

Serum Nitric Oxide in Post-Streptococcal Acute Glomerulonephritis

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ABSTRACT

Background: The role of nitric oxide in renal immune injury has recently become the focus of intense investigations. In various forms of glomerulonephritis, there is increased production of NO in isolated glomeruli and enhanced expression and activation of the inducible isoform of nitric oxide synthetase (iNOS).

Objectives: To study the changes that occur in post-streptococcal acute glomerulonephritis.

Methods: Serum nitric oxide (NO) levels were measured in 20 children with post-streptococcal acute glomerulonephritis (PSAGN) during their initial presentation. Diagnosis of PSAGN was based on the presence of haematuria, transient hypocomplementaemia and positive laboratory evidence of recent streptococcal infection. Patients were compared to 20 healthy children of matched age and sex.

Results: Patients in the acute attack had significantly higher levels of NO when compared to controls (median = 38.45 Vs 30.45 $\mu\text{mol/l}$ respectively, $p = 0.033$). Serum NO levels were correlated significantly with serum complement 3 (C3) ($r = -0.574$, $p = 0.008$), and proteinuria ($r = 0.698$, $p = 0.001$). No significant correlation was detected between serum NO levels and serum creatinine, hypertension (systolic and diastolic), degree of haematuria or oedema. Out of the studied 20 cases; 16 cases were reassessed 3 months after normalization of their complement level and a significant drop in NO was found ($p = 0.039$).

Conclusions: We suggest that NO has a role in the pathogenesis of PSAGN mostly through mediation of immunologic injury to the glomeruli.

INTRODUCTION

The role of nitric oxide in renal immune injury has recently become the focus of intense investigations. In various forms of glomerulonephritis, there is increased production of NO in isolated glomeruli and enhanced expression and activation of the inducible isoform of nitric oxide synthetase (iNOS)⁽¹⁾ iNOS-derived NO release is sustained and of high output and can reach supraphysiologic levels within nephritic glomeruli thereby causing oxidative injury.

This form of injury can occur by NO itself⁽²⁾ or by the potent oxidant peroxynitrite, which is formed by the interaction of NO with superoxide⁽³⁾.

PATIENTS AND METHODS

Twenty Egyptian children (group 1) with diagnosis of PSAGN were included in the study. They were 12 males and 8 females with their ages ranging from 2.5 to 13 years (median 5 years). These patients represented all patients with diagnosis of PSAGN

admitted to the Pediatric Nephrology Unit in Mansoura University Children Hospital, Egypt, in the period from August 2001 to November 2001. Diagnosis of PSAGN depended on presence of haematuria, transient hypocomplementaemia and positive laboratory evidence of recent streptococcal infection. Out of the 20 studied cases, 16 patients (group 2) were reassessed 3 months after normalization of their serum complement levels. They were 10 males and 6 females with their ages ranging from 2.5 to 13 years (median 4.5 years). Both groups were compared to 20 healthy children of matched age and sex who served as a control group.

Serum NO Assay: Sera were obtained and stored at -70°C . NO levels were assessed by calorimetric assay (R & D system, INC, USA). This technique determined the total NO based on enzymatic conversion of nitrates to nitrite by nitrate reductase enzyme. The level of nitrite is measured by calorimetric assay. The detection limit for assay of NO was $1.0\ \mu\text{mol/l}$, deconcentration range of the standered curve was 1.0 to $500\ \mu\text{mol/l}$.

Clinical Assessment: The degree of edema was graded on a scale from 1-4, no edema, 1; puffy eye lids, 2; mild edema in the lower limb, 3; and massive edema, 4. The degree of hypertension was also graded on a scale from 1-3; no hypertension, 1; not-severe, 2; and severe, 3⁽⁴⁾.

Urinalysis: haematuria was arbitrarily

graded on a scale from 0 to 4 by light microscopy: negative, 0; 1-5/visual field, 1; 6-20, 2; 21-50, 3; and $> 50/\text{visual field}$, 4.

Urinary protein was quantitated by the pyrogallol red method⁽⁵⁾.

Biochemical Examinations: Serum creatinine was measured by the enzyme method⁽⁵⁾.

Statistics: Data were analyzed by the SPSS 10 for windows. Data showed non-parametric distribution. Tests used included Mann-Whitney, U-Wilcoxon W Test; Spearman Correlation Test; Kendall's tau_b Test; and Wilcoxon Signed Ranks Test.

RESULTS

Patients in the acute attack (group 1) had significantly higher levels of NO when compared to controls (median = 38.45 vs. $30.45\ \mu\text{mol/l}$ respectively, $p = 0.033$); Table (1). NO levels correlated significantly with serum C3 ($r = -0.574$, $p = 0.008$), and proteinuria ($r = 0.69$, $p = 0.001$). No significant correlation was detected between NO levels and serum creatinine, creatinine clearance, hypertension, degree of haematuria, or edema; Table (2). NO levels in group 2 (patients 3 months after recovery) were not significantly different from controls (median 31.85 vs. $30.45\ \mu\text{mol/l}$, $p = 0.17$); Table (1). Serum NO levels were significantly decreased in cases reassessed after 3 months ($p = 0.039$); Table (3).

Table 1: Comparison of NO Levels in Different Groups^a

Median ^b		Z	2T-P
Group 1	Control		
38.45	30.45	- 2.137	0.033*
Group 2	Control		
31.85	30.45	0.164	0.174

^a Using Mann-Whitney U - Wilcoxon Rank Sum W Test

^b umol/l

* = Significant

Table 2: Correlation between NO Levels and Other Parameters in Group 1

	C3 ^a	Proteinuria ^a	Cr ^a	Cr Cl ^a	SBP ^b	DBP ^b	Haema ^b	Edema ^b
NO	r = -0.57	r = 0.69	r = 0.24	r = -0.12	r = -0.32	r = -0.29	r = -0.25	r = 0.03
	p = 0.008*	p = 0.001*	p = 0.25	p = 0.65	p = 0.07	p = 0.06	p = 0.4	p = 0.65

^a using Spearman correlation test

^b using Kendall's tau_b correlation test

Cr = Serum Creatinine

Cr Cl = Creatinine Clearance

SBP = Systolic Blood Pressure

DBP = Diastolic Blood Pressure

Haem = Haematuria

* = Significant

Table 3: Comparison of NO Levels During Activity of Disease and After Improvement^a

+ve Ranks	-ve Ranks	Ties	Z	2 T-P
Active < improved	Active > improved	Active = improved		
3	13	0	-2.068	0.039*

^a using Wilcoxon Matched Pairs Signed Ranks Test

• = Significant

DISCUSSION

Nitric oxide is an indispensable molecule with a variety of biologic functions. It has been shown to regulate multiple cellular functions, including smooth muscle relaxation, neurotransmission and macrophage induced cytotoxicity as well as cell proliferation and apoptosis⁽⁶⁾. In the kidney, the presence of NO under physiologic conditions was confirmed by microdialysis studies⁽⁷⁾. Low concentrations of NO, released by glomerular endothelial cells, play a pivotal role in regulating microvascular hemodynamics, in concert with antagonistic vasoactive substances such as angiotensin II and endothelin⁽⁸⁾. Under pathologic conditions, NO generation in the glomeruli is markedly enhanced because of the induction of NO synthetase (NOS) expression in intrinsic glomerular cells and infiltrating activated macrophages⁽⁸⁾. Induction of nitric oxide synthetase depends probably on locally

produced cytokines⁽⁹⁾. The exact role of NO in the pathophysiology of GN is unclear; NO could have both toxic as well as protective effects. This may depend on the NOS isoform generation⁽¹⁰⁾. It has been found that enhanced production of NO was closely related to the induction of proteinuria in GN. Moreover, blocking NO synthetase expression with the specific inhibitor L-NMMA profoundly attenuated proteinuria in experimental models of GN which indicates that NO was involved in the disturbance of the glomerular permeability barrier, leading to proteinuria⁽¹⁾. Evaluation of our cases revealed significant increased serum NO during the acute attack which significantly dropped after improvement. NO levels correlated significantly with severity of the illness as manifested by the degree of proteinuria and the level of complement consumption. These findings strongly support a possible pathophysiologic role of NO in PSAGN.

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