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Original Research Article

Serum nitric oxide levels in chronic renal failure patients on maintenance hemodialysis

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Abstract

Background: Nitric oxide (NO) is an endothelium derived vasodilator. NO regulates renal function through modulation of vascular tone. With the progressive development of renal insufficiency, it remains unclear whether endogenous NO production is increased or decreased in the kidney.

Aim: This study was carried out to evaluate NO levels and its correlation to routine parameters of renal dysfunction in patients of chronic renal failure (CRF) on maintenance hemodialysis (MHD) in comparison to healthy controls.

Material and Methods: 30 CRF patients on MHD with serum creatinine levels >2.5 mg/dl were included in the study along with 30 healthy controls. Serum NO was estimated by spectrophotometric method using cadmium reduction. Routine renal function tests; blood urea nitrogen (BUN) and creatinine were performed by standard clinical chemistry procedures. The between-group differences and between-variable correlations were studied by the independent sample t-test and Pearson correlation analyses, respectively. The receiver operating characteristic curve (ROC) analysis was performed to obtain the sensitivity, specificity and area under curve (AUC) values for serum NO.

Results: The serum NO levels were found to be significantly increased ($p<0.01$) in CRF on MHD (96.5± 26.22 µmol/l) as compared to the controls (40.57 ± 13.36 µmol/l). NO output correlated with serum creatinine ($r=0.615$, $p<0.01$) and BUN ($r=0.584$, $p=<0.01$) in the CRF group. The ROC analysis on serum NO discriminated between CRF patients and controls with good sensitivity (93.3%), specificity (96.1%) and AUC (0.95) results at a cut-off value of 72.5 µmol/L.

Conclusions: Our study findings of increased serum NO level and its significant positive correlations with BUN and creatinine in CRF patients on MHD suggests an altered endothelial function in CRF patients on MHD. This increase in serum NO has been found to be useful in discriminating patients from controls.

Keywords: Nitric oxide, Chronic renal failure, Hemodialysis, Kidney dysfunction.

1. Introduction

Kidney failure is a global health problem with increasing incidence, prevalence and poor outcomes.[1] In India, for every one billion population there would be 1,50,000 new cases of Chronic Renal Failure (CRF) every year. Currently, dialysis and kidney transplants are used to extend the lives of the kidney failure patients. Every year, CRF has forced about 200,000 people to be on dialysis and about 87,000 people to have a kidney transplant.[2]

Oxidative stress due to increased Free radicals and decreased antioxidants has been reported to be a main factor in pathogenesis of chronic renal disease.[3] Nitric oxide (NO) is a labile radical gas and is one of the important molecules of human biology in maintenance of health and disease. Among several vital functions of NO, vasodilator function has been well documented. However, cytotoxicity due to excess NO has also been reported. This duality of NO's beneficial and detrimental effects has

created extraordinary interest in this molecule and the need for a detailed understanding of NO. [4] Although it has been suggested that NO may be important in the pathophysiology of chronic renal diseases, the precise molecular mechanisms involved have not been elucidated. Though there are various studies reporting serum NO levels in CRF patients, there is scarcity of literature on NO levels in CRF patients who are on maintenance hemodialysis (MHD).[5-7] Therefore, in the present study, we aimed at evaluating the serum levels of NO in CRF patients on MHD in comparison with healthy controls. We also aimed at studying correlations between serum NO and routine kidney dysfunction parameters like blood urea nitrogen (BUN) and creatinine. Finally, the discriminating ability of serum NO for CRF patients on MHD was also studied by receiver operating characteristic curve analysis.

2. Material and Methods

The present study was conducted on a patient group comprising of 30 patients (age >25 years) diagnosed with CRF due to chronic glomerulonephritis and hypertension who were on maintenance hemodialysis (MHD) with serum creatinine levels >2.5 mg/dl. This patient group was compared to a healthy group including 30 controls. All the study participants were recruited from the 'Mahatma Gandhi Memorial Hospital, Warangal, Telangana, India. The study was conducted after obtaining Institutional Ethical Committee clearance and informed consent was obtained from the study participants.

Five ml of whole blood samples were drawn into plain tubes to obtain serum samples after centrifugation at 4000rpm for 10 minutes. The obtained serum samples were used for biochemical analysis for estimating nitric oxide, blood urea nitrogen and creatinine values. Nitric oxide was spetrophotometrically estimated by the method of Cortas and Wakid.[8] Blood urea nitrogen was estimated by using urease/glutamate dehydrogenase coupled enzymatic technique. [9] Serum creatinine estimation was carried out in fully automated clinical chemistry analyzer using commercial kits based on kinetic Jaffe's method.

2.1Statistical analysis

The data obtained were analyzed by using SPSS 17.0. The results were expressed as Mean and standard deviation (SD) for all normally distributed data, unless otherwise specified. Independent sample t-test was used to assess the between-groups statistical significance. And, the correlations between variables were studied in the patient group by using 'Pearson correlation' analysis. The receiver operating characteristic curve (ROC) analysis was performed on serum NO for sensitivity, specificity and the area under ROC curve (AUC) values. A p-value of <0.05 was considered to be statistically significant.

3. Results

The mean and SD values of study variables between the patient and control groups were presented in Table 1. There was a significant increase in the BUN, serum creatinine and serum NO values in the patient group when compared to healthy controls ($p<0.01$). The increase in serum NO levels found in the patient group showed a significant and positive associations with both BUN ($r=0.58$, $p<0.01$) and creatinine ($r=0.61$, $p<0.01$) levels. Results of this correlation analysis were presented in Table 2. As shown in the Fig.1, the ROC analysis on serum NO discriminated between CRF patients and controls with good sensitivity (93.3%), specificity (96.1%) and AUC (0.95) results at a cut-off value of 72.5 µmol/L.

Table 1: Results of study variables between patient and control groups

Variable (Mean±SD)	Controls (n=30)	Patients (n=30)	p-Value
BUN (mg/dL)	11.9±2.86	86±20.62	<0.01
Creatinine (mg/dL)	0.99±0.23	9.98±2.74	<0.01
NO (µmol/L)	40.57 ± 13.36	96.5± 26.22	<0.01

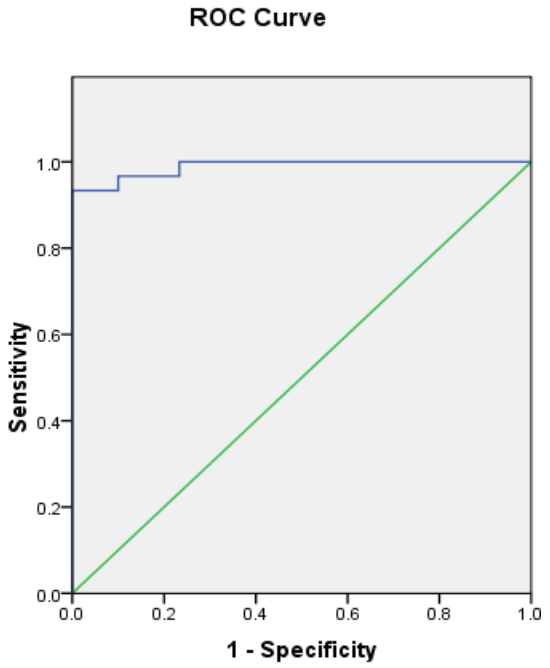
BUN: blood urea nitrogen, NO: nitric oxide

Table 2: Correlations of NO with BUN and Creatinine in the patient group

Correlation between	Correlation coefficient (r)	p-Value
NO & BUN	0.584	<0.01
NO & Creatinine	0.615	<0.01

BUN: blood urea nitrogen, NO: nitric oxide

Figure 1: The ROC analysis on nitric oxide results



4. Discussion

The results of our present study indicated a significant increase in serum NO levels in CRF patients on MHD when compared to that of healthy controls. Similarly, the BUN and creatinine levels were also found to be significantly high in the patient group as compared to controls (Table 1).

Since NO is eliminated by the kidneys, its elevated levels may represent declined renal function. There is no appreciable fall in serum NO despite the subjects being on regular sessions of dialysis. It is therefore possible that there are other factors which contribute to the increased NO levels. It is well known that MHD as a therapeutic option has several limitations such as; endogenous production of NO in the kidneys, insitu formation during hemodialysis and the involvement of inflammatory cytokines. [10-12] The process of dialysis triggers a series of events that increase the levels of inflammatory cytokines as TNF α, IL-I and interferon γ (INF γ) by neutrophils and monocytes. The high plasma levels of these inflammatory cytokines in patients on haemodialysis appear to be additional reasons for enhanced formation of NO in uraemia, as IL-1 and TNF α are potent inducers of iNOS where in NO is released in micromolar amounts in cellular systems.[12] Serum creatinine and urea levels often fall marginally after these sessions. In the subjects of CRF on MHD the mean serum creatinine and serum urea continued to remain respectively high. In line with this, we have also found a significant positive correlation of serum NO with BUN and creatinine (Table 2).

During the process of haemodialysis there could occur an increase in NO production due to haemo incompatibility of the dialyzing membrane. Also there is an increase in the blood turbulence and shear stress due to the mechanical components of the dialyzer. This activates both the platelets as well as leukocytes augmenting the release of NO from them. The use of heparin may be an additional factor in this enhanced production.[11] Increased urea levels could be expected to have an inhibitory effect on NO synthesis. Amongst the three possible sources namely; the neutrophils, monocytes and the platelets the inhibitory effect of urea can be effective only in cells with an intact nucleus which has the protein synthetic machinery- neutrophils and macrophages.[11] The platelets on the other hand has only a constitutive isoform of the enzyme, eNOS. It could therefore step up production of NO despite high levels of urea in the environment. Intraplatelet cGMP levels have found to be higher in uraemic patients both in dialysed and undialysed.[12] Since cGMP is the second messenger for NO production, this could have activated NO synthases and hence increased serum NO levels.

In view of observation on increased serum NO levels, we have also tested it by ROC analysis. Our results depicted in Figure 1 shows that the elevated serum NO could be useful in discriminating CRF patients on MHD from controls. At a cut-off values of 72.5µmol/L, the respective sensitivity, specificity and AUC values are; 93.3%, 96.1% and 0.95. Therefore, the increased NO levels observed in our study and its positive association with the routine kidney dysfunction markers may reflect the cytotoxic effects of NO indicative of oxidative stress, inflammation in renal disease and may be also due to dialysis procedure.

5. Conclusion

In conclusion, our study findings in accordance with previous evidence indicated an increased levels of serum NO and its significant positive correlations with BUN and creatinine in CRF patients on MHD. This increase in serum NO has been found to exhibit better sensitivity, specificity and AUC values for discriminating patients from controls. Future studies with large sample sizes are needed to substantiate the findings of our case-control study.

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