Online publication ahead of print, AJMHS Vol 65, 2024

The Current State of Knowledge on the Role of Folic Acid in Neural Tube Defects

Bruno Zappacosta[1*], Malvina Hoxha[2]

[1] Department of Biomedical Sciences, Faculty of Medicine,
Catholic University Our Lady of Good Counsel, Tirana, Albania
[2] Department of the Chemical-Toxicological and Pharmacologic Evaluation of Drugs, Faculty of Pharmacy,
Catholic University Our Lady of Good Counsel, Tirana, Albania

Abstract

Background: Neural tube defects (NTDs) are important human birth defects with a prevalence of about 1/1000 pregnancies; some of them are incompatible with life, such as anencephaly. Spina bifida or meningocele, are responsible for childhood mortality and severe disabilities, without considering the significant effects on families, and on health care systems.

Aim: The aim of this study is to report the current state of knowledge on the role of folic acid in neural tube defects.

Methods: We conducted a review to report all the up-to-date knowledge on the role of folic acid in NTDs prevention.

Results: The etiology of NTDs is complex and multifactorial, however, the relevance of folic acid (FA) in the prevention of many NTDs has been universally accepted. FA supplementation (0.4-0.8 mg/daily) is greatly recommended for child-bearing age women, starting from the periconceptional period, considering that the critical period for the development of the embryonic nervous system is represented by the early weeks of pregnancy. Unfortunately, only a small percentage of women follow these recommendations and, for this reason, several nations have introduced a mandatory grain fortification policy to assure an optimal folate

Address for correspondence: Bruno Zappacosta*, Department of Biomedical Sciences, Faculty of Medicine, Catholic University Our Lady of Good Counsel,Tirana, Albania. Address: Rr. Dritan Hoxha, Tirana, Albania. Email: b.zappacosta@unizkm.al

status and, as a consequence, a significant NTDs prevention.

Conclusion: However, further studies are requested to identify potential health risks associated to higher circulating FA concentrations.

Keywords: Folic acid; Neural tube defects; Pregnancy; Prevention.

INTRODUCTION

Neural tube defects (NTDs), the second most common congenital defects, include congenital malformations of the central nervous system (CNS) occurring during early embryogenesis (1). Some of them, such as anencephaly are incompatible with life after birth. Spina bifida, encephalocele, myelomeningocele, are in any case responsible for childhood mortality, or severe health problems and disabilities, without considering the significant effects on patients and their families, as well as on the health care systems (2).

The prevalence of NTDs is about 0.5-1/1000 pregnancies in western countries (3), but in Northern China, India, and in low- and middle-income countries is 3-4 fold higher (4). The pathogenesis of NTDs is based on the failure of the neural plate closure to form the neural tube, normally occurring in the early weeks of pregnancy (5). The critical gestational periods for anencephaly and spina bifida are the 35th and the 42nd gestational days respectively (6). In addition, depending on the level and on the grade of the defective closure, several clinical pictures are possible. The aim of this review is to report the current state of knowledge on the role of folic acid in neural tube defects.

METHODS

Study Design

We conducted a review to report all the up-to-date knowledge on the role of folic acid in NTDs prevention.

Literature search

We searched in PubMed, Scopus, Medline, Embase databases using the following key words: “Folic acid and neural tube defects”; “Folic acid and NTD”; “Folate and neural tube defects”; “Folate and neural tube closure”; “Folate and preventing neural tube defects”; “Folate metabolism and neural tube defects”, to identify all studies that indicated the association between FA and NTDs. After data extraction we reviewed the titles and the respective abstracts for all records. All authors independently reviewed the full texts to further assess if the selected studies fulfilled the eligibility criteria, verifying the results and removing duplicates. No disagreements were noted between the authors.

Inclusion and exclusion criteria

The eligibility criteria for inclusion were: All original studies (case control, or cohort studies), randomized controlled trials (RCTs), reviews, systematic reviews, opinions, reporting the implication of folic acid in NTDs prevention. The literature search was not restricted by the year of publication. Exclusion criteria comprised studies reporting only NTDs prevention, without mention of folic acid supplementation role in NTDs. We excluded studies published in any other language rather than English.

RESULTS

The etiology of NTDs is complex and multifactorial, likely resulting from the interactions between genetic and environmental factors (7). Genetic studies have focused on

several genes related to folate one carbon metabolism such as methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MS), methylenetetrahydrofolate dehydrogenase (MTHFD1), reduced folate carrier (RFC1). The polymorphism C677T of MTHFR gene, characterized by a thermolability dependent reduced enzyme activity, is usually associated with lower concentrations of folic acid (8,9), and has been associated with higher frequency of NTDs, especially in some populations such as Irish (10). G1958A polymorphism of MTHFD1 gene is also associated with an enhanced risk of NTDs, because of a failure in purine synthesis. Moreover, MTR A2756G (11), and the RFC1 A80G polymorphisms are also considered as risk factors for NTDs.

Among the environmental factors, folate deficiency has been considered as the main risk factor for NTDs(12). Folate supplementation in the periconceptional period, and during the first trimester of pregnancy has in fact proved to reduce the risk of NTDs, as well as of other adverse outcomes of pregnancy, such as health defects, preterm delivery, and fetal growth retardation (13). This effect is attributed to its involvement as a cofactor in nucleic acid synthesis and methylation, through the main methylating product S-adenosylmethionine (SAM), in the one-carbon metabolism. Moreover, following its involvement in one-carbon transfer reactions, folate can influence epigenetics, amino acid homeostasis, and redox defense (14).

Folate, poly-glutamic 5-methyl-tetrahydrofolate (THF), is a water-soluble B vitamin (B9), highly unstable, not synthesizable by animal organism and naturally present in a variety of foods such as leafy green vegetables, fruits, legumes and liver. The predominant form of folate in plasma is 5-methyl-THF. A synthetic form of folate is represented by folic acid (FA) that is more stable and has a bioavailability about 70% higher than that of the natural form (15). FA is normally present in supplements, multivitamin products and fortified foods. Folic acid is reduced to 5,6,7,8—THF and methylated at intestinal level by dihydrofolate reductase (DHFR). The efficacy of folate in one-carbon metabolism reactions, is strictly dependent on its interaction with vitamin B12 and vitamin B6.

Preconception and prenatal maternal lifestyle, inadequate nutrition, diabetes mellitus, obesity, smoking and drugs interfering with folate metabolism are other relevant risk factors for NTDs.

With regard to the complex etiology of NTDs, the epigenetic modifications, both structural and functional usually occurring in the periconceptional period are considered as other potential mediators in the onset of NTDs (16). An increased risk for NTDs could in fact derive from a combined effect of inherited genes with several environmental factors. Inadequate maternal folate status, as well as that of other methyl donors involved in one-carbon metabolism such as choline and methionine, could impair methylation of cytosine in DNA, influencing

gene expression without altering the DNA sequence. DNA methylation is in fact tightly dependent on the availability of methyl donors through SAM, folate and other B vitamin. Although it has been widely proved that FA supplementation can prevent many NTDs through the epigenetic regulation, the exact mechanism is not completely clarified. The same folate dependent altered DNA methylation has been recently reported as a risk factor for other neurodevelopmental disorders such as Autism spectrum disorders (ASDs), likely as a consequence of an impaired neurotransmitters synthesis(17).

A field of great debate among experts is the most efficient FA dose for the prevention of NTDs. Two interesting intervention trials tested two different dosages and formulations of FA; in the first, the Hungarian randomized controlled trial, the women were supplied with a micronutrient combination, a multivitamin containing 0.8 mg FA. The results showed a prevention of 90% of the first occurrence of NTDs (18). The second study that used 0.4 mg FA, showed a reduction of about 79% in the risk of NTDs (19).

The relevance of folate in the etiology of NTDs (20,21) brought to the recommendation of 0.4-0.8 mg FA daily intake to women of childbearing age, whereas 4 mg daily of FA supplementation was indicated to prevent NTDs recurrence (22).The body folate status is usually evaluated through folate measurements in serum samples, reflecting recent intakes of folate, and/or in red blood cells (RBC), which is more reliable because it indicates the long-term status, deriving from the measurement of folate stores in tissues. In order to warrant an optimal protection against NTDs, the optimal folate RBC concentration is 906 nM according to the results obtained in 1995 by Daly et al.(23), versus1500 nM suggested by Crider et al. (24). However, these RBC concentrations might be reached in a very long interval (25). Nevertheless, higher RBC folate concentrations did not seem to have additional benefits (26). Moreover, a large debate has been going on for years about the correct maternal dosage to reach an optimal prevention. As interestingly observed by Chitayat et al., (27), the pharmacokinetic and pharmacodynamic factors can significantly influence the dosage of FA supplementation necessary to obtain an optimal folate status. FA dosage might be different on the basis of personal or clinical conditions, partly confirming the observations of Wald et al., who observed that not always it's possible to reach RBC folate concentration of 906 nM with 0.4 or 0.8 mg of daily folate supplementation (28).

However, other conditions can make it difficult to reach an optimal folate status when it is most needed: in case of unplanned pregnancy (about 50% of pregnancies), maternal folate status may not have been correctly supplemented in the periconceptional period, or women could be less motivated to take folic acid. In the presence of some genetic polymorphism, such as the MTHFR 677C-T variant, an adequate folate intake it's crucial to compensate for an impaired one carbon metabolism (29). The dietary folate intake,

characterized by few vegetables and fruit, might be very low, and the compliance with folate supplementation is often inadequate, or not observed. Interestingly, this aspect seems to be more frequent in rural areas (30) and in low educational level population.

In 1998 the US Government established the mandatory fortification of enriched cereal grain products with 140 mg of FA per 100 g (31), with the aim to reach a more adequate NTDs prevention. This fortification policy has been adopted in subsequent years by several countries. A significant fall in NTDs prevalence was reached between pre-fortification and post-fortification, 10.7/10000 pregnancies versus 7/10000 pregnancies respectively in USA, and 15.8/10000 versus 8.6/10000 in Canada (32).

Some authors consider the extension of the fortification policy to the European countries other than Kosovo and Moldova, as an effective opportunity to dramatically reduce the prevalence of NTDs compared to the minimal FA supplementation (33,34,35,36). In addition, according to Mills et al., the mandatory FA fortification would help in reducing folate deficiency in the general population as observed in US after fortification: the folate deficiency dropped from 3.5% to <1%.

Other authors don't agree on this point, emphasizing the potential risk connected to higher dosages of FA such as worsening of neurological symptoms (37), cognitive decline, and cancer especially in older subjects (38), even if a recent metanalysis by Vollset et al. didn't find an enhanced rate of bowel cancer after FA supplementation (39).

However, the mandatory FA fortification, in addition to a recommended voluntary supplementation with FA, and to a richer diet in vegetables and fruit, especially in case of pregnancy, doesn't exclude a possible higher maternal folate concentration. An interesting debate is growing in regard to the potential risk following the higher FA intake pre or during pregnancy and due to the presence of circulating unmetabolized FA (UMFA), not only for NTDs, but also for other neurological disorders (40). The excess of FA circulating in the plasma, until its removal through the kidneys, results from the low activity of DHFR usually present in humans, which might further inhibit the enzyme DHFR(41).Different authors could speculate that high concentrations of UMFA could lead to a lower 5-MTHF concentration in the cell, an impaired transformation to methionine, and an altered methylation process.

High concentrations of UMFA can derive not only from higher maternal FA intake, but also from an impaired one carbon metabolism, with or without vitamin B12 involvement (42).

Some possible detrimental effects of UMFA have been observed and include reduced cytotoxicity of natural killer cells in animal studies (43), neurological and cognitive disorders (44), and cancer.

UMFA, however, is not usually present in serum when supplementation is not exceeding 400ug/day (45).

DISCUSSION

NTDs represent common congenital anomalies, whose prevalence shows geographical and socio-economic variations, strictly associated with high rate of mortality, or severe disability (46,47).

Currently, the exact causative mechanisms of NTDs are not fully understood, but it's however confirmed their multifactorial origin. Low educational level, socioeconomic factors, unhealthy lifestyle, antifolate therapies, in addition to genetic predisposition can be responsible for an inadequate maternal folate status.

Starting from the preliminary observations of Smithells et al. (20), according to which NTDs could be strictly connected to an undernutrition status, many studies have reported for decades the importance of folate supplementation in NTDs prevention(16,22,48).

The beneficial effects that maternal supplementation with FA plays in the prevention of NTDs in the periconceptional period is likely depending on the control of epigenetic modifications and on the regulation of cell proliferation (25), considering the relevant role that folate plays in the biochemical pathways of the one carbon metabolism to provide methyl groups for methylation.

Although the incidence of these birth defects is significantly decreased in the past decades as a consequence of a better nutrition and ultrasound screening (49), in low- and middle-income countries it still remains high.

The prenatal diagnosis of NTDs through ultrasound screening, often leading to an elective termination of pregnancy, has significantly contributed towards an essential reduction of the prevalence of these birth defects, but probably, the ethical and psychological consequences of this dramatic resolution, are not always deeply evaluated.

However, notwithstanding the frequent health policies campaigns to recommend voluntary periconceptional supplementation with FA (0.4 mg), or FA (0.8 mg)daily (50,51), the rate of women of childbearing age adhering to this recommendation is still too low. On the other side, conflicting opinions about the beneficial and detrimental effects of the mandatory FA fortification of grain products hinder its universal diffusion (52,53).

In some countries it has been suggested a voluntary FA supplementation in addition to the consumption of natural folate richer diet, but these recommendations didn't prove to be able to reduce NTDs prevalence, leaving a significant part of childbearing age women unprotected, as shown by low serum and RBC folate concentrations(54, 55). In addition, the double fortification of salt with folic acid and iodine is a new pathway to guarantee total population coverage (56).

Of note, the main strength of this paper is that the main findings reported in the results section are based in a very large number of studies carried out in statistically significant study samples, and in different nations. Despite the language

"criteria" limitation of this study, which included studies published only in English, the study findings are not influenced considering the very high number of articles analyzed.

CONCLUSION

FA supplementation, might not always be effective in preventing NTDs, probably because of a FA resistance in some cases (57,58). The multifactorial origin of NTDs could require a more complex preventive approach. In addition, low circulating levels of vitamin B12 have been associated with an enhanced risk of NTDs, hence an integrated supplementation of FA and vitamin B12 could be beneficial to prevent a significant number of NTDs (59). However, a recent study reported that folic acid does not have a masking effect in vitamin B12 deficiency anemia (60).

Despite mandatory flour fortification remains the most efficient solution to assure prevention for NTDs to a large number of childbearing age women, especially to those in critical socio-economic conditions, further studies are necessary to clarify the potential detrimental effects of a generalized supplementation with FA.

Acknowledgements: None declared.

Conflict of Interest Statement: The author declares that have no conflict of interest.

REFERENCES

1. Greene NDE, CoppAJ.Neural tube defects. Annu Rev Neurosci 2014; 37: 221-42.

2. Botto LD, Moore CA, Khoury MJ, Erickson JD.Neural-tube defects. N Engl J Med 1999; 341(20): 1509-19.

3. Mitchell LE.Epidemiology of neural tube defects. Am J Med Genet Semin Med Genet 2005; 135C(1):88-94.

4. Naggan L, MacMahon B. Ethnic differences in the prevalence of anencephaly and spina bifida in Boston, Massachusetts.NEngl J Med 1967; 277(21): 1119-23.

5. Practice Bulletin No 187: Neural tube defects. Obstet Gynecol 2017; 130: e279-e290.

6. Czeizel AE, Dudás I, Paput L, Bánhidy F. Prevention of neural-tube defects with periconceptional folic acid, methylfolate, or multivitamins? Ann Nutr Metab 2011;58(4):263-71.

7. Copp AJ, Stanier P, Greene ND. Neural tube defects: recent advances, unsolved questions, and controversies. Lancet Neurol. 2013;12(8):799-810.

8. Crider KS, Zhu JH, Hao L, Yang QH, Yang TP et al. T genotype is associated with folate and homocysteine concentrations in a large, population-based, double-blind trial of folic acid supplementation. Am J Clin Nutr 2011;93(6):1365-72.

9. Nauman N, Jalali S, Shami S, Rafiq S, Große G, Hilger AC et al. Low maternal folate concentrations and maternal MTHFR C677T polymorphism are associated with an increased

risk for neural tube defects in offspring: a case-control study among Pakistani case and control mothers. Asia Pac J ClinNutr 2018;27(1):253-60.

10. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: aHuGE review. Am J Epidemiol 2000;151(9):862-77.

11. Wang Y, Liu Y, Ji W, Qin H, Wu H, Xu D et al. Analysis of MTR and MTRR polymorphisms for neural tube defects risk association. Medicine (Baltimore) 2015; 94(35): e1367.

12. Wolff T, Takacs Witkop C, Miller T, Syed SB, U.S. Preventive Services Task Force. Folic acid supplementation for the prevention of neural tube defects: an update of the evidence for the U.S. Preventive Service Task Force. Ann Intern Med 2009; 150(9): 632-9.

13. Tamura T, Picciano MF, McGuire MK. Folate in pregnancy and lactation. In: Bailey LB, editor. Folate in health and disease, 2nd ed. Boca Raton (FL): CRC Press, Taylor and Francis Group; 2010; 111–31.

14. Ducker GS, Rabinowitz JD. One-carbon metabolism in health and disease. Cell metabolism 2017;25(1):27-42.

15. De-Regil LM, Pena-Rosas JP, Fernandez-Gaxiola AC, Rayco-Solon P. Effects and safety of periconceptional oral folate supplementation for preventing birth defects. Cochrane Database Syst Rev 2015;14(12):CD007950.

16. Liu HY, Liu SM, Zhang YZ. Maternal folic acid supplementation mediates offspring health via DNA methylation. Reprod Sci 2020; 27(4):963-76.

17. Modabbernia A, Velthorst E, Reichenberg A. Environmental risk factors for autism: an evidence-based review of systematic reviews and meta-analyses. Mol Autism 2017; 8(1): 13-29.

18. CzeizelAE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 1992; 327(26): 1832-5.

19. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H et al. Prevention of neural-tube defects with folic acid in China. China-U.S. collaborative project for neural tube defect prevention. N Engl J Med 1999; 341(20):1485-90.

20. Smithells RW, Sheppard S, Wild J, Schorah CJ. Prevention of neural tube recurrences in Yorkshire: final report. Lancet 1989; 2(8661):498-9.

21. Bower C, Stanley FJ. Periconceptional vitamin supplementation and neural tube defects; evidence from a case-control study in Western Australia and a review of recent publications. J Epidemiol Community Health 1992; 46(2): 157-61.

22. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet 1991; 338(8760):131-7.

23. Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM. Folate levels and neural tube defects. Implications for prevention. JAMA 1995; 274(21):1698-1702.

24. Crider KS, Devine O, Hao L, Dowling NF, Li S, Molloy AM et al. Population red blood cell folate concentrations for prevention of neural

tube defects: Bayesian model. BMJ 2014; 349: g4554.

25. van Gool JD, Hirche H, Lax H, De Schaepdrijver. Folic acid and primary prevention of neural tube defects: a review.Reprod Toxicol 2018; 80:73-84.

26. Clarke R, Bennett D. Folate and prevention of neural tube defects. BMJ 2014; 349: g4810.

27. Chitayat D, Matsui D, Amitai Y, Kennedy D, Vohra S, Rieder M et al. Folic acid supplementation for pregnant women and those planning pregnancy: 2015 update. J Clin Pharmacol 2016; 56(2):170-5.

28. Wald NJ, Law MR, Morris JK, Wald DS. Quantifying the effect of folic acid. Lancet 2001; 358(9298):2069-73.

29. McNulty H, Ward M, Hoey L, Hughes CF, Pentieva K. Addressing optimal folate and related B-vitamin status through the lifecycle: health impacts and challenges. Proc Nutr Soc 2019; 78(3):449-62.

30. Rosenthal J, Reeve ME, Ramirez N, Crider KS, Sniezek J, Vellozzi C et al.Red blood cell folate insufficiency among nonpregnant women of childbearing age in Guatemala 2009 to 2010: prevalence and predicted neural tube defects risk. Birth Def Res AClin Mol Teratol 2016; 106(7):587-95.

31. Food US and Administration Drug. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. Final rule. 21 CFR Parts 136, 137 and 139. Fed Regist 1996;61:8781-9.

32. Williams J, Mai CT, Mulinare J, Isenburg J, Flood TJ, Ethen M et al. Updates estimates of neural tube defects prevented by mandatory folic acid fortification-United States, 1995-2011. MMWR Morb Mortal Wkly Rep 2015;64(1):1-5.

33. Bestwick JP, Huttly WJ, Morris JK, Wald NJ. Prevention of neural tube defects: a cross-sectional study of the uptake of folic acid supplementation in nearly half a million women.PloS One 2014;9(2):e89354.

34. Mills JL, Dimopoulos A. Folic acid fortification for Europe? BMJ 2015;351:h6198.

35. Mills JL, Dimopoulos A. Authors' reply to Smith and colleagues. BMJ 2016; 352:i746.

36. Kancherla V, Botto LD, Rowe LA, Shlobin NA, Caceres A, Arynchyna-Smith A, Zimmerman K, Blount J, Kibruyisfaw Z, Ghotme KA, Karmarkar S, Fieggen G, Roozen S, Oakley GP Jr, Rosseau G, Berry RJ. Preventing birth defects, saving lives, and promoting health equity: an urgent call to action for universal mandatory food fortification with folic acid. Lancet Glob Health 2022;10(7):e1053-e1057. doi: 10.1016/S2214-109X(22)00213-3.

37. Institute of Medicine Food and Nutrition Board. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline. Washington, DC: National Academies Press; 1998.

38. Smith AD, Refsum H, Selhub J, Rosenberg IH. Decision on folic acid fortification in Europe must consider both risks and benefits. BMJ 2016; 352:i734.

39. Vollset SE, Clarke R, Lewington S, Ebbing M, Halsey J, Lonn E et al. Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomized trials: meta-analyses of data on 50,000 individuals.Lancet 2013;381(9871):1029-36.
40. Choi JH, Yates Z, Veysey M, Heo YR, Lucock M. Contemporary issues surrounding folic Acid fortification initiatives. Prev Nutr Food Sci 2014;19(4):247-60.
41. Kao TT, Wang KC, Chang WN, Lin CY, Chen BH, Wu HL et al. Characterization and comparative studies of zebrafish and human recombinant dihydrofolate reductases—inhibition by folic acid and polyphenols. Drug Metab Dispos 2008; 36(3):508-16.
42. Egorova O, Myte R, Schneede J, Hagglof B, Bolte S, Domellof E et al. Maternal blood folate status during early pregnancy and occurrence of autism spectrum disorder in offspring: a study of 62 serum biomarkers.Mol Autism 2020; 11(1):7-22.
43. Sawaengrsi H, Wang J, Reginaldo C, Steluti J, Wu D, Meydani SN et al. High folic acid intake reduces natural killer cell cytotoxicity in aged mice. J Nutr Biochem 2016; 30:102-7.
44. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Circulating unmetabolized folic acid and 5- methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors. Am J Clin Nutr 2010; 91(6):1733-44.
45. Kelly P, McPartlin J, Weir DG, Scott JM. Unmetabolized folic acid in serum: acute studies in subjects consuming fortified food and supplements. Am J ClinNutr 1997; 65(6):1790-5.
46. Ferembach D. Frequency of spina bifida occulta in prehistoric human skeletons. Nature 1963;199:100-1.
47. Kuttner RE. Prehistoric spina bifida occulta. JAMA 1978;240(24):2631.
48. Oakley GP Jr. The scientific basis for eliminating folic acid-preventable spina bifida: a modern miracle from epidemiology. Ann Epidemiol 2009;19(4):226-30.
49. Evans MI, Llurba E, Landsberger EJ, O'Brien JE, Harrison HH. Impact of folic acid fortification in the United States: markedly diminished high maternal serum alpha-fetoprotein values. Obstet Gynecol 2004;103(3):474-9.
50. Mitchell LE, Adzick NS, Melchionne J, Pasquariello PS, Sutton LN, Whitehead AS. Spina bifida. Lancet 2004;364(9448):1885-95.
51. Hoxha B, Hoxha M, Domi E, Gervasoni J, Persichilli S, Malaj V, Zappacosta B. Folic Acid and Autism: A Systematic Review of the Current State of Knowledge. Cells 2021; 3;10(8):1976.
52. Ismail S, Eljazzar S, Ganji V. Intended and Unintended Benefits of Folic Acid Fortification—A Narrative Review. Foods 2023, 12, 1612.
53. Petch S, McAuliffe F, O'Reilly Sh, Murphy C, Smith SC, Campos DA, Khattak H, Mahmood T. Folic acid fortification of flour to prevent neural tube defects in Europe – A position statement by the European Board and college of obstetrics and gynaecology (EBCOG).European Journal of

Obstetrics & Gynecology and Reproductive Biology 2022; 279: 109-111.

54. Khoshnood B, Loane M, de Walle H, Arriola L, Addor MC, Barisic I et al. Long term trends in prevalence of neural tube defects in Europe: population based study. BMJ 2015;351:h5949.

55. Zappacosta B, Persichilli S, Iacoviello L, Di Castelnuovo A, Graziano M et al. Folate, vitamin B12 and homocysteine status in an Italian blood donor population. Nutr Metab Cardiovasc Dis 2013;23(5):473-80.

56. Kancherla V, Tsang B, Wagh K, Dixon M, Oakley GP Jr. Modeling shows high potential of folic acid-fortified salt to accelerate global prevention of major neural tube defects. Birth Defects Res 2020; 112: 1461–74.

57. Blencowe H, Cousens S, Modell B, Lawn J. Folic acid to reduce mortality from neural tube disorders. Int J Epidemiol 2010;39 (1):110-21.

58. Wald NJ. Folic acid and neural tube defects: Discovery, debate and the need for policy change. J Med Screen 2022;29(3):138-146. doi: 10.1177/09691413221102321. Epub 2022 Jun 23. Erratum in: J Med Screen. 2022 Sep;29(3):147.

59. Molloy AM, Kirke PN, Troendle JF, Burke H, Sutton M, Brody LC et al. Maternal vitamin B12 status and risk of neural tube defects in a population with high neural tube defect prevalence and no folic acid fortification. Pediatrics 2009;123(3):917-23.

60. Berry RJ. Lack of historical evidence to support folic acid exacerbation of the neuropathy caused by vitamin B12 deficiency. Am J Clin Nutr 2019; 110: 554–61.