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The Role of Nitric Oxide Synthase Inhibitor: ADMA in the Patients of Acute Kidney Injury Undergoing Haemodialysis

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ABSTRACT
Acute kidney injury (AKI) is characterized by a rapid decline in kidney function and is associated with significant morbidity and mortality. Nitric oxide (NO) and its endogenous inhibitor, asymmetric dimethylarginine (ADMA), play critical roles in renal physiology and pathophysiology. This study investigated the effects of haemodialysis on NO and ADMA levels in AKI patients. This case-control study included 300 participants: 50 healthy controls and 250 AKI patients undergoing haemodialysis. AKI patients were categorized into five groups based on dialysis stage (before dialysis, after first, second, third and fourth dialysis). Plasma ADMA and NO concentrations were measured using ELISA. NO levels were significantly elevated in AKI patients compared to controls ($p<0.0001$) and showed dynamic changes across different dialysis stages, with the highest levels observed before dialysis. ADMA levels were also significantly elevated in AKI patients ($p<0.0001$) but did not show a consistent pattern of reduction during haemodialysis. Haemodialysis effectively reduces plasma NO concentrations in AKI patients, suggesting its potential utility in removing excess NO. However, ADMA clearance appears to be less efficient. The persistent elevation of ADMA despite haemodialysis may have implications for vascular health and kidney disease progression. Monitoring NO and ADMA levels during haemodialysis could provide valuable insights into treatment response and disease prognosis.

INTRODUCTION

Acute kidney injury (AKI) is a silent crisis in healthcare, contributing to significant morbidity and mortality worldwide. This complex clinical disorder is characterized by a sudden decline in kidney function, often with reduced urine output (oliguria), developing rapidly over hours or days. AKI frequently affects hospitalized and critically ill patients with potentially devastating consequences. Although some individuals regain kidney function, many face long-term dialysis dependence or irreversible renal impairment[1]. Alarmingly, AKI occurs in 5% of all hospitalized patients and its associated mortality rate ranges from 25% to over 90%[2]. For those with severe AKI requiring dialysis, the mortality rate exceeds 50%[3]. Despite extensive research, a recent meta-analysis of over 700 studies revealed that the prognosis for AKI has not shown significant improvement[4]. This highlights the urgent need for a deeper understanding of the pathophysiology of AKI and the development of more effective therapeutic strategies[5,6]. In the pursuit of earlier AKI detection and outcome prediction, researchers have investigated various plasma and urinary biomarkers[7]. These biomarkers have shown promising sensitivity and specificity in diagnosing AKI and may even help predict short-and long-term outcomes, including mortality and the need for dialysis[8]. While these biomarkers hold great potential for early diagnosis and risk stratification, their ability to predict recovery after established AKI requires further investigation. This highlights the need for continued research to identify biomarkers that can accurately assess the potential for renal recovery. In addition to these biomarkers, researchers are exploring the roles of various vasoactive factors and cytokines in AKI pathogenesis[9,10], aiming to uncover further insights into the complex mechanisms underlying this condition. Nitric oxide (NO), a recognized vasodilator and cardio-protector, is an endogenous molecule produced by many cells through the action of nitric oxide synthase (NOS). NOS converts L-arginine to NO and L-citrulline. NO plays a vital role in maintaining renal health by regulating blood flow, glomerular filtration and renin secretion[11]. However, under the oxidative stress often present in AKI, excessive NO production or impaired antioxidant defences can lead to the formation of reactive oxygen species (ROS), potentially contributing to kidney damage[12]. Among the endogenous inhibitors of nitric oxide synthase (NOS) are methylated arginines such as N -monomethyl-L-arginine (L-NMMA) and asymmetric dimethylarginine (ADMA)[13]. ADMA is an intracellular amino acid analog formed by post-translational methylation of arginine.While ADMA is normally present in plasma, urine and various tissues, it is primarily cleared by the kidneys through metabolic conversion to citrulline and dimethylamine, catalyzed by dimethylarginine dimethylaminohydrolase (DDAH). However, by inhibiting NOS, ADMA can impair NO synthesis, contributing to endothelial dysfunction and decreased vessel elasticity. Importantly, ADMA levels are markedly elevated in chronic kidney disease (CKD) and end-stage renal disease (ESRD)[14]. ADMA has emerged as a potential biomarker predicting higher mortality and faster progression of kidney injury in CKD[15], suggesting its potential relevance in the context of AKI. This study investigates the dynamic interplay between NO and ADMA in the context of AKI and hemodialysis. We aim to determine whether hemodialysis effectively removes excess NO and ADMA from the circulation in patients with AKI. Furthermore, we will explore the relationship between NO and ADMA levels at different stages of hemodialysis to better understand their roles in AKI pathophysiology and recovery. Materials and Methods This case-control study, approved by the Institutional Ethics Committee (IEC-15/2016-17), was conducted at the Department of Biochemistry, Dr. Vasantrao Pawar Medical College, Hospital and Research Centre, Nashik. A total of 300 participants were enrolled, including 50 healthy controls (Group I) and 250 AKI patients undergoing haemodialysis. were recruited from the Department of Medicine and categorized into five groups based on dialysis stage: before dialysis (Group II), after first dialysis (Group III), after second dialysis (Group IV), after third dialysis (Group V) and after fourth dialysis (Group VI).

Inclusion and Exclusion Criteria: Inclusion criteria were adults (>18 years) with AKI. Exclusion criteria were AKI with comorbidities such as diabetes mellitus, coronary heart disease, chronic kidney disease, multiple organ failure, cancer and severe acute respiratory syndrome (SARS).

Sample Collection: After obtaining informed consent, 10 ml of antecubital venous blood samples were collected from all participants following aseptic precautions. Samples were allowed to clot and then centrifuged. Separated plasma and serum were stored at -20°C until analysis.

Biochemical Analysis: Plasma ADMA and Nitric oxide concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) method.

Statistical Analysis: Statistical analysis was performed using SPSS version 21.0. Data were expressed as mean±standard deviation (SD). One-way ANOVA was used to compare groups. Pearson's correlation coefficient was used to assess correlations between NO and ADMA levels.

RESULTS AND DISCUSSIONS

It shows statistically significant increases in the serum NO and ADMA levels in the AKI patients, as compared to those in the healthy controls ($p<0.0001$). Serum NO

Table 1: Biochemical Parameters (No and ADMA) of Control Group Compared to AKI Group

Biochemical Parameter	Control Group (Group I) Mean±SD N=50	AKI group (Group II) Mean±SD N=50	T test	p Value
Nitric Oxide (NO)	35.86±26.27	95.25±37.44	9.24	<0.0001
Asymmetric Dimethyl arginine (ADMA)	0.50±0.31	3.52±1.82	11.66	<0.0001

Table 2: Shows Serum Nitric Oxide (NO) and Asymmetric Dimethyl Arginine (ADMA) Level Before and After Haemodialysis Stages

Parameters	Before Dialysis (Group II)	After First Dialysis (Group III)	After second Dialysis (Group IV)	After Third Dialysis (Group V)	After Fourth Dialysis (Group VI)	T test	P value
Serum NO level (Mean±SD)	95.25±37.43	43.11±40.27	38.20±43.39	43.92±44.00	24.06±22.46	-2.672	0.0001
plasma ADMA level (Mean±SD)	3.52±1.82	3.36±1.59	3.34±1.34	4.22±1.66	3.48±1.88	-26.632	0.0001

and ADMA showed a significant positive the AKI patients (Tables 1/Fig-1 and 2). (Table no. 2) shows plasma levels of NO and ADMA before and after dialysis. It was observed that serum NO levels was significantly increased before dialysis. while decreases significantly after first and second dialysis and rose significantly after third dialysis. Concentration of NO in AKI patients after fourth hemodialysis was significantly lower in relation to first to third dialysis stages.

Plasma Levels of NOS Inhibitor: ADMA was significantly higher in AKI before HD, as compared to control group. It was observed that after first, second and fourth dialysis, the plasma level of ADMA is slightly decreased. And increased levels were seen after third dialysis stage. There was no appreciable fall in the plasma ADMA, despite the subjects being on regular sessions of dialysis.(Tables 2 /Fig 1 and 2).

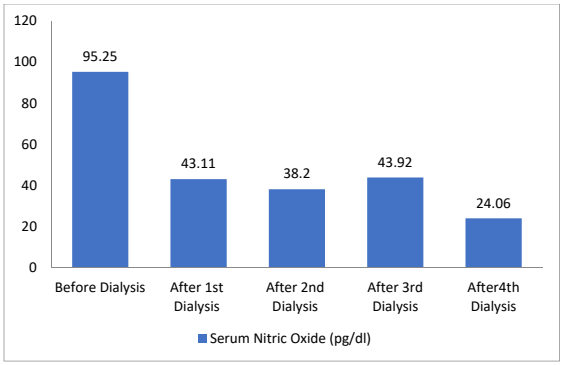

Fig 1: Serum Nitric Oxide Levels Before and After Dialysis

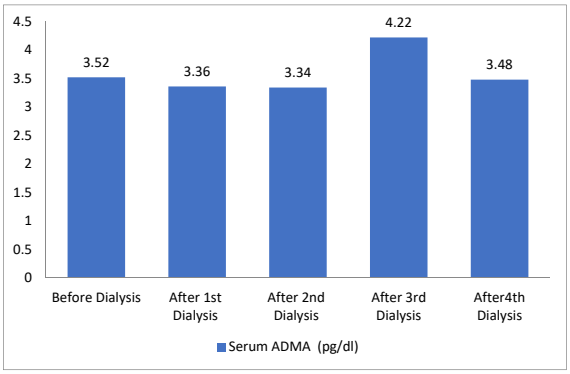

Fig. 2: Plasma ADMA Levels Before and After Dialysis

Plasma concentrations of NO were greater in pre-HD patients (P<0.001) versus controls, reflecting in (Table 1 and fig 1). Plasma NO levels decreased significantly and were longer different from values immediately post-HD (fig. 1), post-HD remained slightly elevated versus normal values. Plasma levels of the NOS inhibitor, ADMA, were elevated approximately 3 times in pre-HD plasma versuscontrols (Table 1). Plasma ADMA values were not significantly less in HD patients versus controls (Table 2) and did not expected change during HD (fig 2), whereas pre-HD plasma ADMA concentrations were high versus controls (P<0.001., Table 2).

This study provides compelling evidence that haemodialysis significantly reduces serum nitric oxide (NO) levels in patients with acute kidney injury (AKI). This finding supports our hypothesis that haemodialysis can effectively remove excess NO from the circulation in AKI. While serum NO levels were markedly elevated in AKI patients before haemodialysis, we observed a significant decrease in NO concentrations following the first and second dialysis sessions. Interestingly, NO levels showed a resurgence after the third dialysis, before declining again after the fourth session. This dynamic pattern suggests a complex interplay between NO production, clearance and the haemodialysis process itself. In addition to the dynamic changes in NO, we also observed significantly elevated levels of asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor, in AKI patients compared to healthy controls. Although ADMA levels showed some fluctuations during the haemodialysis sessions, there was no consistent trend of significant reduction, indicating that ADMA clearance by haemodialysis may be less efficient than that of NO. Our observation of decreased NO levels during haemodialysis is consistent with the findings of Mårtensson[16], who reported a similar reduction in plasma NO concentration during HD. They also noted that NO was generated within the dialyzer, possibly due to enhanced cytokine production by cells stimulated during the HD procedure[16]. This local NO production within the dialyzer could contribute to the observed fluctuations in NO levels throughout the haemodialysis sessions. Although NO is a small, defeasible molecule that can be cleared by dialysis, its dynamics in AKI patients appear to be more complex

than simple elimination. Several factors likely contribute to the observed fluctuations in NO levels. These may include increased endogenous NO production due to inflammation and oxidative stress, up regulation of the L-arginine/NO pathway, immune system activation by the dialysis procedure and platelet activation due to uraemia[17]. Notably, while NO has important physiological roles, excessive NO can be cytotoxic, particularly in AKI, where it can contribute to oxidative and nitrosative stress, potentially exacerbating kidney injury[17]. Some studies, such as Matavulj[18], have suggested that increased NO production in renal failure might have compensatory effects, like mitigating uremic complications and haemodialysis hypotension. However, our findings, along with those of Dejanova[19], who observed higher NO levels in haemodialysis patients and attributed this to the haemodialysis membrane and/or impaired renal excretion, indicate a more complex relationship. Conversely, Blicharz[20] reported a decrease in NO concentration with the duration of haemodialysis, aligning more closely with our observations after the first and second dialysis sessions. These contrasting findings highlight the need for further research to fully elucidate the role of NO in AKI and haemodialysis. In addition to NO itself, the dynamics of its endogenous inhibitor, ADMA, are crucial. Vallance[21] first identified the critical role of ADMA in disrupting the L-arginine/NO pathway, demonstrating that ADMA accumulation in patients with end-stage renal disease (ESRD) can lead to NOS inhibition and endothelial dysfunction. ADMA competes with L-arginine for binding to NOS, reducing NO production and impairing endothelium-dependent vasodilation. This can contribute to cardiovascular complications in renal failure, including hypertension, atherosclerosis and thrombosis. While some studies have reported a decrease in ADMA concentrations during dialysis (e.g., Kielstein[22], Yaman[23]), others have found no significant change or even an increase (e.g., Kielstein[24], Asmarawati[25], Vo[26]). This variability may be due to differences in dialysis modalities, patient populations and measurement techniques. Our study contributes to this debate by demonstrating that while plasma ADMA levels were significantly elevated in AKI patients before haemodialysis, there was no consistent or significant reduction in ADMA concentrations throughout the four haemodialysis sessions. This persistent elevation of ADMA, despite dialysis, may have important implications for vascular health and the progression of kidney disease. Interestingly, while some ADMA is removed during dialysis, its clearance is less efficient than expected based on its molecular weight and solubility. This may be due to protein binding and cellular release of ADMA. Furthermore, a 'rebound' phenomenon has been observed, where circulating ADMA concentrations increase after dialysis, often exceeding pre-dialysis levels. This rebound effect could contribute to the persistent elevation of ADMA in patients with renal failure[27,28]. Elevated ADMA levels have been consistently observed in early stages of renal disease and are associated with accelerated disease progression and increased mortality. Several mechanisms contribute to the detrimental effects of ADMA in renal pathology, including: reduced glomerular blood flow and ultra filtration due to decreased NO bioavailability increased vasoconstrictor and blood pressure due to impaired endothelial-dependent relaxation., disturbances in intra glomerular hemodynamic., heightened oxidative stress and the induction of renal fibrosis. Moreover, elevated ADMA can lead to renal parenchyma damage, further compromising NO production by reducing the expression and activity of DDAH, the enzyme responsible for ADMA degradation[27]. Ischemia, inflammation and oxidative stress, hallmarks of conditions like sepsis, promote the accumulation of ADMA[28]. Under these pathological conditions, ADMA is released due to increased protein degradation and impaired DDAH activity. This results in a substantial increase in plasma ADMA concentrations. Notably, intracellular ADMA levels are considerably higher than plasma levels due to active transport mechanisms. Therefore, elevated plasma ADMA may indicate even greater intracellular accumulation, potentially exacerbating pathological effects within tissues. High ADMA levels can trigger a cascade of detrimental effects, including endothelial dysfunction, platelet aggregation, leukocyte adhesion, increased vascular permeability, red blood cell deformity, oxidative stress and sympathetic activation[28].

Clinical Implications: The findings of this study have potential implications for the management of AKI patients undergoing haemodialysis. The dynamic changes in NO levels suggest that monitoring NO concentrations during haemodialysis might provide valuable insights into the patient's response to treatment and overall vascular health. Furthermore, the persistent elevation of ADMA despite haemodialysis highlights the need for further research into ADMA-lowering therapies in this population. Strategies to enhance ADMA clearance or inhibit its production could potentially improve outcomes in AKI patients.

Limitations: This study had a limited sample size, which may not fully represent the diverse AKI population. More detailed studies with larger and more diverse cohorts are needed to confirm these findings and assess their generalizability.

CONCLUSION

This study investigated the dynamics of nitric oxide (NO) and its endogenous inhibitor, asymmetric dimethylarginine (ADMA), in acute kidney injury (AKI) patients undergoing haemodialysis. Our findings demonstrate that haemodialysis effectively reduces plasma NO concentrations, supporting its potential role in removing excess NO in pathological conditions characterized by NO overproduction. However, the study also highlights the complex interplay between NO production, clearance and the haemodialysis process itself, as evidenced by the fluctuating NO levels observed across different dialysis sessions. Further more, our results indicate that haemodialysis may not effectively remove ADMA, as evidenced by the persistent elevation of ADMA levels despite treatment. This finding underscores the need for further research to explore ADMA-lowering therapies and to elucidate the complex interplay between ADMA, NO and haemodialysis in the context of AKI. The persistent elevation of ADMA in AKI patients undergoing haemodialysis may have significant clinical implications, particularly regarding vascular health and long-term kidney function. Monitoring NO and ADMA levels during haemodialysis could provide valuable insights into disease progression and treatment response. Future research should focus on developing targeted interventions to modulate NO and ADMA levels, potentially leading to improved outcomes in AKI patients.

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