Evaluation of IL-6 and TNFα in Iraqi Patients with Acute Renal Failure

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ABSTRACT

Background and objective:-

Acute kidney injury (AKI) is estimated to occur in about 20–200 per million
population in the community, 7–18% of patients in hospital, and approximately
50% of patients admitted to the intensive care unit (ICU) Importantly, AKI is
associated with morbidity and mortality; an estimated 2 million people
worldwide die of AKI every year. we aimed to evaluated IL-6 & TNFα in Iraqi
patients with acute renal failure.

Methods:- This study included 60 cases and classified as 30 patients with acute
renal failure. these patients with age range (15 years and above) who were
admitted to the Kadimyiah Teaching Hospital, Baghdad Teaching Hospital, Ibn
Al-Bitar center for cardiac surgery during the period from December 2020 to
March 2021 and compared with 30 apparent healthy controls with age range
(15 years and above). They were clinically diagnosed under the supervision of
consultant committee. Laboratory diagnosis was depended on using
spectrophotometry for detection biochemical tests. IL-6 & TNFα levels were
estimation by Enzyme Linked Immuno-sorbent assay (ELISA).
Results:- The current study showed that majority of patients with acute renal failure within age group above 60 years which account (43.3%) and most of these patients were females (53.3%). Furthermore preponderance diseases associated with acute renal patients were heart disease which comprised (50%) of these cases. The statistical analysis showed highly significant increase in serum levels of urea and creatinine in AKI patients as paralleled with healthy groups (P≤0.001). Moreover, the result refers to Highly significant increase in serum levels of IL-6 & TNFα in AKI patients as paralleled with healthy groups (P≤0.001).

Conclusion: Highly significant elevated of pro-inflammatory cytokines which include IL-6&TNFα in acute renal diseases indicated these cytokines participate in the pathophysiology of reduced renal function and role of these cytokines as principle mediators of inflammatory reaction in renal damage and these cytokines could be potential therapeutic targets.

keywords : IL-6 ; TNFα ; Iraqi Patients ;Acute Renal Failure.

INTRODUCTION

Acute kidney injury (AKI) is a multiphasic disease that represents a global concern. The onset of AKI increases the risk of developing chronic kidney disease (CKD) or end-stage renal disease (ESRD). Furthermore, CKD affects up to 16% of the global population and pre-existing CKD significantly increases the risk of new AKI episodes.( Mehta et al., 2017; Chawla et al., 2014).

Acute kidney injury (AKI) in hospitalized patients is associated with unacceptably high mortality rates (in the range of 30% to 50% in most recent series for dialysis-requiring AKI).( Hoste, &Schurgers ,. 2008; Lameire et al., 2005).
At present, therapies for AKI are limited to supportive care, such as dialysis. A number of major impediments exist to developing therapies for AKI. First, biomarkers that diagnose AKI before an increase in serum creatinine are needed (Zhou et al., 2006; Waikar & Bonventre., 2007). Because serum creatinine is a marker of glomerular filtration rate and therefore of established AKI, substantial kidney injury may have occurred by the time serum creatinine increases. Second, the pathogenesis of AKI in humans is complex and involves the endothelial and epithelial cell compartments, as well as inflammatory cells. Finally, AKI may have a detrimental impact on other organs, particularly the lung. (Rabb et al., 2001; Faubel ., 2008).

However, considering that AKI is also an inflammatory process, determination of the proteins involved in its pathogenesis may provide unique tools for its assessment and prediction before considerable renal damage has been established. Interleukins (ILs) are inflammatory cytokines believed to exercise a fundamental role in the pathophysiology of AKI. (Nechemia-Arbely et al., 2008). In patients with established AKI, serum interleukin (IL)-6, IL-8, IL-1β, IL-10 and tumor necrosis factor-α (TNF-α), were increased. (Kathleen et al., 2009; Simmons et al., 2004).

Interleukin-6 (IL-6) is an inflammatory cytokine that is elevated in the plasma soon after an insult and peaks while still in the acute phase of the critical illness. (Remick et al., 2002; Nylen & Alarifi ., 2001). Serum level of IL-6 is associated with clinical outcome or organ dysfunction severity in critically ill patients. (Oda et al., 2005; Shimazui et al., 2017; Quispe et al., 2016) and may be useful for predicting the development of AKI. (Zhang et al., 2015; Liu et al., 2009).

Tumor necrosis factor (TNF-α) is one of an important proinflammatory cytokine and essential factor of inflammatory tissue injury which release by
doi:10.4708/jphr.32.3.3

Dendritic cells (DCs) in the renal interstitium. It has important immune regulatory functions. Most researchers reported a role of TNF in acute and chronic renal disease pathogenesis. Thus, after renal injury the early proinflammatory mediator is TNF-α. (Singbartl et al., 2019). Serum levels of TNF-α may be useful as an early biomarker to predict severity of AKI. (Fatani et al., 2018).

AKI can lead to CKD and if unchecked can lead to end-stage renal disease (ESRD). So the purpose of this investigation is to outline some of the evidence linking immune and inflammatory mechanisms to the progression of renal disease and may be reach to therapies that have anti-inflammatory or immunosuppressive effects in the treatment of renal disease by evaluation of some cytokines include IL-6, TNFα in sera of patients with acute renal failure.

**MATERIALS AND METHODS**

The current study included 30 patients with acute renal failure, these patients with age group (15 years and above) who were admitted to the Kadimyiah Teaching Hospital, Baghdad Teaching Hospital, Ibn Al-Bitar center for cardiac surgery during the period from December 2020 to March 2021. These patients were diagnosed as having acute renal failure based on previous medical reports, laboratory tests and clinical examination by consultant nephrologists. The results of those patients were compared with (30) healthy subjects with age group 15 years and above as a control group. The control group subjects were selected as healthy individuals without a history of kidney disease, current or previous kidney stones and not suffering from diabetes mellitus or hypertension depending on previous medical reports and laboratory investigation. Venous blood sample (5ml) were taken from each patient and healthy individuals. We collected serum according to the technique low speed centrifugation at 3000 × g at 4°C, for 15 min. The serum was
removed, aliquoted and stored at -20 °C until time of assay. We used this serum for detection of biochemical parameters include (urea, creatinine, sodium, potassium and calcium) in addition for cytokines analysis which include IL-6, TNFα.

Laboratory diagnosis was depended on using auto-analyzer known as Cobas c 311 , Germany for estimation serum urea and creatinine while serum calcium, potassium, sodium were measured by using auto-analyzer known as Beckman coulter au480, USA. in addition IL-6, TNFα were identified by ELISA test, Human reader, Germany. Methods was conducted according to the instructions of manufacturing companies leaflet.

Data analysis: In this study Chi-square test was used to detect the significances between variables. All the statistical analysis was done by SPSS program (version-20 ). Data was presented as Mean ± Stander error. P-value was considered significant when < 0.05.

RESULT

The current study involved 60 samples divided into two groups (30 acute renal failure and 30 healthy apparent controls). Age of the study population ranged from 15-90 years. the acute renal failure patients can be seen most clearly by looking at the age frequency which monitor the peak distribution in acute renal failure under investigation which represented (43.3%) were distributed among age intervals above (60) years whereas (40%) of the cases found in age intervals (40-60) years and (16.7%) of them were distribution in age group (under 40) years. There are highly significant differences between the incidences of the different age groups (P<0.001) among acute renal patients as shown in Table (1).

In same table the study groups were categorized according to gender in acute 16 (53.3%) were female and 14 (46.7%) male. According to gender there was
non significance difference at (p=0.107) . also this table demonstrated other diseases associated with study groups which included that highest frequency of acute 15 (50%) was among heart diseases, followed by diabetic mellitus (DM) 10 (33.3%) and hypertension (HT) 5 (16.67%).

Table (1) :- Distribution of study groups according to clinical characteristics (Age, gender and other diseases).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute group</td>
<td>NO</td>
<td>%</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Under 40</td>
<td></td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>40-60</td>
<td></td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>Above 60</td>
<td></td>
<td>13</td>
<td>43.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.0095**</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>14</td>
<td>46.7</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>16</td>
<td>53.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.1073 NS</td>
<td></td>
</tr>
<tr>
<td>Other Diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetic mellitus (DM)</td>
<td></td>
<td>10</td>
<td>33.3</td>
</tr>
<tr>
<td>Hypertension (HT)</td>
<td></td>
<td>5</td>
<td>16.67</td>
</tr>
<tr>
<td>Heart Diseases</td>
<td></td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>30</td>
<td>99.9</td>
</tr>
</tbody>
</table>

In present study regarding acute renal group, results of biochemical tests in comparing with control group involved mean urea for acute was (157.18± 16.548) but mean urea for control (28.35±1.42), mean creatinine for acute was (5.42±0.76) whereas mean creatinine for control (0.668±0.03), mean S.K for
acute was (4.77±0.27) while mean S.K for control (4.21±0.08), mean S.Ca for acute was (9.11±0.08) then mean S.Ca for control (19.22±0.10), mean S. Na for acute was (138.71±0.41) but mean S. Na for control (139.12±0.89). The results found highly significance of urea and creatinine at (p=0.001), while the other biochemical tests there were non significant as shown in Table (2).

**Table (2) : Determination of biochemical tests between Acute and Control groups.**

<table>
<thead>
<tr>
<th>Studied Groups</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urea</td>
</tr>
<tr>
<td>Acute group</td>
<td>157.18 ±16.548</td>
</tr>
<tr>
<td>Control group</td>
<td>28.35 ±1.42</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001**</td>
</tr>
</tbody>
</table>

** (P≤0.01) Highly Significant , NS= Non significant

Results of IL-6 level between acute group and control group were seen in Table (3) which appear that mean for acute group was (73.34±4.56) but mean for control group was (13.02±0.34) Statistically, Highly significant difference at (p=0.0001).while the results of TNF-α in both groups included mean for acute group was (167.14±29.42) whereas mean for control was (66.55±0.45) which revealed that Highly significance difference at (p=0.0036). **figures (1) , (2) confirmed this data.**

**Table (3) : Estimation of cytokines in the sera of acute renal patients in comparison with control group.**
<table>
<thead>
<tr>
<th>Studied Groups</th>
<th>Mean ± SE</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IL-6</td>
<td>TNF-α</td>
<td></td>
</tr>
<tr>
<td>Acute group</td>
<td>73.34 ±4.56</td>
<td>167.14 ±29.42</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>13.02 ±0.34</td>
<td>66.55 ±0.45</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001 **</td>
<td>0.0036 **</td>
<td></td>
</tr>
</tbody>
</table>

** (P≤0.01) Highly Significant.

Figure (4-1) :- Estimation of IL-6 levels between acute and control groups
Our study showed that The overall correlation between cytokines, biochemical testes (urea, creatinine, S.K, S.Ca and S.Na) were listed in the Table (4) below. This table revealed that there no significant association between the cytokines and the parameters previously mentioned among acute renal group.

**Table (4) : Correlation between Cytokines and biochemical parameters among Acute renal patients.**

<table>
<thead>
<tr>
<th>Pearson Correlation</th>
<th>Biochemical parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urea</td>
</tr>
<tr>
<td>IL-6</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>C/S</td>
</tr>
<tr>
<td>TNF-α</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td>C/S</td>
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</tbody>
</table>

*R*= Pearson correlation coefficient, C/S= correlation significant
DISCUSSION

Acute kidney injury (AKI) is estimated to occur in about 20–200 per million population in the community (Tao et al., 2013; Chawla, 2014). In the current study, the peak of age frequency of acute renal failure group was found in age group more than (60) years which constituted (43.3%) as shown in the table (1) this results agree with (Libin et al., 2021) which revealed a positive relationship between the AKI incidence and age among the older adults aged 75 years or older. However, the incidence of AKI was higher in the elderly patients. Possible explanations for this include depleted nephron reserves, increased susceptibility to exposure to nephrotoxic agents, impaired ability to repair injuries, polypharmacy, and multiple comorbidities, specifically CKD (Khan et al., 2017; Sullivan et al., 2017; Rosner et al., 2009). Furthermore, elderly individuals are susceptible to severe infection and are therefore more likely to develop septic acute tubular necrosis (ATN) and experience mortality (Rosner et al., 2018).

In a previous study, age was an independent predictor of mortality risk in AKI patients (Bouchard et al., 2015) On the contrary, some studies have reported that age was not an independent risk factor for mortality in AKI patients >60 years of age (Yokota et al., 2017; Silveira et al., 2018) The mortality rate of elderly patients with AKI has been reported to be 18%–61% (Yokota et al., 2017, Kayatas et al., 2014) this wide range could be due to the heterogeneity of the study populations, availability of a critical care unit, standard of care administered, or research methodology used.

Our investigation shown that The AKI group included more females (53.3%) than male (46.7%) as seen in table (1) which is consistent with the trends in previous study (Wanjak et al., 2020).
The association between heart failure (HF) and renal dysfunction is well known; while HF increases the risk of renal insufficiency, chronic kidney disease (CKD) increases the risk of hospitalization and mortality (Smith et al., 2006).

Our observation shown that AKI group was associated with (50%) heart diseases as shown in table (1). this results appeared some extent compatibility with other study (Jose et al., 2020). Which demonstrated that (44.7%) of AKI related to heart failure. another study by Damman et al., 2014 which detected that 50% of heart failure patients had reduction in glomerular filtration rate (GFR).

However, it was found that 20-30% incidence of AKI take place during treatment for heart failure (Forman., 2004; Smith., 2003). this outcomes less than our study. the broad ranges in prevalence of CKD and AKI among heart diseases are related to varying definitions, differences in the observed time at risk, and the heterogeneity of selected populations being studied.

It is now sufficiently clear that renal dysfunction occurs frequently in all phenotypes of HF, and when present, it is associated with higher mortality and morbidity. The cause of renal dysfunction is multifactorial, but reduced renal perfusion and venous congestion are prominent factors (Kevin and Jeffrey, 2015).

Multiple studies have shown that diabetes alone is an independent risk for acute kidney injury (AKI) (Saran et al., 2017; James et al., 2015) the incidence of AKI was found to be higher in diabetic patients undergoing surgery (Hertzberg et al., 2015), taking certain medications(Oliveira et al., 2009), with sepsis/septic shock (Venot et al., 2015) and even without precipitating events. (Girman et al., 2012; Mittalhenkle et al., 2008). In this respect, the present study shown that (33.3%) of AKI group had diabetic mellitus as noted in table (1). This results agree with study by (Prabhu et
al., 2021) which concluded that incidence of AKI was 31.1% in patients with diabetic kidney diseases (DKD). Study by (Meda et al., 2018) which revealed that acute kidney injury is a sudden decrease in kidney function with or without kidney damage, occurring over a few hours or days in Diabetes and heart conditions.

Pavkov et al., (2018) who demonstrated that persons with diabetes accounted for ~40% of all AKI hospitalizations, with absolute increases over time in AKI hospitalizations being larger among persons with diabetes than among persons without diabetes. In a retrospective study of 94 patients admitted to an intensive care unit with severe diabetic kidney ketoacidosis (DKA) 47 patients (50%) presented with AKI on admission (Orban et al., 2014) . this study disagree with our data . The variations in the incidence between our and the other study can be explained by differences in sample size, study design.

Hypertension is a frequent finding in both acute and chronic kidney disease, particularly with glomerular or vascular disorders (Bakris & Ritz., 2009). Our data had been detected that (16.6%) of acute renal patients group had hypertension as noted in table (1). This results appeared some extent compatibility with other study(Chi-yuan et al., 2016) which demonstrated that AKI was associated with 22% of hypertension . in contrast , study by Seon et al., 2019 who shown that (30.1%) of AKI had hypertension. Furthermore , it has been found that prevalence of hypertension was 85% in post-renal AKI followed by renal AKI 75% and pre-renal AKI 30% (Magdalena et al., 2019). This variation between our study and these studies may be related to difference in sample size, geographical distribution, genetic factor.
The current study shown that mean of urea and creatinine increase among AKI group in comparing with control group as seen in Table (2) . this results compatible with other study (Charles., 2008) which demonstrated that increase Serum creatinine and blood urea nitrogen (BUN) have typically been used to diagnose AKI . furthermore Ho et al., (2012) who showed that measurement of serum creatinine, within 6 h of completion of cardiac surgery, significantly improved the prediction of AKI .

In addition Keren et al., 2017 who detected that a rise of >15% in serum creatinine  over baseline, taken within 2 hours of arrival to ICU for the development of AKI and he recommended early serum creatinine accurately predicts acute kidney injury post cardiac surgery. a progressive daily rise in serum creatinine  is diagnostic of AKI and Urea nitrogen may increase but BUN may be misleading because it is frequently elevated in response to increased protein catabolism resulting from surgery, trauma, corticosteroids, burns, transfusion reactions, parenteral nutrition, or gastrointestinal or other internal bleeding. (Anna Malkina., 2020).

However , our investigation revealed that mean of S. K increase while mean of S. Ca and S. Na decrease in AKI patients when comparing with control group as seen in table (2). These observation in line with other studies (Gianmarco ., 2021; Achim et al., 2018) were confirmed that Electrolyte disturbances are common in patients with acute kidney injury (AKI) and should be corrected. In particular, hyperkalaemia above 6–6.5 mmol/L (especially with electrocardiogram changes) constitutes a medical emergency and warrants immediate intervention. Both hypo- and hypernatraemia may which observed that hyponatremia had been found in acute renal failure patients. it has been suggested that normal ranges of serum sodium and potassium levels using in clinical sittings are determined
mainly based on healthy subjects, whether these values are applicable to AKI patients with complex comorbidities is still unknown (Polcwiartek et al., 2018).

According to previous reports (Xu et al., 2019; Peres et al., 2015) more AKI patients suffered the hyponatremia (Na+ < 135 mmol/L) than hypernatremia (Na+ > 145 mmol/L) (36% vs 6%) when admitted into ICU. These results similar with our data. However, electrolyte disturbances are common disorders in the hospitalized population. Serum sodium (Na) imbalance is frequently observed in the hospital setting. Dysnatremia conditions (including hyponatremia [Na < 135 mEq/L] and hypernatremia [Na > 145 mEq/L]). As the main organ involved in water metabolism and homeostasis, the kidney is generally the main culprit for such disorders. Defective urine dilution with disproportionately high water intake causes hyponatremia. On the other way around, disorders involving urine concentration with inadequately low water intake cause hypernatremia. Therefore, it is not surprising that kidney diseases, especially acute kidney injury (AKI), characterized by an abrupt reduction in renal function, are commonly associated with these pathological conditions. (Gianmarco, 2021).

Also study by Charat., (2020) who identified that hypercalcemia occur in AKI and arise via several mechanisms such as volume depletion from polyuria (decreased collecting duct cells response to vasopressin) and direct alterations of intravascular tone. Besides, persistent hypercalcemia can lead to calcium deposits in the kidneys or nephrocalcinosis. These findings dissimilarity with our data may be attributed to the difference in sample size, methodology, normal range in different laboratories in multiple countries.
Cytokines play important roles in the inflammatory response and may induce organ dysfunction when released in excess. (Cohen., 2002) . The current study we analyze the pro-inflammatory cytokines of IL-6 ,TNFα in AKI patients . we found that level of IL-6 were higher in AKI patients than control group with highly significant difference between them as documented in table (3) . Ours results were in accordance with previous findings (Takashi et al., 2019; Yael Nechemia-Arbel et al., 2008). These studies found that AKI severity was significantly higher in the group with a higher IL-6 level and determined the Serum IL-6 level was also associated with renal recovery in survivors. Our work revealed that an IL-6–mediated inflammatory response contributes in part toward the generation of renal injury. Study by Min et al., 2018 which identified in adult cardiac surgery patients, increased plasma IL-6 levels were associated with AKI development, and the risk of AKI increases with increasing IL-6 levels. Moreover, Serum level of IL-6 is associated with clinical outcome or organ dysfunction severity in critically ill patients. (Shimazui et al., 2017) and may be useful for predicting the development of AKI . (Zhang et al., 2015).

In same table our results shown that highly significant elevated in TNFα among AKI in comparing with control group . these result agree with previous study (Cristina et al., 2019). which described that AKI patients exhibited significant higher levels of TNFα in plasma as compare with non-AKI patients. also similar finding were find by Lin Sun et al., 2015 who detected that TNF-α causes renal injury in the diabetic mellitus.

In this regard, a number of mechanisms have been proposed by which TNF-α may induce renal injury directly or indirectly via the elaboration of other inflammatory cytokine. TNF-α may stimulate the production of endothelin-1, leading to dysregulation of vascular tone resulting in reduced intrarenal blood flow and glomerular filtration rate. It may also disrupt

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endothelial intercellular junctions and thus adversely affect the integrity of the glomerular filtration barrier, thereby causing an increase in its permeability and albuminurria. In addition, TNF-α may also have direct cytotoxic effects on glomerular podocytes and tubular cells and thus may induce apoptosis and cell death (Lin et al., 2015). However, our data shown non significant correlation between cytokines and biochemical tests among AKI patients as seen in table (4). these results agreement with other study (Liliane et al., 2020).

CONCLUSION

Our conculsion stated that acute renal patients were prevalent among those Iraqi females with age above 60 years. elevated Pro- inflammatory cytokines (IL-6,TNFα) in acute renal diseases indicated these cytokines participate in the pathophysiology of reduced renal function and role of these cytokines as principle mediators of inflammatory reaction in renal damage and these cytokines could be potential therapeutic targets. so we recommended further studies with large Iraqi samples to shed light and confirm the role of cytokines (IL-6,TNFα) in acute and chronic renal patients.

REFERENCES


5- Charat Thongprayoon, Wisit Cheungpasitporn, Api Chewcharat. Impact of admission serum ionized calcium levels on risk of acute kidney injury in hospitalized patients. Scientific Reports. 2020; Volume 10, Article number: 12316


9- Chi-yuan Hsu, Raymond K. Hsu, Jingrong Yang, Juan D. Ordonez, et al. Elevated BP after AKI. JASN March, 2016; 27 (3) 914-923.


