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Exploring The Role Of Inflammatory Mediators In The Patients Of Acute Kidney Injury Undergoing Haemodialysis.

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ABSTRACT

Acute kidney injury (AKI) is a critical condition characterized by a sudden decline in renal function with significant morbidity and mortality. The objective of the study was to assess the differential status of inflammatory markers like plasma Neutrophil gelatinase-associated lipocalin (NGAL), Tumor necrosis factor -alpha (TNF-α), and Interleukin-10 (IL-10) in AKI patients before and after haemodialysis. In the present study 300 participants were recruited. Fifty were Controls without AKI (Group- I) while 250 were AKI patients. These 250 AKI patients were further divided in Group II (Before Dialysis), Group III (After First Dialysis), Group IV (after Second dialysis), Group V (after third dialysis) & Group VI (after fourth dialysis) containing 50 each. Inflammatory markers were estimated by ELISA method. A significantly increased levels of inflammatory markers were observed in AKI patients before dialysis, whereas NGAL level is significantly decreased, after dialysis. Plasma TNF- α levels were significantly decreased after second dialysis, but after Groups III, V, and VI show particularly high TNF-α levels. The plasma IL-10 levels were increased after haemodialysis as compared to pre-dialysis. The findings of the study provided insight regarding the inflammatory response. The evaluation of decreased NGAL levels after dialysis coupled with elevated TNF-α and IL-10 activity may help in assessing progression of AKI. Evaluation of these biomarkers may provide useful prognostic information also predict long-term kidney outcome and mortality.

Keywords: Acute Kidney Injury (AKI), Neutrophil gelatinase-associated lipocalin (NGAL), Tumor necrosis factor -alpha (TNF-α), Interleukin-10 (IL-10)

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INTRODUCTION

Acute kidney injury (AKI) is characterized by a rapid decline in kidney function within hours, which encompasses both injury and impairment of renal function. AKI is not considered a pathological condition of single organ failure, but a syndrome in which the kidney plays an active role in the progression of multi-organ dysfunction [1].

The prevalence of AKI in the population is estimated to be as high as 5–7% [2]. The incidence of AKI in the intensive care unit (ICU) is significantly higher affecting approximately 25% of patients and is associated with a high mortality rate of 50–80%. In a recent multinational study involving nearly 30,000 critically ill patients, found that 5.7% developed AKI requiring renal replacement therapy with a strikingly high mortality rate of 60.3% [3]

Acute Kidney Injury (AKI) risk factors have been associated with exposure related factors and susceptible related factors, susceptibilities are generally shared risk factors such as certain demographic and genetic predisposition, whereas exposure related factors are specific patient-related risk factors. such as septic shock, cardiac surgery, radiocontrast agents, nephrotoxic drugs and liver transplantation etc [4]. Changes in kidney function are detected by a change in biomarkers, The most common biomarker being serum creatinine (SCr). Serum creatinine is an imperfect biomarker for recognizing AKI, given that an increase in SCr often lags (48–72 hours) behind the onset of injury. In addition, SCr is not in a steady-state condition in critically ill patients, leading to inaccurate estimates of glomerular filtration rates (eGFRs). Using an imperfect biomarker for AKI definition, recognition, and management may affect patient outcomes. Despite improvements in renal replacement therapy (RRT), AKI outcomes are not optimal [5].

Hence, Kidney injury biomarkers are needed to improve early AKI detection and will likely replace SCr in the definition and staging of AKI. Kidney injury biomarkers, including Neutrophil Gelatinase-Associated Lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1), TNF -Alpha (TNF- α), Interleukin-18 (IL-18), Interleukin-10 (IL-10), Liver-type Fatty Acid Binding Protein (L-FABP), Insulin-like growth Factor Binding Protein 7 (IGFBP-7), and Tissue Inhibitor of Metalloproteinase-2 (TIMP-2), may be elevated before SCr increase, enhancing the detection of kidney damage without functional change (Murray 2014; Haase 2012). The combination of damage and functional biomarkers may enhance the detection, differential diagnosis, and subsequent management of AKI. Neutrophil gelatinase-associated lipocalin NGAL) is a 25-kDa protein from the lipocalin family. It is up-regulated after ischemic or nephrotoxic AKI, is detected in the urine 3 hours post-injury, and peaks 6 hours post-injury. Injury to the kidney is mitigated by NGAL through the inhibition of apoptosis and increased proliferation of renal tubule cells. [6]

Pro-inflammatory cytokines like Tumor necrosis factor- α (TNF- α), play a role in coordinating the inflammatory response by stimulating the synthesis of other proinflammatory cytokines, adhesion molecules, and anti-inflammatory cytokines, particularly Interleukin-10 (IL-10) that inhibits the secretion of IL-1β, TNF-α,and IL-6, thus regulates pro-inflammatory cytokines production. The balance between pro- and anti- inflammatory cytokines affects the clinical outcome of various inflammatory conditions including AKI [7]. Neutrophil gelatinase-associated lipocalin (NGAL) has emerged as a promising biomarker for early detection of AKI, reflecting tubular injury. Furthermore, the dysregulation of inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) plays a crucial role in the pathogenesis of AKI. As TNF-α and IL10 are considered inflammatory response modulators. Thus the relationship between NGAL, TNF-α, and IL-10 in AKI patients compared to healthy individuals remains to be elucidated. The current study sought to assess some markers in AKI patients before and after dialysis and a control group. This investigations was carried out for acquiring the better understanding of the biochemical changes linked to AKI with different dialysis stages, possibly identifying prognostic values and enhancing patient outcome.

MATERIAL AND METHODS

The present study was carried out in the Department of Biochemistry, Dr. Vasantrao Pawar Medical college, Hospital and Research Center Nashik. In this case-control study 300 subjects were included. Out of these 300 subjects, 50 healthy volunteers (Group I) were included as controls, and 250 AKI, patients were selected from department of Medicine, on the basis of different stages of dialysis.

Control Group I: 50 Normal healthy persons.

Study Group: 250 patients of AKI undergoing dialysis. These subjects will be sub-divided into following groups.

Group II: 50 AKI Patients before dialysis.
Group III: 50 Patients undergoing first dialysis.
Group IV: 50 Patients undergoing second dialysis.
Group V: 50 Patients undergoing third dialysis.
Group VI: 50 Patients requiring more than three dialysis stages,

Inclusion criteria: Subjects adults (>18 years) of either gender from Acute kidney injury.

Exclusion Criteria: AKI with Diabetes mellitus, Coronary heart diseases Chronic kidney diseases, Multiple organ failure patients, Cancer and Patients with Severe Acute Respiratory Syndrome (SARS) patients were excluded from the study.

The study was approved by institutional ethics committee (Ref. Inward IEC-15/2016-17, Dated 17/06/2016). Informed consent was obtained from all the participants prior to their inclusion in the study. Data confidentiality and privacy were ensured throughout the research process. After obtaining informed consent**,**10 ml antecubital venous blood sample were collected after all aseptic precautions, using sterile needles and syringes, from healthy volunteers and AKI patients of five study groups. Samples were allowed to clot at room temperature in a clean dry sterile plain and EDTA bulb for 45 min and then centrifuged for 15 minutes at 3000 rpm. Sample were stored in two separate aliquots at -20^{0}C until further analysis. Separated sample were used for estimation of NGAL, TNF-alpha and IL-10.Plasma levels of NGAL, TNF-α, and IL-10 were estimated by using enzyme-linked immunosorbent assay (ELISA) methods.

Statistical analysis

The statistical analysis was performed by using SPSS ,version 21.0 software package of social science program . The data was expressed in terms of mean ±SD. Comparison among groups were performed using the one-way ANOVA test.

RESULTS

Table 1: Shows plasma levels of NGAL, TNF-α and IL-10 in healthy control group I compared to AKI cases

Biochemical Parameter	Control (Group I) Mean ±SD N=50	AKI patients before dialysis (Group II) Mean ±SD, N=50	T test	P Value
NGAL	65.460 ± 40.34	576.15 ± 290.7	12.43	<0.0001
TNF α	11.05 ± 7.56	120.9 ± 108.1	7.25	<0.0001
IL10	1.25 ± 1.21	19.9 ± 15.47	8.60	<0.0001

Significant value(p<0.0001)

Table 2: Shows Plasma NGAL, TNF α and IL- 10 level before and after dialysis stages.

Parameters	Group II Before Dialysis	Group III After First Dialysis	Group IV After second Dialysis	Group V After Third Dialysis	Group VI After Fourth Dialysis	Significance
Serum NGAL level (Mean ± SD)	576.15 ± 290.7	632.3 ± 229.3	545.5 ± 220.66	317.7 ± 146.2	225.58 ± 159.5	< 0.0001
Serum TNFα level (Mean ± SD)	120.9 ± 108.1	144.6 ± 132.7	90.67 ± 67.34	155.9 ± 98.95	175.84± 98.7	< 0.0001
Serum IL-10 level (Mean ± SD)	19.9 ± 15.47	25.1 ±15.9	20.6 ± 15.19	17.3 ± 11.6	27.89 ± 19.4	< 0.0001

Significant value (p<0.0001)

Table no. 1 Shows, plasma levels of NGAL, TNF-α and IL-10 in healthy control (group I) compared to AKI patients before dialysis (group II.)

The plasma levels of NGAL, TNF-α and IL-10 were significantly elevated in AKI patients compared to healthy controls ($p < 0.0001$). This analysis revealed positive correlations between inflammatory markers and AKI severity, as well as adverse clinical outcomes such as mortality and need for haemodialysis dependence.

Table 2 presents, Plasma NGAL, TNF α and IL- 10 level before and after dialysis stages.

The mean NGAL (Neutrophil Gelatinase-Associated Lipocalin) levels across different patient groups. It is the significantly elevated in AKI patients (Groups II-VI) compared to the control group (Group I), as evidenced by the highly significant p-value ($p < 0.0001$). This indicates that NGAL is a strong marker for acute kidney injury. Additionally, there is a general trend of decreasing NGAL levels with increasing dialysis sessions, suggesting that dialysis may help reduce NGAL levels and potentially improve kidney function. However, even patients requiring multiple dialysis sessions (Group VI) still have significantly higher NGAL levels than controls, highlighting the potential for persistent kidney damage even with treatment.

Mean TNF-α (Tumor Necrosis Factor-alpha) levels across all AKI patient groups (II-VI) compared to the controls (Group I), as evidenced by the low p-value ($p < 0.0001$). This suggests that TNF-α plays a role in the inflammatory response associated with acute kidney injury. Interestingly, there is no clear linear relationship between TNF-α levels and the number of dialysis sessions. Groups III, V, and VI show particularly high TNF-α levels, potentially indicating persistent inflammation despite dialysis.

Mean IL-10 (Interleukin-10) levels across all AKI patient groups (II-VI) compared to the controls (Group I), evidenced by the low p-value ($p < 0.0001$). This suggests the involvement of IL-10, an anti-inflammatory cytokine, in the response to acute kidney injury. Notably, while there is a general trend of elevated IL-10 in AKI patients, the levels do not consistently decrease with more dialysis sessions. Group VI, with patients requiring extended dialysis, even shows the highest mean IL-10 level.

DISCUSSION

Acute Kidney Injury (AKI) receives inadequate attention from many clinicians which can lead to serious consequences, due to the reversibility of the condition evidenced by the higher level of serum creatinine in many patients. Most of the cases of AKI may be at greater risk of long-term sequelae due to the development of permeant renal injury that affects renal microvascular structure and function, thus augmenting the risk of chronic kidney disease. In addition, AKI confers prolonged hospital stay and increased morbidity, mortality and progression to chronic kidney diseases ,by increasing the risk of diseases. Therefore, measurement of these inflammatory mediators are very important in AKI patients because these markers can tell about progression of the renal injury.

In the present study, we found that NGAL (Neutrophil Gelatinase-Associated Lipocalin) levels across different patient groups is significantly elevated in AKI patients (Groups II-VI) compared to the control group (Group I), as evidenced by the highly significant **F** statistic and p-value ($p < 0.0001$). This indicates that NGAL is a strong marker for acute kidney injury. Additionally, there's a general trend of decreasing NGAL levels with increasing dialysis sessions, suggesting that the response of the patient towards the treatment. It is observed that patients requiring multiple dialysis sessions (Group VI) still have significantly higher NGAL levels than controls. This highlights the potential for persistent kidney damage even with treatment. This shows the utility of NGAL as a diagnostic and prognostic biomarker in acute kidney injury, particularly in monitoring treatment response and identifying patients at risk for long-term complications The finding of our study align with those of Cruzet et al [2], who demonstrated that plasma NGAL can accurately confirmed the diagnosis of AKI upto 48 hours before a clinical diagnosis can be made based on established AKI definitions. According to Catherina et al [8]. children who underwent cardiopulmonary bypass and developed AKI, had a three-fold increase in plasma NGAL levels. However. rise in serum creatinine was delayed by two to three days. Research conducted by Constantine and his team [9] reveled that serum NGAL levels were elevated in patients admitted to the ICU, suggesting that NGAL may be a valuable biomarker for identifying patients at risk of AKI or other critical illness. Hemodialysis (HD) patients with residual renal function have significantly lower serum

NGAL concentrations compared to anuric individuals. Thus, NGAL levels can provide insight into the degree of kidney function deterioration in patients with end-stage renal disease (ESRD). In the group of patients treated with HD, apart from renal dysfunction, low-grade inflammation, which is more intensified in anuric individuals, can contribute to increased serum NGAL level. Thus, NGAL can act as an inflammatory marker in HD patients, especially when hemodialyzed with the use of CVPC, especially in those who are mostly susceptible to infections [10]. Inflammatory conditions trigger the release of proinflammatory cytokines such as TNF-α, which plays a crucial role in modulating the immune response and serving as a key messenger in the inflammatory process. The markedly elevated TNF-α levels in different dialysis stages compared to controls underscores the role of TNF-α as a key inflammatory marker in acute kidney injury. It must also be taken into account that adsorption of cytokines occurs mainly in the first minutes of HD and may not reflect a substantial amount of cytokines removal. and HD membranes may increase cytokines production by activating mononuclear cells [11]. This pro-inflammatory response is followed by compensatory increase in the production of anti-inflammatory mediator including IL-10, that inhibits production TNF-α, thus regulates the release of proinflammatory process and facilitate tissue repair [12]. The discovery of elevated TNF-α concentrations in haemodialysis patients aliens with previous research showing that TNF-α plays a significant role in the inflammatory response associated with haemodialysis. The finding of increased serum TNF-α, levels during the haemodialysis session, is in agreement with the result by Bukan *et al* who also observed a similar rise in serum TNF-α before and after dialysis [13]. The subsequent research suggest that , the elevated levels of TNF-α in haemodialysis patients may be due to uraemia as impaired renal function may be one of the major factors associated with increased serum levels of TNF-α. Uremia -dependent factors may selectively influence the secretion of TNF-α with damaged cells [14]. These findings suggest that renal mesangial cells could be responsible for the increased TNF-α production observed in patients with end-stage renal failure. Alternatively, a possible role of increased TNF-α levels in the development of renal impairment before dialysis should be considered. In fact, some authors have recently shown that TNF-α infused in rats can induce metabolic acidosis and acute renal failure [15]. Moreover, Rhee, McGoldnck and Meuwissen [16]. reported the presence of a neutrophil stimulatory reduced oxygen species (ROS) factor in serum from non-dialyzed uremic patients and suggested that it is specifically associated with renal dysfunction. Interestingly, during the first dialysis session TNF-α maintained its initial level. These findings may suggest that repeated dialysis sessions and/or accumulation of dialysis-related factors are necessary to induce or to amplify the secretion of these cytokines [13] along with this, Insulin resistance, volume overload and obesity have been proposed as important causes of elevated TNF-α levels in haemodialysis [17]. High concentration of pro-inflammatory cytokines have been reported to correlate with the prognosis [18].

It has been found that IL-10 is an anti-inflammatory cytokine plays a very important role in AKI caused by different aetiologies [19]. In our study, IL10 levels were significantly higher in dialysis patients than the healthy controls, these increased levels, indicating a potential role of IL-10 in the inflammatory response to dialysis. Numerous studies have consistently reported elevated level of IL10 observed in dialysis patients seems to be a counterregulatory mechanism to control chronic inflammation associated with uremia and the dialysis technique. Tang et al [20], reported that IL-10 containing extracellular vesicles significantly (EVs) improved tubular damage and inflammation associated with ischemia / reperfusion injury and effectively reduced the transition to chronic kidney disease. In addition, Andres-Hernando A et al [21] showed that IL-10 can suppress inflammatory response at both systemic and local levels. caused by AKI. Thus, confirming the role of IL-10 in antagonizing inflammatory response and in AKI renal protection. These studies suggest that IL-10 could be associated with renal tubular cell death and renal inflammatory response in AKI progression, thereby preventing the conversion of AKI to CKD. Therefore, IL-10 could serve as a potential therapeutic target for AKI [19].

Therefore, we proposed these biomarkers of AKI, which may predict long-term kidney outcome and mortality and prognostic interventions, and the prediction of the need for dialysis.

CONCLUSION

The findings of the present study demonstrate new insight on the status of inflammatory markers in AKI patients. The elevated plasma levels of Neutrophil gelatinase-associated lipocalin (NGAL), Tumor necrosis factor alpha (TNF-α), and Interleukin-10 (IL-10) in AKI patients compared to healthy individuals, suggesting an inflammatory response and renal injury mechanisms in AKI. Neutrophil gelatinase-associated lipocalin (NGAL) emerges as a promising biomarker for AKI severity and prognosis. Further

research is warranted to elucidate the intricate interactions between NGAL, TNF-α, and IL-10 in AKI pathogenesis and explore their potential as therapeutic targets. In haemodialysis patients, increased levels of inflammatory markers usually predict a poor outcome.

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