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# Correlation between Muscle Tone and Motor Delay and Biochemical Levels of Carbohydrates in Urine

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors DN designed the study and wrote the protocol. Author AA wrote the first draft of the manuscript, managed the literature searches, analyses of the study performed the spectroscopy analysis and managed the experimental process. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** To establish a possible correlation between children's clinical status and carbohydrates in their urine samples as they could be a useful additional tool for objective assessment of muscle tone and motor development.
**Place and Duration of Study:** The Department of Child, Adolescent and Developmental Neurology, University Children's Hospital in Ljubljana between December 1, 2011 and August 31, 2013.
**Methodology:** The retrospective part of the study comprised children aged 0-5 years (average age, 29.3 months) with motor developmental delay and/or abnormal muscle tone. A group of healthy individuals comprised the prospective part. Besides clinical assessment, electrochemical detection of ten sugars in the urine samples was performed using ion chromatography combined with electrochemical detection and tandem mass spectrometry.
**Results:** 72.0% of 82 hospitalised children had a disorder of muscle tone and/or DQ < 70, 28.0% had a pathological urine sample and an abnormal clinical examination, while 44.0% of children had

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a clinical abnormality and a normal urine sample. In the control group, 22.7% had pathological urine samples. There was a statistically significant difference between patients and control subjects in the value of fucose (p = 0.020), sucrose (p = 0.051) and ribose (p = 0.000).
**Conclusion:** A significant correlation was found between abnormalities in clinical status and pathological urine samples (p = 0.002). Absolute and relative values of specific carbohydrates and the number of elevated sugars showed a strong correlation with disease severity.

*Keywords: Carbohydrates in the urine samples; muscle tone; motor delay; metabolic disorder; children aged 0-5 years.*

## ABBREVIATIONS

- *IC-ECD, IC-MS/MS: ion chromatography combined with electrochemical detection and tandem mass spectrometry*
- *DCADN: Department of Child, Adolescent and Developmental Neurology, University Children's Hospital, Ljubljana, Slovenia*
- *DQ: developmental quotient*
- *MRC scale: Medical Research Council Scale for assessment of motor (muscle) power*

## 1. INTRODUCTION

Muscle tone is a continuous contraction of muscle fibres that maintains a constant length of resting muscles. It represents the resistance of muscle fibres to passive stretching [1]. Phasic muscle tone is a response to a high-intensity stretch and is seen as a rapid contraction of muscles. Postural muscle tone is low-level tonic motor activity of resting muscles [2].

Muscle tone is controlled by neurological inputs coming from supraspinal structures located in the CNS and from motor units that represent a stretch reflex [3]. A sensory receptor of the reflex arch represents a muscle spindle that acts as a stretch receptor. The muscle spindle provides critical information about movement position and velocity. Myelinated sensory group Ia neurons and group II neurons terminate on the non-contractile central region of a muscle spindle, while their polar parts are made of contractile elements and are innervated by γ- motoneurons. Extrafusal muscle fibres are innervated by α-motoneurons [4].

Hypotonia means a decrease of the muscle tone in resting muscle. Muscle resistance against passive stretch is decreased. This presents clinically with muscle weakness, decreased muscle strength and often with decreased tendon reflexes [3,5]. The cause of hypotonia can be peripheral (lower motor neuron disease) or it can lie in the CNS (upper motor neuron disease) [6].

Hypertonia is a condition of increased muscle tone in resting muscle. The main reason is an upper motor neuron lesion. Excitatory impulses descending from the CNS prevail over inhibitory impulses, resulting in hyperactivity of α- and γ-motoneurons in the anterior spinal cord. Tendon reflexes are often hyperactive [7].

Asymmetrical muscle tone is a condition where muscle tone in different parts of the body is unequal; either qualitatively (hypotonia, hypertonia) or quantitatively. It is often the result of an upper motor neuron lesion [8].

Motor impairment is a developmental neurological disorder that affects motor coordination. Due to weak or disorganised connections between neurons, information coming into the CNS is wrongly processed [9].

Motor developmental delay means that developmental milestones are not reached at the expected time. It usually becomes evident in the first six to eighteen months and is expressed as a developmental quotient (DQ) [10]. An infant's development should follow certain milestones. In 2006, WHO undertook research [11], which showed that normal developmental milestones have a broader spectrum regarding when they should be achieved. Based on these findings, WHO recommended the use of limits that determine the oldest age at which attainment of a milestone is still acceptable. When these are exceeded, the child is said to have motor developmental delay.

Metabolic disorders can affect carbohydrate turnover. Abnormalities in functional enzymes can lead to incorrect metabolism of

carbohydrates, which influences muscle tone, skeletal abnormalities, causes developmental delay and intellectual impairment, or can even cause death. It is possible to precisely measure the quantity of different carbohydrates in the urine, which can assist in the diagnosis and follow-up of diverse metabolic conditions [12].

## 2. METHODOLOGY

### 2.1 Patients and the Control Group

Ninety children who were admitted to our Department (DCADN) between December 1, 2011 and August 31, 2013, were included in the main group of the study. Eight children were excluded because they exceeded the age limit of five years. Our final study group comprised 82 children; 48 males (58.5%) and 34 females (41.5%). The average age was 29 months (SD 13 months). The age range was 8 to 64 months.

Each child underwent complete clinical evaluation with a detailed medical history (family history, pregnancy, Apgar score, personal health history, current issues, allergies, medications, results of previous examinations, nutritional supplements) and clinical examination, including neurological examination. The motor developmental quotient (DQ) was also calculated. Besides clinical diagnostics, at the same time ten sugars in urine samples were electrochemically detected using ion chromatography combined with electrochemical detection and tandem mass spectrometry. Urine samples were taken under fasting conditions in the morning before the children had their first feed.

The control group for the study included forty-four children, aged up to five years. None of them had any neurological disorders, abnormalities in muscle tone or motor developmental delay. All urine samples were taken between June 1, 2013 and August 31, 2013 to measure ten carbohydrates using ion chromatography combined with electrochemical detection and tandem mass spectrometry. Urine samples were collected at the University Children's Hospital and at six community Health Centres in Ljubljana, Slovenia.

### 2.2 Assessment of Muscle Tone, Muscle Strength and Motor Developmental Quotient

Muscle tone was defined on clinical grounds. Hypotonia was detected on inspection as the children were lying in a frog-legged posture. On passive stretching of their limbs, diminished muscle resistance and joint hyperflexibility were felt. When a child was pulled to the sitting position, head lag was observed, while older children had difficulty keeping their head upright in the ventral position. In the shoulder suspension test, the examiner had the feeling that the child would slip through his/her hands. In the ventral suspension test, the child was not able to maintain limb posture against gravity. When the child's hand was placed across his/her neck to reach the opposite arm, the elbow crossed the midline ("scarf sign"). The popliteal angle was more than 90°.

Hypertonia was diagnosed when increased muscle resistance to stretching was felt. When the child was lying, his/ her legs were flexed or even crossed (scissoring sign). In the ventral suspension test, the child's limbs and head were excessively flexed. When the child was pulled to the sitting position, his head was pulled backwards. When a child's hand was placed across the neck to reach the opposite arm, the elbow could not cross the midline. The assessed popliteal angle was less than 90°.

As our research target were children up to five years of age, it was not possible to assess their muscle strength on the basis of the MRC scale. Children's muscle strength was defined as appropriate/normal or decreased, when no resistance was detected against passive movement of limbs and their movements against gravity were poor.

After clinical evaluation, the motor developmental quotient, which defines a child's motor abilities over time, was calculated. Two basic data were required for DQ calculation – the child's motor abilities and his/ her chronological age, in months. They were divided and multiplied by 100. When calculating DQ for premature infants, their chronological age must be adjusted until the age of two years. DQ between 80 and 120 is defined as normal motor development. If DQ was between 70 and 80, it was necessary to re-examine the child and to recalculate the DQ. Motor developmental delay was established when the motor quotient was below 70. Mild motor delay was defined as DQ between 50 and 70. DQ below 50 signified global motor developmental delay, which is defined as a delay in at least two of five developmental categories – gross motor development, fine motor development, speech, cognitive function and social-behavioural abilities [13].

### 2.3 Laboratory Analysis

Beside clinical diagnostics, ten sugars (fucose, sucrose, arabinose, galactose, glucose, xylose, fructose, ribose, lactose, mannose) were electrochemically detected in the urine samples using ion chromatography combined with electrochemical detection and tandem mass spectrometry (IC-ECD, IC-MS/MS). This is a sensitive and selective method used for profiling carbohydrates in biological samples.

### 2.4 Statistical Analysis

Each urine sample from hospitalised children and from the healthy control group was analysed using the IBM *SPSS Statistic 21* statistical program. For each of ten carbohydrates, the average value, median, standard deviation, and minimal and maximal values were calculated. Both groups were compared using a non-parametric Student's t-test or Mann-Whitney test with reliability $p < 0.050$. If the difference was statistically significant, values were compared with the Bonferrony test. The predictive value of each carbohydrate in the urine sample was determined using the ROC curve and the surface below it was calculated by the DeLong method. The surface area under the ROC curve, being 0.8, was taken as the statistically reliable predictive value.

## 3. RESULTS

### 3.1 Clinical Status

Twenty-six of 82 children had normal muscle tone, 43 had hypotonia, 10 had hypertonia and 3 showed asymmetrical muscle tone. Forty-two had adequate motor development and 40 had motor developmental delay (DQ < 70), as shown in Table 1.

### 3.2 Urine Samples

Seventy-three children were hospitalised once, eight twice and one child was hospitalised three times. Urine samples were obtained during each hospitalisation. Ten different carbohydrates (fucose, sucrose, arabinose, galactose, glucose, xylose, fructose, ribose, lactose, mannose) were measured in 136 urine samples; 36 had an elevated level of at least one sugar. Twenty-six of these samples belonged to children with changes in muscle tone and/or developmental delay, while ten were detected in the control group of 44 children without any known neuromuscular disease.

66 urine samples from children hospitalised at KOOMRaN were normal (71.7%), 16 urine samples had elevated level of one carbohydrate, 5 urine samples had elevated level of two carbohydrates and 5 urine samples had elevated level of three carbohydrates. Children from the control group had 34 normal urine samples (77.8%) and 10 urine samples with elevated level of one carbohydrate (22.2%).

Three of 26 pathological urine samples belonged to the same child, so 24 children had a pathological urine sample. In pathological urine samples of children hospitalised at KOOMRaN fructose was elevated 15 times, sucrose two times, galactose five times, glucose 13 times, fructose 12 times, ribose three times, lactose once and mannose two times. Arabinose and xylose never exceeded normal values. In the control group, all ten pathological urine samples had an elevated level of one carbohydrate (fucose eight times and sucrose twice).

All ten carbohydrates were distributed normally. There was a statistically significant difference between patients and control subjects in the value of three carbohydrates in urine sample – fucose [mean 1.43 (standard deviation 1.68) vs. 2.07 (1.35), $p = 0.020$], sucrose [mean 1.72 (3.31) vs. 3.82 (9.99), $p = 0.051$] and ribose [mean 2.90 (4.86) vs. 0.15 (0.14), $p = 0.000$]. There was no significant difference between groups in arabinose [mean 1.70 (1.76) vs. 2.04 (1.33), $p = 0.252$], galactose [mean 4.85 (7.36) vs. 3.43 (4.40), $p = 0.228$], glucose [mean 13.80 (50.25) vs. 4.13 (2.24), $p = 0.209$], xylose [mean 1.94 (2.55) vs. 2.40 (2.70), $p = 0.321$], fructose [mean 4.75 (7.66) vs. 3.03 (3.50), $p = 0.152$], lactose [mean 3.64 (5.40) vs. 4.67 (5.39), $p = 0.287$] and mannose [mean 2.03 (6.51) vs. 0.40 (0.41), $p = 0.100$].

The correlation between muscle tone, DQ and analysed urine samples is presented in Table 2 and in Table 3.

Table 4 shows the muscle tone and DQ of children who had a pathological urine sample.

## 4. DISCUSSION

Developmental disabilities are estimated to affect 5-10% of children [14]. Serious developmental disabilities affect approximately 2% of school-age

children, which is ten times higher than the estimated prevalence of cerebral palsy, and are lifelong conditions that incur substantial financial and social costs [15]. There is considerable uncertainty regarding the evaluation of infants and young children with GDD with respect to the appropriate extent of laboratory investigations [16]. Laboratory investigations relevant to establishing the possible underlying aetiology include metabolic studies that screen for specific inborn errors of metabolism, although extensive studies involving large numbers of individuals with significant GDD have yielded 0.6% and 1.35% diagnostic clues when using non-selective screening protocols respectively [17]. However, in one study it was confirmed that a stepwise approach rather than a routine screening approach can increase the diagnostic yield 10-folds [18]. In this study, the goal was to examine the usefulness of neurometabolic testing in patients with unexplained developmental delay. Abnormal metabolites in the serum or urine were not in themselves diagnostic, but pointed to the need for further testing.

Our research was focused on establishing the correlation between muscle tone, impairment of motor development in infants and small children and their biochemical levels of carbohydrates in a urine sample. We are well aware that several methods exist for assessing tone and there is an excellent method for assessment and follow-up of comprehensive neurological development from birth until the age of six years [19]. However, for the purpose of this study, we evaluated muscle tone mainly by observing the infant's posture and movement, by feeling the resistance offered by passive movement, changing his/her position and by observing subsequent movements (active tone) and evaluating changes in tone [20].

Analysis of ten different carbohydrates in urine samples revealed that a child with a pathological urine sample also has abnormal muscle tone and/or motor developmental delay ($p = 0.002$). These findings contradict the finding of Papavasilliou AS et al. regarding abnormal metabolites in urine; however, in their study they did not check for sugars in the urine [17].

**Table 1. Absolute and relative numbers of children with normal or abnormal muscle tone correlated with DQ in the research group**

| | | Muscle tone | | | | Total |
|---|---|---|---|---|---|---|
| | | Normal | Hypotonia | Hypertonia | Asymmetrical | |
| DQ | Normal | 23 (28.0%) | 13 (15.9%) | 4 (4.9%) | 2 (2.4%) | 42 (51.2%) |
| | < 70 | 3 (3.7%) | 30 (36.6%) | 6 (7.3%) | 1 (1.2%) | 40 (48.8%) |
| Total | | 26 (31.7%) | 43 (52.5%) | 10 (12.2%) | 3 (3.6%) | 82 (100%) |

**Table 2. Absolute and relative number of urine samples with normal or pathological urine sample correlated with muscle tone in a group of hospitalised children**

| | | Carbohydrates | | Total |
|---|---|---|---|---|
| | | Normal | Elevated | |
| Muscle tone | Normal | 20 (21.7%) | 10 (10.9%) | 30 (32.6%) |
| | Hypotonia | 33 (35.9%) | 14 (15.2%) | 47 (51.1%) |
| | Hypertonia | 9 (9.8%) | 2 (2.2%) | 11 (12, 0%) |
| | Asymmetrical | 4 (4.3%) | 0 (0.0%) | 4 (4.3%) |
| Total | | 66 (71.7%) | 26 (28.3%) | 92 (100.0%) |

**Table 3. Absolute and relative number of urine samples with normal or pathological urine sample correlated with DQ in a group of hospitalised children**

| | | Carbohydrates | | Total |
|---|---|---|---|---|
| | | Normal | Elevated | |
| DQ | Normal | 32 (34.8%) | 14 (15.2%) | 46 (50.0%) |
| | < 70 | 32 (34.8%) | 14 (15.5%) | 46 (50.0%) |
| Total | | 64 (69.6%) | 28 (30.4%) | 92 (100.0%) |

**Table 4. Absolute number of children with a specific clinical picture and pathological urine sample. Relative numbers stated in bracket are calculated for the whole study group of 82 children**

| | | Muscle tone | | Total |
|---|---|---|---|---|
| | | Normal | Abnormal | |
| | Normal | 1 (1.2%) | 4 (4.9%) | 5 (6.1%) |
| DQ | < 70 | 9 (11.0%) | 10 (12.2%) | 19 (23.2%) |
| Total | | 10 (12.2%) | 14 (17.1%) | 24 (29.3%) |

However, the hypothesis that children with either abnormal muscle tone or motor developmental delay have significantly elevated values of carbohydrates in urine samples could not be confirmed (for abnormal muscle tone $p = 0.472$ and for motor developmental delay $p = 1.000$). Both the specificity and sensitivity of urine sample analysis for defining abnormalities in children's clinical status are not high enough for this test to be considered reliable (for abnormal muscle tone the sensitivity was 0.26 and specificity 0.67; for defining decreased DQ the sensitivity was 0.30 and specificity 0.70.

A study of Piccardo et al. [21] proved that the quantity of eliminated sugars correlates well with the severity of certain diseases, and that a specific sugar can provide important information about a person's clinical status. Using the Student' t-test, we proved a statistically reliable difference in fucose ($p = 0.020$), sucrose ($p = 0.051$) and ribose ($p = 0.000$) levels between the group of children who were hospitalised and the healthy control group. Also all children with an elevated level of fructose were assessed as being hypotonic due to an "upper motor neuron disease" and 83.3% of them had motor developmental delay. In the control group, fructose was normal in all samples. This excludes the possibility that children with normal muscle tone and normal DQ have elevated levels of fructose.

With a rising number of elevated carbohydrates, children's muscle tone and motor development were more severely affected. A urine sample with an elevated level of at least three sugars showed a statistically reliable evidence of abnormal muscle tone and motor developmental delay. However, a normal urine sample does not exclude changes in muscle tone and decreased DQ because 27 children with abnormal clinical status had all ten carbohydrates in a urine sample within the normal range.

Besides determining absolute values of a specific carbohydrate in a urine sample, their relative values were also analysed. Previous research [22,23] showed a stronger correlation between the relative values of carbohydrates in a urine sample and the patient's clinical status. We found that all children with the same relative values of all ten carbohydrates had the same clinical status, even though absolute values of sugars were different. This clearly showed the importance of looking at relative values of sugars in urine and not just searching for the ones that exceeds normal value.

Developmental assessment is often criticised for not being very applicable to young infants and children and for its poor ability to predict degrees of normality in individual children [24]. It is important to be aware that early detection of possible developmental delay must lead to early intervention, which is still the only significant management available to these infants and children. Analysis of carbohydrates in urine opens up new research options that could assist in the detection of additional underlying causes of developmental delay as a consequence of congenital metabolic disorders.

## 5. LIMITATIONS OF THE STUDY

However, we are well aware of certain drawbacks of our work. Assessments of muscle tone and DQ are subjective and depend on the clinical experience of the examiner. Clinical evaluation would be more accurate if each child underwent assessment from two examiners. If their evaluation differed, a third examiner could be involved. A broader research group would also diminish the possible difference between examiners' assessments. As our study group comprised children up to 5 years of age, it was not possible to influence their urine secretion and some of the collected urine samples were not suitable for analysis. Ultimately we were able to collect 136 urine samples. The number of children included in our research must be increased.

In the future, we could follow these children and see if their clinical picture and sugars in the urine change. Vitamins, minerals and neurophysiotherapy are often prescribed when a child is found to have motor developmental delay or abnormal muscle tone. We intend to analyse urine samples after a period of time to see if any change in muscle tone and DQ due to the use of vitamins, minerals and neurophysiotherapy is reflected in the elimination of carbohydrates in the urine.

## 6. CONCLUSION

We found a significant correlation between the change in muscle tone and/or delayed motor development and elevation of carbohydrates in a urine sample. Level of the specific carbohydrate and relative values of all ten carbohydrates in a urine sample showed strong correlation with the severity of motor developmental delay and/or change in muscle tone. Elevated levels of at least three carbohydrates were a reliable factor for prediction of changes in muscle tone and motor developmental delay.

## CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

## ETHICAL APPROVAL

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

The study with the title "Correlation between muscle tone and motor delay and biochemical levels of carbohydrates in urine" received ethical approval from the National Medical Ethics Committee of the Republic of Slovenia (NMEC), number 102/02/13.

Patients or their carers have given informed consent to the research and to publication of the results.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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