X. ABSTRACT

Introduction. Health is determined by endogenous and exogenous factors. Both the former and the latter exert an impact on the human independently and with diverse intensity. It is currently assumed that nutrition is a major environmental factor affecting human health and that anti-health behaviours can have near and distant consequences. Results of many recent studies seem to confirm that the intrauterine period is the first important time in the human life. The lifestyle of a pregnant woman has a significant impact on the course of pregnancy, labour and the post-partum period, as well as on child's health after birth and in the subsequent years of life.

Folic acid and group B vitamins are particularly important for the normal development of the foetus. Their adequate concentration allows for normal DNA synthesis, erythropoesis and the development of the nervous system. Folic acid is a co-factor of DNA and RNA biosythesis enzymes, takes part in the synthesis of methionine, is actively involved in histidine transformation, regulates growth, functioning and division of cells, ensures normal erythropoesis, has anticarcinogenic properties. It has a role in homocysteine transformations. An appropriate level of this folate prevents neural tube defects and megaloblastic anaemia caused by prolonged maturation of erythrocytes in the bone marrow.

Homocysteine as a sulphuric amino acid is associated with metabolism of vitamins B and takes part in biochemical transformations of methionine and cysteine. The level of homocysteine depends on many factors, such as genetic predisposition, age, sex, nutrition, the use of stimulants, and liver and kidney efficiency. The level of homocysteine increases with folic acid deficiency.

The level of homocysteine in blood plasma is a major marker of folic acid deficiency. Hyperhomocysteineamia is an independent risk factor of atherosclerosis, and the elevated level of homocysteine can be found in patients with diagnosed coronary disease. Its high level may impair physiological processes in the cell and cause a number of serious cardiovascular and neurological diseases, cancers, as well as pregnancy pathology and some congenital defects.

The results of the conducted study indicate that women in the procreative period do not always have a proper diet and they frequently fail to take vitamins according to current recommendations.

Study objective. The aim of the current study was to assess the level of folic acid and homocysteine in the umbilical cord blood of newborns after birth depending on chosen factors. Verification and analysis of environmental factors concerning the nutritional mode of women during pregnancy and vitamin supplementation in the pre- and post-conception period constituted an important part of the study.

The research hypothesis posed in the study was that in the study population of pregnant women the levels of folic acid and homocysteine depend on a number of environmental factors, including first of all dietary habits. The mode of nutrition ignoring current recommendations may increase the risk of an abnormal course of pregnancy and foetal development, which may affect the child's health condition after birth and in the later period.

The following research questions were formulated:

1. What is the level of folic acid and homocysteine in umbilical cord blood serum?
2. Is there any corelation of mother's age, marital status, education, job, place of residence, financial status and dietary behaviour with the levels of folic acid and homocysteine in umbilical cord blood serum?

3. What are the levels of folic acid and homocysteine depending on supplementation (before and during pregnancy)?
4. Is there any correlation of newborn's birthweight and sex with the levels of folic acid and homocysteine?
5. What is the birthweight in correlation with health behaviours of mothers?
6. What were the sources of information pertaining to the recommended mode of nutrition for pregnant women?

Material and methods. The study was conducted between March and August 2014 on newborn babies and their mothers having their babies in the Obstetric-Gynaecological Ward of the Independent Public Centre of Healthcare in Sanok. The study project was approved by the head of the Independent Public Centre of Healthcare in Sanok, head of the Obstetric-Gynaecological Ward and the Bioethics Committee of the Medical University of Lublin (No *KE-0254/66/2014*).

The group of mothers consisted of 88 healthy women aged 18–43 years, having their babies by natural labour. Most study women were married, coming from villages and small towns with the population of 31,000 to 50,000 inhabitants, with secondary or higher education and considering their socioeconomic conditions to be good. The newborn babies were from full-term pregnancy, born in good condition and with normal body weight. All the children from current pregnancies were healthy and without congenital defects.

The inclusion criteria were as follows: obtaining the informed consent of the patient to participate in the study, good health condition, natural labour, participation in all stages of the study, completeness of the obtained data and proper quality of the umbilical cord blood for biochemical analysis. The exclusion criteria included abnormal course of pregnancy and systemic diseases in the mother, lack of informed consent of the mother to participate in the study, patient's withdrawal from at least one stage of the study, cesarean section and haemolysis of the collected umbilical cord blood making laboratory tests impossible.

The umbilical blood (mixed venoarterial) was collected with mother's consent, directly after labour and cord separation, before placenta delivery, from the clamped umbilical cord. Tests were performed in the Scientific Laboratory attached to the 3rd Chair of Paediatrics, Medical University of Lublin. The collected umbilical cord blood was used to determine two parameters: folic acid and homocysteine (Hcy) with the use of commercial sets of reagents.

The original questionnaire was the research tool of the current doctoral thesis. It contained 43 questions, both open and closed. The questionnaire included questions concerning basic anthropometric data (i.e. body weight and height of the mother before pregnancy, body weight of the mother before labour, BMI of the mother before pregnancy, birthweight of the baby) and socioeconomic data (i.e. age, marital status, education, financial status, place of residence, occupational activity). There were also basic questions associated with the past obstetric history and health condition (number of pregnancies and labours, the course of pregnancy, chronic diseases, health condition of children after birth), as well as the current pregnancy and the condition of the newborn (pregnancy course and duration, Apgar score after delivery).

The assessment of dietary knowledge among study pregnant women and their dietary behaviour was performed based on the history questionnaire and referred to preferences in the frequency and type of consumed meals with the respective food products, type and amount of consumed liquids, dietetic changes made, the use of stimulants, diet supplementation with vitamins and minerals in the perinatal period and giving up certain products not recommended in pregnancy. Questions about smoking, i.e. frequency and the number of cigarettes, were also included.

The results were subjected to statistical analysis. Parameters of the analysed values measured in the nominal scale were characterised based on the number (n) and percentage (%). The range of values (Min,. Max.), the arithmetic mean (M), median (Me), standard deviation (SD) and confidence interval (95% CI) were calculated for quantitative features. The Kolgomorov-Smirnov test was used to assess the distribution of the analyzed parameters. Due to the oblique distribution of the measurable parameters studied or because of variance impurity, nonparametric tests were applied to assess the significance of differences between the study groups. The following statistical tests were used: chi^2, Kolgomorov-Smirnov test, t-Student test, U Manna-Whitney test for equality of distributions, and Spearman's correlation coefficient. Values at $p < 0.05$ indicating statistically significant correlations or differences were considered significant (with 5% risk of conclusion validity error). The calculations included the following programs: spreadsheet program EXCEL 2007 (Microsoft) and STATISTICA 10 (StatSoft, Inc.)

Results. The women most frequently modified their diets in the first and second pregnancy, and those who declared modification of their dietary behaviour admitted that their diet underwent both qualitative and quantitative changes (56.8%), only quantitative (14.8%) or only qualitative (9.1%). The mothers-to-be eliminated some food products from their diet, taking into account their own health and the child's well-being (42%), whereas the remaining women (58%) did not. The good thing was that the women did not have their meals in mass nutrition facilities or 'fast food' type restaurants, but in vast majority they prepared their meals at home. The most common culinary technique was cooking, less frequent was baking or frying.

It can be concluded that despite nutrition modifications in the procreation period, the changes introduced were not always beneficial for the mother and foetus. Most pregnant women involved in the study used traditional diet (72.7%), although a large group of women (27.3%) preferred dietary limitations with regard to chosen food components. They used low-fat diet (10.2%), high-protein diet (9.1%), diabetic diet (5.1%) and vegetarian diet (2.3%). No woman consumed vegan diet. The use of these diets resulted either from previous behaviours or from changes in nutrition due to pregnancy. The most common dietary errors among pregnant women involved low intake of cereal products, milk and dairy products, fish as well as snacking between main meals, mainly sweets. Women preferred wheat bread, poultry and pork, often drank tea and strong coffee.

The assessment of the nutritional state of the women showed that the mean body weight before pregnancy was 60.88 ± 10.85 kg, and BMI 22.64 ± 3.75 kg/m^2, which indicates normal values in the majority of the women studied (68.2%). The women with abnormal body weight were more frequently overweight than underweight. The mean weight gain during pregnancy in the study group was 13.32 kg.

The women involved in the study used multivitamin dietary supplements and single-ingredient products. Multivitamin and multi-microelement preparations as well as those containing folic acid, Omega-3 acids and iron were assessed. Most of the study women used vitamin-mineral supplementation in pregnancy (77%), but some did not (23%). The supplementation was usually started from the beginning of pregnancy (22.7%), from month 2 of pregnancy (20.5%), from month 4 (13.6%), from month 3 (10.2%), and in single cases after month 5 of pregnancy.

Dietary supplementation had an effect on the level of folic acid and homocysteine in the serum of the umbilical cord blood. A higher level of folic acid in the umbilical cord blood serum was noted in the newborn babies of mothers taking vitamin preparations during pregnancy ($M = 18.30 \pm 9.49$ ng/ml) as compared to the mothers who did not use the supplementation ($M = 12.44 \pm 10{,}29$ ng/ml, $p < 0.05$). The mean level of homocysteine was higher in the umbilical cord blood serum of the neonates born to mothers not taking vitamin

supplementation in the perinatal period (M = 9.31 ± 3.53 μmol/l) as compared to the mothers using the supplementation (M = 6.97 ± 2.18 μmol/l, $p < 0.01$).

The study revealed many irregularities in the use of preparations containing folic acid and in the time when supplementation was started. Although the majority of the study women admitted taking folic acid preparations (88%), over 50% of them started taking the supplements after conception (55%), and only 33% still before pregnancy. Some mothers did not take folic acid at all (12%). Pregnant women involved in the study usually started the supplementation from month 1, month 2 or month 3 of pregnancy.

The statistical analysis showed that the mean level of folic acid in the umbilical cord blood was 16.97 (± 9.92 ng/ml), and the level of homocysteine 7.50 (± 2.71 μmol/l).

A negative statistically significant correlation was observed between the level of folic acid and the level of homocysteine in the umbilical cord blood serum ($Rs = - 0.3174$, $p < 0.05$). It can be thus concluded that the levels of folic acid and homocysteine were interrelated.

The level of folic acid depended on pregnancy duration, which was confirmed by negative statistically significant correlation between the level of folic acid and pregnancy duration ($Rs = - 0.23$, $p < 0.05$). The mean level of folic acid was higher (21.45 ± 8.58 ng/ml) in the neonates born before week 38 of gestation as compared to its level (16.05 ± 9.98 ng/ml) in pregnancies terminated later than week 38, the difference being statistically significant ($p < 0.05$). Pregnancy duration had no effect on the serum level of homocysteine in the umbilical cord blood.

However, the level of folic acid in the umbilical cord blood serum did not depend on mothers' age, body weight before pregnancy and labour, body mass gain during pregnancy, on which pregnancy it was, body height, BMI of women before pregnancy and birthweight of the newborn.

The mean level of folic acid in the umbilical cord blood serum in female neonates (19.51 ± 9.94 ng/ml) was higher in comparison with the mean level found in male newborn babies (14.65 ± 9.43 ng/ml), the difference being statistically significant ($p < 0.05$). No statistically significant differences were found between the level of homocysteine in the umbilical cord blood serum and the sex of the newborn. In male babies, the level was 7.66 ± 2.86 μmol/l, in female babies 7.33 ± 2.56 μmol/l, and the difference was statistically insignificant.

The level of folic acid in the umbilical cord blood serum was higher in babies whose mothers did not smoke during pregnancy (M = 17.54 ± 9.82 ng/ml) as compared to those born to smoking mothers (M = 10.34 ± 9.25 ng/ml), the difference being statistically significant ($p < 0.05$). In the newborn babies of mothers who did not smoke during pregnancy a positive correlation was found between the week of gestation and child's birthweight ($Rs = 0.3844$, $p < 0.05$) and negative correlations were noted of the level of folic acid in the umbilical cord blood serum with the week of gestation ($Rs = - 0.3476$, $p < 0.05$) and with the level of homocysteine ($Rs = - 0.3240$, $p < 0.05$).

Considering frequent conflicting results of studies assessing the levels of folic acid and homocysteine in the umbilical cord blood serum in neonates depending on various environmental factors, further research conducted on a larger group of patients and assessing other factors seems justified to explicitly state what vitamin and microelement supplementation could ensure proper development of the foetus, infant and adult in the successive years of life.

Conclusions

1. The current study revealed that the diet consumed by the majority of pregnant women underwent both qualitative and quantitative changes. However, despite dietary modifications in the procreative period, the changes did not always comply with the principles of rational nutrition designed for pregnant women.

2. The most common dietary errors among pregnant women included low intake of cereal products, milk and dairy products, and fish, as well as snacking between main meals, particularly sweet consumption. Women preferred wheat bread, poultry and pork, often drank tea and strong coffee.
3. The majority of women used multivitamin supplementation, including folic acid, but they started taking the supplements late, usually in pregnancy, which could result in low vitamin saturation of the mother and foetus.

4. The knowledge among women on the adequate dietary behaviour during pregnancy was most frequently obtained from the Internet, books, magazines and from a gynaecologist/ obstetrician.

5. Higher level of folic acid in the umbilical cord blood was found in female neonates, born to older mothers with shorter pregnancy duration, women having higher education, unemployed, taking vitamin supplementation during pregnancy and not smoking when pregnant.

6. The level of homocysteine was higher in the umbilical cord blood of babies born to mothers who did not take vitamin supplements, younger mothers, and those with primary or vocational education or unemployed.

7. The birthweight did not depend on the level of folic acid and homocysteine in umbilical cord blood serum. Birthweight significantly correlated with pregnancy duration, with the number of pregnancy and with smoking during pregnancy.

8. Rational nutrition and well-balanced diet during pregnancy determines normal development of the foetus, pregnancy and mother's health, as well as the health of children in the subsequent years of life. Therefore, it is necessary to include nutrition in the program of perinatal care and to conduct maternal education.

Key words: nutrition in pregnancy, supplementation, folic acid, homocysteine, umbilical cord blood.