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# Glycosaminoglycan levels in dried blood spots of patients with mucopolysaccharidoses and mucolipidoses

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**Conflict of Interest:**

Francyne Kubaski, Yasuyuki Suzuki, Kenji Orii, Roberto Giugliani, Heather J. Church, Robert W. Mason, Vũ Chí Dũng, Can Thi Bich Ngoc, Seiji Yamaguchi, Hironori Kobayashi, Katta M. Girisha, Toshiyuki Fukao, Tadao Orii, and Shunji Tomatsu declare that they have no conflict of interests.

**Contributions to the project:**

Francyne Kubaski has contributed to the concept and planning of the project, collection of data, data analysis, the draft of the manuscript, and reporting of the work described.

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## Abstract

Mucopolysaccharidoses (MPSs) and mucolipidoses (ML) are groups of lysosomal storage disorders in which lysosomal hydrolases are deficient leading to accumulation of undegraded glycosaminoglycans (GAGs), throughout the body, subsequently resulting in progressive damage to multiple tissues and organs. Assays using tandem mass spectrometry (MS/MS) have been established to measure GAGs in serum or plasma from MPS and ML patients, but few studies were performed to determine whether these assays are sufficiently robust to measure GAG levels in dried blood spots (DBS) of patients with MPS and ML.

**Material and methods—**In this study, we evaluated GAG levels in DBS samples from 124 MPS and ML patients (MPS I = 16; MPS II = 21; MPS III = 40; MPS IV = 32; MPS VI =10; MPS VII = 1; ML= 4), and compared them with 115 age-matched controls. Disaccharides were produced from polymer GAGs by digestion with chondroitinase B, heparitinase, and keratanase II. Subsequently, dermatan sulfate (DS), heparan sulfate (HS-0S, HS-NS), and keratan sulfate (mono-sulfated KS, di-sulfated KS, and ratio of di-sulfated KS in total KS) were measured by MS/MS.

**Results—**Untreated patients with MPS I, II, VI, and ML had higher levels of DS compared to control samples. Untreated patients with MPS I, II, III, VI, and ML had higher levels of HS-0S; and untreated patients with MPS II, III and VI and ML had higher levels of HS-NS. Levels of KS were age dependent, so although levels of both mono-sulfated KS and di-sulfated KS were generally higher in patients, particularly for MPS II and MPS IV, age group numbers were not sufficient to determine significance of such changes. However, the ratio of di-sulfated KS in total KS was significantly higher in all MPS patients younger than 5 years old, compared to age-matched controls. MPS I and VI patients treated with HSCT had normal levels of DS, and MPS I, VI, and VII treated with ERT or HSCT had normal levels of HS-0S and HS-NS, indicating that both treatments are effective in decreasing blood GAG levels.

**Conclusion—**Measurement of GAG levels in DBS is useful for diagnosis and potentially for monitoring the therapeutic efficacy in MPS.

### Keywords

mucopolysaccharidoses; mucolipidoses; glycosaminoglycans; tandem mass spectrometry; hematopoietic stem cell transplantation

## 2. Introduction

Mucopolysaccharidoses (MPSs) are a group of lysosomal storage disorders (LSDs) caused by a deficiency of lysosomal hydrolases responsible for the catabolism of glycosaminoglycans (GAGs)[1, 2]. Mucolipidoses (ML) are related diseases caused by a deficiency of N-acetylglucosaminyl-1-phosphotransferase. This enzyme deficiency produces unphosphorylated lysosomal enzymes, which leads to inhibition of reuptake of enzymes and accumulation of GAGs and lipids. MPSs and ML are classified according to the enzyme deficiency (Table 1).

The MPSs and ML are progressive LSDs that share many clinical features such as: coarse faces, neurological impairment (MPS I, II, III, VII and ML II), skeletal dysplasia (all, but maybe mild in MPS III), hepatosplenomegaly, joint rigidity, and heart valvular disease [3]. MPSs are usually asymptomatic at birth, and the initial signs and symptoms appear with progression of the disease during the first one or two years of age. Mucolipidoses II (ML II; I-cell disease) is fatal during childhood or the first decade of life, and can even produce intra-uterine fractures, while ML III has a milder somatic phenotype with slower progression throughout childhood but leads to severe neurodegeneration with a fatal outcome during adulthood [2, 4].

ML II and III are caused by impaired trafficking of several lysosomal enzymes [2, 5]. The prevalence of ML is variable among different populations: 0.3 cases per 100,000 live births in Australia, 0.16 per 100,000 live births in Portugal, and 0.08 per 100,000 live births in the Netherlands [6, 7]. The incidence in Quebec, Canada is very high, 1:6,184, due to a founder effect [8]. The combined incidence of MPSs is 1:25,000 live births, and therefore more common than ML [9].

Enzyme replacement therapy (ERT) is available commercially in practice for MPS I, II, VI, and IVA [10–13]. Hematopoietic stem cell transplantation (HSCT) is recommended for MPS I [14, 15]. Several studies indicate that HSCT will also improve outcomes for MPS II [16–18], MPS IVA [19, 20], MPS VI [21] and MPS VII [22].

Levels of GAGs in patients with MPS have been studied for several decades. Initially, total urinary GAGs were measured using a variety of dye methods [23–34]. Although these methods were useful and cost-effective; they gave high false-positive rates [35], could not be easily applied to measure GAGs in blood and/or tissues due to the presence of proteins and other interferent molecules, and could not distinguish specific GAG(s) [36]. Measurement of total urinary GAG using a dimethylmethylene blue (DMMB) method did not distinguish a substantial number of MPS IVA patients from age-matched controls [37–41]. The development of ELISA methods in early 90’s made it possible to measure HS and KS in blood and urine of MPS and ML patients [40, 42–44]. We used an ELISA method to show that KS levels in blood are elevated not only in MPS IV, but also in other types of MPS and ML [44]. However, ELISA assays are expensive and cannot distinguish subclasses of HS and KS. Since 2001, protocols have been developed for GAG analysis using tandem mass spectrometry (MS/MS). Two main branches of GAG detection methods by MS/MS have been developed: detection of digested disaccharides (direct or labeled with aniline) [45–50]

and chemically depolymerized GAGs by methanolysis and/or butanolysis [51–56]. Such MS/MS methods have been used to measure specific GAGs in blood and urine of MPS and ML patients [51, 54, 55, 57–65]. MS/MS provides a sensitive, specific, and reproducible GAG analysis and allows measurement of several GAGs simultaneously, indicating its potential for use in mass screening, prognosis, and monitoring therapeutic effect in patients with MPS and ML. More recently, MS/MS methods have been developed to measure GAGs in dried blood spots (DBS) [57, 61, 66, 67].

In this study, we have simultaneously determined levels of dermatan sulfate (DS), heparan sulfate (HS-0S, HS-NS), and keratan sulfate (mono, di-sulfated, and ratio di-sulfated in total KS) in DBS of control subjects and patients with MPS I, II, III, IV, VI, VII; and ML II and III by liquid chromatography tandem mass spectrometry (LC/MS/MS). We have also evaluated GAG levels in ERT and HSCT treated patients with some types of MPS.

## 3. Material and Methods

### 3.1 Enzymes and standards

Chondroitinase B, heparitinase, keratanase II, chondrosine (internal standard-IS), and the unsaturated disaccharides: heparan ΔDi-0S [2-acetamido-2-deoxy-4-O-(4-deoxy-a-L-*threo*-hex-4-enopyranosyluronic acid)-D-glucose] (HS-0S), heparan ΔDi-NS [2-deoxy-2-sulfamino 4-O-(4-deoxy-α- L-*threo*-hex-4-enopyranosyluronic acid)-D-glucose] (HS-NS), chondro Δ Di-4S [2-acetamido-2-deoxy 3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-D-sulfo-galactose] (Di-4S), mono-sulfated KS [Galβ1-4GlcNAc(6S)], and di-sulfated KS [Gal(6S) Galβ1-4GlcNAc(6S) were all provided by Seikagaku Co (Tokyo, Japan). Stock solutions of HS-0S (100 μg/mL), HS-NS (100 μg/mL), Di-4S (250 μg/mL), mono- and di-sulfated KS (1,000 μg/mL), and IS (5 μg/mL) were prepared in dd$H_2$0 (Millipore Milli-Q Reference A+ System).

### 3.2 Samples

Whole blood was collected with EDTA by venipuncture and 150 μL of blood was spotted onto filter paper to create DBS. DBS from 106 untreated MPS and ML patients (MPS I = 7; MPS II = 21; MPS IIIA = 12, MPS IIIB = 17, MPS IIIC = 6, MPS III (undefined) = 2; MPS IVA = 28, MPS IVB = 2; MPS VI = 7; MLII = 3; ML III=1), 18 treated MPS (MPS I with ERT = 6, MPS I with ERT+HSCT = 2, MPS I with HSCT = 1; MPS IIIA with HSCT = 1, MPS IIIB with HSCT = 2; MPS IVA with HSCT = 2; MPS VI with ERT = 2, MPS VI with HSCT = 1; MPS VII with HSCT = 1), and 115 control subjects. Diagnosis of MPS and ML was made with enzyme assay.

DBS from MPS patients were provided by Shimane University (Japan), Gifu University (Japan), St. Mary`s Hospital (UK), and Kasturba Medical College Manipal University (India). Control samples were obtained from 15 volunteer subjects from Hospital de Clínicas de Porto Alegre (Brazil) and from subjects who had blood draws for clinical testing for non-metabolic disease from Shimane University (Japan). Informed consent was obtained at each Institute for all patient and control samples according to IRB approval at each institute..

All de-identified samples were shipped to Nemours/AIDHC and stored at −20 °C until the GAG assay was conducted. This study was approved by the Nemours IRB (protocol # 281498).

### 3.3 Sample preparation

Two disks (3.3mm) were cut from each DBS sample with a DBS puncher (PerkinElmer®; Waltham, MA) and placed into wells of a 96 well Omega 10K filter plate containing 100 μL of 0.1% BSA. Samples were pre-treated as previously described by Kubaski et al., 2016 [48, 67–70] using a standardized method for extraction of GAGs from DBS. Briefly, samples were incubated with enzymes (chondroitinase B, heparitinase and keratanase II) to digest polymeric GAGs to their constituent disaccharides at 37°C overnight. The next day, disaccharides were collected by centrifugation of digests through a filter plate for 15 min at 2,500 g into receiver plates prior to LC/MS/MS analysis.

### 3.4 LC/MS/MS

The apparatus consisted of a 1290 Infinity LC system with a 6460 triple quad mass spectrometer (Agilent Technologies, Palo Alto, CA). Disaccharides were separated on a Hypercarb column (2.0 mm i.d. 50 mm length; 5 μm particles; Thermo Scientific, USA), thermostated at 60 °C. The method was modified from that developed by Oguma et al. [49]. The mobile phase was a gradient elution of 148 mM of ammonia (pH 11.0) (solution A) to acetonitrile 90% acetonitrile (solution B). The flow rate was 0.7 mL/min, and the gradient was as follows: 0 min 100% solution A, 1 min 50% solution A, 2 min 50% solution A, 2.20 min 0% solution A, 2.60 min 0% solution A, 2.61 min 100% solution A, 5 min 100% solution A. The mass spectrometer was operated with electrospray ionization in the negative ion mode (Agilent Jet Stream technology) with drying gas temperature 350 °C, drying gas flow 11 L/min, nebulizer pressure 58 psi, sheath gas temperature 400 °C, sheath gas flow 11 L/min, capillary voltage 4,000 V, nozzle voltage 2,000 V. Specific precursor and product ions, *m/z,* were used to quantify each disaccharide respectively (IS, 354.3, 193.1; DS, 378.3, 175.1; mono-sulfated KS, 462, 97; di-sulfated KS 542, 462; HS-NS 416, 138; HS-0S 378.3, 175.1) [49, 54, 71, 72]. DS was measured as Di-0S after digestion of Di-4S by a 4S-sulfatase present in the preparation of chondroitinase B. The injection volume was 5 μL with a running time of 5 min per sample. Ratio of di-sulfated KS in total KS was calculated as di-sulfated KS divided by (mono-sulfated KS + di-sulfated KS) × 100%.

### 3.5 Statistical analysis

For KS (mono and di-sulfated KS, ng/mL; ratio di-sulfated KS in total KS, %) analyses, patients were grouped by age as follows: 0–5 (years), 5–10 (years), 10–15 (years), 15–30 (years), and >30 years. Age-matched data was summarized using mean and standard deviation (SD). Untreated patients were compared to controls and treated patients (ERT, HSCT, and/or ERT+HSCT) using student *t-test* at the level of significance of 0.05 performed using Graphpad Prism 7.0a.

## 4. Results

Ages of the patients with MPS were as follows: untreated MPS I (mean: 3 ± 2 years; range: 3 months to 5 years; n = 7), MPS I with ERT (mean: 26 ±18 years; range: 1.1 to 37.9 years; n = 6), MPS I with HSCT (12.8 years; transplant age = 2.5 years, n = 1), MPS I with ERT +HSCT (ages 4 and 15 years; transplant age: 2.3 and 3.8 years; n = 2); untreated MPS II (mean: 9 ± 7 years; range: 1.1 to 29 years; n = 21); untreated MPS III (mean: 13 ± 8 years; range: 1 to 34.2 years; n = 37), MPS IIIA with HSCT (ages 8 and 17 years, transplant age: 7 and 12 years; n = 2), MPS IIIB with HSCT (age: 21, transplant age: 2; n = 2); untreated MPS IVA (mean: 15 ± 14 years; range: 7 months to 56 years; n = 28), MPS IVA with HSCT (ages: 25.7 and 26.3 years; transplant ages: 4 and 15 years old; n = 2); untreated MPS IVB (ages 9 months and 48 years; n = 2); untreated MPS VI (mean: 6 ± 7 years; range: 2 months to 22 years; n = 7), MPS VI with ERT (ages: 3.5 and 9.4 years; n = 2), MPS VI with HSCT (age: 21.9 years, unknown transplant age; n = 1); MPS VII with HSCT (age: 30 years; transplant age: 12 years; n = 1); untreated ML (mean: 6 ± 6 years; age range: 0.2 to 14 years; n = 4); controls (mean: 12 ± 12 years; age range: 1 month to 57 years; n = 115).

### 4.1. Distribution of GAG levels in untreated MPS and ML patients compared to controls

No age-dependent differences in DS, HS-0S or HS-NS were detected in control subjects, or MPS and ML patients. Consequently, levels of these GAGs in all untreated patients with each MPS or ML were compared with levels in control subjects.

Mean levels of DS were higher than controls in untreated MPS I ($p = 0.006$), MPS II ($p < 0.0001$), MPS III ($p = 0.04$) (Fig. 1). Levels of DS in 6 of 7 MPS I, 20 of 21 MPS II, 2 of 37 MPS III, 3 of 6 MPS VI, and 3 of 4 ML were higher than the mean + 2SD of the control group. DS was not significantly higher in untreated MPS IV patients.

Levels of HS-0S were higher than controls in untreated MPS I ($p = 0.004$), MPS II ($p < 0.0001$), MPS III ($p < 0.0001$), MPS VI ($p = 0.03$) (Fig. 2A). Levels of HS-0S in 5 of 7 MPS I, 20 of 21 MPS II, 24 of 37 MPS III, 4 of 6 MPS VI, and 3 of 4 ML were higher than the mean + 2SD of the control group.

Levels of HS-NS were also higher than controls in untreated MPS I ($p = 0.006$), MPS II ($p < 0.0001$), MPS III ($p < 0.0001$), MPS IV ($p = 0.04$), and MPS VI ($p = 0.03$) (Fig 2B). Levels of HS-0S in 6 of 7 MPS I, 19 of 21 MPS II, 30 of 37 MPS III, 10 of 30 MPS IV, 4 of 6 MPS VI, and all 4 ML were higher than the mean + 2SD of the control group.

Di-sulfated KS levels vary with age in control samples, being high from newborn until 15 years of age and then decreasing with age (Table 2a). Although the numbers of patients in each age group is low, di-sulfated KS levels are also lower in older patients. Di-KS levels in MPS patients were generally higher than in age-matched controls (Table 2a) although only 12 of 21 MPS II, 10 of 37 MPS III, 12 of 30 MPS IV, and 4 of MPS VI were more than mean + 2SD of the control groups.

Mono-sulfated KS levels also varied with age in the control group, with lower levels after the age of 15 years (Table 2b). Levels of mono-KS were generally higher in MPS patients

than in age-matched controls, although differences were only significant in older MPS III and IV patients (Table 2b). Only 12 of 21 MPS II, 7 of 37 MPS III, 4 of 30 MPS IV, 2 of 6 MPS VI were more than mean + 2SD of the age-matched control groups.

The ratio of di-sulfated KS in total KS gradually increased with age in the control group (Table 2c). As seen previously in newborn samples [67], this ratio was higher in DBS from MPS I, II, and III patients aged 0 – 5 than in age-matched controls. This was also the case for MPS IV children in this age group. Differences were not significant in older patients.

### 4.2. GAG levels in untreated vs. treated patients with MPS and ML

Levels of HS-0S and HS-NS were generally lower in all treated patients compared to untreated patients although due to the limited sample size, only the ERT treated MPS I patients showed a significant reduction in these GAGs (Fig 2). Levels of DS were less affected by treatment although lower levels were seen in HSCT treated MPS I and VI patients (Fig 1). Two MPS IVA patients were treated by HSCT. Patient 1 was 25 years old (transplanted at 4 years of age) and patient 2 was 26 years old (transplanted at 15 years of age). In patients 1 and 2 respectively, levels of di-sulfated KS were 21 ng/mL, 4 ng/mL; levels of mono-sulfated KS 56 ng/mL, 79 ng/mL; and ratio of di-sulfated KS 27% and 5%. Levels of mono-sulfated, di-sulfated KS, and ratio of di-sulfated KS were more similar to age-matched controls than untreated MPS IV patients (Table 2).

## 5. Discussion

We have demonstrated the usefulness and significance of assay of disaccharides from DBS by LC/MS/MS as a diagnostic approach and therapeutic monitoring tool for MPS and ML. We measured several GAGs (DS, HS, and KS) simultaneously and found that the majority of untreated MPS and ML patients had higher levels of at least one GAG compared to age-matched controls. We have also shown a reduction of GAGs in patients treated with ERT and/or HSCT in MPS I, VI, and VII when compared to other untreated patients, suggesting the potential use of this method for treatment monitoring. Samples from individual patients pre- and post-therapy were not available for this study.

An important consideration when selecting a method to analyze GAG levels is related to the choice of specimens. Urine has been extensively used in clinical practice due to convenience for collection and accessibility [32, 41, 64, 73]. DBS may offer a useful alternative to urine for measurement of GAGs, including easy access of specimens, simplicity of transport, and potential for use in multiple assays including measurement of enzyme activity. DBS samples are routinely used for newborn screening for several metabolic disorders [74].

Limitations for use of DBS are related to procedures for DBS preparation. Although each 3.3 mm disc from a DBS corresponds to approximately 3.6 μL of blood [75], variations in sample collection methods amongst different centers might affect blood volume per spot. Also, protocols are typically standardized for serum or plasma use, and consequently, extra validation is required for use of DBS. Another important consideration for clinical use is that measurements of GAGs in DBS will reflect the concentration of analytes in both the plasma and the cellular fraction of whole blood, whereas the cells are removed in serum or plasma.

Consequently levels of GAGs in DBS may not directly correspond with levels in plasma or serum. Another disadvantage of DBS is that the volume of sample is small and may not be sufficient for early stage research that typically requires more samples for protocol validation [76].

As expected, and described previously in serum (or plasma) and urine samples [40, 43, 57, 59, 61, 77], the analysis of DBS samples revealed that MPS I, II, III, VI, and ML patients had an elevation of DS compared to controls (Fig. 1). These patients also had elevated HS-0S and HS-NS, although elevations were not statistically significant for the ML patients. The data support the use of DBS to measure DS and HS levels to screen for MPS and ML patients. DS was not significantly elevated in DBS from MPS IV patients, but levels of HS-NS were elevated, indicating that these patients may also be detected in a screen for HS in DBS.

Levels of KS are age-dependent, and results of this study confirm that levels of KS are lower in both controls and patients more than 15 years old. MPS II and MPS IV patients generally had higher levels of mono- and di-sulfated KS than controls. However, due to limited numbers of patients in each age group, most differences were not statistically significant. By contrast, the ratio of di-sulfated KS in total KS was significantly higher than controls for all MPS patients (I, II, III, and IV) in the 0 – 5 year age group. These results are consistent with our previous findings that the ratio of di-KS in total KS is elevated in newborn DBS of MPS I, II, and III patients [67]. This ratio was also higher in the ML patients, consistent with our previous observation of a patient with ML II who had elevated KS levels [44].

It is well-established that each type of MPS results in characteristic accumulated of specific GAG(s) based on the deficient enzyme (Table I, [1]). More recent studies show that secondary elevations of other GAGs also occur. For example, elevation of KS level in blood and urine is diagnostic for MPS IV since the deficient enzyme is directly involved in catabolism of KS; but KS is also elevated in several other types of MPS [55, 58, 77–79]. Notably, in the current study the secondary KS elevation in DBS of MPS II patients is as high as the levels seen in patients with MPS IVA. Elevation of the ratio of di-KS in total KS is not a specific biomarker for MPS IV, but is a marker in newborns and young patients for several forms of MPS including I, II, III, and IV.

Although MPS are progressive disorders that often take years to present clinically, there is considerable evidence from both humans [80–83] and animal models [84, 85] that biochemical storage commences in the fetus. Results of this study showing elevation of GAGs in young patients support these observations.

It is well known that total urinary GAG is reduced by ERT [10, 11, 13, 86, 87]. However, studies examining the effects of either ERT or HSCT treatment on specific GAG levels are limited. We previously showed that HS levels are more effectively reduced by HSCT than by ERT in MPS II patients [88]. In the present study, we evaluated the effect of ERT and/or HSCT on specific GAGs in MPS I, III, VI, and VII. For all treated patients, levels of HS-0S and HS-NS were similar to control levels, indicating efficient reduction of GAGs in blood for both types of treatment and each form of MPS. The results are less clear for DS, with

values remaining high for the two ERT treated MPS VI patients and the HSCT-treated MPS VII patient. More extensive studies are required with many patients treated with ERT and/or HSCT to determine whether levels of HS, and possibly other GAGs, are consistently reduced and whether this reduction translates into better outcomes for patients.

Because most MPS patients are not diagnosed at birth, DBSs of newborn MPS patients are rare and consequently it is difficult to define cutoff values of GAGs for newborn screening. In this study, we show that as levels of DS and HS do not vary significantly with age, most older patients still have levels of DS and HS that are distinguishable from controls using a cutoff of mean + 2SD of the controls. With a limited number of newborn MPS samples, we were able to define cutoffs for HS and DS that effectively discriminated MPS I, II, and III patients from controls [67]. Previous studies have reported the validity of GAG measurement in urine and CSF in which a good discrimination between MPS patients and controls were seen [51, 52, 54–56, 64]. However, some of the older patients in the present study were indistinguishable from the controls. The reason for low levels of GAGs in our patients is not known, but management of their condition by palliative care or anti-inflammatory treatments could lower GAG levels in the bloodstream. It is also possible that their genotype and phenotype were less severe with lower GAG accumulation.

In conclusion, this study has demonstrated that DBS, when available, can be used as a convenient source of patient samples for rapid and simultaneous measurement of multiple GAGs by MS/MS and that they could be used for diagnosis of severe forms of MPS and ML and, potentially, also for therapeutic monitoring. Furthermore, it is important to note that MPS patients with attenuated phenotypes are likely to have lower GAG levels and that this could be a potential limitation, with potential false-negative results when using DBS for diagnosis and/or treatment monitoring on those patients. Longitudinal studies should be conducted in order to elucidate the feasibility of GAG monitoring with DBS samples.

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## Highlights

- DBS is feasible for GAG measurement by tandem mass spectrometry;
- Untreated patients had higher GAG levels compared to control samples;
- Treated patients had lower GAG levels compared to untreated patients;
- GAG in DBS seem to be feasible for diagnosis and therapeutic monitoring;

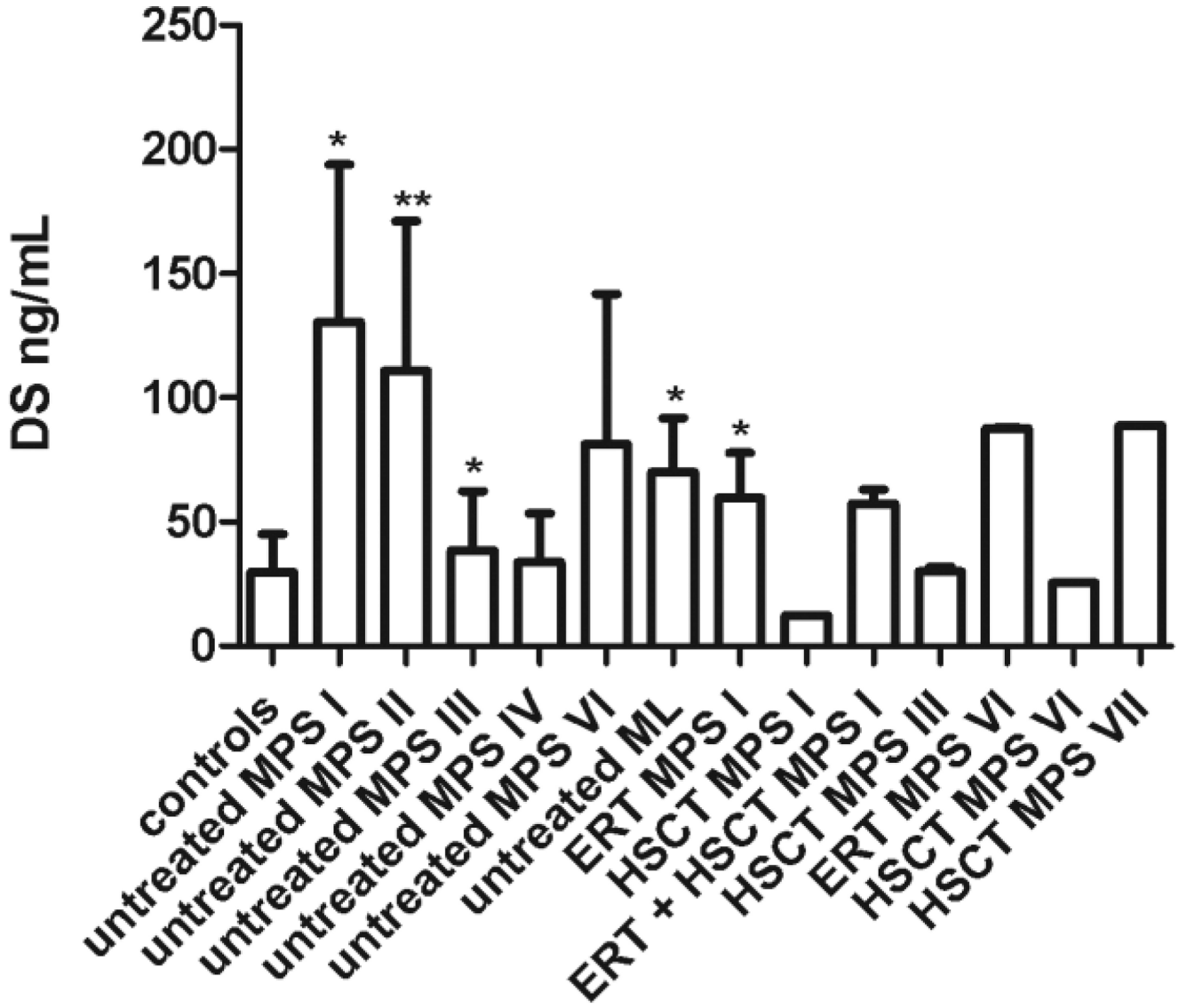


**Figure 1. Dermatan sulfate (DS) levels in treated vs. untreated patients**

Untreated patients were compared with controls; treated patients were compared with untreated. Values that were significantly increased in untreated patients or decreased by treatment are marked *$p < 0.05$; **$p < 0.0001$

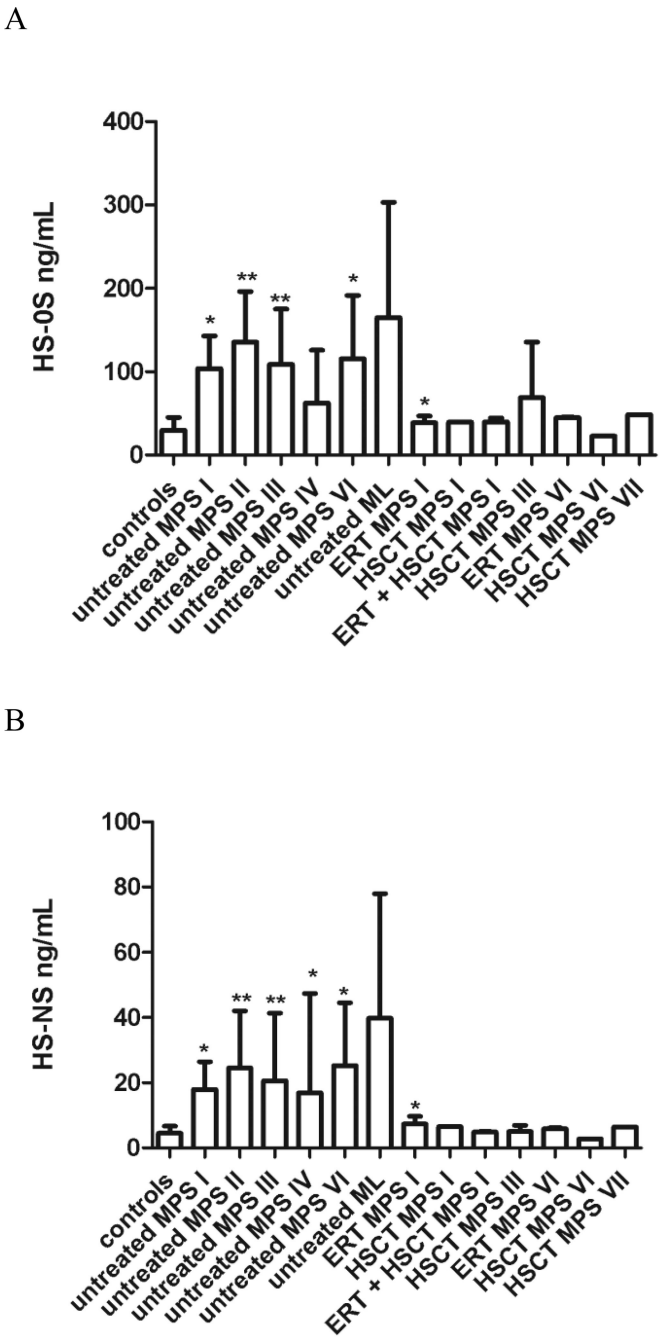


**Figure 2. Heparan sulfate (HS-NS and HS-0S) levels in treated vs. untreated patients**

Untreated patients were compared with controls; treated patients were compared with untreated. A shows levels of HS-NS and B shows levels of HS-0S as mean and standard deviation. Values that were significantly increased in untreated patients or decreased by treatment are marked *$p < 0.05$; **$p < 0.001$

**Table 1**

Classification of MPS and ML

| MPS | Enzyme deficiency | GAG or sphingolipid accumulated |
|---|---|---|
| MPS I | α-L-Iduronidase (IDS) | DS & HS |
| MPS II | Iduronate-2-sulfatase (IDS) | DS & HS |
| MPS IIIA | Heparan-N-sulfatase | HS |
| MPS IIIB | α-N-acetylglucosaminidase | HS |
| MPS IIIC | Acetyl-CoA- α-glucosaminidase acetyltransferase | HS |
| MPS IIID | N-acetylglucosamine-6-sulfatase | HS |
| MPS IVA | N-acetylgalactosamine-6-sulfate sulfatase | C6S & KS |
| MPS IVB | β-galactosidase | KS |
| MPS VI | N-acetylgalactosamine-4-sulfatase | DS |
| MPS VII | β-glucuronidase | DS, CS, HS |
| MPS IX | Hyaluronidase | HA |
| ML II | N-acetylglucosaminyl-1-phosphotransferase | GAGs & sphingolipids |
| ML III | N-acetulglucosamine-1-phosphotransferase | |

DS: dermatan sulfate; CS: chondroitin sulfate; HS: heparan sulfate; HA: hyaluronan; KS: keratan sulfate

**Table 2**

Levels of KS in controls and untreated patients

**A. Di-sulfated KS (ng/mL)**

| Age (years) | Controls | MPS I | MPS II | MPS III | MPS IV | ML |
|---|---|---|---|---|---|---|
| Newborns | 27 ± 47 | 39 ± 20 | 84 | 29 ± 7 | n/a | n/a |
| 0–5 | 33 ± 21 | 55 ± 30 | 245 ± 474 | 92 ± 88 | 107 ± 139 | 42 |
| 5–10 | 40 ± 20 | n/a | 84 ± 41 | 44 ± 23 | 126 ± 200 | 25 |
| 10–15 | 41 ± 19 | n/a | 295 ± 557 | 106 ± 165 | 497 ± 869 * | 46 |
| 15–30 | 21 ± 11 | n/a | 122 | 44 ± 20 * | 38 | n/a |
| >30 | 19 ± 15 | n/a | n/a | 56 | 56 ± 37 | n/a |

**B. Mono-sulfated KS (ng/mL)**

| Age (years) | Controls | MPS I | MPS II | MPS III | MPS IV | ML |
|---|---|---|---|---|---|---|
| Newborns | 128 ± 161 | 172 ± 82 | 208 | 86 ± 27 | n/a | n/a |
| 0–5 | 188 ± 75 | 216 ± 127 | 398 ± 415 | 257 ± 186 | 332 ± 408 | 100 |
| 5–10 | 152 ± 63 | n/a | 313 ± 163 | 106 ± 49 | 225 ± 190 | 115 |
| 10–15 | 140 ± 69 | n/a | 560 ± 720 | 315 ± 383 | 259 ± 177 | 182 |
| 15–30 | 67 ± 32 | n/a | 317 | 121 ± 60 * | 96 | n/a |
| >30 | 49 ± 24 | n/a | n/a | 143 ± 85 | 123 ± 61 * | n/a |

**C. Ratio of di-sulfated KS in total KS (%)**

| Age (years) | Controls | MPS I | MPS II | MPS III | MPS IV | ML |
|---|---|---|---|---|---|---|
| Newborns | 17 ± 6 | 26 ± 15 *** | 27 ± 12 *** | 28 ± 11 *** | n/a | n/a |
| 0–5 | 15 ± 8 | 23 ± 5 * | 26 ± 10 * | 25 ± 5 *** | 23 ± 4 ** | 30 ± 2 |
| 5–10 | 21 ± 7 | n/a | 21 ± 1 | 24 ± 4 | 29 ± 8 * | 18 |
| 10–15 | 23 ± 5 | n/a | 26 ± 8 | 30 ± 9 * | 32 ± 21 | 20 |
| 15–30 | 23 ± 7 | n/a | 28 | 26 ± 4 | 29 | n/a |
| >30 | 28 ± 7 | n/a | n/a | 29 | 24 ± 9 | n/a |

* $p < 0.05$;

** $p < 0.0005$;

*** $p < 0.0001$

Newborn data from Kubaski et al., 2016.