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DIETARY INTAKE OF FOLATE AND THE FREQUENCY OF ITS DEFICIENCY IN CHILDREN WITH TYPE 1 DIABETES MELLITUS AND HEALTHY CHILDREN

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Adequate folate intake is essential for a child's growth. There is lack of information about the prevalence of this nutrient deficiency in the Ukrainian population, including children. The aim of the study was to evaluate the dietary intake of folate and determine the frequency of folate deficiency in children with Type 1 diabetes mellitus (T1D) and healthy children. Determination of folate in serum was performed by ELISA. Folate level <3 ng/ml was diagnosed as a folate deficiency. Among all observed children the folate deficiency was diagnosed in 23 (32.9%): in 6 (17.1%) patients with T1D and in 17 (48.6%) healthy children ($P \leq 0.01$). The mean level of serum folate in patients with T1D was (5.09 ± 2.16) ng/ml and (3.72 ± 1.87) ng/ml in healthy children ($P \leq 0.01$). The average daily intake of folate with food was (138.68 ± 70.37) μg, without difference between T1D (12.00 ± 3.51 yr.) and healthy groups (10.83 ± 3.24 yr.), and it was more than two times lower than age requirements (300 μg/day). However, it was self-reported that 15 (48.9%) children of T1D group received vitamin supplementation one time in six months, while in healthy children only 6 (17.1%) children received vitamins ($P \leq 0.01$). In conclusion, the frequency of folate deficiency is high in the pediatric population. Nutrition does not provide the necessary intake of folate, which indicates the need for additional folate supplementation.

K e y w o r d s: folate, deficiency, dietary intake, diabetes mellitus, children.

Nutrient deficiencies are common worldwide and show high variability. A lack of nutrients, including iron, calcium, zinc, folate and vitamins B_{12}, C, D and E, has been reported among people of different age categories. It has been described that many diseases are the result of nutritional deficiencies [1-3].

Folate is a vitamin that is crucial for DNA and RNA replication during cell division and DNA methylation in the formation of red blood cells and nerve cells [4- 5].

Folate cannot be synthesized by the human body so maintaining normal levels depends on its external intake. Therefore, it is important to pay special attention to maintaining adequate folate status in childhood and adolescence, which represent critical periods of particular vulnerability due to higher demands during intensive growth and psychological and neurocognitive development.

Sufficient nutrient levels are important for children with socially significant diseases, including Type 1 diabetes mellitus (T1D). Due to the growing incidence of T1D, this pathology remains a problem not only for diabetologists but also for pediatricians and general practitioners, who are increasingly faced with the problem of optimal care for children with this disease [6].

T1D is a chronic condition that requires regular insulin administration and diligent patient effort to maintain health. Treatment is aimed at reducing hyperglycemia while minimizing the risk of hypoglycemia. Complex glucose balance is affected by food, insulin doses, body stress, physical activity and dozens of other factors. Educating patients and

families regarding the everyday needs of life with a chronic illness is key for good outcomes. With proper care and support, children and adolescents with T1D can expect a long and fulfilling life [7, 8].

However, over time, children with T1D may develop systemic consequences. It is believed that endothelial dysfunction, which is a precursor to vascular disease, begins to form in the early stages of T1D and is associated with folate status [9].

Folic acid deficiency can arise from multiple causes, including inadequate dietary intake. The fact that folate, which occurs naturally and is found in a variety of foods, has the ability to break down quickly during preparation and storage, its instability increases the risk of impaired folate status [10].

Diseases such as celiac disease, tropical sprue, short bowel syndrome, amyloidosis, gastric bypass, mesenteric vascular insufficiency, achlorhydria or congenital deficiencies of enzymes required in folate metabolism can inhibit transport mechanisms of folate across the intestinal wall resulting in a deficiency. Methotrexate, phenytoin, sulfasalazine and trimethoprim intake can antagonize folate utilization, inhibit its absorption or conversation to its active form resulting in folate deficiency. Alcoholism, pregnancy, hemolytic anemia and dialysis can also result in folate deficiency [11].

Therefore, sufficient folic acid intake is essential for the growth of a healthy child's body, especially for a body with chronic pathology. Because there is no information on the prevalence of folic acid deficiency among children in Ukraine, this study was designed to evaluate dietary folate intake and determine the frequency of folate deficiency in children with T1D and in healthy children.

Materials and Methods

The case group included 35 children with T1D aged 6 to 17 years. Blood samples were taken during a routine follow-up examination in Ternopil Regional Children's Clinical Hospital, Ternopil, Ukraine, early in the morning before insulin injection. The study was approved by the Bioethics Commission of Ternopil National Medical University under No. 60 on 01.09.2020. Parents or lawful caregivers and patients aged 18 years or more gave written consent for the abovementioned diagnostic procedures and for participation in this research project.

The control group consisted of 35 healthy children who matched the age and gender of the case group. Blood samples were taken during a routine follow-up examination on an empty stomach. The exclusion criteria were any acute or chronic diseases.

Alimentary folate supply was determined using a questionnaire. The questionnaire contained a list of foods that contain folate. Each child aged 10 to 17 years, under the supervision of their parents, reproduced their weekly diet, indicating the portion of a certain product and the frequency of intake during the week. The amount of folate was calculated taking into account its content in 100 grams of the product. Young children (from 6 to 9 years) were helped to reproduce the weekly diet by their parents. We calculated the average weekly and average daily nutrient intake with each food item individually and as a whole.

In all patients, physical examination was performed. Anthropometric measurements carried out in all children included weight (kg) and height (cm). Using these values, the body mass index (BMI) was calculated using the standard equation (the body mass in kilograms divided by the square of the body height in meters).

The project was carried out between March 2021 and June 2021 in order to even out the influence of the seasonal factor on the level of folate (physiologically caused by an increase in the consumption of fruit, vegetables and herbs by the population in the climate of Ukraine).

Determination of folate in serum was performed by enzyme-linked immunosorbent assay (Monobind, AccuBind ELISA Microwells, USA) in all children. Folate deficiency in children was indicated by level of folate ≤3 ng/ml in the blood according to guidelines for the diagnosis and treatment of cobalamin and folate disorders (2014, Aug.) [12].

Whereas folic acid deficiency can occur subsequent to vitamin B_{12} deficiency due to an impairment of methionine synthase resulting in the trapping of folate as methyltetrahydrofolate [11], we determined the level of B_{12} in serum by enzyme-linked immunosorbent assay (Monobind, AccuBind ELISA Microwells, USA) in all children. All children with Type 1 diabetes underwent a blood test to rule out anemia as the most common manifestation of folic acid deficiency.

Statistical analysis was carried out using the statistical package STATISTICA 10.0 and table editor Microsoft Excel 2003. Continuous variables were expressed as mean and standard deviation (SD). The comparison of frequency parameters was performed using the Chi-square test. Spearman's correlation co-

efficient was used to determine the rank measure of association. The differences between the parameters were statistically significant at $P \leq 0.05$, $P \leq 0.01$.

Results and Discussion

The study included 35 children with T1D and 35 healthy children. Table 1 shows the basic characteristics of the studied groups by age, gender, anthropometric indicators and data on the time of occurrence and duration of T1D of the case group.

The revealed significant difference in weight and height is related to the age of children in the studied groups, but such changes did not affect the BMI indicator – 15 (42.6%) children with T1D and 11 (31.4 %) healthy children showed normal BMI.

Folate is found in a variety of foods, including vegetables (especially dark green leafy vegetables), fruit and fruit juices, nuts, beans, peas, seafood, eggs, dairy products, meat, poultry and cereals. Spinach, liver, asparagus and Brussels sprouts are among the foods with the highest folic acid content [13]. Table 2 shows the number of children who consume a particular product containing folate during the week.

The presence of foods such as beef liver, sprats, pork, spinach, soft cheese and ice cream in the weekly diet was significantly lower among children with T1D than among healthy children. It is worth noting that foods rich in folate (liver, beef, cod liver, peas, spinach, nuts) [13] were included on the diet list of fewer than one in five respondents in both groups. However, dairy products, bread, buckwheat, oat flakes, eggs, pork, chicken, apples and oranges prevailed; although, the consumption of these food items in children is below dietary recommendations.

The average daily intake of folate with food was (138.68 ± 70.37) μg, without difference between groups, and it was more than two times lower than age requirements (300 μg/day) [14, 15].

However, 15 (48.9%) children with T1D received vitamin supplementation one time in six months, while in group with healthy children only 6 (17.1%) received vitamins ($P \leq 0.01$). A comparison of mean folate levels in children who received extra vitamins and those who did not receive them, did not reveal a significant difference between children with T1D and healthy children. No correlation was found between food intake and plasma folate levels.

Among all observed children, the folate deficiency was diagnosed in 23 (32.9%): 6 (17.1%) patients with T1D and 17 (48.6%) healthy children ($P \leq 0.01$). The mean level of serum folate in children with T1D was (5.09 ± 2.16) ng/ml and (3.72 ± 1.87) ng/ml in healthy children ($P \leq 0.01$).

In the present study, no children were identified with vitamin B_{12} deficiency (plasma cobalamin <200 pg/ml), and the median cobalamin concentration was (523.87 ± 235.1) pg/ml in children with T1D and (407.72 ± 206.5) ng/ml in healthy children ($P \leq 0.05$).

Furthermore, in the process of targeted questioning, 14 (40.0%) children with T1D and 4 (11.4%) healthy children noted the presence of increased irritability ($P \leq 0.01$) and 3 (8.6%) in each study group indicated impaired concentration. However, these complaints cannot be considered as specific to folate deficiency.

Table 1. Basic characteristics of the children with Type 1 diabetes and healthy children

Parameters	Children with T1D, n = 35	Healthy children, n = 35
Female/Male	15/20	12/23
Age, years (mean ± SD)	12.00 ± 3.51	10.83 ± 3.24
Weight, kg (mean ± SD, min-max)	45.34 ± 16.4 (18-74)*	34.98 ± 12.13 (20-60)
Height, m (mean ± SD, min-max)	1.53 ± 0.21 (1.10-1.80)*	1.39 ± 0.15 (1.18–1.77)
BMI, kg/m^2 (mean ± SD, min-max)	18.51 ± 3.35 (13.83–25.33)	17.38 ± 3.29 (13.17–28.26)
Age of the diagnosis, years (mean ± SD, min-max)	6.33 ± 3.82 (0.5–13)	–
Disease duration, years (mean ± SD, min-max)	4.91 ± 3.85 (1 week–14 years)	–
HbA1c, % (mean ± SD, min-max)	8.83 ± 2.77 (5.5-15.8)	–

*$P \leq 0.01$

Table 2. Characteristics of consumption of food containing folate

Product	Diabetics		Healthy children	
	n (%)	Frequency of consumption per week	n (%)	Frequency of consumption per week
milk	21 (60.0%)	4-5	27 (77.1%)	4-5
ice cream	3 (8.6%)*	1	16 (45.7%)	1-2
hard cheese	34 (97.1%)**	4-5	30 (85.7%)	4-5
soft cheese	20 (51.7%)**	2-3	28 (80.0%)	2-3
sour cream (30%)	17 (48.6%)*	2-3	31 (88.6%)	2-3
almonds	9 (25.7%)	2-3	7 (20.0%)	1
hazelnut	8 (22.9%)	2-3	7 (20.0%)	2
walnut	16 (45.7%)	2-3	21 (60.0%)	1-2
bread (wheat)	35 (100.0%)	6-7	32 (91.4%)	5-6
beans	9 (25.7%)	1-2	12 (34.3%)	1-2
orange	28 (80.0%)	4-5	27 (77.1%)	3
lettuce leaves	17 (48.6%)**	2-3	9 (25.7%)	2-3
kiwi fruit	18 (51.4%)	2-3	16 (45.7%)	2
apple	34 (97.1%)	5-6	35 (100.0%)	4-5
spinach	1 (2.9%)*	2	9 (25.7%)	3-4
fig	16 (45.7%)*	1-2	29 (82.9%)	1-2
buckwheat	34 (97.1%)	3-4	33 (94.3%)	2-3
oatmeal	21 (60.0%)	4	22 (62.9%)	2
peeled peas	1 (2.9%)*	1	11 (31.4%)	2
chicken egg	31 (88.6%)	3-4	33 (94.3%)	3-4
veal	19 (54.3%)	1-2	14 (40.0%)	2-3
pork	27 (77.1%)*	2-3	34 (97.1%)	2-3
chicken	35 (100.0%)	3-4	32 (91.4%)	2-3
caviar	4 (11.4%)	1	3 (8.6%)	1
cod liver	0	0	3 (8.6%)	1-2
sardines (canned)	4 (11.4%)	1	7 (20.0%)	1
sprats	2 (5.7%)*	1	10 (28.6%)	1
sea fish	20 (57.1%)	1-2	21 (60.0%)	1-2
beef liver	3 (8.6%)**	1	8 (22.6%)	1
pork liver	7 (20.0%)	1	6 (17.1%)	1-2

*$P \leq 0.01$; **$P \leq 0.05$

All children with T1D underwent a blood test to rule out anemia as the most common manifestation of folic acid deficiency. In this group, no children were identified with anemia and the average value of hemoglobin was (13.1 ± 0.9) gm/dl, erythrocytes – (4.4 ± 0.5) million cells/μl

There are only individual reports that demonstrate the folate status in patients with T1D [9]. Most of those reports confirm normal or elevated serum folate concentrations in T1D patients after additional folic acid intake. Recent studies of folate status in a healthy population of children aged 4–11 years have

shown that the average folate value was 8.6 ± 4.6 ng/ml, and folate deficiency was 4.6% [16]. Among Norwegian preschoolers, 13% had a folate concentration <10 nmol/l, which was interpreted as folate deficiency [17].

The prevalence of folate deficiency in our study was significantly higher than was previously reported, and the average folate levels in this study were lower compared to literature data. However, such results could be affected by the mandatory consumption of products fortified with folic acid by the Norwegian population, which is absent in Ukraine. For example, in the United States and other countries, folic acid fortification of food products has been carried out since 1998 and has reduced the population spread rate of deficiency from 16 to 0.5% [17]. Observations conducted in adults with T1D showed that folic acid intake significantly reduced some markers of glycemic control [18]. Observations in adults with Type 1 and Type 2 diabetes mellitus have shown that folic acid significantly reduces some markers of glycemic control [19, 20], so folic acid deficiency adversely affects the underlying disease, especially in children. At the same time, no children were identified with vitamin B_{12} deficiency. The absence of vitamin B_{12} deficiency is in accordance with the aforementioned study among Norwegian children [17].

Along with the survey on child nutrition, the financial condition of the family was clarified. It was found out that 25 (35.7%) of all surveyed children were from families with low-income. Consequently, an equally important factor remains that, despite some reduction in income-related poverty in recent years, malnutrition remains widespread. Iron, vitamin A, folic acid and zinc deficiencies are common worldwide, especially in children from low-income areas [1]. However, according to national dietary surveys, the consumption of fruits and vegetables in Norwegian children is below dietary recommendations [17]. Low public awareness of the daily needs of folic acid intake and the importance of folic acid for the body also affects the provision of a balanced diet for children, both healthy and sick [21, 22].

In addition, our data suggest that children have a lower folate intake through foods than recommended; although, we did not find an association between average daily intake of folate with food and folate status. An increased consumption of foods rich in folate could be a feasible approach to improve folate status. This also emphasizes the importance of developing healthy dietary patterns in children for the prevention of diseases and complications of diseases later in life.

The current study has some limitations. Little-known biochemical values in children are difficult to interpret due to limited age-specific reference values, which is why the cutoff values for defining deficiencies are not clear.

During the survey, respondents indicated the approximate portion size of the product that they consumed. Thus, the folate alimentary supply questionnaire is not precise enough, as it depends on the accuracy of the respondents' answers.

According to literature data, the fully combined folic acid status should include the determination of serum folate, vitamin B_{12}, methylmalonic acid and homocysteine levels. Red blood cell (RBC) folate levels are a useful index of body stores and can help access the duration of deficiency because serum folate is an indicator of short-term status, while RBC folate is an indicator of long-term status [11]. Using only two biomarkers (serum folate, vitamin B_{12}), as we did in our research, could limit the validity of this indicator.

*Conclusion*s. The frequency of folate deficiency is high in the pediatric population. Each third child aged 6-18 years had folate deficiency, mostly due to an unbalanced diet and insufficient food intake. The frequency of deficiency was higher in healthy children than in children with diabetes due to lack of vitamin supplementation in healthy children. Nutrition does not provide the necessary intake of folate, whichindicates the need for its additional supplementation.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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СПОЖИВАННЯ ФОЛАТІВ ТА ЧАСТОТА ЇХ ДЕФІЦИТУ У ДІТЕЙ З ЦУКРОВИМ ДІАБЕТОМ 1 ТИПУ ТА ЗДОРОВИХ ДІТЕЙ

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Достатнє споживання фолатів має важливе значення для росту дитини. Наразі відсутня інформація про поширеність дефіциту фолатів серед населення України, зокрема дітей. Метою дослідження було оцінити споживання фолатів з їжею та з'ясувати частоту дефіциту фолієвої кислоти у дітей з цукровим діабетом 1 типу (ЦД1) і здорових дітей. Визначення фолатів у сироватці крові проводили методом ІФА. Рівень фолатів <3 нг/мл діагностували як дефіцит фолієвої кислоти. Серед дітей, яких спостерігали, дефіцит фолатів діагностовано у 23 (32,9%): у 6 (17,1%) пацієнтів із ЦД1 та у 17 (48,6%) здорових дітей ($P \leq 0{,}01$). Середній рівень фолатів у сироватці крові у дітей із ЦД1 дорівнював (5,09 ± 2,16) нг/мл і (3,72 ± 1,87) нг/мл у здорових дітей ($P \leq 0{,}01$). Середнє добове споживання фолієвої кислоти з їжею становило (138,68 ± 70,37) мкг, без різниці між групами, і було більш ніж у 2 рази нижче вікових вимог (300 мкг/добу). Проте 15 (48,9%) дітей з ЦД1 повідомляли про отримання 1 курсу вітамінних добавок протягом останніх 6 місяців, тоді як серед здорових дітей таких було 6 (17,1%, $P \leq 0{,}01$. Таким чином, частота дефіциту фолієвої кислоти є високою в педіатричній популяції. Харчування не забезпечує необхідного рівня фолієвої кислоти, що свідчить про необхідність її додаткового надходження.

Ключові слова: фолієва кислота, дефіцит, аліментарна забезпеченість, цукровий діабет, діти.

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