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Oxidative Status in Children with Post-streptococcal Acute Glomrulonephritis

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This study was done to assess the oxidative status in children with post-streptococcal acute glomerulonephritis (PSAGN). For this purpose, 32 children with PSAGN during the activity of the disease (group I) and 16 children out of them, 3 months after control of the disease activity (group II) were studied. In addition, 16 age-and sex-matched healthy children were taken as controls (group III). Plasma malondialdehyde (MDA) levels, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were measured in the studied children. Compared to controls, group I patients had significantly higher plasma MDA levels (2.8 ± 0.01 Vs 1.6 ± 0.01 nmol ml^{-1}, $P < 0.0001$), depressed activity of both SOD (178.6 ± 2.1 Vs $226. \pm 6.9$ U ml^{-1}) and GSH-Px (5.5 ± 0.2 Vs 8.1 ± 0.4 U ml^{-1}) ($P < 0.0001$). Group II patients showed significant decrease in MDA concentration (1.9 ± 0.01 nmol ml^{-1}, $P < 0.0001$) and rise in SOD and GSH-Px activities (222.7 ± 0.4, 7.9 ± 0.2 U ml^{-1} respectively; $P < 0.0001$) compared to group I patients. However, plasma MDA levels were still significantly higher in group II patients than in group III ($P = 0.006$). During the activity of the disease, GSH-Px activity was negatively correlated with the degree of proteinuria ($r = -0.31$, $P = 0.04$) with tendency to correlate with hypertension ($r = -0.28$, $P = 0.08$) and creatinine ($r = -0.29$, $P = 0.09$). In conclusion, there is evidence of oxidative stress during acute phase of PSAGN that could be correlated with the severity of active glomerular disease.

Key words: Oxidants-antioxidants, post-streptococcal acute glomerulonephritis, children

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Introduction

A radical may be defined as any atom or molecule that has one or more unpaired electrons (Halliwell and Gutteridge, 1984; Fantone and Ward, 1985). Physiologically low concentrations of free radicals derived from oxygen (ROS) like the superoxide radical $O^{\cdot}_2$ are commonly formed by all aerobic cells. Excess radicals react with polyunsaturated fatty acid of the cell membrane generating huge range of products (Halliwell and Gutteridge, 1989). Many of these are aldehydes like malondialdehyde (MDA). Defense mechanisms have been established protection from the deleterious effects of excess radicals. Superoxide dismutase (SOD) form hydrogen peroxide (H_2O_2) out $O^{\cdot}_2$ radical that then can be converted to H_2O and O_2 by the catalase system (CAT). Additionally H_2O_2 can be detoxified by peroxidases, mainly the glutathione peroxidase (GSH-Px).

Excess toxic ROS can be generated within the kidney by infiltrating neutrophils and macrophages during respiratory bursts (Halliwell and Guttering, 1989) or by resident renal cells (Baud *et al.*, 1980). In the kidney, proximal tubular cells and to a lesser extent glomeruli show activities of copper-zinc (Cu-Zn) and manganese (Mn) SOD, CAT and GSH-Px (Steinert *et al.*, 1986; Yang *et al.*, 1988).

ROS participate in the pathogenesis of various renal diseases including inflammatory lesions such as glomerulonephritis, interstitial nephritis, ischemic reperfusion injury, hemolytic uremic syndrome and toxic nephropathies and possibly in the progression of chronic renal failure (Turi *et al.*, 1997). However, other groups (Webb *et al.*, 1985; Bertolatus *et al.*, 1991) did not confirm the role of oxidative injury in glomerular diseases.

This study was planned to assess the oxidative stress in children with PSAGN during the activity of the disease and after the clinical resolution by measuring plasma MDA levels and SOD and GSH-Px activities.

Materials and Methods

Patients were recruited successively from Division of Pediatric Nephrology, Mansoura University Children's Hospital, Mansoura, Egypt.

Study included thirty-two patients with PSAGN: 8 males, 24 females, aged 6.7 ± 0.59 years during the acute attack (group I). The diagnosis of PSAGN depends on the presence of hematuria, transient hypocomplementemia and evidence of preceding streptococcal infection. Patients were studied very early during the course of the disease. The severity of hypertension was assessed according the task force on blood pressure in children (Anonymous, 1987). Twenty patients had severe hypertension, 5 patients had non-severe, while 7 patients had normal blood pressure. Hematuria was microscopic in 7 patients and macroscopic in 25 patients. Eight patients had nephrotic proteinuria (more than 1 g m^{-2} d^{-1}), 17 patients had non-nephrotic range proteinuria while proteinuria was absent in 7 patients. Out of the studied patients, 16 children were reassessed after 3 months (group II). At that time, C3 levels returned to normal levels, non-of them had hypertension or proteinuria but microscopic hematuria was still present in 5 patients. In addition 16 age-and sex-matched children were served as healthy controls (group III).

All the subjects were exposed to the following investigations:

Assay of plasma lipid peroxides: The lipid peroxide content of plasma was determined by measuring the thiobarbituric acid-reactive substances expressed as malondialdehyde (MDA) equivalents (nmol ml^{-1}). This method was described by Matsumoto *et al.* (1981) in which precipitates were extracted from the plasma (0.3 ml) using 1/12 N sulfuric acid (2-4 ml) and 10% phosphotungstic acid (0.3 ml). The precipitates were then resuspended in distilled water (4.0 ml) with the addition of 1% thiobarbituric acid (1.0 ml). The reaction was allowed to take place in a boiling water bath for 1 h and color intensities of reaction products were determined at 535 nm and the results were expressed in MDA equivalents (nmol ml^{-1}). Freshly diluted tetraethoxypropan that produces MDA after hydrolysis, was used as standard.

Assay of glutathione peroxidase activity: (Kits from Randox laboratories ltd. UK). Glutathione peroxidase (GSH-Px) activity in whole blood sample was measured by the method of Paglia and Valentine (1967) in which GSH-Px catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbency at 340 nm was measured. The enzyme activities were expressed in U/ml of whole blood.

Assay of superoxide dismutase: (Kits from Randox laboratories ltd. UK). 0.5 ml of whole blood sample was centrifuged for 10 min at 3000 rpm and then plasma was aspirated off. Erythrocytes were washed four times with 3.0 ml of 0.9% NaCl solution with centrifugation for 10 min. at 3000 rpm after each wash. The washed centrifuged erythrocytes were then made up to 2.0 ml with cold distilled water.

The assay of SOD was based on the method of Crapo *et al.* (1978) and Wooliams *et al.* (1983). This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2- (4- iodophenyl) -3- (4- nitrophenol) -5- phenyl tetrazolium chloride to form a red formazan dye. The reaction products were determined at 505 nm. The superoxide dismutase (SOD) activity was measured by the degree of inhibition of this reaction. Percentage inhibition of sample was used to obtain units of SOD from standard curve and expressed in U/ml of whole blood.

Statistical analysis: The analysis of data was done by using SPSS computer package (version 9). Using Kolmogorov-Smirnov test assessed the distribution of data. Data was found to be normally distributed. Data was expressed as mean $\pm$ standard deviation. Comparison between group I or group II and group III was done by using unpaired t test, while comparison between group I and II was done by paired t tests. Pearson correlation was used to assess the correlation between serum creatinine and MDA levels and SOD and GSH-Px activities while Kendall's tau-b test was used to assess the correlation between the last three parameters and degree of hypertension and proteinuria.

Results

Data (Table 1) showed that group I patients had significantly higher plasma MDA levels (2.8 ± 0.01 Vs 1.6 ± 0.01 nmol ml^{-1}, $P < 0.0001$) and depressed activity of both SOD (178.6 ± 2.1 Vs 226 ± 6.9 U ml^{-1}) and GSH-Px (5.5 ± 0.2 Vs 8.1 ± 0.4 U ml^{-1}) as compared to controls. However, plasma MDA levels (1.9 ± 0.01) were still significantly higher in group II patients than in group III (1.6 ± 0.01). While no differences in SOD and GSH-Px were present.

Group II patients showed a significant decrease in MDA concentration (1.9 ± 0.01 nmol ml^{-1}, $P < 0.0001$) and a significant rise in the activity of SOD (222.7 ± 0.4 U ml^{-1}) and GSH-Px (7.9 ± 0.2 U ml^{-1}) compared to group I patients (Table 2). During the activity of the disease, GSH-Px activity was negatively correlated with the degree of proteinuria ($r = -0.31$, $P = 0.04$) with tendency to correlate with hypertension ($r = -0.28$, $P = 0.08$) and creatinine ($r = -0.29$, $P = 0.09$) (Table 3).

Discussion

A disturbance of the balance between oxidative stress and antioxidant defense mechanisms plays a major role in the pathomechanism of glomerular diseases (Turi *et al.*, 1997). In this study, an evidence of the presence of an oxidative stress in children with PSAGN was demonstrated. Patients have higher levels of plasma MDA levels (as an indicator of lipid peroxidation), and lower activity of the antioxidants SOD and GSH-Px during the activity of the disease.

Table 1: Comparison between plasma MDA levels and SOD and GSH-Px activities in patients with PSAGN (group I or II) versus controls (group III)

Parameters	Group I (n = 32)	Group II (n = 16)	Group III (n = 16)
MDA (nmol ml^{-1})	2.8 ± 0.01*	1.9 ± 0.01**	1.6 ± 0.01
SOD (U ml^{-1})	178.6 ± 2.1*	222.7 ± 0.4	226.4 ± 6.9
GSH-Px (U ml^{-1})	5.5 ± 0.2*	7.9 ± 0.2	8.1 ± 0.4

*: P < 0.0001 **: P = 0.006

Table 2: Comparison between plasma MDA levels and SOD and GSH-Px activities in group I versus group II

Parameters	*Group I (n = 32)	*Group II (n = 16)
MDA (nmol ml^{-1})	2.8 ± 0.01	1.9 ± 0.01
SOD (U ml^{-1})	178.6 ± 2.10	222.7 ± 040
GSH-Px (U ml^{-1})	5.5 ± 0.20	7.9 ± 0.20

Values represent Mean ± S.D *: P < 0.0001

Table 3: Correlation between plasma MDA levels and SOD and GSH-Px activities and some clinical and Lab parameters in group I patients

	Hypertension*		Proteinuria*		Creatinine**	
Parameters	r	P	r	p	r	p
MDA (nmol ml^{-1})	- 0.03	0.87	0.25	0.11	0.29	0.81
SOD (U ml^{-1})	0.01	0.9	- 0.19	0.21	- 0.32	0.07
GSH-Px (U ml^{-1})	- 0.28	0.08	- 0.31	0.04	-0.29	0.09

MDA: Malondialdehyde, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase * Kendall's tau-b test ** Pearson test

These results are in agreement with the findings of Tauri *et al.* (1997) who reported that elevated plasma MDA levels and lower GSH-Px activities in children with PSAGN. Similarly Kashem *et al.* (1994) demonstrated that circulating polymorphonuclear leukocyte (PMNL) of mesangial proliferative glomerulonephritis patients with or without mesangial IgA deposits generate $O^{\cdot}_2$ radical in significantly greater amounts than the control group.

PSAGN is characterized by diffuse proliferation of endothelial and mesangial cells as well as PMNL infiltration (Heptinstall, 1974). Granular deposits of IgG and C3 are detected by immunofluorescence in the majority of patients (Feldman *et al.*, 1966).

Resident renal cells (Baud *et al.*, 1980), in addition to PMNL (Rehan *et al.*, 1986), can be the source of ROS in this study. Cultured glomerular mesangial cells have been shown to produce ROS when exposed to complement membrane attack complexes C5b-9 (Steinert *et al.*, 1986).

Indeed, in several models of inflammation including a model of glomerulonephritis in the mouse, SOD has been shown to markedly reduce neutrophil infiltration (McCord *et al.*, 1980). Johnson *et al.* (1988) have reported that four to ten days after infusion of H_2O_2 a marked proliferative glomerular lesion develops with about a 30 to 59% increase in the resident glomerular cells. From these two observations, one could speculate that ROS could share in the pathogenesis of the histological changes of PSAGN. It's known that histologic resolution of PSAGN may be delayed than the clinical resolution of the disease (Hinglais *et al.*, 1974). This may explain the persistence of high plasma MDA levels in patients with clinically inactive disease.

Similar to other cells, renal cells respond to with increased antioxidant activities (Pimstone *et al.*, 1971). Cultured rat glomerular mesangial cells incubated with H_2O_2 were shown, to have Mn-SOD activity significantly elevated over the level of untreated cells (Yoshioka *et al.*, 1994). The Mn-SOD mRNA level in rat mesangial cells was significantly elevated after exposure to H_2O_2 (Yoshioka *et al.*, 1994). However the low levels of antioxidant activity in the patients of this study could be due to exhaustion of this system or due to down regulation of antioxidant gene expression.

Many studies suggest that ROS, by altering glomerular vascular permeability, may play a role in proteinuria (Johnson *et al.*, 1986; Rehan *et al.*, 1986). Kashem *et al.* (1994) reported a positive correlation between the amount of O_2 production and the severity of proteinuria in patients with proliferative glomerulonephritis. These observations could explain the negative correlation between GSH-Px activity and the degree of proteinuria in the patients of this study.

The weak correlation between the decline in kidney function and the activity of SOD and GSH-Px in this study could be explained by the findings of Yoshioka *et al.* (1988), who showed that ROS generated by neutrophils evoked a profound constrictive response in the glomerular microcirculation and that the effects of ROS were localized primarily on the efferent arteriole and the glomerular capillary. Basci *et al.* (1987) postulated that ROS produced by infiltrating leukocytes in glomerular diseases was associated with increased cyclic AMP. Increase cyclic AMP content in glomeruli caused a fall in the glomerular ultrafiltration coefficient (Dworkin *et al.*, 1983).

Many mediators as proteates and cation protein participate in the pathophysiologic changes in PSAGN (Johnson *et al.*, 1994). This fact could explain the absence of correlation between plasma MDA level and the parameters of the severity of the disease. Oxidant injury in PMNL-dependent glomerulonephritis is mediated primary by H_2O_2 (Rehan *et al.*, 1986). This could explain why the parameters of the severity of the disease in patients (present study) correlate mainly with GSH-Px (H_2O_2 scavenger) rather than SOD.

In conclusion, children with PSAGN have an oxidative stress due to imbalance between oxidants and antioxidants. These results may open doors for the use of antioxidants in the management of PSAGN. Further studies are recommended to clarify the mechanisms of depressed antioxidant activity in these children.

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