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REVIEW

Anencephaly in Slovakia and Czech Republic: embryogenesis, risk factors, epidemiology and preventative approaches

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ABSTRACT
Anencephaly, a fatal anomaly of the central nervous system, belongs to the group of defects of the neural tube (NTDs). It is considered the most common congenital NTD, characterized by concurrent absence of a significant portion of the brain and cranial vault. This deformity occurs between days 23 and 26 after fertilization due to improper closure of the neural tube at its cranial end. Many genetic, epigenetic, and non-genetic factors (nutritional, environmental and geographical factors, parental socioeconomic status) contribute to the etiology of this disease. Despite significant advances in treatment and preventive measures, NTDs continue to pose a significant health and financial burden on patients and society as a whole. This study aimed to examine the incidence of anencephaly in Slovakia compared to the Czech Republic between 2012 and 2020. The authors seek to elucidate the reasons behind the higher incidence of this disease in Slovakia as compared to the Czech Republic, explore the male predominance of anencephaly in Slovakia, and investigate whether the prevention standards used in Slovakia differ from those employed in other countries *(Tab. 1, Fig. 2, Ref. 129)*. Text in PDF www.elis.sk
KEY WORDS: neural tube defects, anencephaly, risk factors, folic acid, food fortification

Introduction

Congenital anomalies are defined as the presence of one or more defects in the development of individual organs. They occur during embryonic and fetal phases of development as a result of maternal exposure to environmental factors or genetic mutations. Primary birth defects arise from genetic errors in the developmental process, while secondary malformations occur when environmental factors disrupt the normal intrauterine developmental process. They can be caused by substances such as drugs, alcohol, and smoking or exogenous influences such as temperature or environmental pollutants. These substances have the potential to disrupt the placental barrier and induce changes in fetal development. NTDs are congenital anomalies resulting from abnormal development of the central nervous system (CNS), encompassing a range of malformations such as spina bifida, encephalocele, anencephaly, and many others. Despite the increasing availability of improved diagnostic and therapeutic techniques for the prevention, management, and treatment of congenital malformations, these diseases remain a significant problem in the contemporary world.

Embryogenesis

NTDs stand out as the most common congenital malformations in humans, originating during embryonic development and potentially affert the quality of life from birth. Despite ongoing efforts, the pathogenesis of NTDs remains elusive. Current studies suggest that genetic, epigenetic, and non-genetic factors likely contribute to this anomaly (1). Gaining a thorough understanding of the mechanisms involved in neural tube closure is essential for pinpointing the exact causes of NTDs (2, 3).

Neural tube development is a multistep process regulated by genes and influenced by multiple environmental factors. This process involves an interplay of gene-gene, gene-environment, and gene-nutrient interactions (4). In vertebrate embryos, two types of neuroepithelial cell division occur: apicobasal and planar. The development of cell polarity is crucial in the process of neurulation because it is involved in the closure of the neural tube (5). Neurulation is a mechanical process that takes place during early embryogenesis of the fetus. Its goal is the formation of the neural tube, which is the precursor of the brain and spinal cord (6). Neurulation, as a series of systematic, morphological, and structural changes, begins with the formation of the notochord, which induces

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differentiation of ectoderm to neuroectoderm. The dorsal thickening of embryonal ectoderm gives rise to the formation of the neural plate, a flat and open neural epithelium that deepens into the neural groove. The edges of the neural plate are raised, neural folds are formed at its lateral ends and the neural groove is formed in the midline (7). The two neural folds continue to mutually approach each other until they finally fuse in the midline, firstly in the cervical region, near the 5th somite, then the fusion continues both cranially and caudally until the neural tube is open at two points only. At the cranial end, this place is referred to as *neuroporus anterior*, and at the caudal end as *neuroporus posterior* (8, 9). The fusion of the neural folds leads to the formation of the neural tube, the development of which is completed in the first month of pregnancy. The period between days 23 and 26 of pregnancy is the phase when the closure of the neural tube becomes complete, and its lumen transforms into the neural canal (9, 10). The cephalic and caudal open ends of the neural tube communicate with the amniotic cavity through the cranial and caudal neuropores. The cranial neuropore closes at the 18–20-somite stage (approximately on day 25) and the posterior neuropore closes around two days later, at the 25-somite stage (i.e., on day 27) (11). Failure to close the neural tube at its cranial end in the region of *neuroporus anterior* results in anencephaly, while failure to close the caudal end in the region of *neuroporus posterior* results in spina bifida (12, 13). These conditions may occur due to impaired cell fate, cell adhesion, and neural tube closure mechanisms (9). Anencephaly is always associated with acrania, and the absence of the calvaria leads to degeneration of the brain, whilst the brainstem remains intact (8, 9). Furthermore, abnormal brain structure and vascularization contribute to the destructive process characterized by angiogenesis. At the macroscopic level, the remaining part of the brain has a mushroom-like appearance (9).

Clinical and morphological features

The National Institute of Neurological Disorders and Stroke explains the symptoms of this diagnosis as follows: a child born with anencephaly is usually deaf, blind, does not perceive their surroundings, and is unable to feel pain. Reflex responses such as breathing and responses to sound or touch may occur (14, 15). Due to the presence of the brainstem, children with anencephaly have almost all of the primitive reflexes of a newborn, respond to auditory, vestibular, and painful stimuli, have spontaneous limb movements, and the pupils can react to light (15–17). This means that the child can move, smile, suck, and breathe without the help of devices (18). Since the fetus cannot swallow, a hydramnion is formed (19).

Clinical signs of anencephaly can vary depending on the severity of the disorder and the presence of other birth defects (Fig. 1). Common symptoms of anencephaly include an absence of scalp and skull in the head region, absence of brain tissue, with the brainstem and cerebellum present but not the cerebrum. Other notable features include microcephaly, abnormal facial features such as a flattened nose, low-set ears and eyes, small jaw, abnormal head shape (e.g., pointed head), abnormally shaped eyes, no nose or a small, abnormally shaped nose, abnormalities of the spine (e.g., scoliosis or kyphosis), chest and rib cage abnormalities, rib deformities, and abnormalities of the heart and other organs. In their study, Salari et al (20) claim that an anencephalic child has a frog-like appearance, bulging eyes, and a large tongue.

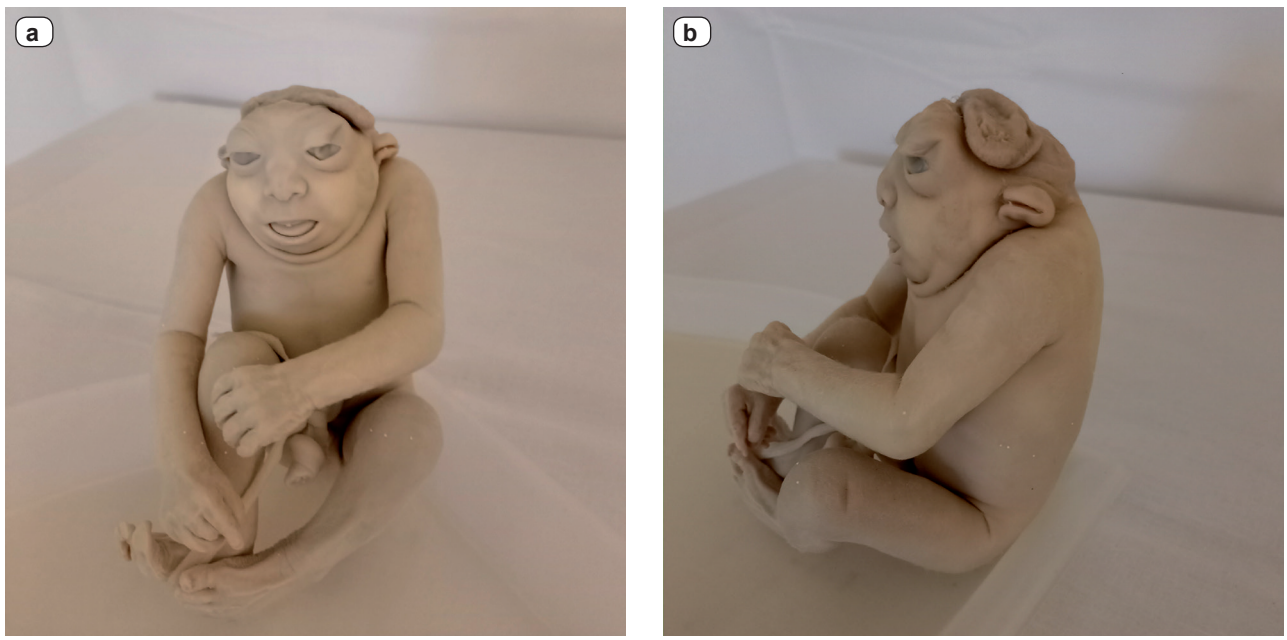

Fig. 1. Anencephalic male fetus. (a) front view (b) side view. Indicators of the anomaly are bulging eyes (described as frog eyes), short necks, and prominent ears. Abnormalities of the trunk and limbs are absent (Archive of the Institute of Anatomy, Faculty of Medicine, Comenius University, Bratislava).

Diagnostics

Prenatal diagnosis comprises methods and procedures for detecting serious congenital developmental defects in the unborn individual early on. A diverse range of presently available prenatal examinations and techniques includes both, the invasive methods such as amniocentesis, placental biopsy, chorionic villus biopsy, fetal blood sampling, and fetoscopy as well as non-invasive methods such as maternal blood sampling and ultrasonography (US).

Ultrasonography

Imaging diagnostics through ultrasound examination is the key method for identifying prenatal abnormalities of the CNS. Anencephaly can be detected by transvaginal ultrasound as early as during the first-trimester screening (typically between weeks 11 and 13 +6 days), as well as during the second and third trimesters (21, 22). If, during the ultrasound examination, an absence of the upper part of the cranial vault is observed along with an absence of brain tissue in the place of the cerebral hemispheres, the first suspicion of anencephaly arises (18, 23–26). In cases of anencephaly, the hemispheres of the brain are replaced by masses of connective and vascular tissue interspersed with neuroglial tissue, and disorganized choroid plexuses located at the base of the skull, referred to as cerebrovascular or angiomatous stroma. The "Mickey Mouse" sign refers to irregular floating masses located above the fetal orbital paths in a coronal view of the head, lacking any cranial structure above them (27). Another important feature of anencephaly detected via US is the presence of fibrous amniotic bands. Sepulveda et al (28) described the "Turkish turban" sign in a study using transvaginal and three-dimensional (3D) US imaging during the first trimester of pregnancy. This feature indicates the formation of an amniotic band around the outer base of the developing calvaria. As previously mentioned, anencephaly can be detected with 100% accuracy using the US during the second trimester. However, this diagnosis cannot be completed during the first trimester due to insufficient development of the corpus callosum and cerebellum, which can only be identified in the second trimester. Anencephaly describes a failure in the closure between the midbrain and the forebrain, while normal fusion is present in the cerebellum, pons, and medulla oblongata (29). The "frog's eye" indication shows the presence of orbital structures associated with abnormal cortical formation (27). Sonographic examination also helps in detecting polyhydramnios, a common feature of anencephaly. Excessive fluid behind the baby's neck and in the amniotic sac may indicate a potential chromosomal disorder or heart defect (30). Anencephaly is associated with abnormalities not only in the CNS but also in other organs and tissues. Alongside anencephaly, malformations such as cervical rachischisis (failure of fusion of the neural arches), cleft palate, heart and lung defects, talipes equinovarus, overlapping fingers, cradle legs, hexadactyly, and other musculoskeletal defects often occur. However, one umbilical artery can be identified as early as week 10 of pregnancy. Ossification can be detected at week 11 of pregnancy, typically affecting the frontal, parietal, and main parts of the occipital bones (31).

Karyotyping

Karyotyping is an older diagnostic method for anencephaly. It is based on staining the chromosomes with a dye and examining the structures of the chromosomes under a microscope. Scientists have discovered a link between anencephaly and the 13th chromosome. In this diagnosis, the genes responsible for the correct development of the brain are deleted and the remaining parts of the chromosome are tied at the ends, forming a circle (18).

Alpha-fetoprotein

The second important diagnostic marker is the determination of alpha-fetoprotein levels in the mother's serum in the second trimester (30, 32). This protein, whose peak levels occur at the end of the first trimester, is produced by the fetal liver and yolk sac. It enters the amniotic fluid through diffusion across the skin of the fetus and during fetal urination. Alpha-fetoprotein enters maternal circulation through diffusion across the placenta, and its high level in the mother's blood can signal the presence of NTDs.

Biopsy of chorionic villi, amniocentesis

Other diagnostic screening methods include chorionic villus biiopsy, which involves examining a small tissue sample from the placenta which is tested for genetic abnormalities. Amniocentesis is performed to determine the levels of fetal allantoic fluid proteins and acetylcholinesterase. Another examination considered when anencephaly is suspected involves measuring homocysteine levels in maternal plasma (30). In the postnatal period, the diagnosis is made through physical examination. All the following criteria are necessary for establishing a positive diagnosis: a large portion of the skull and scalp is absent, the cerebral hemispheres are missing, and the hemorrhagic fibrotic mass of degenerated brain tissue is exposed to the external environment (25). The orbits and eyes of anencephalic fetuses are well-developed. This malformation gives patients the frog-like appearance.

Risk factors

The pathogenesis and etiology of anencephaly have a multifactorial origin, indicating the involvement of several genes in the interaction with various factors such as environmental risk factors and maternal nutritional factors (33).

1) Non-genetic factors

These factors indirectly affect neural tube development by modulating gene functions (34).

A) Nutritional factors

Maternal nutrition plays an important role in the embryonic development of the fetus, which is associated with the observation that most congenital deformities occur predominantly in families with a lower socioeconomic status.

Folic acid (FA), vitamin B12

Low levels of the B-vitamin folate have been reported in fetuses with NTDs, prompting a clinical investigation into FA sup-

plementation. Many studies have confirmed that FA supplementation before conception and during the first trimester of pregnancy can play an important role in the prevention of anencephaly (35, 36). Folate is a coenzyme that participates in the methylation process of homocysteine (Fig. 2). It also supports the synthesis of purines and pyrimidines. Lack of folate leads to the inability to make proteins and deoxyribonucleic acid (DNA) properly and also changes the expression of some genes (35). For these reasons, women of reproductive age are recommended to include a folate supplement in their diet (35, 37). The effective dose is 0.4–0.8 mg per day (38, 39). Some research has suggested that not only folates but also choline and vitamin B12 metabolism is involved in NTDs. It has been shown that reduced vitamin B12 content and increased total choline or homocysteine in the mother's blood are associated with an increased risk of NTDs (40).

Trace elements

Trace elements are microelements that a person needs in small quantities for proper functioning and development. Disruptions in the homeostasis of essential trace elements during pregnancy can lead to congenital developmental malformations (35). Higher levels of manganese in maternal blood during pregnancy increase the risk of NTDs (41). Also, low levels of selenium in the mother's plasma and serum have been implicated in the development of NTDs (42, 43). Alkaline earth metals such as barium, thorium, and cesium have also been linked to NTDs (44–46). The case studies of Abebe et al (47) conducted in northeastern Ethiopia confirmed that alcoholism, smoking, maternal infections during the first trimester of pregnancy, obesity, or caffeine can also act as risk factors contributing to the development of anencephaly.

B) Environmental factors

An environmental cause is any factor unrelated to genes that increases the probability of a birth defect in individuals who have been exposed. These factors comprise issues such as fetal infection, maternal illness, lack of nutrients, consuming drugs, exposure to hazardous chemicals, air pollution, radiation, and other external influences. It is worth noting that various birth defects can be caused by a variety of risk factors.

Maternal hyperthermia

Due to factors such as fever, hot water baths, saunas or whirlpools, the mother's body temperature can rise as high as 40°C during pregnancy, which represents another risk factor for the development of NTDs (34). Many *in vivo* and *in vitro* studies have demonstrated the neural tube's heightened sensitivity to elevated temperature, impacting various developmental processes such as cell differentiation, migration, apoptosis, and proliferation (33). Both teratogenic and mutagenic effects of hyperthermia have been proven through clinical cases and animal studies (48–52). Many studies have confirmed that maternal exposure to hyperthermia during the first trimester can lead to improper closure of the cranial part of the neural tube. Hyperthermia can therefore be correlated with the development of anencephaly (53). In their study, Salih et al (51) proved that the combination of sauna, febrile illnesses, and hot tub usage during pregnancy increases the risk of developing NTDs up to 6 times.

Pesticides

To meet the rising demand for agricultural products, new procedures dependent on the use of pesticides are being introduced. Pesticides are non-biodegradable, and their residues can easily enter the human body through milk and meat of cattle, vegetables, fruits, and water, representing an increased risk for pregnant women. By accumulating in adipose tissue and the placenta, pesticides induce reproductive and developmental disorders with cancerous and teratogenic potentials (54–56). A 2001 study by Muñoz revealed that mothers exposed to pesticides such as methyl parathion during the first trimester of pregnancy were five times more likely to give birth to a child with anencephaly than mothers not exposed to this pesticide (18).

Arsenic (As)

Currently, there has been a notable increase in the worldwide use of As. On the one hand, As has found its application in the metalworking industry, pesticide production or coal combustion. On the other hand, the contamination of the air, water, and soil with inorganic As is on the rise. Among Europeans, the consumption of arsenic-contaminated food accounts for up to 95% of As absorption (57). The teratogenic and toxic effects of As pose the greatest risk for the development of NTDs by promoting the formation of reactive oxygen species while concurrently inhibiting antioxidant enzymes (58–60). As triggers epigenetic alteration and gene mutation, inhibits DNA methylation by reducing the activity of S-adenosylmethionine (61) and disrupts the microvascular structure of the placenta, which leads to impaired transport of nutrients and molecules (59–62).

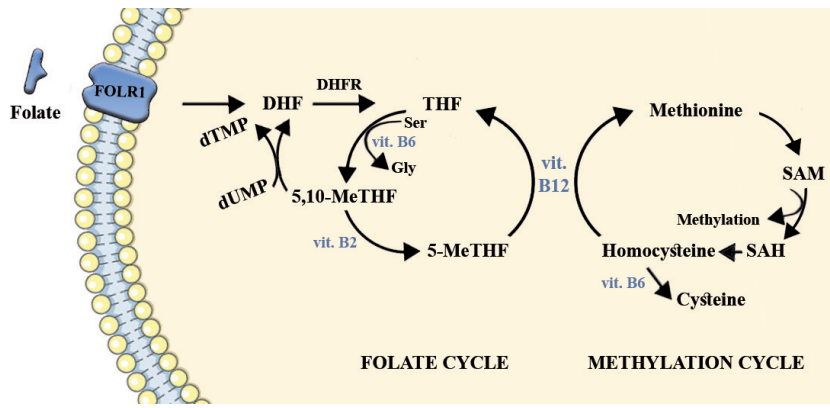

Fig. 2. Folate metabolic pathway. FOLR1 – folate receptor 1; DHF – dihydrofolate; DHFR – dihydrofolate reductase; THF – tetrahydrofolate; 5,10-MeTHF – 5,10-methyleneTHF; dUMP – deoxyuridine monophosphate; dTMP – deoxythymidine monophospate; 5-MeTHF – 5-methyleneTHF; SAM – S-adenosylmethionine; SAH – S-adenosylhomocysteine.

Polycyclic aromatic hydrocarbons (PAH)

PAH are substances that result from anthropogenic activity, particularly from incomplete combustion of wood, oil, coal, and petrol (63). These environmental pollutants have adverse effects on human health, including an increased risk of NTDs. The mechanisms underpinning the association between PAH and NTDs are still not fully understood. It is hypothesized that one possible mechanism could involve reduced global DNA hypomethylation (64).

Antibiotics, anti-seizure drugs

Currently, several groups of drugs in medicine are known to potentially induce the development of defects in the human embryo or fetus, especially when used in the early stages of pregnancy (65, 66). Antibiotics prescribed for the treatment of urinary tract infections have been associated with NTDs (66). The use of sulfonamides, antibiotics given in the treatment of infections caused by gram-positive and gram-negative bacteria, plasmodia, and toxoplasma, have teratogenic effects that can lead to the development of anencephaly (57). Antiepileptics represent another risk group potentially leading to NTDs, with valproate, carbamazepine, and phenytoin altering the absorption of folate, while consequently causing its deficiency in the blood (67).

Infections, metabolic diseases

Several other factors can negatively affect pregnancy, such as various infectious (viral, parasitic) and metabolic diseases (diabetes mellitus, obesity, thyreopathy) that can cause congenital developmental malformations (113).

2) Epigenetic factors

The epigenetic mechanism of gene regulation brings about stable phenotypic changes without changing the DNA nucleotide sequence. Epigenetic regulators play a key role in universal gene regulation. Several studies have shown a correlation between mutations in epigenetic regulators and an increased risk of NTDs (68–70, 117). Changes in DNA methylation, chromatin remodeling, and histone modification can increase the likelihood of NTDs (68, 71). An increased risk of NTDs due to reduced methylation has been reported in many studies to be related to one-carbon folate metabolism (72, 73). Bu et al (74) and Dunwoodie et al (75) claim in their studies that mutations in GCN5 and CITED2 impair acetyltransferase (HAT) activity and thereby increase the risk of developing NTDs. Knockout mice deficient in p300-HAT displayed NTDs, suggesting a vital role of this enzyme in neural tube closure (76). Cheng et al (77) and Vega et al (78) found in their studies that NTDs at the cranial end can be caused by mutations in histone deacetylase (HDAC4 and SIRT1). Mutations in many enzymes modifying chromatin structure may also be associated with NTDs (79, 80).

3) Genetic factors

During embryonic development, neurulation takes place in both mice and humans through two phases, primary and secondary, occurring between embryonic days 8.5 and 10.5 in mice (corresponding to days 22–23 and 26–30 of gestation in humans). The neural tube is an embryonic precursor that develops into the spinal cord and brain through the coordinated action of several signaling pathways, including planar cell polarity (PCP) signaling, sonic hedgehog (Shh) signaling, bone morphogenetic protein (BMP) signaling, inositol metabolism, and retinoid signaling. The process of neural tube closure within a specific time window is regulated by canonical Wnt signaling, fibroblast growth factor (FGF) signaling, tumor growth factor (TGF-β) signaling, notch signaling, receptor tyrosine kinase-like orphan receptor (ROR) signaling, and folate-methionine metabolic signaling pathways. Genes linked to these signaling pathways play a crucial role in epigenetic modifications such as acetylation and methylation, as well as in the organization of chromatin, regulation of the cell cycle, and the actin cytoskeleton (34). NTDs can follow patterns of inheritance with direct evidence of autosomal recessive inheritance (81). As reported by Bonnard et al (82), homozygous inactivation of the NUAK2 kinase leads to anencephaly in humans. Animal models suggest a possible association with TEAD2 transcription factor deficiencies (83). The presence of certain genetic variants that affect folate and FA metabolism can also affect the risk of anencephaly. The C677T polymorphism in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene affects folate synthesis, increasing the risk of spina bifida and anencephaly in the fetus (84, 85). Also, mutations at the level of genes such as McMARCKS, which is active during the development of the neural tube, and the FOXN1 gene, which is important for the proper development of the CNS, can lead to the development of anencephaly. These claims were described in a 1996 study suggesting that a protein encoded by the McMARCKS gene may play a role in closing the neural tube. The McMARCKS protein is stored in the cell membrane, which regulates the binding of actin filaments between cells. Chen et al (18) from Rockefeller University in New York, examined embryos with normal expression patterns of the McMARCKS gene and found that the brain developed normally without signs of anencephaly or other NTDs. They then created mutants that expressed a non-functional McMARCKS protein and found that all embryos were anencephalic. They concluded that McMARCKS plays a major role in neural tube closure. A study by Amorosi et al (18) describes how the authors investigated mutations of the FOXN1 gene. This gene encodes a transcription factor that controls the expression of genes responsible for the development of the CNS. The author and his colleagues concluded that the mutation of the FOXN1 gene led to improper neural tube formation and the development of anencephaly (18).

Prognosis

Anencephaly is a fatal disease (86). Most fetuses with this diagnosis are spontaneously miscarried or are stillborn, while those born alive succumb within hours or days of birth to cardiac and respiratory arrest (15). There is no cure or standard treatment for anencephaly. Owing to prenatal diagnosis, anencephaly is one of the few accepted indications for terminating pregnancy in its third trimester.

Epidemiology

Anencephaly occurs in newborns of any race or ethnic origin. NTDs more often affect the Hispanic population, especially in Latin American countries. Each year, approximately 300,000 children are born with NTDs, resulting in 88,000 deaths and 8.6 million disabilities during their lifetime (87). The frequency of anencephaly varies over time and geographically. For example, the prevalence rate of this condition in northern Iran was recorded at 12 per 10,000 births from 1998 to 2005 (88). In contrast, 2.81 per 10,000 births were reported in Texas, the United States, between 1999 and 2003 (89). The prevalence of anencephaly, as estimated from data collected from EUROCAT member countries during the period 2000–2010, was 3.52 per 10,000 births (90). Bhide et al (91) documented the prevalence of anencephaly in India at 2.1 per 1,000 births through 19 studies. A meta-analysis by Bitew et al (92) reported a prevalence of 63.3 per 10,000 births in Ethiopia. Research has shown that, overall, women are more affected by these diseases compared to men (93). When different ethnic groups are considered, individuals of Mexican descent living in America have significantly higher rates of NTDs and anencephaly. The International Birth Defects Monitoring System reported that in 2002, Mexico had the highest recorded rate of anencephaly in the world. Both maternal and paternal exposures to agricultural pesticides are thought to be a potential contributing factor in the development of this disease (94, 95). From 1998 to 2017, an estimated 95,213 pregnancies with NTDs were reported among 104 million births in 28 EU countries, resulting in a prevalence of 0.92 per 1,000 births (96).

Šípek et al (97) commented on the prevalence of anencephaly in the Czech Republic during the period 1994-2007, which was reported as 0.12 per 10,000 live births. In Slovakia, according to the Institute of Health Information and Statistics, the number of reported NTD cases ranged from 0.52 to 0.59 per 1,000 live births. Nevertheless, it is likely that this figure may be underestimated due to incomplete reporting of abortion cases. The prevalence of live births with NTDs averages 0.28 per 1,000 (98).

Preventative approaches

The optimal approach to reduce the burden caused by NTDs lies in primary prevention, which, given the multifactorial etiology of the disease, includes a range of different measures. Its main principles are as follows:

1. *Support for planned parenthood*

Planned parenthood is a fundamental recommendation in the prevention of NTDs. Studies have shown that young mothers (under 18 years of age) may have an increased risk of developing anencephaly (99-101). Further recommendations largely depend on the factors related to the reproductive stage of the woman.

2. *Protection from harmful factors*

During pregnancy, several potentially harmful factors affect the embryo and later the fetus. Teratogens are various factors that can trigger the development of congenital malformations. Unfortunately, even the common abuse of certain substances, such as tobacco, alcoholic beverages, and drugs, is associated with a whole range of negative effects on intrauterine development. Another group of harmful substances, such as chemicals or high doses of ionizing radiation, can be encountered in a workplace setting (102).

3. *Pharmacotherapy in pregnancy*

The first risk group comprises women with chronic diseases requiring continuous treatment, such as hypertension, diabetes mellitus, or epilepsy. The second risk group involves occasional pharmacotherapy during pregnancy, especially during early pregnancy when a woman may not even be aware that she is pregnant.

4. *Vitamin supplementation*

Although in our circumstances, pregnant women do not typically suffer from a quantitative or qualitative vitamin deficiency, in the area of vitamin supplementation, it is essential to emphasize the beneficial impact of using FA. Numerous studies have demonstrated that this substance decreases the risk of NTDs by up to 72% (4, 103–105, 116). Dietary supplementation with FA is recommended for all pregnant women in the amount of 0.4 mg per day (106) or 4 mg daily after a previously affected pregnancy (107). Although dietary supplementation with FA significantly contributed to the decrease in the incidence of NTDs, it has not reached the point of 100% prevention of these defects because up to one-third of these defects are resistant to the effects of FA (108, 114). Another fact that plays a key role in the prevention of this disease is food fortification (115). Since 1998, mandatory fortification of food with FA has been introduced in the USA and Canada. All North American countries, Australia, and some South American countries have implemented similar rules. In the Czech Republic, fortifying food with FA is voluntary. In these countries, the incidence of NTDs increased significantly after the introduction of fortification (109).

A study by Molloy et al (110) describes a relationship between lower levels of circulating vitamin B12 and an increased risk of developing NTDs. Also, Smithells et al (111) claimed in their study that lower maternal vitamin C levels are associated with the development of NTDs. On the other hand, it is also necessary to mention the potential danger impending from excess of some vitamins, for example, vitamin A, which has proven teratogenic effects (112).

Future perspectives

Studies have demonstrated that while a large number of factors, including genetic, epigenetic, nutritional, and environmental factors, are related to the occurrence of NTDs, the underlying mechanism remains poorly understood, posing significant challenges for targeted prevention and treatment of NTDs (117).

The multifactorial complexity of NTDs implies that analyzing data from such studies will present a major challenge. Moreover, investigators will need to integrate genetic data with information on

epigenetic, environmental, and nutritional factors to obtain a more comprehensive understanding of the causes of individual NTDs (118). Advances in whole-genome sequencing, exome-whole sequencing, and next-generation sequencing may help researchers in beginning to comprehend the genetic and epigenetic basis of NTDs in humans (119, 120). The use of proteomics and other "omics" technology can propel the identification of novel biomarkers and molecular networks implicated in NTDs, which is an indispensable step in the improvement of patient management (121, 122).

As FA plays a vital role in the development of NTDs, folate supplementation during gestation is recommended to reduce the risk of NTDs (123). Many excellent reviews on folate metabolism have appeared over the past decade (124–127). There is an abnormal methylation caused by FA deficiency in NTDs. Cao et al (128) reviewed the research progress on the etiology and mechanism of NTDs induced by methylation modification caused by FA deficiency (128). He and Li anticipated that the field of folate supplementation is poised to evolve from a one-size-fits-all approach to a personalized, precise, and poly-path strategy, crucial for meeting individual needs (129). In the future, the application of bioinformatics tools and explainable artificial intelligence may be applied to understand the molecular mechanisms of NTDs, which may also help in comprehending their relevance in regulating other cellular processes.

Results

The authors analyzed the incidence of anencephaly in Slovakia and the Czech Republic between 2012 and 2020. The data utilized for this analysis were provided by the National Health Information Centre of the Slovak Republic in Bratislava and the Institute for Health Information and Statistics of the Czech Republic in Prague.

In the Czech Republic, one case of anencephaly was diagnosed annually in 2012, 2014, 2016, and 2019, three cases in 2013, and 2018, four cases in 2015, and none in 2017 and 2020. Over the period from 2012 to 2020, a total of 14 cases of anencephaly were diagnosed, involving 7 male and 7 female children. The highest incidence of cases in the Czech Republic was reported to take place in the South Moravian region, the Ústí region, and the Moravian-Silesian region.

In Slovakia, one case of anencephaly was diagnosed annually in 2015, 2016, and 2019, two cases in 2012, 2017, 2018, and 2020, five cases in 2013, with up to 7 cases in 2014. Over the period between 2012 and 2020, a total of 23 cases of anencephaly were diagnosed in Slovakia, involving 15 male and 8 female children. The highest incidence of cases in Slovakia was reported in the Košice region, the Bratislava region and the Prešov region. Table 1 describes the method of pregnancy termination in the aforementioned diagnosed cases of anencephaly.

Tab. 1. Diagnosed cases of anencephaly in children born in the Czech Republic and Slovakia in the years 2012–2020 according to the outcome of the pregnancy (National Centre for Health Information of Slovakia, National Health Information System of the Czech Republic).

	Czech Republic	Slovakia
Stillborn	2	0
Live birth, death in childbirth	4	2
Death within 7 days after birth	4	19
Death later than 7 days after birth	1	0
Live birth, cause of death unknown	3	0
Without information	0	1
Living children	0	1
Total	**14**	**23**

Concluding remarks

Anencephaly is a fatal developmental anomaly characterized by the absence of the cerebral hemispheres and cranial vault. It represents the most prevalent congenital disorder of CNS. This publication aims to analyze the incidence of anencephaly in Slovakia and the Czech Republic over a period from 2012 to 2020.

The data indicate a higher incidence of anencephaly in male births in Slovakia during this period, which is in contrast to the countries of the Western world where anencephaly predominantly affects females at a rate 37 times higher compared to males. Sexual dimorphism is a notable feature in several isolated birth defects, with NTDs exhibiting one of the most significant differences between the sexes.

While various potential causes have been proposed, such as differences in growth and development rates between male and female embryos, higher prenatal mortality in males, epigenetic phenomena, and X chromosome inactivation, the answer to this question remains elusive.

The authors conducted a comparative analysis of diagnosed cases of anencephaly in Slovakia and the Czech Republic. The data revealed that during the investigated period, the incidence in Slovakia was nearly twice as high as that in the Czech Republic. These findings suggest a potential issue with anencephaly prevention in Slovakia.

Currently, Slovak food manufacturers fortify only a selected range of products with folic acid, such as Flora vegetable spreads, Relax juices, Vitalinea yoghurt drinks, and some cereal products. However, the current level of fortification appears insufficient. It is recommended that fortification efforts be intensified to effectively reduce the incidence of anencephaly.

Conflicting perspectives among Slovak millers and lack of crucial information have resulted in flour producers in Slovakia rejecting the proposed amendment to the Food Code for almost three years. This amendment was intended to make it compulsory to fortify all flours with FA. Millers have opted not to support this progressive measure supported by the World Health Organization and the European Union, citing financial burdens as their primary concern. Consequently, their decision has thus left Slovakia without the cheapest, simplest, and most effective protection that can be provided to pregnant women and their future children.

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