

Propionylcarnitine and methionine concentrations in newborns with hypospadias

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Introduction. Of interest is if factors like maternal diet can influence the risk of hypospadias-affected pregnancy. Increased propionylcarnitine (C3) is regarded as a biomarker of vitamin B12 deficiency. The retrospective study was undertaken to determine whether increased propionylcarnitine and low methionine in newborns are associated with hypospadias.

Material and methods. 41 newborns with hypospadias and 90 control newborns without congenital anomalies were investigated. Whole blood propionylcarnitine and methionine concentrations were measured using tandem mass spectrometry.

Results. The mean concentration of propionylcarnitine was higher in newborns with hypospadias compared with newborns without congenital anomalies ($p = 0.026$). The mean methionine level in cases was insignificantly lower than in controls.

Conclusion. There appears to be an association between decreased vitamin B12, as indexed by an increase of propionylcarnitine, and hypospadias in the investigated group of patients.

Key Words: propionylcarnitine ◊ methionine ◊ vitamin B12 ◊ MS/MS ◊ hypospadias

INTRODUCTION

Hypospadias represents a significant proportion of all birth defects, affecting approximately 1 in 125–200 male newborns, and appears to be increasing [1]. It occurs as a result of abnormal urethral closure at around 8 to 14 weeks of gestation. The presence of this abnormality has consequences on physical and psychological development and imposes a substantial social burden. In addition, relatively little is known about the causes of hypospadias, and therefore there are no established effective strategies for reducing occurrence of this congenital anomaly [2]. Of interest is that factors like maternal diet can influence the risk of hypospadias-affected pregnancy [2–6].

Recently, Carmichael et al. [5] observed that increased maternal intake of methionine and vitamin B₁₂, which are involved in methyl groups (one-carbon) metabolism, was associated with reduced risk of hypospadias. Moreover, epidemiological stud-

ies implicate impaired one-carbon metabolism in preeclampsia and hypertension in pregnancy [7]. There is increased risk of hypospadias in offspring among women whose pregnancies were complicated by those disorders [1, 2]. One-carbon metabolism is an unusually complex metabolic network, composed of a set of several interlocking cycles. The essential amino acid methionine is required for protein synthesis as a precursor for cysteine and as a donor of methyl groups. Methionine synthase catalyzes the re-methylation of homocysteine to methionine (CH₃-homocysteine). This is the only reaction in humans in which both vitamin B₁₂ and folate are co-factors [8].

One of the most important advancements in neonatal screening is the analysis of amino acids and carnitines in whole blood drops using tandem mass spectrometry (MS/MS)[9]. High levels of propionylcarnitine (C3) may serve as a sensitive indicator of vitamin B₁₂ deficiency. There are published reports

on deficiency of vitamin B₁₂ in newborns, secondary to maternal deficiency, detected by acylcarnitine profiling through newborn screening for inborn errors of metabolism [10, 11]. Currently, methionine and propionylcarnitine assessments are included in all MS/MS-based newborn screening programs [10]. Identification of metabolic profiles predictive of specific congenital anomalies would help refine nutritional intervention strategies and reduce the risk of abnormal embryogenesis [12]. Hence, the present study was undertaken to assess the associations between levels of whole blood methionine and propionylcarnitine in routine neonatal screening and the risk of hypospadias.

Study population & methods

Study population

Patients with non-syndromic (isolated) glandular and distal forms of hypospadias attending our Institute and unrelated healthy children of similar age from three local primary care pediatricians were considered for the study. Inclusion criteria were as follows: (i) singleton pregnancy, (ii) gestational age at delivery ≥ 37 weeks, and (iii) delivery in the years 2004–2009 in hospitals located in the area covered by the MS/MS Newborn Screening Program provided by our Institute. Most newborn blood specimens (“Guthrie cards”) were collected three days after birth.

Case eligibility was determined using detailed medical records. Finally, we performed a retrospective analysis of methionine and propionylcarnitine screening results of 41 patients with hypospadias and 90 healthy male controls without congenital anomalies. The protocols of the study were approved by the local ethics committee.

Mass spectrometry

Two 3.5 mm dried blood drops (equivalent to 7.5 μL of blood) were punched into a 96 well microtiter plate. Methanolic internal amino acid and carnitine standard solution (100 μL) was added with an 8-channel pipette. The microtiter plate was shaken over a 20-min. extraction. The extract was transferred to a second microtiter plate and dried. The residue was butylated with butanol-HCl at 65°C for 20 min., then dried and dissolved in 50 μL of methanol and water 50:50 mixture with 0.02% formic acid. Samples were analyzed using a tandem mass spectrometer (SCIEX Api 3200, Concord, Canada) equipped with a liquid chromatography autosampler. The working ranges were 1.0–200.0 $\mu\text{mol/L}$ of methionine and 0.01–50 $\mu\text{mol/L}$ of propionylcarnitine.

Statistical methods

Differences in the whole blood concentration of methionine and propionylcarnitine between the groups were tested using *t*-test. Confounding variables were gestational age at birth and birth weight. Correlations were tested with Pearson correlation analysis. Statistical significance was claimed for any test result at or below the accepted type I error rate of 0.05.

RESULTS

Mean gestational age at delivery of study cases and controls were the same (39 weeks). Mean birth weight (SD) of hypospadias newborns and controls were 3.680 (0.370) kg and 3.630 (0.370) kg, respectively, $p = 0.496$. The mean (SD) concentration of propionylcarnitine was higher in newborns with hypospadias compared with newborns without congenital anomalies; 3.52 (1.50) $\mu\text{mol/L}$ *vs.* 2.91 (1.13) $\mu\text{mol/L}$, $p = 0.026$, respectively. We could not demonstrate a difference in the concentrations of methionine between cases compared with controls [30 (9) $\mu\text{mol/L}$ *vs.* 31 (9) $\mu\text{mol/L}$; $p = 0.497$]. There were no significant correlations between concentrations of propionylcarnitine or methionine and clinical variables such as birth weight or gestational age at delivery.

DISCUSSION

To our knowledge, this report is the first study to investigate methionine and propionylcarnitine concentrations in patients with hypospadias. The molecular mechanisms of abnormal penile development are poorly understood. The relatively high and fluctuating frequency of hypospadias is attributed to the sensitivity of urogenital development to environmental insult [1, 3], which stresses the need for further etiological research.

We found an association between increased level of propionylcarnitine and the risk of hypospadias in newborns. The presented results support the hypothesis that there is a link between maternal intake of certain nutrients involved in the metabolism of methyl groups and the risk of hypospadias [5]. Some experimental evidence exists in favor of the ameliorative effects of vitamin B₁₂ on teratogen-induced congenital anomalies in rodents [13, 14]. However, results of human studies on periconceptional supplementation with cobalamin and other group B vitamins and occurrence of hypospadias, as well as association between mother’s vegetarian diet and hypospadias are conflicting, which indicates the difficulty and complexity of a study of hypospadias [4, 5, 15]. The overwhelming

majority of hypospadias cases remain unexplained and the birth defect may be a highly heterogeneous condition subject to multiple genetic and environmental factors. There is no doubt that hypospadias is regulated by a number of factors, rather than by single factors. Androgen and androgen receptors have been thought to play a crucial role in male external genital development [2]. Interestingly, in a dose-dependant manner, cobalamin influences the proliferation of androgen-dependent cell lines [16]. Susceptibility to vitamin B₁₂ status might depend not only on the low level of cobalamin, indexed as increased propionylcarnitine, itself, but also on individual sensitivity modulated by genetic background, including gene polymorphisms and epigenetic modifications.

While conducting further studies, it should be kept in mind that neonatal methylmalonic acidemia with associated increased propionylcarnitine may result not only from acquired vitamin B₁₂ deficiency, but also from inborn errors of metabolism (e.g. methylmalonyl-CoA mutase deficiency).

Our null-findings concerning methionine may be affected by the sample size, which might not be large enough to directly detect modest differences of amino acid levels in the study and the control groups. We were not able to access additional information on newborn feeding and maternal life style factors. Since the mother is the environment for the developing embryo, interactions between genetic factors and nutrients in early pregnancy are assumed to be involved in the pathogen-

esis of malformations. In our study, assessments of methionine and propionylcarnitine levels were performed after delivery and only in newborns, which may have lowered the possibility of finding pertinent teratogenic feto-maternal changes. This study also displays some notable strengths. The MS/MS results used in our study were obtained from a population based newborn screening program and we were able to distinguish between different phenotypic hypospadias types so that only isolated cases were included. Cases and healthy controls without congenital anomalies were under our observation for at least one year. The investigated population was ethnically homogenous, mostly omnivorous, and from an area where pre-conceptional diet supplement use is uncommon.

In conclusion, this study constitutes the first report on methionine and propionylcarnitine levels in patients with hypospadias. This study provides initial data indicating a potential association between low maternal vitamin B₁₂ and hypospadias risk. It is important to stress the need for further studies of metabolic profiles of hypospadias-affected children, which might shed a new light on intervention strategies to reduce the risk of urogenital anomalies.

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