# Inflammatory and oxidative status in neurogenic bladder children after meningomyelocele

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## ABSTRACT

**Introduction:** Neurogenic bladder (NB) most often is caused by meningomyelocele (MMC) and manifests with various lower urinary tract dysfunctions. Condition of NB is worsened by inflammatory process or oxidative status imbalance.
**Purpose:** To estimate of urinary uric acid (UA), hs-CRP, thiol status in association with NB function in MMC patients.
**Materials/Methods**: 33 MMC children and 20 healthy individuals were included into the study. The first daytime urine samples were collected from all examined participants and urinary thiol status, hs CRP and UA were measured.
**Results**: MMC children presented higher urinary UA level. The median hs-CRP level were also higher in MMC patients compared to the reference.

Thiol status were lower in MMC individuals compared to reference group. We found positive correlation between serum creatinine, serum UA and urine creatinine and negative between serum creatinine and GFR. Correlations between urinary UA and physical development parameters, renal function, hsCRP, thiol status and urodynamic findings in MMC and reference groups were found.
**Conclusions**: UA is a marker potentially having direct effect on the bladder function. Disturbed oxidative status and increased markers of inflammation may be a potentially modifiable factors affecting function of lower urinary tract in MMC children.
**Key words**: uric acid, thiol staus, C-reactive protein, urodynamics, bladder function

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# INTRODUCTION

Neurogenic bladder (NB) is most often caused by meningomyelocele (MMC) and manifests with various lower urinary tract dysfunctions [1]. It is well known that many factors influence NB function (prostanoids, ATP, nitric oxide (NO), cytokines, immunoglobulins, free oxygen radicals, nerve growth factor) and in these can modify glomeruli and tubule function causing renal failure [2-6]. The condition of NB is worsened by inappropriate function during urinary tract infections (UTI), high intravesical pressure caused by detrusor overactivity or dysfunctional voiding. Irregular catheterization and the inflammatory process is a background of these abnormalities. Recent studies have demonstrated that an elevated serum UA level is also associated with systemic inflammatory mediators in various clinical conditions [7-9]. It has been shown that soluble UA can induce vascular smooth muscle cell proliferation in vitro [10], predict development of albuminuria in diabetic patients [11] or hypertension [12,13] and is involved in cardiovascular events through the inflammatory mechanism [14]. On the other hand, UA as a natural antioxidant provides an antioxidant capacity in human blood [15]. There are some suggestions that antioxidants, e.g. ascorbic acid, alfatocopherol, urine acid and bilirubin, may minimize tissue damage mostly in adults and in experimental data [16-18]. High-sensitivity C-reactive protein (hs-CRP) is a very well-characterized marker of low-grade inflammation [19]. Oxidative stress markers increased in the early stages of infection in many disorders [20]. 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.

Taking the above into account (hs-CRP as marker of inflammation and thiols as antioxidants), it is worth to examining if UA plays a role as a marker of inflammation or as an antioxidant. However, the data estimating this effect and interrelations are not available.

Therefore, the aim of this study was to estimate urinary UA, hs-CRP, and thiol status (TS) in association with NB function in patients after meningomyelocele.

# MATERIALS AND METHODS

33 children and adolescents aged median 8.87 (1.83-18) yrs. (15 boys; 18 girls) with urodynamically confirmed diagnosis of NB after meningomyelocele were included in the study. Twenty healthy individuals (7 boys; 13 girls, median age:11 (1-17) yrs.) without any history of nephrological and nervous system diseases (including UTIs in the past) were enrolled as a reference (R), they were recruited from healthy volunteers selected during examination before vaccination at primary physician's office and from the hospital staff's children. The healthy subjects were on a standard diet without any vitamins, drugs or diet supplements. Health status was determined based on the patients' medical past histories, parental reports and routine laboratory tests to rule out the presence of acute and chronic inflammation.

Patients who met all the following inclusion criteria were enrolled in the study: 1. Age: 1-18 years, 2. MMC patients with neurogenic bladder confirmed in cystometry, 3. Normal blood pressure for age, centile of height, and gender, 4. normal renal function (creatinine level in normal range, GFR>90ml/min/1.73m$^2$), 5. no clinical and laboratory signs of infection 6. informed consent form signed by the patients and their parents. Patients with hypertension, a history of gouty arthritis, renal stones, UTIs, diabetes mellitus, any other infections, treated with allopurinol, antibiotics and anti-inflammatory drugs were excluded.

The non-catheterized NB patients (n=7) and children from the reference group underwent uroflowmetry (3 times to precise the outcomes), and averaged outcome was calculated. Most NB patients (n=26) can't empty their bladders by themselves so filling during cystometry was terminated when the infusion volume was the same as the patient obtained from everyday CIC, because our intention was to imitate bladder function as in the natural environment. Additionally, urodynamic work-up included: in cystometry: detrusor pressure at overactivity (Pdet overact), intravesical pressure at maximum cystometric capacity (Pves CC), and bladder wall compliance (Comp). Urodynamic findings were classified as: neurogenic detrusor overactivity (NDO), areflexic bladder (AB), and neurogenic detrusor-sphincter discoordination (NDSD).

Patients were divided into 4 groups according to Hoffer's scale (HS), which assesses physical activity (1HS- wheelchair-bounded patient (n=19), 2HS – therapeutic walkers (n=5), 3HS – household walkers (n=3), 4HS – community walkers (n=5).

The age, gender, height, weight, body mass index (BMI), blood pressure (BP), and underlying comorbidities were recorded.

The first daytime urine samples were collected from all examined participants and stored at -80ºC for further analysis. Urinary thiol protein (sulfhydryl) status was measured by enzyme-linked immunosorbent assay (ELISA) according to manual instruction (Immundiagnostik AG Stubenwald-Allee 8a, 64625 Bensheim, Germany). Urinary TS levels were calculated from total thiol levels adjusted for protein concentration in urine and

expressed in μmol/g protein. Urinary hs-CRP levels were measured by ELISA according to the manufacturer's instructions (Immundiagnostik AG, Germany), and then adjusted for urine creatinine concentration and expressed as hs-CRP/creat ratio (ng/mg creatinine). The biochemical work-up included: in urine: creatinine concentration and urine osmolality, UA excretion expressed as 1. UA/kg body mass, 2. UA/100 ml GFR 3. UA/m$^2$ body surface) [21, 22]; in serum: concentrations of creatinine (measured by Jaffe reaction), urea and UA. Urinalysis was performed to exclude subjects with hematuria and leukocyturia. The glomerular filtration rate (GFR) was calculated using Counahan-Barratt Equation (eGFR): GFR = 0.43 x L (cm)/ Scr (mg/dl), L – length, Scr – serum creatinine level. Glomerular hyperfiltration (GHF) was defined as eGFR>140ml/min [23].

All the participants demographic and biochemical data were statistically analyzed and expressed as median with minimum and maximum. The Mann-Whitney U and Kruskal-Wallis tests were used for the comparisons and the Spearman test for correlations between parameters in both studied groups. Statistical analysis was performed using Statistica 10.0. A p-value of less than 0.05 was considered statistically significant.

Informed consent was obtained from all individual participants included in the study.
The study was approved by the Ethics Committee of the Medical University of Bialystok in accordance with the Declaration of Helsinki.

## RESULTS

The clinical and biochemical characteristics of the study participants and comparisons between the two groups are enclosed in Table 1.

**Table 1.** The median values and ranges of basic demographical data and examined parameters in MMC children and reference group. Comparisons between both studied groups

| Parameters (SI) | MMC | Controls | Comparison |
|---|---|---|---|
| | Median (minimum-maximum) | | |
| Female/male | 15/18 | 7/13 | 0.25 |
| Age (years) | 8.87 (1.83-18) | 11(1-17) | 0.21 |
| Height (cm) | 1.2 (0.82-1.67) | 1.54 (0.76-1.9) | <0.001* |
| Weight (kg) | 29.54 (6.81-92) | 38.5 (8.1-71) | 0.01* |
| BMI (kg/m$^2$) | 15.91 (9.08-37.19) | 18.08 (12.02-24.51) | 0.65 |
| S creatinine (mg/dl) | 0.31 (0.18-0.77) | 0.51 (0.2-0.85) | <0.001* |
| S urea (mg/dl) | 27 (11-42) | 31(16-40) | 0.48 |
| S UA (mg/dl) | 3.86 (1.32-7.33) | 3.94 (3.35-5.25) | 0.74 |
| U creatinine (mg/dL) | 51.65 (15.84-111.54) | 129.55(63.56-244.05) | <0.001* |
| U UA (mg/kg body mass) | 13.4( 5.69-42.86) | 9.57(3.33-21.11) | 0.03* |
| U UA (mg/m$^2$) | 610.32(268.81-1754.5) | 486.46(202-02-985.48) | 0.047* |
| U UA (mg/100 ml GFR) | 0.18( 0.07-0.65) | 0.2(0.09-0.42) | 0.58 |
| U  UA (g/24h) | 0.3 (0.13-0.91) | 0.34 (0.1-0.52) | 0.35 |
| eGFR (ml/min/1.73m$^2$) | 180.1 (93.26-310) | 143.16 (110-240) | 0.04* |
| Urine Osmolality(mOsm/kgH$_2$O) | 711.5 (357-1177) | 690 (391-1200) | 0.67 |
| 24h urine collection (ml) | 660 (100-1500) | 925 (400-1500) | 0.21 |
| hs-CRP/crea (ng/mg creatinine) | 8.52 (3.32-23.58) | 3.72 (1.53-12.66) | <0.001* |
| Thiol status | 51.16(0.00-633.33) | 221.55(99.5-1293.1) | <0.001* |
| MMC- meningomyelocele patients; S-serum; U-urine; UA-uric acide; e-GFR- Counahan- Barratt Equation Significant value: *p< 0.05; **p<0.01 | | | |

Overall median age of all children with MMC was 8.87 (1.83-18) years. The age, gender, and BMI of the studied children did not differ from the reference group (p=0.21; p=0.28; p=0.65, respectively). Statistically significant differences were found in the parameters of physical development (body weight p=0.01 and height p<0.001), which were the result of the disease. The MMC children demonstrated lower muscle mass (due to limbs paralysis) or excess body weight resulting from lack of physical activity (wheelchair-bound patients). Moreover, differences in body length were caused by distortions and malformations of the bone structure.

When compared to the reference group, MMC children presented significantly higher urinary levels of UA in mg/kg body mass (p=0.03) and UA in mg/m$^2$ of body surface (p=0.047). We

did not find differences between the MMC and the reference group in median urinary UA in mg/100ml GFR (p=0.58) and median total urinary UA excretion (mg/24h) (p=0.35). Details are shown in Table 1. There were no differences in these parameters between boys and girls nor between the Hoffer's scale. Urinary UA levels in mg/100 ml GFR in younger children were lower than in older children, and the difference was statistically significant (p<0.001). Median hs-CRP levels were

also significantly higher in MMC patients compared with controls (8.91 ng/mg crea (3.82-23.58); 3.72 (1.53-12.66), respectively) (p<0.001). There were no differences in hs-CRP between boys and girls (p=0.68) nor between catheterized and non-catheterized children (p=0.22). There were no significant differences in median hs-CRP levels between patients from the different Hoffer's scale groups. The detailed data are included in Table 2.

**Table 2.** Table 1. Urinary hs-CRP and thiol status (TS) in patients with MMC depending on the Hoffer's scale (HS)

| | 1HS | 2HS | 3HS | 4HS |
|---|---|---|---|---|
| | **Median (minimum-maximum)** | | | |
| hs-CRP/crea | 9.1 (2.9-22.5) | 10.9 (7.2-22) | 8.0 (4.06-9.8) | 6.5 (3.5-11.1) |
| TS | 35 (0-428.57) | 75 (8-444.4) | 31.6 (12-51.2) | 189 (8-633.33) |
| 1HS- wheelchair dependent patient; 2HS - moving with difficulties; 3HS - need support during moving; 4HS - moving without problems | | | | |

TS was lower in MMC individuals compared with the reference group (median 51.16 and 221.55, respectively) (p<0.001). There were no differences in TS between boys and girls (p=0.52) nor between non- and catheterized children (p=0.65). The younger children (under 10 years old) had statistically significantly lower TS (median 17umol/g creat (0-633.3) compared with the older ones (median 88.89 umol/gcreat) (p=0.03). There were no such differences between the age groups in healthy children (younger-median 196.5 umol/gcreat (185.18-1293.1); older - median 252.2umol/g creat (99.5-1200) (p=0.9). Children from various Hoffer's scale groups differed in median TS. The highest values were seen in

patients from HS4 group, and there were no statistically significant differences compared with the controls. Patients from HS1, HS2 and HS3 had statistically significant lower median TS compared with the reference. The detailed data are included in table 2.

We found positive correlations between serum creatinine, serum UA (r=0.482, p<0.05), and urine creatinine level (r=0.475, p<0.05); negative correlations were revealed between serum creatinine and GFR (r=-0.865, p<0.05). Correlations between urinary UA and parameters of physical development, renal function, hsCRP, TS and urodynamic findings in MMC patients and the reference group are presented in Table 3.

**Table 3.** The correlations between urinary uric acid (UA) and parameters of physical development, biochemical parameters, hs CRP, thiol status and urodynamic findings

| Parameters | Urinary Uric Acid | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | g/24h | | mg/kg/24h | | mg/100ml GFR | | mg/1.73m$^2$ | |
| | MMC | Ref | MMC | Ref | MMC | Ref | MMC | Ref |
| Age (years) | 0.378* | 0.069 | -0.727* | -0.520* | 0.579* | -0.39 | -0.593* | -0.376 |
| Height (cm) | 0.45* | -0.331 | -0.723* | -0.882* | 0.594* | 0.042 | -0.557* | -0.751* |
| Weight (kg) | 0.544* | -0.319 | -0.751* | -0.93* | 0.576* | 0.118 | -0.528* | -0.763* |
| BMI | 0.583* | -0.276 | -0.606* | -0.592 | 0.474* | 0.224 | -0.37* | -0.455* |
| S creat. | 0.305 | -0.036 | -0.558* | -0.622* | 0.724* | 0.517* | -0.479* | -0.455* |
| SUA | 0.147 | 0.771 | -0.491* | 0.142 | 0.324 | 0.771 | -0.42* | -0.314 |
| hs-CRP | -0.444* | 0.157 | 0.077 | 0.198 | -0.527* | -0.04 | -0.03 | 0.282 |
| TS | 0.26 | -0.127 | -0.181 | -0.045 | 0.491* | -0.12 | -0.157 | -0.066 |
| 24h urine collection | 0.51* | 0.601* | -0.577* | 0.212 | 0.618* | 0.278 | -0.392 | 0.326 |
| Pdet urg | 0.191 | NA | 0.505 | NA | 0.342 | NA | 0.596* | NA |
| CC | 0.419* | NA | -0.509* | NA | 0.466* | NA | -0.332 | NA |
| Compliance | 0.374 | NA | -0.314 | NA | 0.53* | NA | -0.165 | NA |
| BMI- body mass index; SUA- serum uric acid; TS- thiol status; Pdet urg –detrusor pressure at urgency; CC-cystometric capacity; NA- non analyzed; * p<0.05 | | | | | | | | |

## DISCUSSION

To the best of our knowledge, this study is the first clinical evaluation of urinary levels of hs CRP, urinary UA, and thiol status in children and adolescents with neurogenic bladder after meningomyelocele. The findings of this cross-sectional study in children with neurogenic bladder are as follows: 1. Hs CRP levels in the examined group of children were significantly higher than in healthy controls. 2. Thiol status in the MMC children was significantly lower than in the reference group 3. UA excretion in mg/kg body mass and in mg/m$^2$ were increased in MMC patients compared with controls. There were no differences between MMC patients and the reference group in total urinary UA excretion (mg/24h) and based on GFR (mg/100ml GFR).

So far, there are no perfect methods for estimating UA excretion in children. From the many methods, the most universal are estimations based on excretion counted per kg of body mass and body surface, and less popular is per 100 ml of GFR [21,22,24].

It seems that in neurogenic bladder children, estimation based on GFR is the most adequate because of the many difficulties regarding with 24h urine collection and improperly counted surface of the body caused by minor or major disproportions in weight and length of the body in this group of children. Moreover, UA excretion based on GFR seems to be most appropriate because of hyperfiltration, which has been demonstrated in these children. Elevated serum UA level may activate a cascade of inflammation in many tissues and may be suspected of being responsible for such neurological signs as: poor muscle control or moderate mental retardation [25].

We did not find hyperuricemia in our patients compared with the reference, but hyperuricuria was found in MMC children. Balasubramanian [26], in an experimental study designed on Wistar Rats, described that increased urinary UA affects changes in the metabolic state of these animals and via unknown humoral factor increases serum glucose, insulin and total cholesterol.

It is more likely that UA may also be responsible for regulation mechanisms in the human bladder as we demonstrated correlations between urodynamic parameters and UA excretion. This may indicate that urinary UA may play an important role in affecting bladder function in MMC children. This observation requires further research.

On the other hand, hyperuricemic levels may have a neuroprotective action as it was stated by Auinger et al. [27], who demonstrated slower Huthington disease progression in patients with higher UA levels and 30% reduction in the risk of developing Parkinson's Disease in patients with a history of gout, independent of age, sex, prior comorbid conditions, non-steroid anti-inflammatory drugs, and diuretic use. Elevated urinary UA in MMC patients may suggest its role in such a specific condition, which is connected with abnormal innervations in neurogenic bladder.

UA provides up to 60% of the antioxidant capacity in human blood [15]. There are no studies estimating the role of UA as an antioxidant in urine. In our study, we revealed decreased urinary TS in MMC patients. If UA plays a role as an antioxidant we could expect increased UA levels in urine.

We found negative correlations between thiol status and UA excretion (g/24h collection, per kg of body mass, per body surface), but they were not statistically significant. A statistically significant positive correlation was detected between thiol status and UA excretion based on GFR.

It is important to note that in our survey urinary UA correlated with parameters of physical development, including BMI.

Similarly, in the study by Lyngdoh et al. [28], the association between uric acid and inflammatory cytokines appeared to be dependent on BMI.

Furthermore, we diagnosed decreased urinary thiol status in younger children compared with the older ones. There were no such differences in the reference group. It can suggest an interrelation between thiol status and UA. More questions remain to be addressed and await answers.

Interestingly, thiol status was higher in patients with better physical activity classified by Hoffer's scale. It is more likely that better physical activity results in better bladder function, which is reflected in higher thiol status in these patients.

Positive correlations between serum UA levels and CRP were demonstrated in many studies [27, 28].

Most reports on the influence of UA on inflammation markers are experimental. There have been no studies describing such correlations in urine. In our study, positive correlations between hsCRP and urinary UA may suggest a relationship between elevated urinary UA levels and inflammation and its effect on bladder function.

In summary, we bring direct evidence that urinary UA and inflammatory marker based on hsCRP are increased in contrast with thiol status, which is decreased in MMC children.

Our study has limitations in its cross-sectional nature, allowing us to find correlations between uric acid, markers of inflammation, and antioxidant status in neurogenic bladder, but not to assess the cause-effect relationships.

Another limitation of this study is that the single measurement of all parameters may not accurately reflect long-term inflammation status. Finally, we could not be sure if elevated UA and hs CRP levels came from local circulating production.

## CONCLUSIONS

1. UA is a marker that potentially has a direct effect on bladder function
2. Disturbed oxidative status and increased markers of inflammation may be potentially modifiable factors affecting lower urinary tract functioning in MMC children.

## Acknowledgments

## Conflicts of interest

The authors have no conflict of interest to disclose.

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