The Assessment of Thiol Status in Children with Neurogenic Bladder Caused by Meningomyelocele

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Purpose: Oxidative stress can cause tissue damage in many diseases. Oxidative status depends on the balance between total oxygen radical absorbance capacity and antioxidants. Neurogenic bladder (NB) is a special state where oxidative status can influence urinary tract function. We decided to measure antioxidant (thiol) status in patients with NB and assess the effect of NB on the urinary antioxidant status and to correlate it with urodynamic findings.

Materials and Methods: The investigation was conducted on two groups. The first group, constituted of 41 children with NB. The second group, consisted of 20 healthy children with no abnormality in urinary and nervous systems. The antioxidant status was assessed based on the enzyme-linked immunosorbent assay of thiols.

Results: The median value of urinary protein thiol level was significantly lower in NB patients than in reference group [median 48 (0.0-633.33) and 221.55 (0.17-1293] μmoL/g protein, respectively ($P < .01$). We found out the statistically significant differences in urinary thiol level between patients with and without overactivity ($P = .017$) and between catheterized and non-catheterized patients ($P = .048$).

Conclusion: This study demonstrates that antioxidant status in patients with NB decreased and the level of thiol status depends on the grade of bladder overactivity. Oxidative stress may be involved in the pathophysiology of bladder dysfunction related to neurogenic damage.

Keywords: urinary bladder, neurogenic, overactive; spinal dysraphism, complications; meningomyelocele; pyrroles.

INTRODUCTION

Oxidative stress can cause tissue damage in many children and adults diseases. Hyperglycemia in diabetic adult patients can increase the levels of free radicals.[1] Other authors have described an increase in oxidative stress in pregnant woman, patients with rheumatoid arthritis and bladder carcinoma and in children with cerebral palsy.[2-5] Oxidative status depends on the balance between total oxygen radical absorbance capacity and antioxidants as a compensatory reaction of the body. Among many antioxidants, thiols (sulfhydryl groups) may play an important role. There are some notifications about plasma or urinary thiol status in pediatric nephrological problems. An impaired antioxidative system has also been observed in patients with nephrotic syndrome, primary glomerular disease, and in patients with proteinuria or renal failure.[6-10]

Neurogenic bladder (NB) is an exceptional, incurable state, depended on the range of nervous system damage. To assess the bladder function, the urodynamic study should be performed. In this procedure we can measure intravesical pressure during storage and voiding phase. Most of the patients with NB have dysfunctional voiding so our measurement is based only on storage phase. The time of observation in this procedure is quite short and the outcomes cannot be accurate. We don't know what is going on with bladder function during whole day and/or night time? There are many factors involved in the control of bladder function. In healthy children, firstly it stayed under central nervous system (CNS) control. Each levels of CNS (brain, spinal cords, and peripheral ganglia) are involved in this control. There are some suggestions demonstrating that oxidative status influences urinary tract function. Most of them demonstrate destroyed balance in plasma reactive oxygen species (ROS) and antioxidants (e.g. ascorbic acid, α-tocopherol, uric acid and bilirubin) which may minimize tissue damage mostly in adults and mainly in an experimental data.[11-15]

Patients with NB are still significant group among dialyzed patients. There is a need to look for the reliable examinations for early detection of lower urinary tract deterioration. We have no doubts that early identification of the risk factors of chronic renal failure development should have priority, from the healthy and the economical point of view too.

Till now, there were only a few studies which examined urinary antioxidant status in patients with NB. Most of them were concentrated on the adult patients.[16,17] None of them assessed the thiol status. Hence, we decided to measure antioxidant status in the urine of children with NB based on the assessment of urinary thiol status and compare it with healthy subjects. The aim of our study was to investigate the urinary antioxidant status in patients with NB due to meningomyelocele (MMC) and to correlate it with bladder function.

MATERIAL AND METHODS

The study was carried out in the department of pediatrics and nephrology, medical university of Bialystok, Poland. Patients with urodynamically confirmed diagnosis of NB were included in the study. Finally, 41 NB patients aged median 9.0 (0.7-17.5) years old were enrolled in the study (group 1). Twenty healthy individuals aged median 9.5 (3-17) years old without any nephrological and CNS diseases history were enrolled as a reference group (group 2). This group was recruited from healthy elementary, middle and secondary school pupils, obtained from 2007 to 2009 in the OLAF study: "Elaboration of reference blood pressure ranges for Polish children and adolescents" PL0080 OLAF. The material from the younger control subjects (aged 3-6 years old) was obtained from healthy children attending to day care or nursery school. The healthy subjects were on normal diet without any vitamins, drugs or diet supplements.

The eligible cases (group 1) were male and female patients aged 1-18 years old with NB due to MMC and with voiding dysfunction for at least one year prior to screening. Patients in MMC group underwent cystometry and patients in non-catheterized group underwent uroflowmetry. All of them had normal renal function [glomerular filtration rate (GFR) of > 90 mL/min/1.73m^2] and normal serum creatinine levels. According to the findings of urodynamic study oxybutynin was administered if necessary.

Patients with urinary tract infection (UTI) in the past 4 weeks or other infections were excluded from the study. The non-catheterized patients with NB and children from the control group all underwent uroflowmetry study 3 times and to increase precision, the results were averaged and compared with the urinary thiols concentrations.

Most of NB patients cannot empty their bladders by themselves so we were terminating the infusion during cystometry when the volume of solution was the same as obtain from everyday clean intermittent catheterization (CIC). It was our intention to imitate bladder function as in natural environment. NB children received medications according to the urodynamic study results. The urodynamic work-up included

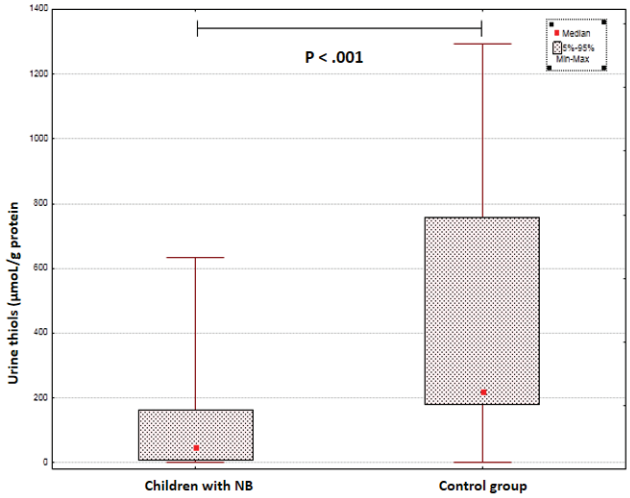

Figure 1. The comparison of urine thiol levels between patients with neurogenic bladder (NB) and reference group.

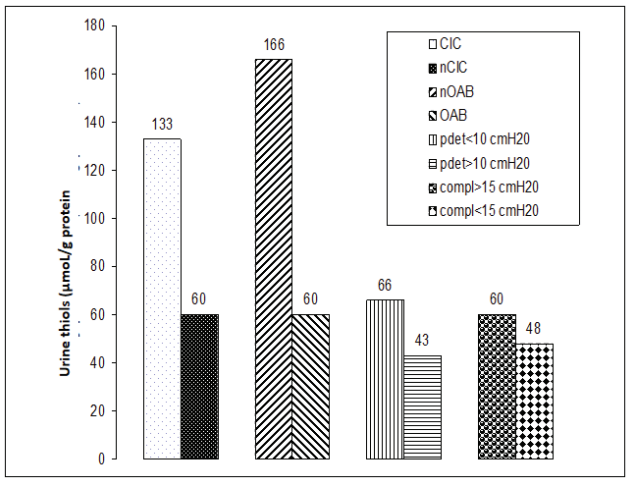

Figure 2. The data of median urine thiols in patients with neurogenic bladder in various urodynamic conditions.

the measurements of following parameters: detrusor pressure at overactivity (Pdet overact), detrusor pressure at maximum cystometric capacity (Pdet CC), bladder wall compliance, and electromyography (EMG) of sphincter at beginning (EMG 1) and at the end (EMG 2) of the filling phase. Anticholinergic drugs were administered if the patient had detrusor overactivity. Informed consent was obtained from all subjects and their parents for all procedures connected to obtaining biological material.

The first daytime urine samples were collected from all examined patients and urinary total protein, creatinine, microalbumin and osmolality were determined. Urinary thiol (sulfhydryl) status was measured by enzyme-linked immunosorbent assay (ELISA) according to manual instruction, (Immundiagnostik AG Stubenwald-Allee 8a, 64625 Bensheim, Germany). Urinary protein and creatinine levels were also measured in 24-hour urine samples by an automated clinical analyzer. Urinary thiol levels were calculated from the total thiol levels adjusted for protein concentration in urine and expressed in µmol/g protein. Serum proteins, albumin and creatinine concentrations were determined in both groups. The GFR was calculated using Schwartz formula. The study was approved by the Ethics Committee of Medical University of Bialystok in accordance with the Declaration of Helsinki. The OLAF study was approved by The Children's Memorial Health Institute Ethics Committee.

Statistical Analysis

The demographic and biochemical data of NB patients was statistically analyzed and expressed as median with minimum and maximum range compared with reference group. Since the antioxidant parameters were not as per the Gaussian distribution, Mann-Whitney *U* test was used for the comparisons between 2 groups. Spearman's coefficient of correlations (*r*) were calculated to look at the possible association between thiol parameters and biochemical and urodynamic one. All statistical analyses were performed using Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA). A *P* value of less than .05 was considered statistically significant.

RESULTS

Characteristics of studied subjects are shown in Table 1. There were no statistically significant differences in demographic characteristics of studied groups such as age, gender and body mass index. There were differences in the physical development parameters resulted from the principal disease. The children with MMC had lower muscular mass (due to paralysis of the limbs) or excess body weight resulting from the lack of activity during the lifespan (the children were wheelchair dependent). Moreover, the differences in the height are often caused by distortion and malformations of the bone structure. The median time of follow up of the NB patients was 6 (0.5-15) years. Nine from 41 patients were non-catheterized. The catheterized subjects were emptying their bladder with median urination frequency of 4 (3-5) times per day with night brake.

In present study we compared the parameters of the kidneys

Table 1. The characteristics of study groups and comparison between patients with neurogenic bladder and reference group.*

Variables	Group 1	Group 2	*P*
Age (years)	9.0 (0.7-17.5)	9.5 (3-17)	.32
Gender			
Male	19	8	NA
Female	22	12	NA
Height (cm)	134 (70-170)	152 (89-176)	.004
Weight (kg)	29 (6.2-92)	43 (16-70)	.021
Body mass index (kg/m^2)	17.01 (8.83-17)	18.13 (12-24)	.75
Oxybutynin administration			
mg/day	3.75 (1.25-10)	-----	NA
mg/kg body weight	0.17 (0.1-0.42)	-----	NA
OAB treatment/none treatment	16/41	-----	NA
Serum creatinine (mg/dL)	0.32 (0.19-0.77)	0.51 (0.2-083)	.00
Urine creatinine (mg/dL)	53.17 (14.58-149)	100.17 (63-244)	.000
GFR mL/min/1.73m^2 body surface	233 (102-303)	162.91 (110-330)	.017
Urine osmolality	715 (314-1177)	685 (391-1130)	.85
Microalbuminuria	5.5 (0.1-386.9)	1.4 (0.3-51.8)	.47
Serum protein (g/L)	7.08 (5.8-8.13)	7.55 (6.62-8.14)	.12
Serum albumin (g/dL)	4.63 (3.61-5.47)	4.79 (4.41-5.23)	.067
Urine protein (mg/dL)	10 (0-128)	0 (0-2)	.000
Urine protein (mg/24-hour)	60 (0-560)	5.11 (1.06-10.05)	.02

Keys: OAB, overactive bladder; GFR, glomerular filtration rate; NA, not applicable.
* Data are presented as median (range).

function (urinary and serum creatinine, urinary excretion of the protein, urine osmolality, GFR and microalbuminuria) and serum albumin and protein levels. Laboratory findings and comparisons are shown in Table 1. We observed statistically significant differences in the serum creatinine, GFR and creatinine and protein excretion (in morning sample and 24-hour urine collection). There were differences between study groups in the serum albumin concentrations values but the results were not statistically significant. We did not find differences in presence of microalbuminuria and serum protein levels between study groups (P = .47 and P = .12, respectively).

As it has been shown in Figure 1, the urinary thiol level was significantly decreased in NB patients compared to the control group and the related numerical data were, median 48 (0.0-633.33) and 221.55 (0.17-1293) µmol/g protein, respectively (P < .001). This reduction was not related to UTI in the past, the follow-up duration and bladder wall thickness, or the dose of administered treatment.

Urodynamic findings are shown in Table 2. We revealed statistically significant differences in most uroflowmetry parameters between study groups. Urinary thiol level correlated negatively with voided volume (r = -0.450, P < .05) and positively with residual urine (r = 0.25, P < .05). The thiol status levels in various urodynamic conditions in NB patients are shown in Figure 2. We found statistically significant differences in urinary thiol levels between patients with and without bladder overactivity (P = .017) and between catheterized and non-catheterized patients (P = .048). We also found differences between urinary thiols concentrations between patients with normal and low bladder compliance (P = .33) and between patients with low (< 10 cmH2O) and high (> 10 cmH2O) detrusor pressure during filling phase (P = .48), but the differences were not statistically significant. There were no correlations between urinary thiol and cystometric capacity, EMG activity at the beginning and at the end of filling phase. Apart of outcomes mentioned above we found positive correlation between urinary thiol status and the age of NB patients (r = 0.33, P < .05) and serum protein concentration (r = .355, P < .05).

Table 2. Urodynamic findings and comparison between study groups.*

	CYSTOMETRY							
	Pdet, cmH2O	Pdet CC, cmH2O	Bladder wall compliance, mL	EMG 1, micro-volts	EMG 2, microvolts			
NB patients	(4-100) 25	(2-75) 14	(3-70) 10	4.5 (0-25)	7 (0-47)			
	UROFLOWMETRY							
	Time to max flow, s	Delay time, s	Flow time, s	Voiding time, s	Maximum flow rate, mL/s	Average flow rate, mL/s	Voided volume, mL	Residual urine, mL
NB patients	7 (7.0-23.4)	15.6 (3.4-280)	20 (2-129)	28 (3-427)	6.3 (1.4-15)	4.25 (0.5-13)	111.4 (5-275)	80 (5-117.5)
Control group	7 (4-12)	2 (1-3)	17 (10-38)	19.5 (11-41)	22.9 (13.3-41)	18.1 (6-31)	219.5 (104-456)	0 (0-5)
P	.835	< .001	.340	.061	< .001	< .001	.004	< .001

Keys: Pdet, detrusor pressure at overactivity; Pdet CC, detrusor pressure at cystometric capacity; EMG 1- electromyography of sphincter at the beginning of filling phase; EMG 2, at the end of filling phase; NB, neurogenic bladder.
* Data are presented as median (range).

DISCUSSION

The purpose of this study was to evaluate the antioxidant status in urine of patients with MMC and to answer whether oxidative status has an influence on the bladder function. The results demonstrated that the decreased concentration of sulfhydryl groups (-SH) or groups existing as protein bound thiols in the urine of patients with NB is caused by increased oxidation. Our findings are comparable to the data in the literature in various pathological conditions in children. Kazunari and colleagues[18] assessed urinary 8-hydroxy-2-deoxyguanosine (8-OhdG) in patients with idiopathic nephrotic syndrome and reported increased levels of ROS and decreased levels of antioxidants in the active phase of the diseases which were normalized in remission phase. These findings are in agreement with Mishra and Schaefer's study.[19] These findings suggest an important role of oxidative status in the pathogenesis of idiopathic nephrotic syndrome in children. Chien and colleagues[20] in an experimental study revealed that substance P influences NB function by its ability to stimulate ROS generation. Other experimental studies have demonstrated imbalance between ROS and antioxidant ability (as a positive reaction of our body) in neurogenic damage. We did not find any study concerning with measuring of urinary thiol (sulfhydryl) groups in patients with NB. Decreased urinary protein thiols in patients with overactive bladder let us to suspect that oxidative stress is involved in the disturbed bladder function. Thus, could we try to normalize function of the bladder by taking antioxidants? Could we influence the detrusor pressure by antioxidants? Could we change the muscarinic receptor function by decreased oxidative stress?[13] What could be the administration method? Answer to this questions required further, very well-planned and good-organized studies which we are planning. Till now, the literature review shows that there are some therapeutic interventions and numerous bioactive compounds that have antioxidant status benefits but unfortunately still in clinical trials or on animal models.[16]

Our results, focused on positive correlation between urinary protein thiol level and serum protein concentration, raise an interesting question whether higher serum protein concentration can increase urinary protein excretion and in this way affect the oxidative status in NB?

Summarizing, received from uroflowmetry results let us speculate that, although the patients emptying their bladders by themselves (urodynamic findings let them to do that), the bladders do not work correctly. It confirms that non-catheterized NB patients require greater attention or verify recommendations.

The kidney function deterioration is connected with the higher frequency of detrusor overactivity diagnosed in childhood.[21] Thus, the meticulous estimation based on the urodynamic findings in connection with the assessment of oxidative status in childhood can be a very important prediction to later kidney impairment, it is the potential clinical application of our finding. This condition must be fulfilled especially in such exceptional state as NB is, when the prognosis of blad-

der function in NB children mostly remains unclear.

Our study has some limitations. Most of the MMC patients were treating with oxybutynin in different doses and there was no possibility to interrupt therapy. The non-treated group was too small and did not allow us to draw an unequivocal conclusion. Another limitation of this study was a one-time thiol status evaluation in specific patients, which may not accurately reflect NB function. Thus, further studies are necessary to better clarify correlations between bladder function and oxidative status in MMC patients.

CONCLUSION

We concluded that antioxidant status in patients with NB decreased in patients with overactive bladder and the level of thiol status depends on the grade of detrusor overactivity. In addition, oxidative stress may be involved in the pathophysiology of bladder dysfunction related to neurogenic damage.

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CONFLICT OF INTEREST

None declared

REFERENCES

1. Awanti S, Baruah PS, Prakash M. Serum and urine protein thiols in type 2 diabetes mellitus patients. Indian J Physiol Pharmacol. 2009;53:185-8.
2. Umeshchandra S, Umeshchandra DG, Awanti S. Serum protein thiol status in pregnant women with malaria. RJPBCS. 2012;3:114-9.
3. Pedersen-Lane JH, Zurier RB, Lawrence DA. Analysis of the thiol status of peripheral blood leukocytes in rheumatoid arthritis patients. J Leukoc Biol. 2007;81:934-41.
4. Badjatia N, Satyam A, Singh P, Seth A, Sharma A. Altered antioxidant status and lipid peroxydation in Indian patients with urothelial bladder carcinoma. Urol Oncol. 2010;28:360-7.
5. Kulak W, Sobaniec W, Solowej E, Sobaniec H. Antioxidant enzymes and lipid peroxides in children with cerebral palsy. Life Sci. 200;77:3031-6.
6. Karthikeyan K, Sinha I, Prabhu K, Bhaskaranand N, Rao A. Plasma protein thiols and total antioxidant power in perdiatricnephrotic syndrome. Nephron Clin Pract. 2008;110: 10-4.
7. Markan S, Kohli HS, Sud K, et al. Oxidative stress in primary glomerular diseases: a comparative study. Mol Cell Biochem. 2008;311:105-10.
8. Prakash M, Shetty JK, Dash S, et al. Urinary protein thiols in different grades of proteinuria. Indian J Clin Biochem. 2008;23:404-6.
9. Mallikarjunappa S, Prakash M. Urine protein thiols in chronic renal failure patients. Indian J Nephrol. 2007;17:7-9.
10. Nakai K, Yoneda K, Maeda R, et al. Urinary biomarker of oxidative stress in patients with psoriasis vulgaris and atopic dermatitis. J Eur Acad Dermatol Venereol. 2009;23:1405-8.
11. Goulart M, Batoréu MC, Rodrigues AS, Laires A, Rueff J. Lipoperoxidation products and thiol antioxidants in chromium exposed workers. Mutagenesis. 2005;20:311-5.
12. Masuda H, Kihara K, Saito K, et al. Reactive oxygen species mediate detrusor overactivity via sensitization of afferent pathway in the bladder of anesthetized rats. BJU Int. 2008;101:775-80.
13. de Jongh R, Haenen GR, van Koeveringe GA, Dambros M, De Mey JG, van Kerrebroeck PE. Oxidative stress reduces the muscarinic receptor function in the urinary bladder. Neurourol Urodyn. 2007;26:302-8.
14. Azadzoi KM, Yalla SV, Siroky MB. Oxidative stress and neurodegeneration in the ischemic overactive bladder. J Urol. 2007;178:710-5.
15. Kawada N, Moriyama T, Ando A, et al. Increased oxidative stress in mouse kidneys with unilateral obstruction. Kidney Int. 1999;56:1004-13.
16. Jia Z, Zhu H, Li J, Wang X, Misra H, Li Y. Oxidative stress in spinal cord injury and antioxidant-based intervention. Spinal Cord. 2012;50:264-74.
17. Barrington JW, Jones A, James D, Smith S, Stephenson TP. Antioxidant deficiency following clam entrerocystoplasty. Br J Urol. 1997;80:238-42.
18. Kaneko K, Kimata T, Takahashi M, Shimo T, Tanaka S, Tsuji S. Change in urinary 8-hydroxydeoxyguanosine in idiopathic nephrotic syndrome. Pediatr Nephrol. 2012;27:155-6.
19. Mishra OP1, Gupta AK, Prasad R, et al. Antioxidant status of children with idiopathic nephrotic syndrome. Pediatr Nephrol. 2011;26:251-6.
20. Chien CT, Yu HJ, Lin TB, Lai MK, Hsu SM. Substance P via NK1 receptor facilitates hyperactive bladder afferent signaling. Am J Physiol Renal Physiol. 2003;283:840-51.
21. Thorup J, Biering-Sorensen F, Cortes D. Urological outcome after myelomeningocele: 20 years of follow-up. BJU Int. 2011;107:994-9.