated with lymphocyte level (-0.626), while serum CRP level positively correlated with neutrophil level (0.547) and monocyte level (0.291). Also serum uric acid negatively and significantly correlated with lymphocyte level (-0.626), and positively correlated with neutrophil level (0.450) and monocyte level (0.236). Compared with those without CKD, patients with CKD have more eosinophil and granulocytes, CRP, uric acid and fewer lymphocytes. Hyperuricemia is a valuable predictor of a GFR decrease, early detection and prevention on hyperuricemia in CKD subjects are critical.
1. INTRODUCTION

Chronic kidney disease (CKD) embraces a spectrum of different pathophysiologic processes associated with abnormal kidney function and a progressive descent in glomerular filtration rate (GFR). There are two mechanisms of damage in the pathophysiology of CKD: either mechanisms related to the underlying cause (e.g., genetically determined abnormalities in kidney development or integrity, immune complex deposition and inflammation in certain types of glomerulonephritis, or toxin exposure in certain diseases of the renal tubules and interstitium) and second a set of progressive mechanisms, including hyper filtration and hypertrophy of the remaining viable nephrons, that are a common consequence following long-term reduction of renal mass, irrespective of underlying etiology (Longo et al., 2012).

One common marker used to clarify systemic inflammation is the plasma level of CRP, an elevation in CRP also increases the risk for progression of kidney disease in subjects with CKD (Pietro et al., 2009).

Among the components involved in the innate immune inflammatory processes are short pentraxins such as CRP. The systemic acute-phase reactant CRP is liberal in the liver after induction by interleukin-6 (Lavin–Gómez et al., 2011).

An association between serum uric acid and kidney disease is biologically comprehensible. A recently developed rat model of mild hyperuricemia observed mild uric acid elevations, even within the normal limits, can cause hypertension and renal microvascular disease without causing urate crystal deposition in kidneys (Cain et al., 2010).

Studies also suggest that uric acid is an active player in modes related to cardiovascular disease (CVD) and kidney disease development like inflammation, endothelial dysfunction and oxidative stress (Corry et al., 2008).

Hyperuricemia is usually defined as serum uric acid levels >7.0 mg/dL in male and >6.0 mg/dL in female. Uric acid, is produced from metabolism of purine, is degraded in most mammals by the hepatic enzyme called urate oxidize (uricase) to allantoin, then it is freely excreted in the urine. Following filtration, uric acid undergoes both reabsorption and secretion in the proximal tubule, and this process is mediated by a urate/anion exchanger and a voltage-sensitive urate channel. The levels of uric acid also differ significantly within humans as the result of factors that increase generation (e.g: high purine or protein diets, alcohol consumption, conditions with high cell turnover, or enzymatic defects in purine metabolism) or decrease excretion. Dropping in glomerular filtration rate (GFR) boosts the quantity of the serum uric acid, although a significant compensatory boost in gastrointestinal excretion happens. Hyperuricemia also may result from increased tubular absorption. Organic anions such as lactate minimize urate secretion through competing for urate via the organic anion transporter (Busuioc et al., 2007).

Automatic measurement of complete blood count is commonly performed for the routine evaluation of patients with and without CKD. Patients with CKD, especially in advanced stages of kidney disease, have anemia and also much attention has been devoted to its assessment and management. However, the pattern of total and differential leukocyte count has been poorly studied, yet this evaluation may be of critical importance to evaluate the relationship between the differential leukocyte count and CRP in people with and without CKD. Patients with CKD are also displayed to inflammatory disease such as pneumonia, bacteremia, or urinary tract infections. These inflammatory illnesses may promote the
evolution of kidney disease. Otherwise, inflammation of the kidney may by itself encourage progression to ESRD. Whether such a relationship between inflammatory episodes and CKD exists is unknown (Agarwal and Light, 2011).

The purpose of this study is to comparatively evaluate the levels and patterns of leukocyte count, bursts in inflammatory activity (CRP) and serum uric acid in patients with and without CKD.

2. Material Methods

2.1. Preparation and Examination of the Peripheral Blood Smear Differential Leukocyte Count:

Blood smears are prepared with EDTA anticoagulant blood no later than three hours after collection. The stained blood smears were examined with a microscope with an oil-immersion lens. One hundred cells were counted in each slide. The leukocyte types are described according to the morphological nomenclature proposed by (Hawkey and Dennet, 1994) in which the percentage of every white cell recorded including; Neutrophile, Eosinophile, Basophile, Lymphocyte, and Monocyte (Troiano et al., 1999).

2.2. Determination of CRP by ELISA

This assay uses the quantitative sandwich enzyme immunoassay technique specific for CRP (Quantikine® ELISA-USA & Canada/ R&D Systems, Inc.). C-reactive protein was determined and measured by using enzyme linked immunosorbent assay (Bang et al., 2005).

2.3. Determination of Serum Uric Acid

Serum uric acid evaluated by using the Kit (BIOLABIO laboratories- France). Uricase acts on uric acid to produce allantion, carbon dioxide & hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase acts with a chromogen to yield quinoneimine, a red coloured complex. The absorbance measured at 520 nm is proportional to the amount of uric acid in the specimen (Tietz, 2006).

2.4. Statistical Analysis

The results of this study are presented as mean values ± standard error (Mean ± SE) and all statistical analyses were performed using the Statistical Package for Social Science program (SPSS for Windows, version 11.5). A two sample T-test (Unpaired-sample comparison) was performed for comparisons of parameters between the two groups (CKD patients and Control subjects), but for evaluation of the correlation between parameters the Pearson correlation method was used.

3. RESULTS AND DISCUSSION

The results presented in table (1) showed significant reduction (p<0.001) in lymphocyte level (12.52± 0.94) compared to control group (26.0±0.84).

Significant increase was observed for CRP (10.56±0.61 ng/mL) in CKD patients compared with the control group (3.36±0.69 ng/mL) at the level (p<0.001).

Serum uric acid was significantly high in patients with chronic kidney disease (7.05±0.205 mg/mL) when compared to controls (4.3±0.27 mg/mL) at the level (p<0.001).
Table 1: Blood parameter changes in patients with chronic kidney disease when compared with controls at ages 10-30 years old groups, (Mean ± S.E).

| Parameters        | Patient N= 27 | Control N= 8 | Statistical Evaluation |
|-------------------|--------------|--------------|------------------------|
| Neutrophil (%)    | 81.63± 1.01  | 68.64±0.93   | N.S                    |
| Lymphocyte (%)    | 12.52± 0.94  | 26.0±0.84    | P≤ 0.001               |
| Monocyte (%)      | 3.48±0.41    | 2.4±0.28     | N.S                    |
| Eosinophil (%)    | 1.78±0.19    | 1.45±0.21    | N.S                    |
| Basophil (%)      | 0.63±0.11    | 0.64±0.203   | N.S                    |
| CRP (ng/mL)       | 10.56±0.61   | 3.36±0.69    | P≤ 0.001               |
| Uric acid (mg/mL) | 7.05±0.205   | 4.3±0.27     | P≤ 0.001               |

Patients with CKD showed none significantly increased granulocytes and significantly decreased lymphocytes. These results are similar to that of Rajiv and Robert (2011). The present study demonstrates that the baseline leukocyte count differs between patients with CKD and control. One study has reported that among the elderly, low lymphocyte levels are associated with elevated IL-6 and C-reactive protein levels, low levels of erythropoietin, and anemia (Ferrucci et al., 2007). Patients with CKD are often inflamed, and low levels of lymphocytes are shown. In fact, a progressive decrease in renal function is associated with activation and selective loss of T cells and CD4_ cells and a marked increase in CD8_ cells (Litjens et al., 2006).

Tables (2 and 3) showed significant increase in CRP and uric acid level in CKD patients when compared to controls at the level (p< 0.001). Lymphocyte count showed significant reduction (p<0.001) (12.93± 0.86) and (13.46± 0.84) compared to control group (25.2±1.45) and (26.35± 0.7) respectively, while other differential leucocyte count show no significant difference when compared with control group.
Table 2: Blood parameter changes in patients with chronic kidney disease when compared with controls at ages 31-50 years old groups, (Mean ± S.E).

| Parameters       | Patient N= 29 | Control N= 10 | Statistical Evaluation |
|------------------|---------------|---------------|------------------------|
| Neutrophil (%)   | 80.07±1.13    | 70.67±1.6     | N.S                    |
| Lymphocyte (%)   | 12.93±0.86    | 25.2±1.45     | P≤ 0.001               |
| Monocyte (%)     | 4.03±0.38     | 2.33±0.21     | N.S                    |
| Eosinophil (%)   | 2.17±0.21     | 1.5±0.22      | N.S                    |
| Basophil (%)     | 0.63±0.089    | 0.33±0.21     | N.S                    |
| CRP (ng/mL)      | 10.13±0.57    | 2.2±0.4       | P≤ 0.001               |
| Uric acid (mg/mL)| 8.64±0.48     | 4.22±0.29     | P≤ 0.001               |

Table 3: Blood parameter changes in patients with chronic kidney disease when compared with controls at ages more than 51 years old groups, (Mean ± S.E).

| Parameters       | Patient N= 23 | Control N= 13 | Statistical Evaluation |
|------------------|---------------|---------------|------------------------|
| Neutrophil (%)   | 80.62±1.07    | 69.17±0.49    | N.S                    |
| Lymphocyte (%)   | 13.46±0.84    | 26.35±0.7     | P≤ 0.001               |
| Monocyte (%)     | 3.67±0.4      | 2.52±0.23     | N.S                    |
| Eosinophil (%)   | 1.92±0.25     | 1.53±0.12     | N.S                    |
| Basophil (%)     | 0.41±0.103    | 0.47±0.12     | N.S                    |
| CRP (ng/mL)      | 11.04±0.57    | 3.06±0.63     | P≤ 0.001               |
| Uric acid (mg/mL)| 8.03±0.345    | 4.33±0.24     | P≤ 0.001               |

N.S. = non significant , P<0.001 = highly significant

In the present study, serum CRP was increased in CKD patients as compared with control group and these results are similar to results of the study done by (Lavín–Gómez et al., 2011) and (Fox et al., 2010).

The immune system actively participates in the development of vascular disease. The early stages of atherosclerosis are characterized by infiltration of inflammatory cells into the vascular wall, with the involvement of innate immunity factors. CRP (short pentraxins) and pentraxin 3 (PTX3), which is long pentraxins are the components included in the innate immune inflammatory process. After induction by interleukin 6, the systemic acute-phase reactant CRP is released in the liver. The main functions of PTX3 are closely related to those of CRP, but the PTX3 protein is produced at a locoregional level by many kinds of cells (endothelial dendritic cells, smooth muscle...
cells, fibroblasts, neutrophils, monocytes, and macrophages, and also kidney proximal tubular epithelial and mesangial cells). Taken together, these data showing elevations in innate immune and inflammatory markers suggest that, because of the decline in eGFR, CKD patients are in a proinflammatory state that should be intensively treated to prevent undesirable cardiovascular events (Lavín–Gómez et al., 2011). So one proposed explanation for higher CRP concentrations with CKD is that there is diminished filtration of CRP in end-stage renal disease (Westhuyzen and Healy, 2000).

Table (4) showed that CRP negatively and significantly correlated with lymphocyte level (-0.626), while serum CRP level positively correlated with neutrophil level (0.547) and monocyte level (0.291).

In table (5) the correlation between serum uric acid and differential leucocyte count was present. The study observed that serum uric acid negatively and significantly correlated with lymphocyte level (-0.530), and positively correlated with neutrophil level (0.450) and monocyte level (0.236).

| Parameters | Neutrophil | Lymphocyte | Eosinophil | basophil | Monocyte |
|------------|------------|------------|------------|----------|----------|
| CRP        | 0.547**    | -0.626**   | 0.069      | 0.045    | 0.291**  |
| P-value    | 0.001      | 0.46       | 0.63       | 0.001    |

** Correlation is significant at the 0.001 level (2-tailed).
* Correlation is significant at the 0.01 level (2-tailed).
Correlation is non significant at the level > 0.1 (2-tailed).
The immune system actively participates in the development of vascular disease. The early stages of atherosclerosis are characterized by infiltration of inflammatory cells into the vascular wall, with the involvement of innate immunity factors. CRP (short pentraxins) and pentraxin 3 (PTX3), which is long pentraxins are the components included in the innate immune inflammatory process. After induction by interleukin 6, the systemic acute-phase reactant CRP is released in the liver. The main functions of PTX3 are closely related to those of CRP, but the PTX3 protein is produced at a locoregional level by many kinds of cells (endothelial dendritic cells, smooth muscle cells, fibroblasts, neutrophils, monocytes, and macrophages, and also kidney proximal tubular epithelial and mesangial cells). Taken together, these data showing elevations in innate immune and inflammatory markers suggest that, because of the decline in eGFR, CKD patients are in a proinflammatory state that should be intensively treated to prevent undesirable cardiovascular events (Lavín–Gómez et al., 2011).

So one proposed explanation for higher CRP concentrations with CKD is that there is diminished filtration of CRP in end-stage renal disease (Westhuyzen and Healy, 2000). Beyond being a marker of reduced glomerular filtration rate, serum UA level is associated with a faster progression of chronic kidney disease.

There are also evidences showing that hyperuricemia seems to induce high blood pressure, renal afferent arteriopathy, a rise in glomerular hydrostatic pressure, and renal scarring. Iseki et al., 2004 reported that a high level of serum uric acid was more predictive for the development of renal dysfunction than proteinuria. This suggests that a high level of uric acid is a valuable predictor of a GFR decrease.

Recent clinical and epidemiological studies have found that hyperuricemia was associated with the mortality and development of hypertension, cardiovascular diseases, and chronic renal diseases, and hyperuricemia can induce pathological restructure of vessels and vascular nephrosclerosis (Duk and Wei, 2011). Soluble uric acid has important biologic roles such as pro-inflammatory and proliferative effects on vascular smooth muscle cells, induction of the dysfunction of endothelial cells in rats and hyperuricemia may induce systemic inflammation and generation of oxidative stress (Corry et al., 2008). Our research results may explain the discovery that uric acid and CRP correlated in the study.

The present study showed that both CRP and uric acid levels negatively and significantly correlated with lymphocyte level, while correlated positively with neutrophil level and monocyte level.

Uric acid induced the proinflammatory cytokine, monocyte chemotactic protein-1 and de novo expression of CRP in vascular smooth muscle and endothelial cells, which was further shown to be due to direct entry of uric acid into cells with activation of mitogen activated protein kinase and nuclear transcription factor. In addition, uric acid can become pro-oxidative under certain circumstances. The prooxidative effects are primarily mediated by intracellular uric acid and can be shown in endothelial cells, vascular smooth muscle cells, renal tubular cells, adipocytes, and cardiac fibroblasts (Yu et al., 2008).

There is increased inflammatory activity with concomitant activation of the acute-phase response in patients with CKD. It has been suggested that the acute-phase reaction seen in kidney disease and failure. Several mechanisms have been postulated to explain this phenomenon, including induction of cytokine
release by an interaction of mononuclear cells (Vandana et al., 2003).

One potential consequence of the systemic inflammatory response in chronic kidney disease is the development of malnutrition. The impact of cytokines on nutritional status via inducing anorexia and reduced food intake, as well as by modulating protein catabolic rate. Accumulating evidence suggests an association between acute-phase proteins that are indicators of inflammation and alteration in leukocyte number especially lymphocytes and monocytes in the kidney failure. Peripheral blood leukocytes are composed of polymorphonuclear cells, including monocytes as well as lymphocytes (Matthew et al., 2008).

Polymorpho- and mononuclear leukocytes can be activated through advanced glycation end products, oxidative stress, angiotensin II, and cytokines. The release of cytokines, such as tumor necrosis factor, transforming growth factor, superoxide, nuclear factor B, monocyte chemoattractant protein 1, interleukin-1, may be activates Leukocytes and others to participate in the pathogenesis of renal complications (Fu et al., 2005).

The study showed an increase in uric acid level in CKD patients when compared to controls. Recent research has highlighted the pathogenic role of uric acid (UA) in renal and cardiovascular disease.

4. CONCLUSIONS

We conclude that peripheral lymphocyte counts are negatively associated with CRP and uric acid. Serum uric acid levels may provide some clues into the role of peripheral lymphocytes in CKD.

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Conflict of Interest

There is no conflict of interest.

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