Assessment of leptin, c-reactive protein and lipid levels in children and adolescents with myelomeningocele

Ocena stężenia leptyny, białka ostrej fazy i lipidów u dzieci i młodzieży z przepukliną oponowo-rdzeniową

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STRESZCZENIE

Wstęp. Rozwój otyłości oraz miażdżycy może rozpocząć się już w wieku dziecięcym szczególnie u pacjentów z ograniczoną aktywnością ruchową w przebiegu przepukliny oponowo-rdzeniowej (MMC). W diagnostyce, oprócz oznaczenia lipidogramu, stężenia glukozy, białka ostrej fazy (CRP), praktyczne znaczenie ma ocena oznaczonego stężenia leptyny w surowicy krwi. Celem pracy była ocena powyższych wskaźników u dzieci i młodzieży z MMC w zależności od skali Hoffer'a. **Metoda.** Badaniami objęto 84 pacjentów z MMC w wieku od 7 do 17 lat. Wykonano pomiary masy i wysokości ciała, oznaczono stężenia: leptyny, oceniono stan gospodarki lipidowej oraz glikemii i CRP, witaminy D₃, białka całkowitego i albumin. Wyliczono wskaźniki: BMI.

Wyniki. W ocenianej grupie pacjentów 29 pacjentów poruszało się samodzielnie (IV wg skali Hoffer'a), a 34 osoby poruszały się na wózkach (I wg skali Hoffer'a) przy średniej wartości skali Hoffer'a 2,45. Średnie stężenie leptyny w grupie badanej wynosiło 9,45 ng/ml i było wyższe niż w grupie kontrolnej 5,85 ng/ml. Stężenia leptyny zależały istotnie statystycznie od wieku ($p = 0,000$); wzrostu ($p = 0,000$); poziomu aktywności ruchowej wg klasyfikacji Hoffer'a ($p = 0,0017$); BMI ($p = 0,000$); białka całkowitego ($p = 0,0306$). **Wnioski.** Wysokie stężenia leptyny u dzieci z przepukliną oponowo-rdzeniową z nadmiarem masy ciała mogą być wskaźnikiem rozwoju zespołu metabolicznego. Dzieci z otyłością oraz współistniejącymi zaburzeniami metabolicznymi powinny być objęte szczególną opieką ze względu na możliwość wystąpienia wczesnych zmian miażdżycowych

Słowa kluczowe: przepuklina oponowo-rdzeniowa, leptyna, otyłość

ABSTRACT

Introduction. Development of obesity and atherosclerosis can begin at a young age, particularly in patients with limited mobility as a result of myelomeningocele (MMC). In diagnostics, in addition to lipidogram, glucose and C-reactive protein (CRP) levels, the concentration of leptin in blood serum has practical importance. The aim of the study was to assess the above indicators in children and adolescents with MMC depending on Hoffer's scale. **Method.** We studied 84 patients with MMC aged 7–17 years. The following were measured: weight, height, leptin levels, lipid metabolism, glycemia, CRP, vitamin D₃, total protein and albumin. BMI was calculated. In the assessed group of patients 29 patients moved independently (IV on Hoffer's scale) and 34 patients used a wheelchair (I on Hoffer's scale) with a mean value of 2.45 on Hoffer's scale. **Results.** Mean leptin level in the study group was 9.45 ng/mL and was higher compared with the control group (5.85 ng/mL). Leptin levels statistically significantly depended on age ($p = 0,000$); height ($p = 0,000$); level of physical activity according to Hoffer's classification ($p = 0,0017$); BMI ($p = 0,000$); total protein ($p = 0,0306$). **Conclusion:** High leptin levels in children with MMC with excess body mass can be an indicator of metabolic syndrome development.

Key words: myelomeningocele, leptin, obesity

INTRODUCTION

Myelomeningocele (MMC) affects approximately 1 in every 1,000 newborns, making it the second (after Down syndrome) most common birth defect. Statistics show that the incidence of MMC ranges between 2.05 and 2.68 per 1,000 live births in Poland [1]. The occurrence of complications in the form of limb fractures below the level of spinal cord injury is associated with bone trophic impairment, mainly due to different movement and activity pattern (predominance of sedentary lifestyle, significantly limited movement, absence of lower extremity loading) [2]. Incre-

ased fat deposition and body weight can lead to decreased physical activity in the affected children. About half of MMC patients use a wheelchair. Obesity, which is common among MMC children, can be a significant risk factor for type 2 diabetes and atherosclerosis. Reports on risk factors for atherosclerosis in children with MMC are sparse [3]. Furthermore, it is believed that there are three SNP genes associated with glucose metabolism and obesity [4]. Leptin, a hormone, is produced mainly in adipocytes and placental trophoblast cells. It bears high structural simi-

urity to the cytokine family. Structurally, leptin is a protein composed of 167 amino acids. Since it is synthesized in fat tissue adipocytes, it belongs to the group of biologically active adipokines (proteins). The hormone acts via leptin receptors located mainly in the hypothalamus. Once leptin binds to hypothalamic receptors, neurons cease to produce a neurotransmitter – neuropeptide Y, which stimulates appetite. Thus, the hormone reduces hunger and stimulates the sympathetic nervous system. Impaired production of this hormone or insensitivity of its receptors often leads to weight gain and obesity [5–8]. The leptin-encoding gene is located on chromosome 7q31.3 and is referred to as the obesity gene [9]. CRP measurement is used to monitor the course or the risk of inflammatory diseases (bacterial or viral infections). Additionally, it is increasingly used to assess the risk of cardiovascular incidents [10]. Pediatric MMC not only leads to physical disability but can also cause premature development of obesity and atherosclerosis in this patient population.

The aim of this study was to determine whether increased levels of leptin occurred in a group of MMC children with varying degrees of motor impairment according to Hoffer's classification, as well as to determine whether there is a relationship between leptin levels and somatic nutritional status parameters and biochemical indicators of lipid metabolism and glycemia.

METHOD

Subjects

The study was conducted in a group of 84 children with MMC (46 girls, 38 boys) aged 7–17 years (mean age 9.1 years), remaining under the care of the Department of Pediatric Rehabilitation and the Department of Pediatrics and Nephrology at the Medical University of Białystok. Each child underwent physical examination with anthropometric measurements; movement disorders were assessed using Hoffer's classification; BMI was calculated based on body weight and height.

Inclusion criteria: patients with MMC. Exclusion criteria: patients with non-MMC; acute infection; hsCRP values higher than 10 mg/L.

Patients were divided into 4 groups according to Hoffer's scale which assesses physical activity (I – wheelchair-dependent patients, II – therapeutic walkers, III – household walkers, IV – community walkers). Additionally, patients were divided according to the level of lesion based on the neurosurgical data into three groups: 1-thoraco-lumbar (Th-L), 2- lumbar-sacral (L-S), 3- sacral (S).

High-Sensitivity CRP is a test that allows precise determination of low CRP levels not exceeding positive values for inflammation detected using standard CRP testing. Hs-CRP higher than 10 mg/l indicates the need to implement diagnostics for conditions other than cardiovascular diseases. The control group included 20 healthy children with median age 9.12 years (1.42–17.75) with no heart or endocrine diseases.

Biochemistry

Biochemical analyses were performed at the Department of Pediatric Laboratory Diagnostics of the University of Children's Hospital in Białystok, Białystok, Poland. Morning fasting venous blood samples were collected from every child for the measurement of the biochemical parameters. The following tests were performed: 1. Assessment of leptin levels using the enzyme-linked immunosorbent assay (ELISA, BioSource). Acceptable leptin levels 1–100 ng/mL. 2. Serum levels of hs-CRP in children with MMC. 3. Fasting blood tests for total cholesterol, HDL and LDL fractions, triglycerides and glycemia, in accordance with the accepted laboratory methods.

Normal distribution of data was evaluated by a Kolmogorov–Smirnov test. Data were not normally distributed. ($d = 0.262$; $p < 0.01$). Mean test results were compared using the Wilcoxon test. Correlations between leptin levels and variables were assessed using multiple regression analysis. Statistical analyses were done using STATISTICA 10 PL software. A level of $p < 0.05$ was considered statistically significant. The Bioethics Committee of the Medical University of Białystok approved the study.

RESULTS

The study was conducted in a group of 84 children (46 girls, 38 boys) aged 7–17 years (mean age 9.1 years) with MMC. In the assessed group of patients, 29 patients moved independently (IV on Hoffer's scale), and 34 patients used a wheelchair (I on Hoffer's scale), with a mean value of 2.448 on Hoffer's scale. Twenty seven patients (31%) had spinal lesion at thoraco-lumbar level, 52 (59%) had at lumbar-sacral, and 9 (10%) at sacral level.

Overall, children with MMC were overweight and healthy children had a normal body weight. Mean BMI values were 19.08 ± 10.03 in children with MMC and 14.80 ± 8.05 in the control group; however, there was no statistically significant difference ($p = 0.102$, Wilcoxon test). Mean leptin values were 9.44 ± 12.41 ng/mL (0.45–52.77) in children with MMC and 5.85 ± 8.23 ng/mL (0.77–29.41) in the control group. Leptin levels were higher in children with MMC compared with the control group; however, there was no statistically significant difference ($p = 0.398$, Wilcoxon test). A comparison of biochemical parameters in children with MMC and the control group revealed only one significant ($p = 0.033$) difference related to hCRP between MMC and controls (tab. I).

There was a statistically significant correlation between leptin levels and patient age, height, physical activity according to Hoffer's classification, BMI, and total protein. Details are shown in tab. II.

DISCUSSION

The study included 84 patients with MMC aged 7–17 years. In the assessed group of patients 29 patients moved independently (IV on Hoffer's scale), and 34 patients used a wheelchair (I on Hoffer's scale), with a mean value of 2.45 on Hoffer's scale. Mean leptin level in the study group

Table I. Comparison of biochemical blood parameters in children with MMC and controls

Parameters	Mean	Min.	Max.	SD	P-value
Glucose MMC (mg/ml)	88.07	69.00	146.00	13.15	0.465
Glucose control (mg/ml)	86.08	75.00	107.00	8.83	
TG MMC (mg/ml)	88.51	30.00	302.00	52.47	0.176
TG control (mg/ml)	94.45	56.00	243.00	52.83	
Cholesterol MMC (mg/ml)	174.02	106.00	229.00	27.05	0.483
Cholesterol control (mg/ml)	169.63	106.00	203.00	26.03	
HDL MMC (mg/ml)	54.95	39.00	83.00	11.89	0.310
HDL control (mg/ml)	50.91	39.00	6400	8.51	
LDL MMC (mg/ml)	101.35	26.00	160.00	26.00	0.498
LDL control (mg/ml)	92.18	10.00	151.00	39.97	
hsCRP MMC (mg/L)	12.7164	2.72	16.72	2.54	0.033 ^a
hsCRP control (mg/L)	7.09143	1.80	4.53	1.27	

Abbreviations: HDL – high-density lipoproteins; LDL – low-density lipoproteins; MMC – myelomeningocele; TG – triglycerides; hsCRP – high-sensitivity CRP; SD – standard deviation.

^a Significant $p < 0.05$.

Table II. Correlations between leptin levels and Hoffer's scale, sex, height, BMI, laboratory parameters in children and adolescents with MMC

Variables	beta	P value
Leptin vs Hoffer	-0.395	< 0.001
Leptin vs. Age	0.432	< 0.001
Leptin vs. Sex	-0.150	0.164
Leptin vs. Height	0.524	< 0.001
Leptin vs. BMI	0.669	< 0.001
Leptin vs. Vitamin D3	0.044	0.683
Leptin vs. Glucose	0.210	0.054
Leptin vs. TG	0.111	0.326
Leptin vs. Total Cholesterol	0.131	0.345
Leptin vs. HDL	0.009	0.934
Leptin vs. LDL	0.001	0.950
Leptin vs. albumin	0.186	0.284
Leptin vs. total protein	0.320	< 0.05
Leptin vs. hsCRP	0.189	0.301

Abbreviations: BMI – body mass index; hsCRP – high-sensitivity CRP; HDL – high-density lipoproteins; LDL – low-density lipoproteins; MMC – myelomeningocele; TG – triglycerides.

was 9.45 ng/mL and was higher compared with controls (5.85 ng/mL). There was a statistically significant relationship between leptin levels and the level of physical activity according to Hoffer's classification; height, BMI, total protein and hsCRP. According to WHO, the normal BMI range is 5th percentile to less than the 85th percentile [11, 12]. A strong correlation between BMI and the amount of body fat tissue and a weaker correlation with height compared with other indicators was considered by the WHO to be an essential element of the assessment of the protein-energy nutritional status. BMI allows to determine body fat levels. Nutritional status can be assessed based on BMI: malnutrition (low fat content), overweight or obesity (excessive fat content) [13]. Mean BMI in the assessed group of children with MMC was 19 kg/m², including 20 patients with BMI over 25 kg/m². It is currently believed that genetic factors account for 30-40% of the obese phenotype, while 60-70% are other factors [14, 15]. The gene regulating leptin production and secretion is well-known. Leptin is a protein produced by fat cells, which controls hunger in the hypothalamus. Elevated body fat mass increases plasma leptin levels, thus reducing hunger and producing a feeling of satiety. This system determines an individual level of sensitivity to a positive energy balance via a tendency for excessive fat tissue accumulation and reduced metabolic rate [16, 17]. It seems that increased leptin levels in obese patients are due to excess adipose tissue producing this hormone [16, 17].

Kulińska-Szukalska [18] investigated correlations with biochemical indicators in a group of overweight and obese children and showed positive correlations between hormone levels and the levels of triglycerides and glucose. These correlations were not statistically significant in the assessment of patients with MMC. Furthermore, the author [18] observed a tendency for correlations (without statistical significance) between leptin levels and HDL-cholesterol as well as a similar, yet negative correlation with total cho-

lesterol. In our study, statistically significant correlations were demonstrated between leptin levels and total protein.

Wouters et al. [19] found a positive correlation between leptin levels and BMI. Similarly, we also found a statistically significant correlation in the assessed group of patients with myelomeningocele. Our findings are in line with numerous reports in the literature on correlations between leptin levels and BMI [20, 21].

Atherosclerosis in children and adolescents is usually asymptomatic, therefore no clear diagnostic criteria have been developed so far. Assessment of serum cardiovascular risk factors (e.g. levels of lipidogram components, glucose and C-reactive protein – CRP) may be also useful [22]. CRP produced in the liver due to the effects of interleukin 6 is a good marker of inflammation. Apart from its typical use in the assessment of inflammation during infections, the assessments of states predisposing to atherosclerosis is also recommended. There is a positive correlation between CRP levels and weight, BMI, insulin and TG levels, as well as a negative correlation with HDL cholesterol [23]. Its increased levels are found in children with type 1 diabetes [24], obesity, and metabolic syndrome [25]. The only significant difference between MMC and controls was found for hCRP. In our study, there was no significant correlation between total cholesterol, sex, and other biochemical parameters. These results are in agreement with the previous studies [26–28].

In conclusion, high leptin levels in MMC children with excess body mass can be an indicator of metabolic syndrome development. Metabolic disorders observed in our patients indicate the need for early primary and secondary prevention aimed at reducing excess body mass through universal education (mainly on lifestyle changes) as well as special medical care targeted at obese pediatric patients as a group at risk for multiple health problems. The lack of correlation between serum C-reactive protein levels and lipid values may indicate an absence of both inflammation and the risk of the atherosclerotic process.

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