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SURVEILLANCE

Prevention of neural tube defects by folic acid – awareness among women of childbearing age in Slovakia

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Abstract: *Background:* Folic acid deficiency plays a central role in the aetiology of many congenital anomalies including neural tube defects. Protective effect of folic acid on embryo may be acquired only if taken periconceptionally.
Objectives: The aim of the study was to investigate the awareness about folic acid among women of childbearing age in Bratislava, Slovakia.
Methods: There were 130 respondents involved in the research (106 pregnant women, 24 female students of medical faculty). Using questionnaire we acquired following data: pregnancy details, interest in diet before and during pregnancy, recommendations regarding nutrition and supplementation pre- and post-conception, knowledge about folic and other acid in 2004 and 2009.
Results: More than half of the respondents knew the sources of folic acid. The interest in the nutrition facts of the food dropped from 91 % to 58.5 %. The number of pregnant women advised about correct nutrition and folic acid supplementation before and during pregnancy increased from 16 % to 37 %. Planning the next gravidity with folic acid supplementation became greater than 21 % (38 % in 2009). Nevertheless, only 46 % of these women believed that proper food content with folic acid may prevent congenital anomalies. In a group of students planning to take folic acid periconceptionally the number raised up to 62.5 %.
Conclusion: The results revealed low knowledge about the effect of folic acid on developing embryo among women of childbearing age. Effective intervention programs are needed with the aim to improve periconceptional intake of folic acid in 2004 and 2009. The results in both periods show low knowledge about this essential vitamin *(Tab. 1, Fig. 8, Ref. 31)*. Text in PDF *www.elis.sk*.
Key words: neural tube defects, folic acid, prevention.

Congenital malformations are somatic defects present in a child at birth. They occur in 2–3 % of newborns and represent one of the leading causes of neonatal and infant morbidity and mortality. Congenital malformations may effect various organs. The structure development is either stopped, delayed or directed in the wrong way in early embryonic stage. Causes of congenital malformations may be inherited or acquired (as fetal exposure to infections, toxic substances or radiation etc).

Neural tube defects (NTDs) are the second most common birth defect. Current research highlights the important role of folic acid deficiency in the aetiology of neural tube defects and some other congenital malformations.

Definition, occurrence and classification of NTDs

The term neural tube defect applies to malformation of the embryonic brain and/or spinal cord. In Slovakia annually 5 per 10000 live births are affected with congenital malformation of central nervous system. Worldwide it is one child with neural tube defect per 1000 live births (1, 2).

McComb classified neural tube defects to open spinal, closed spinal and cranial defects (3). According to the main defect NTDs could be divided into open and closed, cranial and caudal (4). The major defect of spina bifida is the cleft in vertebral arch. Spina bifida alone is not a neural tube defect but accompanies all open and up to 80 to 90 % of closed NTDs (4).

Open defects represent more serious form of NTDs, where part of neural tube is left uncovered by the skin or protrudes from the spinal column and causes neurodeficiency. In the literature, open defect in the caudal neural tube (lumbal or sacral region) is called *spina bifida aperta*. *Craniorachischisis totalis* is a total defect of neurulation (development of the brain and spinal cord) and is not compatible with life. Patients with *meningo(myelo) cele* include individuals with failure of the posterior neural tube closure. Defect of the spinal cord consist of unfused vertebral arches, pedicles are located laterally and the spinal canal is expanded. In the *myelocele* neural tube is exposed on the surface and has no sac (no covering of meninges) and is situated in the level of the skin. *Meningocele´*s sac is composed of anomalous meninges containing spinal fluid. If the sac contains nerves or spinal cord, it is called *meningomyelocele* (Figs 1 and 2) (5). Most patients with lesions located lower in the spinal cord can

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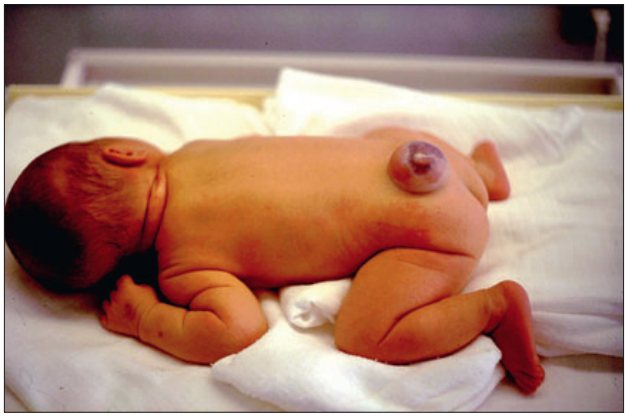

Fig. 1. Newborn with meningomyelocele.

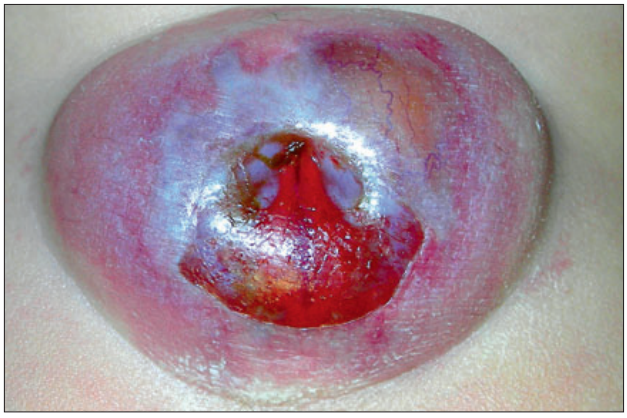

Fig. 2. Open neural plate of meningomyelocele.

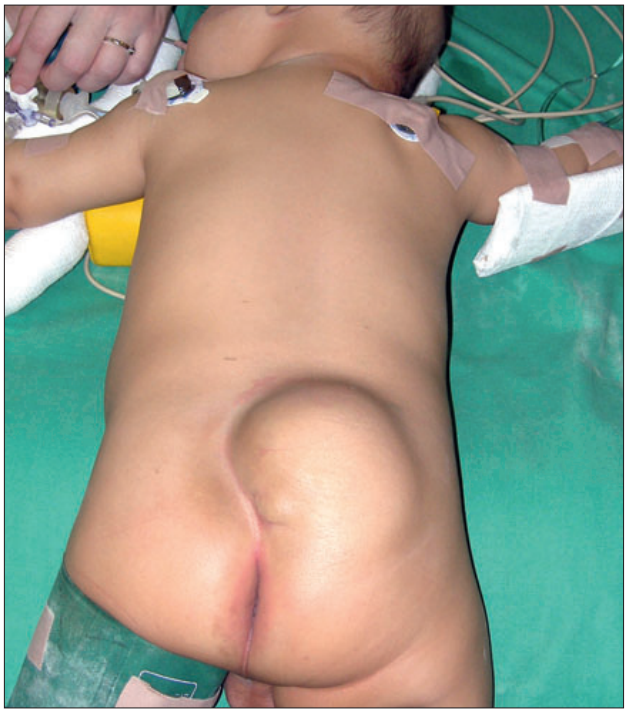

Fig. 3. Child with lipoma.

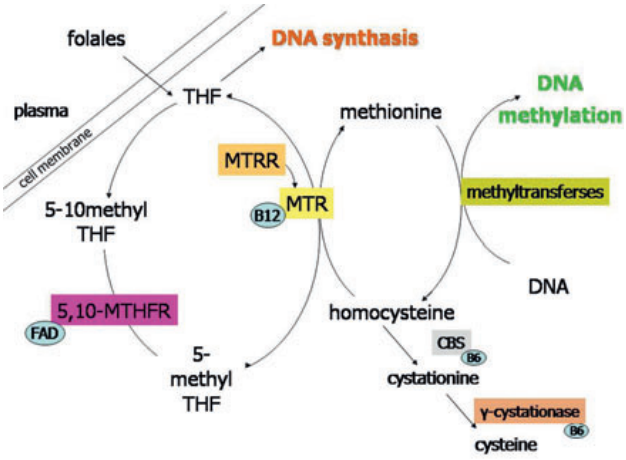

Fig. 4. Folic acid metabolism. THF: tetrahydrofolate; 5,10-methylenTHF: 5,10-methylentetrahydrofolate; 5,10-MTHFR: 5,10-methylenetetrahydrofolate reductase; 5-metylTHF: 5-methyltetrahydrofolate; MTR: methioninsynthase; FAD: flavinadenin dinucleotide; B6: vitamine B6; B12: vitamine B12; MTRR: methioninsynthase reductase, CBS: systationine β-synthase

walk without assistance, while those with a lesion located above are largely immobile (6). Hydrocephalus occurs almost in all patients with open NTDs.

Closed NTDs are covered by the skin of the back, but 80 % of those located in the lumbosacral area present with lesion of the skin in form of accumulation of hair, pigmented naevus, cutaneous dimples or other. Although the neurological deficiency in infants is usually not present, they may have motor or sensory impairment of the lower extremities or abnormalities of the sphincters as well. Closed NTDs are known as *spina bifida occulta* and *spina bifida cystica*. *Spinal lipomas* – lipomatous malformations of the spinal cord are a common cause of cord tethering that can lead to progressive neurological defects (Fig. 3).

Anencephaly is a disorder of cranial closure of the neural tube. Frontal and parietal bones and squamous parts of occipital bone are usually missing, resulting in bare nervous tissue. Most children with this disorder die before or during delivery. Those born alive can survive only for a couple of weeks.

Encephalocele is probably a defect arising after the closure of the cranial neural tube (7). It occurs in the occipital (70 to 80 % of cases), frontal, at least often in the parietal, or temporal region (7).

Embryogenesis

Neurulation (development of the central nervous system) in humans occurs in two phases, a primary and a secondary phase. Primary neurulation refers to folding of an induced neural plate that occurs on the dorsal side of the embryo and results in the formation of the brain and spinal cord. Secondary neurulation refers to sequential processes of canalization and retrogressive differentiation of a massive neural cord, and results in the development of the caudal part of the spinal cord. Thus, neural tube formation is initiated by neural folding and completed by canalization.

Primary neurulation begins during the third week of embryonal life when fetal ectoderm of the shield thickens between pharyngeal membrane and primitive Hensel junction. Neural plate is formed by ectoderm coarsening. Cranial end extends to the sides, caudal end is narrower. Neural plate invaginates along its central axis to form the neural groove, with neural folds on each side. Gradually, the neural folds approach each other in the midline and fuse, converting the neural groove into a neural tube. The fusion begins approximately in the region of the future neck and proceeds in the cranial and caudal directions (beginning of the 4th week of development). Primitive neural tube communicates with the amnionic cavity by way of the cranial (anterior) and caudal (posterior) neuropores. Cranial neuropore closes on the 25th to 26th day, whereas closure of the posterior neuropore occurs about two days later.

As the neural tube separates from the surface ectoderm, the neural crest cells migrate to the sides of the neural tube. The neural crest separates into the right and left part, and migrates to dorsolateral aspects of the neural tube, giving rise to the sensory ganglia of the spinal and cranial nerves. Extended cranial end of the neural tube represents the brain and narrower dorsal part represents basis for spinal cord (8).

Neural tube defects occur when various parts of the neural tube fail to close in embryos 21 to 28 days after conception.

Aetiology of NTDs

Development of the neural tube is a multi-step process controlled by genes and modulated by a number of environmental factors. It involves gene–gene, gene–environment and gene–nutrient interactions. Despite years of intensive research, the exact aetiology of NTDs remains rather complex and poorly understood. It is generally agreed that most NTD cases are of multifactorial origin (9).

NTDs may occur along with congenital heart defects, anus and rectum malformations, omphalocele, tracheoesophageal fistula, diaphragmatic hernia or bladder extrophy. The highest coincidence (up to 25 %) is in patients with anorectal malformation (10, 11) and in children with cloacal extrophy (even up to 100 %) (12). On the other hand concomitance of NTDs with similar anomalies such as orofacial clefts or gastroschisis has not been reported.

Environmental causes of NTDs

Incidence of NTDs is dependent on the geographic areas, socioeconomic status etc. Among the environmental risk factors McLone included: agricultural chemicals, pesticides, cleaning agents and disinfectants, radiation, anaesthetics, nursing, solariums, saunas and fever (increased body temperature), lead, tobacco smoking and obesity > 100 kg (13). Some drugs, especially folic acid antagonists (anticonvulsants, trimethoprim, triamterene and others) significantly increase the risk of NTDs in children of women receiving above mentioned therapy in critical period of development. Folic acid deficiency in diet of women at childbearing age was proven as an independent risk factor for NTDs by large interventional trials.

Genetic causes of NTDs

Neural tube defects exist in isolation in most cases. Genetic causes of NTDs are associated with several point mutations (e.g. cerebrocostomandibular syndrome, Waardenburg syndrome, Fraser syndrome, Meckel-Gruber syndrome) and chromosomal aberrations (trisomy 13 and 18).

Familial occurrence is constantly subject to study. Parents of children born with NTDs are at risk of having subsequent pregnancy complicated by the presence of neural tube defect 10 to 20 times higher. If there is a relative in wider family with the history of NTD, the risk for malformation is 5 to 10 times higher. According to some authors, the parent with NTD does not have higher risk of giving birth to a child with NTD in comparison to the rest of the population.

Swain et al. (1994) focused on congenital malformations in newborns in terms of order of pregnancy. The babies born to mothers of gravidity 4 or more had significantly higher incidence of congenital malformations (40 % of them affecting the CNS) when compared to mothers of lower gravidity (14). It is known that defects in several genes place the embryo at risk of faulty neural tube closure. However, we do not know of any gene that would be independently responsible for NTDs in humans (7, 15).

Experimental models are often used to illustrate the early stages of embryonic development. The aetiology of NTD is studied mostly on mouse models. In the beginning of spinal cord development *Wnt* signaling pathway accounts for dorsalization and posterioration of neural plate, midbrain development and organization of dorzovertebral somites. *Hedgehog* pathway plays similar role. *Hedgehog* induces neural tube plates and notochord and dorsoventral organization of neural tube. Zero mutations of *hedgehog* receptor in mice cause spinal defects and open neural tube defects (17). *HOX* and *PAX* transcription factors take part in neural tube closure. Genes for these proteins were also studied as candidate genes for NTDs in human (9). Neurulation is controlled and driven by convergent extension through the planar cell polarity (PCP) pathway (non-canonical *Wnt/frizzled* pathway) (17). Genes in the PCP pathway include *Frizzled (Fz), Dishevelled (Dsh), Flamingo (Fmi), Prickle (Pk), Diego (Dgo)* and *Strabismus/VanGogh (Stbm/Vang)* (18). Gene mutations for *VANGL 2* in loop-tail mice, which caused craniorachischisis were, also studied for potential role in NTDs in human, however, with various results (19, 20).

Interaction of genotype and environment

Folic acid deficiency is an important factor causing NTDs and other congenital malformations (congenital heart defects, malformations of the urinary tract, limb malformations and others). Folic acid and folates are necessary for producing and maintaining proper DNA structure, DNA methylation, cell division especially in rapidly dividing cells (bone marrow, embryonic cells and other). Folic acid deficiency may cause megaloblastic anemia, vascular disease or cancer, particularly when combined with mutations/polymorphisms in enzymes of folic acid cycle. The incidence of

NTDs depends on presence of mutant alleles in different populations, but also on factors such as lifestyle, intake of micronutrients not only during first trimester of pregnancy, but also before conception. Different genotypes interact together with diversity of eating habits in determining the risk for NTDs in the offspring. Low folate and vitamin B12 level in mothers of children with NTDs and children them self are an independent risk factor for these malformations. The combination of C677T MTHFR polymorphism and low folate status is associated with a greater risk for NTDs than either variable alone (21).

Folic acid deficiency, MTHFR, hyperhomocysteinemia, vitamin B12

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) converts folate to its active form 5-methyltetrahydrofolate, which together with vitamin B12 participates in the conversion of homocysteine to methionine (Fig. 4). Vitamin B 12 represents essential cofactor of MTHFR. Disturbances in methylation cycle (folate cycle and homocysteine cycle) may result in hypomethylation of DNA, chromosomal instability and breaks. The C677T single nucleotide polymorphism (Ala222Val) in the MTHFR gene results in thermolabile variant with 40 % reduction of enzyme activity in heterozygote form (677CT) and 70 % reduction in homozygote form of MTHFR (677TT). The enzyme variants cannot effectively catalyze the conversion of 5,10-methylene tetrahydrofolate (THF) to 5-methylTHF, i.e. the methyl donor for methionine synthase with further decreased level of active tetrahydrofolate in the cells and increased level of homocysteine. Low maternal red blood cell folate has been associated with higher risk of neural tube defect pregnancy (22). Metaanalysis has proven the association of polymorphism C677T MTHFR with higher risk factor for neural tube defects in Europe (23). Second common polymorphism A1298C MTHFR (Glu429Ala) has been associated with NTDs in some populations.

Prevention of NTDs

Quality of life with neural tube defects has improved in last few decades, though complications accompanying everyday are serious, often even life threatening. Large intervention trials conducted in late 1980s and early 1990s have studied occurrence of congenital anomalies after folic acid supplementation periconceptionally (i.e. 2 to 3 months before and until the end of first trimester). The main findings of these trials were 71–91 % reduction of NTDs recurrence (24) and 41–93 % reduction in the first occurrence of NTDs depending on the incidence of NTDs (25, 26). These intervention trials showed the efficacy of multivitamin containing folic acid in the reduction of other structural anomalies as well, mainly congenital heart defects (25–50 %), urinary tract malformations and others.

The aim of the present study was to investigate the knowledge about folic acid among pregnant women in Slovakia, Bratislava. The authors focused on the changes in eating habits before and during pregnancy, periconceptional recommendations and awareness about these recommendations, knowledge about preventive effect of folic acid on birth defects and other diseases in five years period (2004 and 2009).

Methods

Pregnant women were recruited to the study before a routine ultrasound examination at their local obstetrician. The participants were asked to fulfil questionnaire about following data: pregnancy details, expert advice on nutrition and supplementation during pregnancy, preferring certain food during pregnancy, chronic diseases, congenital anomalies in the family, etc. The responders were also asked to report their opinion on possible preventive role of nutrition in the aetiology of some diseases including congenital anomalies, as well as the specific role of folic acid. Women were asked to define the sources of folic acid as well. The questionnaire was taken in 2004 and after five years interval, in 2009.

The study included 106 pregnant women in 2004 and in 2009. In addition answers from 24 female students of medical school were also included in both years, leaving a total of 130 respondents.

Statistical analysis was performed by the Pearson's Chi-square (χ^2) test and Fisher's exact test.

Results

Characteristics of the study population

Age distribution of the study population is provided in Table 1. The majority of women in both years were 21 to 35 years old (76.2 % in 2004 and 76.9 % in 2009) including students.

In the group of pregnant women 70 % of the participants already had at least one child, among these 1 child had a congenital anomaly (in 2004). In 2009 there were 64 % mothers and two were mothers of children born with congenital anomaly. Medical students were not pregnant and did not have any children.

Knowledge about folic acid

The questionnaire taken in 2004 revealed that more than ¾ of the respondents (76 %) heard about folic acid and 66 % also know which foods are rich on this micronutrient. Nevertheless, only 16 % claimed, they were recommended to use some supplements preventively or were aware of the proper food content during pregnancy. Nobody specified what food or which supplement was the responder recommended to use. In the future 17 % of the women plan to use folic acid. Five years later (in 2009) the respondents claimed that they heard about folic acid in 71 %, 57 % also knew where folic acid is found. On the other hand up to 37 % claimed that they were advised to take preventively some medications or eat nutritionally proper food, which represents significant increase in comparison to the group of students ($p<0.012$). In the next gra-

Tab. 1. Age distribution of the respondents (pregnant women/students).

Age (years)	2004 (number)	2009 (number)
≤ 20	6/0	8/0
21−35	75/24	76/24
36−50	20/0	18/0
≥51	5/0	4/0

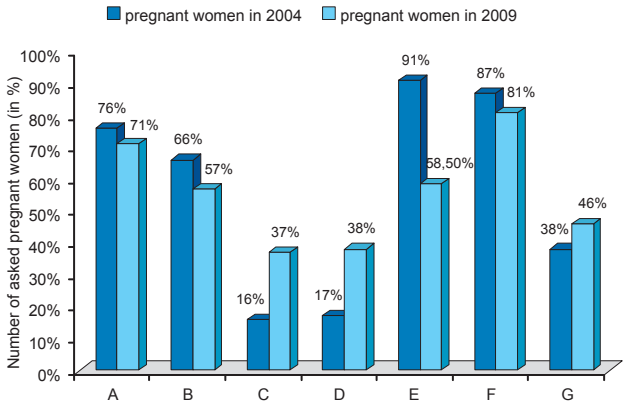

Fig. 5. Knowledge about folic acid - pregnant women results comparison.
A) heard about folic acid; B) know the sources of folic acid; C) were recommended to use supplements or special foods before and during pregnancy; D) plan to use folic acid preventively; E) care about nutrition facts of the foods; F) agree that by proper food content we may prevent some diseases; G) agree that by proper food content we may prevent congenital anomalies.

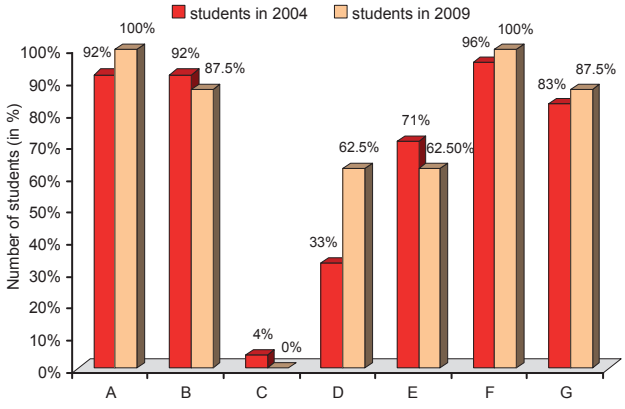

Fig. 6. Knowledge about folic acid – students' results comparison .
A) heard about folic acid; B) know the sources of folic acid; C) were recommended to use supplements or special foods before and during pregnancy; D) plan to use folic acid preventively; E) care about nutrition facts of the foods; F) agree that by proper food content we may prevent some diseases; G) agree that by proper food content we may prevent congenital anomalies.

vidity 38 % of the asked women plan to use folic acid. Ninety one percent were interested in the nutrition facts of the foods and 87 % believed that proper nutrition may prevent some diseases. But only 38 % respondents believe this could prevent congenital anomalies (in 2004). In 2009 the interest about nutrition facts dropped to only 58.5 %, only 81 % of the pregnant women believed the preventive effect of the nutrition. However, 46 % respondents said that nutrition may prevent congenital anomalies (Fig. 5).

Medical students, who are expected to be aware of folic acid, heard about this vitamin in 92 % (in 2004). This percentage also knew which food is rich in this vitamin. 33 % of the students would like to use folic acid periconceptionally. In 2009 more than sixty two percent of the students wanted to use folic acid. In 2004 ninety six percent of the student agreed with the statement about possible preventive effect of folic acid on some diseases, and 83 % claimed that also congenital anomalies are preventable by proper food selection, whereas in 2009 it was up to 87.5 % (Fig. 6).

Risk factors causing congenital anomalies

Among risk factors causing congenital anomalies the pregnant women stated: high maternal age 51 %, higher number of gravidity 5 %, folic acid deficiency 17 %, alcohol 90 %, drugs 92 %, smoking 92 % (in 2004). In 2009 the risk factors causing congenital anomaly included: high maternal age 47 %, number of gravidities 13 %, folic acid deficiency 26.5 %, alcohol 89 %, drugs 98 %, smoking 81 % (Fig. 7). In 2004 all the students claimed the risk factors to be alcohol, drugs and smoking. The fact that folic acid deficiency may cause congenital anomalies 83 % of the students agreed, in 2009 it was 88 %. There was significant increase in the group of pregnant women thinking that with the higher number of gravidity may cause congenital anomaly in the offspring ($p<0.016$) (Fig. 8).

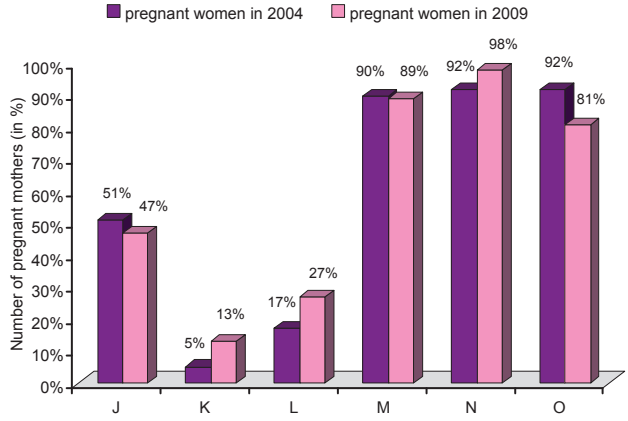

Fig. 7. Risk factors causing congenital anomalies (pregnant women).
J) high mother´s age; K) number of gravidities; L) folic acid deficiency; M) alcohol; N) drugs; O) smoking.

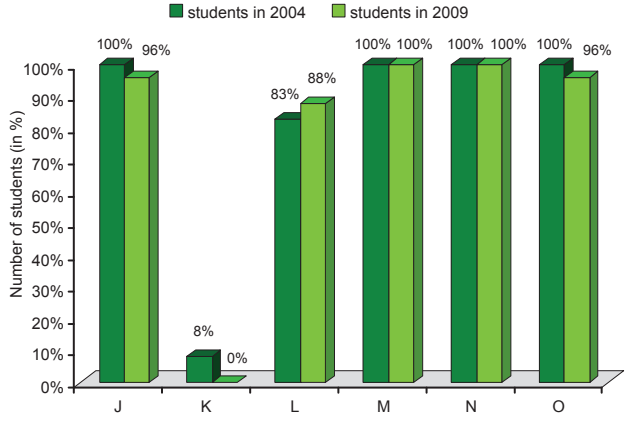

Fig. 8. Risk factors causing congenital anomalies (students).
J) high mother's age; K) number of gravidities; L) folic acid deficiency; M) alcohol; N) drugs; O) smoking.

Discussion

May periconceptional folic acid supplementation prevent neural tube defects? The main factor playing role in answering this question is planning the gravidity. Presented survey shows that only 39 % of the gravidities were planned and more-or-less planned in 31 %. These results proved that more than 70 % of the women could take folic acid preventively before and during the first months of pregnancy, which is comparable with the western European countries (27). As much as 91 % of the respondents were interested in the nutrition facts of the food, nevertheless only 38 % believe that by correct nutrition we may prevent congenital anomalies (in 2004). Five years later (in 2009) it was 58.5 % and 46 %, respectively.

Periconceptional folic acid administration (i.e. 2 to 3 months before and until the end of first trimester) or multivitamin containing folic acid should be advised to all women capable of getting pregnant. The major problem is that only about half of all pregnancies in Slovakia are unplanned. Knowledge of medical staff, common people and especially women of childbearing age about protective effect of folic acid on embryonic development in Slovakia is not sufficient and hence supplementation is not applied in practice. There are four possible ways how to raise folate level in human:

1) *Diet:* natural foods rich in *folates* especially green leafy vegetable, beans, citruses, liver, broccoli etc. The usual daily intake of folates is about 200 mg per day in Slovakia (28) and this consumption is not significantly higher in other developed countries. Higher intake of folates may be seen in vegetarian food which, on the other hand, lacks vitamin B12. Generally, diet rich in folates is important for the prevention of NTDs but cannot alone completely neutralize the genetic predisposition for this congenital anomaly (29).

2) *Supplements* containing *folic acid* (synthetic form of folates) alone or as part of the multivitamins: Experts in the USA recommended that 'all women of childbearing age who are capable of becoming pregnant should consume 4OO micrograms of folic acid per day for the purpose of reducing their risk of having a pregnancy affected by spina bifida or other NTD' in addition to diet rich on natural folates (30). Folic acid supplementation has positive effect on intrauterine embryo development only if started before conception to raise lever of red blood cell folate.

3) *Folic acid must be reduced to the metabolically active form* – tetrahydrofolate. 5-methyl-tetrahydrofolate (5-methyl-THF) is the predominant form of dietary folate found in circulation and is the metabolic form transported into peripheral tissues for cellular metabolism. It is also available commercially as a crystalline form of the calcium salt – *metafolin*. This form may be used either alone or as part of the multivitamins. Recently U.S. food and drug administration (FDA) approved *oral contraception metafolin* for the purpose of reducing the risk of neural tube defect in a pregnancy conceived while taking contraception or shortly after discontinuing (31).

4) *Food fortification*: In 1997 USA and Canada, later Chile initiated flour fortification with folic acid. Their decision was influenced by news that folic acid may reduce not only occurrence of congenital anomalies but also may lower the level of hyperhomocysteinemia and reduce the risk of cardiovascular diseases, cancer and improve cognitive functions in elderly. Another important benefit of flour fortification with folic acid is that fortification enables higher intake of this vitamin in all socioeconomic classes.

For the purpose of reduction of recurrent NTDs it is advised to take 4 mg of folic acid per day periconceptionally. After the 3rd month of pregnancy it is recommended to continue with 400 μg of folic acid per day until the 10th-12th week after delivery. Higher dosage of folic acid (4 mg per day) is suggested in addition to women with epilepsy, insulin dependent diabetes mellitus and women with obesity (BMI over 35 kg/m^2), who are at higher risk of bearing a child with congenital malformation (30).

Conclusion

The prevention of NTD is much better than the medical care. Especially in situation, when the only approach is elective termination of pregnancy after prenatal diagnosis of NTD in foetus. The results of the study revealed low knowledge about effect of folic acid on developing embryo among women of childbearing age. Effective intervention programs are needed with the aim to improve periconceptional intake of folic acid.

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