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Nitric oxide and nicotine amide adenine dinucleotide phosphate oxidase activity before and after renal transplantation

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Abstract

***Background & Aims*:** Renal transplantation has been considered as the best therapeutic strategy for end stage renal disease (ESRD). Oxidative stress induced by the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and nitric oxide has key role in the pathogenesis of ESRD. Therefore, in this study, the propose of this research was to investigate the enzymatic activity of NADPH oxidase (NOX), as well as nitric oxide (NO) levels before and after renal transplantation,

Materials and Methods: Twenty-five patients with ESRD and renal transplantation with age range of 20-60 years was enrolled in this study. Serum samples were collected for measurement of NADPH oxidase and NO levels, before, one hour and 14 days after transplantation, by ELISA technique using a commercial kit. Serum levels of urea and creatinine was also evaluated.

Results: The serum urea and creatinine levels significantly decreased after transplantation ($p<0.05$). research indicated that the serum levels of NADPH oxidase and NO was increased in the three times, one hour and 14 days after transplantation, but this increase was not statistically significant.In addition, NO and NADPH oxidase showed a significant correlation.

Conclusion: This study showed that the serum levels of NADPH oxidase and NO was not significantly changed before and after renal transplantation. In addition, NO had no diagnostic value in renal transplant patients who did not have acute rejection.

***Keywords*:** End-stage renal disease, renal transplantation, nitric oxide, NADPH oxide

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Introduction

End-stage renal disease (ESRD), a global public health problem with an increasing incidence worldwide, is in close association to high morbidity due to lifestyle diseases includingatherosclerosis, diabetes, and hypertension(1). Renal transplantation is considered as the best therapeutic strategy for ESRD patients(2). In fact renal transplantation significantly improves the mortality rate for ESRD as compare to other therapeutic strategies for ESRD such as peritoneal dialysis and hemodialysis(2). Patients with chronic renal failure have defective antioxidant systems, while oxidants levels are increased due to ageing, diabetes, chronic inflammation and incompatibility with dialysis solutions and membranes(3). Evidence suggests that oxidative stress is involved in the pathogenesis of the various renal disease, including ESRD, acute renal failure (ARF), Chronic Renal Failure(CRF), and glomerular damage(3-

4). Oxidative stress impairs immune function and accelerates the process of atherosclerosis in ESRD patients(5). In normal conditions, the amount of free radicals production in the kidney is balanced with the antioxidant defense system, while in the pathologic conditions, production of reactive oxygen species (ROS) increases and leads to serious damage to the structure and function of the kidney(6). In the transplanted individuals due to weakening of the body's defense system,the balance between the oxidant and antioxidant levels is impaired(7). Therefore, it was suggested that oxidative stress is one of the most important mediators of renal damage following immunosuppression(7). In addition to ROS, reactive nitrogen species (RNS) are also involved in enhancing oxidative stress. RNS are derived from nitric oxide (NO) and superoxide produced via the enzymatic activity of inducible nitric oxide synthase (iNOS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, respectively(8). In the kidney, ROS produced through NADPH oxidase activity is mainly responsible for regulating renal blood flow, altering cellular fate and regulating gene expression(9). Superoxide reacts vigorously with NO and limits its vasodilator effect on the arterial occlusion while hydrogen peroxide participates in activating protein tyrosine kinase, phospholipase, and serine threonine kinase(9). Given the importance of NADPH oxidase and NO and their downstream effects in the kidney, we aimed to evaluate the enzymatic activity of NADPH oxidase, as well as NO levels before and after renal transplantation.

Materials and Methods

The study protocol was approved by the ethics committee of the Urmia University of Medical Sciences, and informed consent was given by each patient before enrollment. We enrolled 25 patients with ESRD and renal transplantation patients who had referred to Department Of Renal, Emam Khomeini Medical Research and Training Hospital of Urmia, Iran. The selected age range was 20-60 years. All participants were requested to complete a questionnaire form and then necessities were thoroughly explained to them. Patients who had difficulty during operation for any reason or did not have stable condition, were excluded from the study.

Sample collection and handling:

Blood Sampling from patients selected for kidney transplantation was performed in three steps. The first sample was taken one day before transplantation, and this procedure was repeated in the day of the operation. peripheral venous blood samples were taken into tubes. then samples were centrifuged at 4000 rpm for 10 minutes, and serum was separated at room temperature (25°C). The prepared samples were aliquoted and immediately frozen at −80°C until analysis.

Biochemichal measurement:

Blood creatinine and urea determinations were performed by using commercial kits (Parsazmoon; Iran) by BT3000 autoanalyser (Italy). The serum contents of NADPH oxidase and NO were assessed by enzyme immunoassay procedure. NADPH oxidase and NO levels was measured with a specific human ELISA kit (Bioassay Technology Laboratory, China) according to the manufacturer’s instructions. The absorbance was determined at 450 nm.

Statistical Analysis:

Data were processed by the SPSS version 20. All quantitative data are presented as mean±SD values. ANOVA test was used to analyze of the parameters. Differences were declared significant when $p < 0.05$.

Results

Measurements of urea and creatinine changes were performed to evaluate the success of the transplant. Table 1 shows the mean values of urea and creatinine at different times. As shown in figures 1 and 2, the values of urea and creatinine decreased after transplantation.

The comparison of urea and creatinine values at different times showed a significant difference (p=0.001). However, urea values did not show a significant difference between one hour and 14 days after transplantation (p=0.522). In the case of NADPH oxidase, our results indicated that the serum levels of enzyme was increased in the three times one day before, one hour and 14 days after transplantation, but this increase was not statistically significant (Figure 3).The NO levels is increased in all times. However, this increase was not statistically significant (Figure 4). The correlation between studying parameters was also investigated in our investigation. NO and NADPH oxidase showed a significant correlation (Figure 5). However, as shown in table 2, there was not any significant correlation between parameters in our study.

Table 1: the serum levles of urea and creatinine in three times one day before, one hour and 14 days after transplantation

Parameter	Levels one day before transplantation	Levels 1h after transplantation	Levels 14 days after transplantation
Urea (mg/dL)	125.75±4.8	79.12±2.42	69.12±2.87
Creatinine (mg/dL)	8.58±3.65	4.79±1.83	1.52±0.79

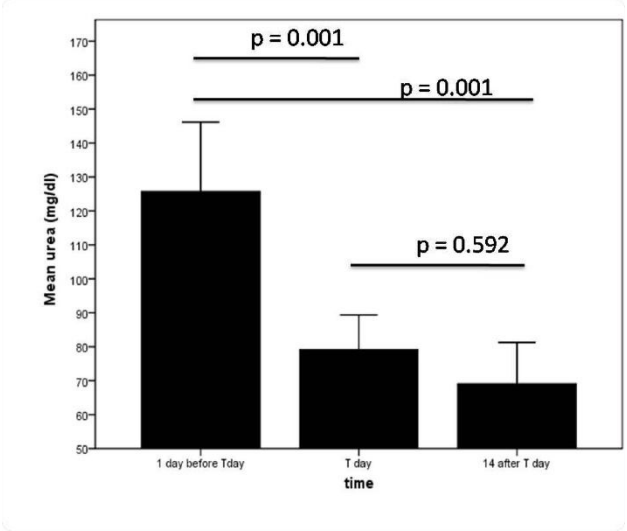

Fig 1: the serum levles of urea in three times one day before, one hour and 14 days after transplantation. T day: Transplantation day

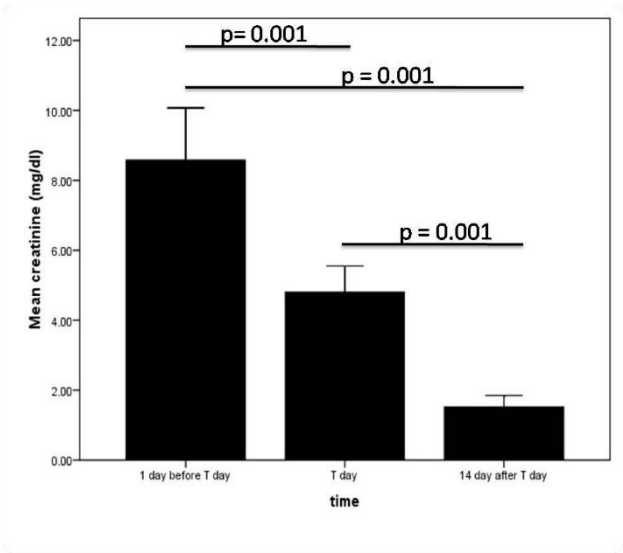

Fig 2: the serum levles of creatinine in three times one day before, one hour and 14 days after transplantation.T day: Transplantation day

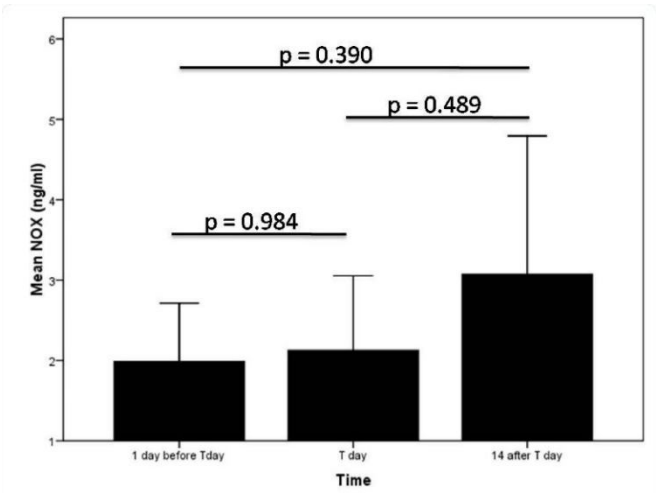

Fig 3: the serum levles of NOX enzymein three times one day before, one hour and 14 days after transplantation. NOX: NADPH oxidase, T day: Transplantation day

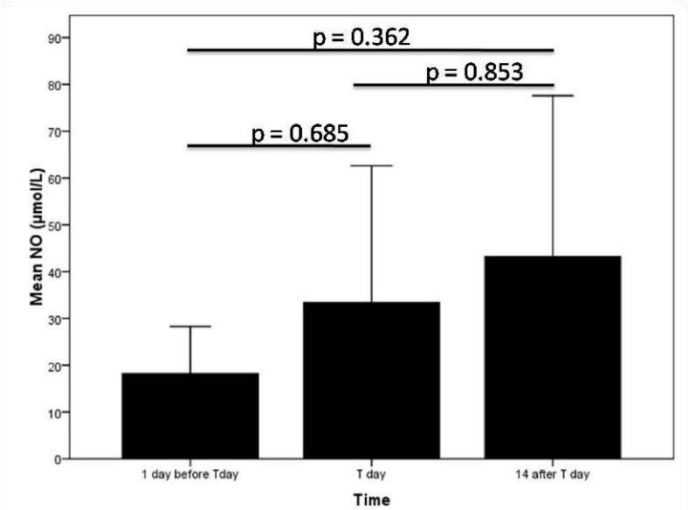

Fig 4: the serum levles of NOin three times one day before, one hour and 14 days after transplantation. NO: nitric oxide,T day: Transplantation day

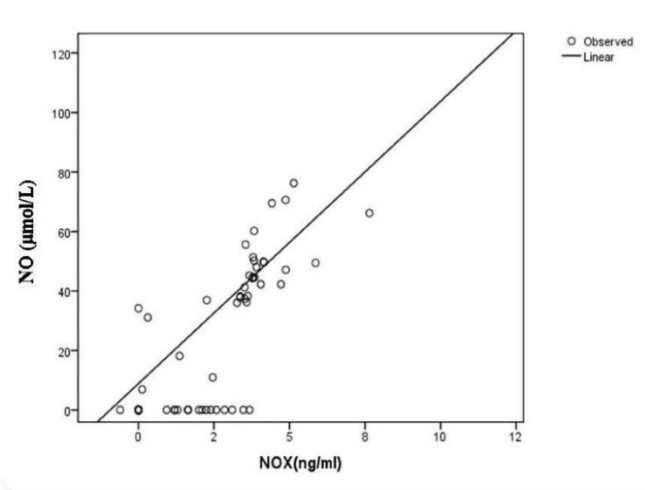

Fig 5: The correlation between NO and NOXin three times one day before, one hour and 14 days after transplantation. NO: nitric oxide, NOX: NADPH oxidase, T day: Transplantation day

Table 2. Correlation analysis between NO/creatinine, NO/urea, NOX/creatinine, NOX/urea at all three times

Parameter	Pearson	Pvalue	Time
NO/Urea	0.827	0.001	Levels1day before transplantation
NO/ Creatinine	0.22	0.301	Levels 1h after transplantation
NOX/Urea	0.475	0.019	Levels 14 days after transplantation
NOX/Creatinine	0.429	0.001	Total

Discussion

ESRD is one of the most important health problems worldwide, and an accumulating body of studies has been conducted to identify the contributing factors in pathogenesis, prognosis and diagnosis of this disorder(10). However, involving biochemical processes was not yet completely understood in this area. In this study, the changes in NO and NADPH oxidase enzyme were measured at three times one day before, one hour and 14 days after renal transplantation in patients. For evaluation the success of renal transplantation, serum levels of urea and creatinine were also measured. Kasiske et al(11).showed that the serum creatinine level was stable at 1.6 mg/dL for 3 months to 10 years, after successful transplantation. Norouzadeh et al(12). also reported that the serum levels of creatinine decreased from 10.83 mg/dL before the transplantation to 4.89 mg/dL in transplantation day and to 1.74 mg/dL at the 14th day after transplantation. In agreement to mentioned studies, our results also showed that the mean of creatinine level decreased from 8.85 to 4.79 and eventually decreased to 1.52 mg/dL. Similar results were observed for serum urea levels, all of which prove the success of renal transplantation. The important part of our study is evaluation of the alternations in the NADPH oxidase levels before and after transplantation. In the kidney, oxidative damage induced by the activity of this enzyme is involved in the regulation of various biological consequences(13). In a study by Khanna et al(7). it was found that the adverse effects of Tacrolimus, an immune suppressor agent, was mediated by the increase in the activity of NADPH oxidase, which was inhibited by anti-TGF-b antibodies. In another study by Djamali et al(14). it was reported that NADPH oxidase was responsible for the development of fibrogenesis in the renal tissue, and inhibition of this enzyme is associated with a decrease in the fibrosis. Although NOX is not the only source of oxidative stress

in the body, other enzymes such as xanthine oxidase, lipoxygenase, cyclooxygenase, and electron transfer chains are also involved as oxidative stress-inducing components; however, NADPH oxidase acts as a major component in increasing oxidative damage(15). The results of our study indicated no statistical significant change in the enzyme activity before and after transplantation, may be due to small size of studied population. Another oxidative stress- related parameter measured in this study was NO. We observed no significant changes in the serum NO levels before and after transplantation, which is in agreement with results of Eftekhar study(12). In contrast to our finding, Bellos et al(16). showed that the serum nitrite and nitrate metabolites levels indicate significant changes in the first ten days of the transplant that can be used as a noninvasive biomarker for the evaluation of the acute renal transplant rejection. Considering that during this study, participants with acute rejection of the transplant were excluded, so the existing contradiction is justifiable. In the study of Smit et al(17).NO synthase (NOS) activity was evaluated in urine of patients with renal transplantation. The results showed that NOS activity in patients with rejection significantly increased. Suzuki et al(18). Found that the serum levels of NO metabolites and NOS activity were much higher, and by inhibiting the activity of NOS with aminoguanidine, the adverse effects of NOS and its metabolites were reduced. However, NO variations in our study were not significant but showed an increasing trend. Increased NO can enhance the blood supply and exacerbate the complications of ischemic reperfusion by vasodilatation(19). In addition to the goals defined for the present study, the results of the data analysis showed a significant correlation between NO and NADPH oxidase values. In this regard, a study was conducted by Zhang et al(20). in 2006. The results of their study showed that NADPH oxidase-produced superoxide directly affects the bioavailability of NO, not the NO levels. In a study by Selenidis et al(21). it was demonstrated that NO caused a permanent inhibition of NADPH oxidase through S-nitrolyzation of enzyme. The correlation between NO and NADPH oxidase before, one hour and 14 days after the transplantation indicated that the correlation between NO and NOX is established before transplantation($p = 0.001$). After transplantation, the correlation disappeared at the first hour and eventually was restored on the 14th day. These results suggested that in stable conditions with any intervention,NO and NOX are a function of each other, and this correlation disappeared during an operation that disrupt homeostasis in many dimensions. In this study, there was not any significant correlation between NO/urea, NO/creatinine, NADPH oxidase/urea and, NADPH oxidase/creatinine.

In conclusion, our study showed that the serum levels of NADPH oxidase and NO was not significantly changed before and after renal transplantation, may be due the small study population, as well as the conditions of participants. In addition, NO had no diagnostic value in renal transplant patients who did not have acute rejection.

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