**Original Article**

# Nitric Oxide Status in Children with Chronic Kidney Disease.

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## Abstract

### Introduction
Children with chronic kidney disease (CKD) are at increased risk of cardiovascular (CVD) morbidity and mortality. Decreased production or reduced bioavailability of nitric oxide (NO) can augment such complications.

### Aim of the study
To assess the levels of Nitric Oxide in the serum of chronic kidney disease children.

### Methods
Case-control cross sectional study was conducted on 55 children as a stratified non-random sample; 30 with CKD on regular hemodialysis (HD) (Group I), 10 with CKD on conservative therapy (Group II) and 15 healthy children (Group III).

### Results
Children with CKD whether on regular HD or on conservative therapy had significant lower serum NO levels ($0.5 \pm 0.2$ and $2.1 \pm 0.5$ ng/ml respectively) as compared to healthy controls ($9.8 \pm 0.9$ ng/ml; $p < 0.001$). Serum NO has significant negative correlations with duration of hemodialysis and with hypertension in Group I.

### Conclusions
Nitric Oxide production is decreased in children with CKD, further decrease with the progression of the disease and correlated with hypertension and duration of hemodialysis.

### Keywords
Chronic renal failure, End stage renal disease, Hemodialysis, Nitric oxide

### Running title
Nitric oxide status in children with chronic kidney disease

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## Introduction

Nitric Oxide is a potent vasodilator of macrovascular and microvascular renal blood vessels predominantly in the afferent arterioles [1, 2]. NO regulates mitochondrial respiration. It enhances water, sodium, and bicarbonate absorption in the proximal tubules [3]. In the cortical collecting duct, NO inhibits sodium conductivity and inhibits $H^+$-ATPase activity [4, 5].

In children with CKD, hypertension is a major cause of cardiovascular morbidity and mortality. Generally, atherosclerosis has been associated with reduced availability of NO. NO production was found to be reduced in all stages of CKD [6].

A deficiency of endogenous vasodilator nitric oxide (NO) has been implicated as a potential cause of increased blood pressure in end-stage renal disease (ESRD) patients [5].

## Methods

A cross sectional case-controlled study comprised fifty-five children; forty patients with CKD diagnosed according to KDIGO [6], and fifteen clinically healthy children serving as controls. The study was approved by the ethics committee of our university and it conforms to standards currently applied in Egypt. All children are enrolled at the Nephrology Unit and Outpatient Clinic. An informed written consent was obtained from the parents of each child before enrollment according to the Declaration of Helsinki. This study was carried from the first of July 2018 to the end of July 2019. It comprised 3 main Groups:

**Group I:** 30 children with end stage renal disease (ESRD) [6] on regular hemodialysis (HD).

**Group II:** 10 children with CKD (Stages 2&3) [6] on conservative therapy.

**Group III:** 15 clinically healthy children age- and sex-matched.

All Groups were subjected to the following measures: Clinical: duration of hemodialysis in Group I, duration of illness in Group II, age, sex and blood pressure (systolic blood pressure SBP and diastolic blood pressure DBP) applied to all groups

**Laboratory:**

- Quantitative determination of serum Nitric Oxide (NO) by classic colorimetrical Griess reaction; In acid medium and in the presence of nitrite the formed nitrous oxide diazotise sulphanilamide and the product is coupled with N-(1-naphthyl) ethylenediamine. The resulting azo dye has a bright reddish-purple color which can be measured at 540nm.
- A predialysis blood sampling of serum creatinine, blood urea nitrogen (BUN) was obtained (no significant difference between NO level before and after dialysis sessions) [7].
- Glomerular filtration rate (GFR) calculated using the Cockcroft-Gault equation [8].

**Data Management and Statistical Analysis**

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 20). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

1- Descriptive statistics:
   a. Mean, Standard deviation (± SD) for numerical data.
   b. Frequency and percentage of non-numerical data.
2- Analytical statistics:
   a. Student T Test was used to assess the statistical significance of the difference between two study Group means.
   b. Chi-Square test was used to examine the relationship between two qualitative variables.

**Correlation analysis (using Pearson's method):** To assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "r" defines the strength (magnitude) and direction (positive or negative) of the linear relationship between two variables.

- r = 0-0.19 is regarded as very weak correlation
- r = 0.2-0.39 as weak correlation
- r = 0.40-0.59 as moderate correlation
- r = 0.6-0.79 as strong correlation
- r = 0.8-1 as very strong correlation

## Results

The study included fifty five children. Group I comprised 30 CKD on regular hemodialysis, 16 female and 14 male with age 159 ± 20months, Group II comprised 10 CKD on conservative therapy, 6 female and 4 male with age 147±14 months, and Group III of 15 healthy age and sex matched children (Table 1).

Serum nitric oxide has mean of 0.5 ± 0.2 ng/ml in Group I, 2.1 ± 0.5 ng/ml in Group II and 9.8 ± 0.9 ng/ml in Group III. On comparing the Groups with each other as regards serum NO, there is significant difference, being lowest in Group I ($p < 0.001$, Table 1).

Table 2 denotes significant difference in SBP ($p < 0.001$) and GFR ($p < 0.001$) between children on hemodialysis (Group I) and on conservative therapy (Group II).

In Group I, serum NO has negative moderate correlation with age ($r = -0.480$, $p = 0.007$) and DBP ($r = -0.441$, $p = 0.015$), very strong with duration of hemodialysis ($r = -0.891$, $p < 0.001$) (Figure 1) and strong with serum creatinine ($r = -0.669$, $p < 0.001$) (Figure 2) and SBP ($r = -0.744$, $p < 0.001$). However, there was a positive very strong correlation between NO and GFR ($r = 0.905$, $p < 0.001$) (Figure 3). Whereas serum creatinine shows positive strong correlation with age ($r = 0.600$, $p < 0.001$) and SBP ($r = 0.725$, $p < 0.001$), very strong with duration of dialysis ($r = 0.808$, $p < 0.001$). There was very strong negative correlation between serum creatinine and GFR ($r = -0.813$, $p < 0.001$) (Table 3).

Similarly, nitric oxide has very strong positive correlation with GFR ($r = 0.830$, $p = 0.003$) and very strong negative correlation with duration of illness ($r = -0.869$, $p < 0.001$) serum creatinine ($r = -0.885$, $p = 0.001$) in CKD children on conservative therapy. Unlike Group I, serum NO has weak to moderate correlation with SBP and DBP ($p = 0.074$, $p = 0.241$ respectively) (Table 4).

**Table1 Sociodemographic and laboratory parameters of the studied groups**

| | | Group I (Hemodialysis) | | Group II (Conservative therapy) | | Group III (Healthy control) | | Chi square test | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | p value | sig. |
| Sex | Female | 16 | 53.3% | 6 | 60.0% | 7 | 46.7% | 0.803 | NS |
| | Male | 14 | 46.7% | 4 | 40.0% | 8 | 53.3% | | |
| | | Mean | SD | Mean | SD | Mean | SD | ANOVA | |
| Age (months) | | 159.3 | 19.8 | 147.3 | 14.3 | 153.4 | 22.8 | 0.233 | NS |
| Nitric Oxide (ng/ml) | | 0.5 | 0.2 | 2.1 | 0.5 | 9.8 | 0.9 | < 0.001* | S |
| Creatinine (mg/dl) | | 8.3 | 0.9 | 2.2 | 0.6 | 0.6 | 0.1 | < 0.001* | S |

**Post Hoc Test:** Group I vs Group II (S, $p < 0.001$), Group I vs Group III (S, $p < 0.001$) and Group II vs Group III (S, $p < 0.001$)

**Table 2 Clinical and laboratory difference between Group I and II**

| | Group I (Hemodialysis) | | Group II (Conservative therapy) | | t test | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | p value | sig. |
| SBP mmHg | 153.5 | 18.8 | 126.0 | 8.4 | < 0.001* | S |
| DBP mmHg | 92.0 | 13.4 | 82.0 | 7.1 | 0.031 | S |
| GFR ml/min/1.73m$^2$ | 7.9 | 1.1 | 60.9 | 6.0 | < 0.001* | S |

SBP; systolic blood pressure, DBP; diastolic blood pressure, GFR; glomerular filtration rate

**Table 3 Correlation coefficient of clinical and laboratory data in Group I**

| Group I (Hemodialysis) N = 30 | | nitric oxide | age | duration of HD | SBP | DBP | GFR |
|---|---|---|---|---|---|---|---|
| Nitric Oxide (ng/ml) | r | | -0.480* | -0.891* | -0.744* | -0.441* | 0.905* |
| | p | | 0.007 | 0.000 | 0.000 | 0.015 | 0.000 |
| Creatinine (mg/dl) | r | -0.669* | 0.600* | 0.808* | 0.725* | 0.350 | -0.813* |
| | p | 0.000 | 0.000 | 0.000 | 0.000 | 0.058 | 0.000 |

*Significant correlation

**Table 4 Correlation coefficient of clinical and laboratory data in Group II**

| Group II (Conservative therapy) N = 10 | | Nitric Oxide (mg/ml) | Age (months) | Duration of illness | SBP | DBP | GFR |
|---|---|---|---|---|---|---|---|
| Nitric Oxide (ng/ml) | r | | -0.541 | -0.869* | -0.334 | -0.407 | 0.830* |
| | p | | 0.106 | 0.001 | 0.346 | 0.243 | 0.003 |
| Creatinine (mg/dl) | r | -0.885* | 0.723* | 0.992* | 0.588 | 0.409 | -0.958* |
| | p | 0.001 | 0.018* | 0.000 | 0.074 | 0.241 | 0.000 |

***Significant correlation**

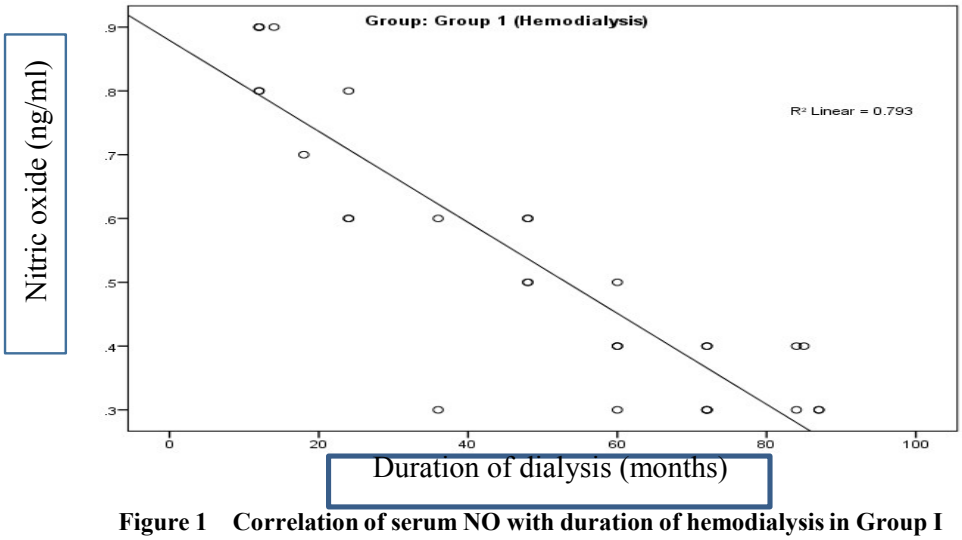


**Figure 1 Correlation of serum NO with duration of hemodialysis in Group I**

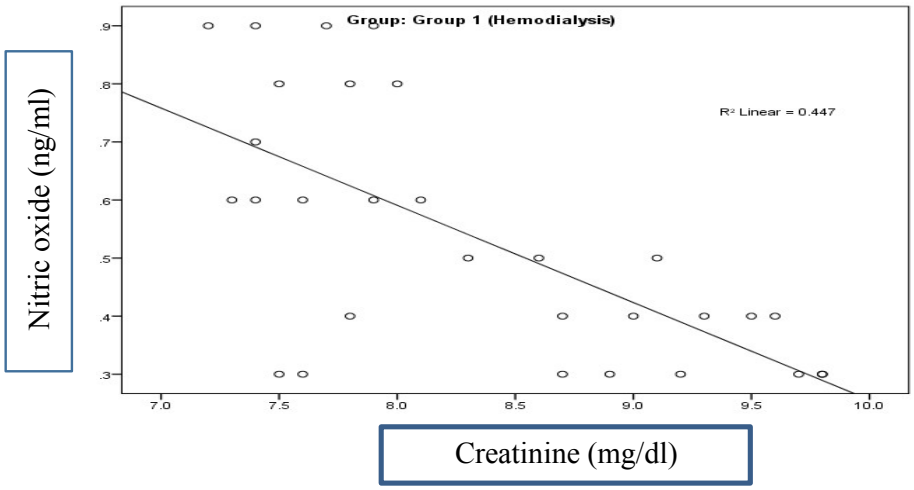


**Figure 2 Correlation of serum NO with serum creatinine in Group I**

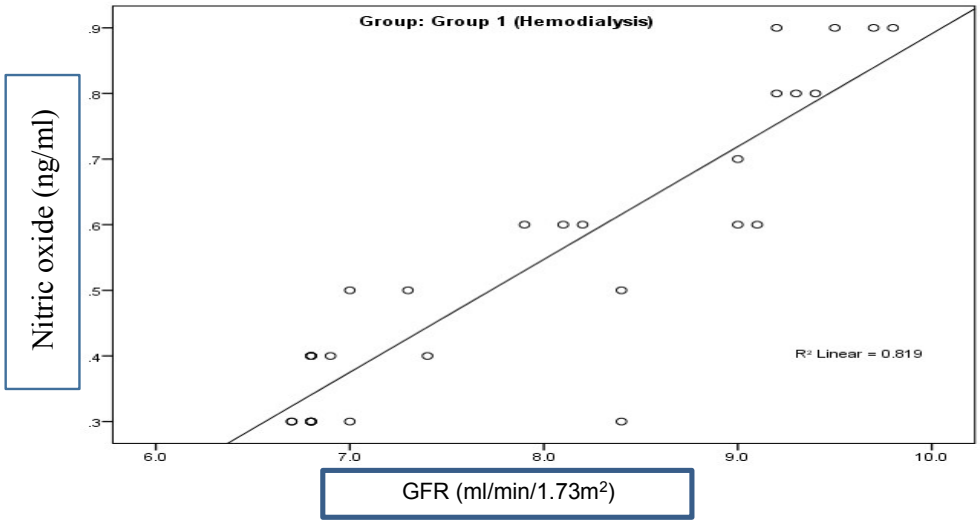


**Figure 3 Correlation of serum NO with GFR in Group I**

## Discussion

In our study, children with CKD on regular HD had significant lower serum NO levels as compared to healthy controls. Moreover, serum NO levels in children with CKD on regular HD were significantly lower in comparison to those on conservative therapy. Reduced NO production in patients with chronic kidney diseases could be ascribed to decreased bioavailability of the substrate for NO synthetase; L-arginine, and increased ADMA, the inhibitor of NO synthetase **[2, 9].** Previous adult studies reported decreased production of NO in CKD patients **[10, 11, 12].** Another study comprised pediatric age with CKD on conservative therapy also reported decreased levels of NO **[3].**

In a study done to investigate the changes in serum NO concentrations in adult hemodialysis patients related to hemodialysis duration, serum NO concentration was statistically different ($p = 0.001$) between the HD group $\leq 5$ years and HD group $\geq 5$ years, and healthy controls. No significant difference ($p = 0.17$) in serum NO level was observed between HD patients with different duration of dialysis therapy **[13].** In our study, inverse correlations were reported between serum NO levels and each of the duration of illness in Group II and duration of HD in Group I (Table 3 and 4). This was concordant with the results of Duranton and colleagues **[14].**

Our study reported negative correlation between serum NO levels and arterial blood pressure in both CKD Groups, being significant in Group I **(Table 3 and 4)**. This coincided with the results of previous studies concerned adult population **[15-19].** Those results shed light on potential pathogenic role of reduced NO production in aggravating cardiovascular morbidity and mortalities in children with CKD and pinpoint a potential of tailored therapies towards this biomarker in CKD.

A study on 13 adult with CKD was conducted, to determine whether 24-hour NOX (NO2 and NO3) excretion (a qualitative index of total NO production) is reduced or not, by Schmidt and Baylis. They found that urinary NOX excretion was low in CKD patients compared with controls despite similar dietary NO intake and systolic blood pressure was higher in CKD patients than in controls (P <

0.05) [20]. As such, these results confirm the results of our study even though urinary and not serum level of NO was assessed.

Serum NO levels correlated inversely with serum creatinine levels in children with CKD whether on regular HD or on conservative therapy ($p < 0.05$ in both Groups). This was in agreement with the results of Meenakashi and Ragni [21].

Youssef DM and colleagues evaluated pulmonary function tests (PFTs), NO level and their correlation in children with ESRD on regular HD. Unlike our study, significant difference was found between patient and control groups regarding NO levels with higher levels in the patient group and also found no significant difference between NO level before and after dialysis sessions. Their rationale was due to possible other factors which contribute to the increased NO levels, which may be an increased endogenous production, the hyperactive L-arginine/ NO synthetic pathway and the activation of the immune system by the dialysis procedure itself, leading to the induction of iNOS and also the platelets which generate more NO due to uremia [7].

Our pilot study reported reduced NO production in children with CKD whether on regular HD or on conservative therapy. The reduction was more significant in children on regular HD. NO levels inversely correlated with duration of illness, hemodialysis and arterial blood pressure. Therefore, NO reduced expression might be a reliable objective prognostic biomarker in predicting premature cardiovascular morbidities and mortalities in children with CKD. NO monitoring may be useful for follow up and may help to improve research and management of CKD disease. NO manipulation could represent a novel strategy for prevention or treatment of cardiovascular complications in CKD children.

## Conclusion

NO production is decreased in children with CKD, further decrease with the progression of the disease and correlated with hypertension and duration of illness and hemodialysis.

## Abbreviations

| | |
|---|---|
| **ADMA** | Asymmetric dimethylarginine |
| **BUN** | Blood urea nitrogen |
| **CKD** | Chronic kidney disease |
| **CVD** | Chronic cardiovascular disease |
| **DBP** | Diastolic blood pressure |
| **ESRD** | End stage renal disease |
| **GFR** | Glomerular filtration rate |
| **HD** | Hemodialysis |
| **iNOS** | Inducible nitric oxide synthase |
| **KDIGO** | Kidney disease improving global outcomes |
| **NO** | Nitric oxide |
| **S** | Significant |
| **SBP** | Systolic blood pressure |

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## Statements

**Ethics approval and consent to participate:**
This study protocol and the consents were approved and deemed sufficient by the Ethical Committee of our institution and informed and written consent was obtained in every case from their legal guardians.

**Consents for publication**
"Not applicable"

**Availability of data and material**
"Not applicable"

**Conflict of interest**
The authors declare no conflict of interest.

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