National Journal of Advanced Research
www.allnationaljournal.com
ISSN: 2455-216X
Received: 20-05-2022, Accepted: 05-06-2022, Published: 21-06-2022
Volume 8, Issue 1, 2022, Page No. 33-38

Role of folic acid in neural tube defects: A review

Rajni Goyal
Department of Home Science, Institute of Integrated and Honors Studies, Kurukshetra University Kurukshetra, Haryana, India

Abstract
Neural tube defects are birth defects of brain and spinal cord. Supplementation of folic acid helps to reduce the risk of neural tube defects(NTDs).The present review was written to analyze the role of folic acid in the etiology of neural tube defects (NTD's),epidemiological aspects related to NTD's and desired levels of folate which prevents NTD's. This problem occurs in first month of pregnancy and generally women are unaware about their pregnancy at that time. To overcome this problem there is a need to stimulate people especially women to consume foods naturally rich in folate. This review is valuable for adolescent girls, women of child bearing age group and pregnant women.

Keywords: folic acid, neural tube defects, brain and spinal cord

Introduction

Folic acid is also called Pteroyl mono Glutamic Acid (PGA) and is commercially available form of the vitamin. The term folate and folic acid are often used interchangeably. Folate is a generic term, which exhibits the biological activity of the parent molecule folic acid. Folic acid participates in synthesis of co-enzyme called tetra hydro folic acid and is required for certain enzyme reactions which catalyze the transfer of one carbon group. It consists of a pteridine nucleus linked through a methylene bridge to a p-aminobenzoic acid and L-glutamic acid residue. It acts as a co-enzyme in several single carbon transfer reactions, which leads to synthesis of components of DNA, RNA and proteins. In food, folate is mainly present as reduced tetrahydro-folate polyglutamate. Folate co-enzyme function as acceptor and donor of one carbon unit (C_1) in number of essential reactions eg. nucleotide and amino acid metabolism. These carbon units are carried by folate only when the folic acid vitamin is present in fully reduced form *i.e*. tetrahydrofolate form (Shane, 1995) [40].

Folic acid refers to the most oxidized, stable and easily absorbable synthetic form of pteroylmonoglutamic acid, which is present in supplements and does not occur naturally in significant qualities. Although it is readily converted in vivo to natural forms Mc Nulty, 1997) [28]. Folic acid is known under different names. L. Wills (1931) [48] proposed that macrocytic anemia in pregnant women in India responded to treatment with either crude liver or yeast extract. Later various workers suggested and described the structure of folic acid (Vitamin M, Vitamin Bc', factor U, norite elute factor).

Folic acid is used to treat the megaloblastic anemia which generally arises during pregnancy or infancy. It is never used to correct the macrocytic anaemia due to vitamin B_{12} deficiency (Herbert, 1987). Megaloblastic anemia is characterized by large, malformed and multisegmented blood cells. Folate deficiency is thought to cause this condition. It prevents proper cell division due to lack of nucleic acid for DNA synthesis during cell division. Since folate is required for nucleic acid synthesis and nucleic acids are required for synthesis of DNA, treatment with folic helps to overcome this condition by allowing the synthesis of nucleic acid.

However, during the last few years, new functions of folate have been discovered. The positive relationship between maternal folate intake during pregnancy and birth weight of infants has been well documented by various studies. Hibbard and Smithells (1965) were the first who postulated that folate is involved in the closure of the neural tube. There are now solid evidences that supplementation of folic acid during the periconceptional period significantly reduces the risk of giving birth to a child with neural tube defects (NTD's)(Czeizel & Dudas, 1992) [6]. The Centers for Disease Control and Prevention estimated that the average annual prevelance of the 2 most common kinds of NTDs, anencephaly and spina bifida, was 6.5 per 10000 live births for the period from 2009 to 2011(Willams *et.al.*, 2015)

It is one of the most congenital abnormalities in the United States, which affects approximately one in 1,000 infants at birth (Elwood, 1972) [11]. An estimated 4000 pregnancies are affected with NTD each year. More than 1/3rd of these pregnancies are electively terminated or spontaneously lost, thus about 2500 infants per year are born with an NTD.

There is strong relationship between maternal folic acid supplementation and about 85% decline in the neural tube defects (Berry & Erickson 1999) [2]. The potential association between folate and neural tube defects is the focus of this review and is subject of intense research.

In response to the findings of several studies establishing the relation between folate intake and incidence of neural tube defects, there is broad international agreement on increasing the amount of folate especially in the diet of women of childbearing age (Scott et. al., 1995) [37]. There are three ways by which folate intake can be increased: by educating women to choose folate-rich foods such as legumes, green vegetables, fruits and fortified foods; with folic acid supplementation in the form of a tablet and lastly by fortification of staple foods.

Development tube or neural tube

Brain and spinal cord are parts of Central Nervous System. Brain is short and wide, situated in the head and spinal cord is long and narrow, situated in the neck and trunk. Brain and spinal cord contain special ectodermal cells interspersed among the neurons. Brain and spinal cord develop in the embryo from the dorsal tract of ectoderm called the neural plate. The edges of the neural plate grow towards one another as neural folds, which finally fuse in the mid-dorsal line, rolling the neural plate into neural tube. The neural tube is wider at anterior end and consequently the neural tube is dilated in this region from the very beginning. This dilated part of the neural tube gives rise to the brain and its remaining narrow part changes into the spinal cord.

Biologic mechanisms of neural tube defects

Neural tube defects are major malformations in which there is a failure of the developing tube to close properly during the 4^{th} week of embryonic life. If the head end of the tube fails to close, the cerebral hemispheres and cranial vault do not develop. This defect is called ancephely. In this condition, there is little or no brain development and this condition is incompatible with life. Affected babies die in utero or shortly after birth.

If the tail end of the neural tube is not completed normally, the lower part of the neural plate remains exposed. Lower back vertebrates fuse and incomplete closure of spinal cord results in defect known as spina bifida. This defect results in paralysis and loss of sensation of the lower half of the body.

Folic acid has received major attention as an etiologic factor for these malformations.

The exact mechanism by which folate exerts its protection from NTD is not known. There are, however, some possible biochemical explanations:

a. The folic acid co-enzyme participates in reactions which lead to the synthesis of purines, thymine and methylated pyrimidine of DNA and DNA is essential for the synthesis of proteins. This emphasizes the fundamental role of folic acid in growth and reproduction (Lindenbaum & Allen, 1995) [27]. Purines and pyrimidines are needed for synthesis of nucleic acid and are vital to all cell nuclei. A deficiency of folic acid at early stage of pregnancy leads to faculty cell division and it could be detrimental to neural tissue as exhibited by neural tube defects. This can also involve delay in replication link progression, uracil misincorporation to DNA and promotion of genomic instability (Rampersaud *et al.*, 2000) [31].
b. Folate acts as substrate donor in the remethylation of homocysteine into methionine. A lack of folate will increase the level of homocysteine and decrease methionine level. This might disturbs the development of neural tube (Steegers Theunissen *et. al.*, 1991) [42]. Additionally, homocysteine associated vascular impairment may lead to placental hypoxia or ischemia (Myatt & Miodovnik, 1999) [30]. Elevated level of homocysteine or its metabolite homocysteic acid may cause direct neurotoxicity. A molecular basis of teratogenic effect of homocysteine has not been demonstrated clearly. However, studies support that homocysteine binds with N-methyl -D- aspartate subtype of the glutamate receptor which is a calcium conducting class of excitatory amino acid receptors involved in development and migration of neural tube. This may lead to abnormal fetal development (Rosenquist *et al.*, 1996) [34]. Embryotoxicity of homocysteine may be attributed to high concentration of S-adenosyl homocysteine (SAH) which binds with high affinity to cellular methyl transferase, thereby reducing the methylation of DNA& RNA (YiP Melnyk and Pogribna, 2000) [50]
c. Folic acid appears to protect against NTD by overcoming a partial block in folate metabolism rather than correcting a nutritional deficiency. The authors provided the first direct evidence of a genetic explanation for NTD by identifying a functional variant of the gene for 5, 10 methylene tetrahydrofolate reductase, which was associated with NTD (Whitehead *et. al.*, 1995) [45]
d. Various vitamins are needed to maintain folic acid in its reduced form which is the active form of folic acid. Ascorbic acid, vitamin B_6 and B_{12} plays a key role in these reactions (Slattery and Jenerich, 1991). This could also explain the observation that low levels of vitamin C and B_{12} are associated with neural tube defects.
e. NTD's arise in those embryos which have delayed DNA synthesis. The delay in DNA synthesis occurs when metabolites necessary for biosynthesis of DNA are present in lower amount. As a result, morphogenesis of central nervous system is delayed and therefore can not be completed correctly (Seller, 1983).
f. Kirbe *et al*. (1992) showed that folate and vitamin B_{12} levels are independent risk factors for NTD. Because vitamin is the co enzyme for methionine synthetase, this enzyme may play role in determining susceptibility to NTD.

Epidemiological aspects related to neural tube defects

Deficiency of folic acid will arise in any individual when the requirement is more than its availability. Such a condition may arise due to result of decreased availability or increased requirement or both. Some of factors responsible for neural tube defects are:

A. Low dietary intake

Sauberlich (1995) reviewed that low intake of folate have been reported in adolescent females and in elderly subjects from low-income sub groups. In general, the folate intake of women in various populations is considerably lower than in men (Table 1)

B. Method of food preparation and cooking

Herbert in 1987 reported that food preparation and cooking methods had a significant effect on amount of folate. Since folate is sensitive to heat and broken down on prolonged exposure to heat and is lost substantially on boiling. This was confirmed by Herbert in 1990 that Indian women had high incidence of folate deficiency as compared to Chinese women. The reason being, Indian women prefer to cook food by prolonged boiling which leads to destruction of folic acid, whereas Chinese prefer rapid stir frying of vegetables which results in more retention of the vitamin.

C. Association with drugs

Senti & Pilch (1985) [39] reported that women (20-44 yr.) who were using oral contraceptive agents have been associated with low folate status. Whitehead *et al*, (1973) [47] conducted a study on effect of oral-contraceptive on folate status of women & reported that use of oral-contraceptive was associated with megaloblastic changes in cells of uterine cervix. However, when folic acid was given to such women, the condition became normal.

Some drugs like methotrexate, aminopterin (cancer chemotherapeutic), pyrimethamine (anti-malarial), trimethoprim (antibacterial agents) acts as antifolate drugs. The use of these drugs during pregnancy has teratogenic effect and should be taken only under medical supervision. Studies have demonstrated an association between the occurrence of NTD and administration of folate antagonists at the time of neurulation. These studies have shown that methothrexate, aminopterin (Feldkamp & Carey, 1993) [12] and 5-flurouracil (Stephens *et. al.*, 1980) increase the risk of spontaneous abortion and are associated with the occurence of NTD in the offspring of mothers undergoing drug treatment in early pregnancy. Treatment of epilepsy with valproic acid (sodium valproate) has been reportedly linked to an elevated risk of NTD in offspring (Lammer *et. al.*, 1987) [24]. Valproic acid blocks the enzyme glutamate formyl transferase which catalyses the conversion of tetrahydrofolate to 5-formyl tetrahydrofolate. Campbell *et al.*, (1986) [3] observed that use of abortifacient aminopterin and more recently use of valproic acid, both of which are folic acid antagonists, results in various types of neural tube defects.

D. Stage of life cycle

One of the important functions of folate co-enzyme is that it plays a specific role in synthesis of DNA, RNA and protein. During pregnancy, lactation & early growth, the demand for folic acid is increased. Mc Nulty *et. al.*, (1995) reported that during pregnancy utilization of folic acid for synthesis of DNA is increased. Thus catabolism of folate leads to maternal folate depletion during pregnancy.

Chanarin (1990) reported that folate requirement will be increased (less commonly) in various pathological conditions like malignancy, increased haematopoiesis and inflammatory condition.

E. Socio-economic factors

Women in lower socio-economic groups have been shown to be at an increased risk to deliver infants with neural tube defects (Leck, 1983) [26].

F. Women with history of NTD's

Women who have delivered an infant with a neural tube defect are at a greater risk of having another infant with this defect (Strassburg *et. al.*, 1983) [44], although the lower prevalence has been universally found in the second birth.

G. Age of mother

Age of mother, whether she is very young or older has been associated with increase in prevalence of neural tube defects. A greater effect has been seen when examining the mother's birth cohort (Janerich, 1972)

H. Region/area specific or genetic factors

Wide variations have been noted in the prevalence of NTD's between countries. The rate of appearance of NTD' is much higher for some groups such as Irish & Welsh (Elwood, 1972) [11] and Sikhs (Baird, 1983) [1] and lower for blacks (Roeper *et. al.*, 1988) [3]. Studies have demonstrated that persons who are migrating to some areas have possibility to adopt NTD's which lies between the rate of the country from which they migrated and those of the country to which they migrate (Barid, 1983).

I. Smoking and alcohal consumption

Sauberlich (1995) reported that serum erythrocyte and folate levels are adversely affected during smoking leading to folate deficiency in smokers. Halsted in 1995 reported that use of alcohal causes folate deficiency, which might be due to 3 reasons:

1. Intestinal malabsorption

2. Increased catabolism of vitamin
3. Increased excretion of vitamin

Thus, combination of any of the above factor may contribute to deficiency of folic acid especially in women of child-bearing age. Consequently, there is hypothesis that dietary intake is associated with development of neural tube defects.

Desired intake of folate which prevents Neural tube defects

Since early 1980's a number of intervention trails examining the impact of periconceptional folic acid on the incidence of NTD's have been published (Table 2). The first intervention trail was conducted by Laurence *et. al.* (1981) who observed that prevalence of NTD was reduced by 58% when group of women were supplemented with 4 mg/day folic acid, however, the study population was small. Later the results of MRC Vitamin Study (1991) provided conclusive evidence of the effectiveness of periconceptional folic acid in the prevention of NTD's. In this study, 1200 women in 7 countries over 8 years participated who had previous history of NTD and study showed that supplementation of folic acid (4 mg) resulted in 72% decrease in prevalence of NTD's.

Later in 1992, Czeizel and Dudas confirmed that folic acid prevents not only the recurrence of NTD but also their occurrence *i.e.* occurring to women for the Ist time. This study was followed by number of studies which confirmed earlier studies. According to study conducted by Jan *et.al.,(*2018) [20] the supplementation of folic acid should be started 5-6 months before conception because if 4 mg folic acid is consumed daily, the optimal folate level which helps to prevent NTD's will reache in approximately 20 weeks.

Desired folate intakes which have been recommended are as follows:

For women with previous history of NTD (recurrent NTD):

Women with a history of NTD in previous pregnancy should be advised to consume 4000 µg of folic acid per day starting 1 month before the onset of pregnancy and through out the first 3 months of pregnancy. (Desposito *et. al.*, 1999) [8]. However, during times in which a pregnancy is not planned, these high-risk women should consume 0.4 mg (400 µg) of folic acid per day.

The Netherlands and the UK formulated comparable recommendations i.e. 4000-5000 ug/d of supplemental folic acid to reduce the recurrent risk of NTD's (EAG, 1992) [10]. However, the use of supplements in doses above 1000ug/d might present some toxic side effects, so folic acid supplementation in doses of 4000-5000 ug/d and higher are only prescribed under medical supervision (Dickinson, 1995) [9].

For Women with no previous history of NTD (concurrent NTD)

All women of child bearing age who had no previous history of NTD should consume 0.4 mg (400 ug) of folic acid daily. An estimated 50% of pregnancies are unplanned (Department of Health 1992) and malformation of NTD occurs in the periods between 24th and 27th day of pregnancy and at this stage women usually are not aware about their pregnancy. Thus any intervention aimed at implementing these recommendations should be targeted at all women of child-bearing age so that optimal folate status will be achieved in those who may become pregnant.

For other high risk persons

Women with a close relative eg. sibling, niece or nephew who has an NTD, risk of having NTD is approximately 0.3 to 1.0%, women with type I diabetes mellitus and women treated with valproic acid risk is approximately 1%. Such women should discuss with their physician before planning a pregnancy about the risk for an affected child. They should also discuss the merits and demerits of increasing folic acid supplementation to 4000ug (Desposito *et. al.* 1999) [8].

Conclusion

Efforts are currently under way to clarify that folic acid is an essential vitamin for prevention of NTD. Evidences suggest that there are a number of stages in the life cycle, which affect the requirement of folic acid and may lead to deficiency of folic acid in women. Most of the studies have been aimed to provide supplements prior to or shortly after conception. However, there is possibility that chronic malnutrition may contribute to these defects rather than an acute deficiency near the conception period. Current folate recommendations need reconsideration so that population reaches a desired folate intake. More research should be focused on the lowest effective dose of dietary folate to prevent NTD. It will be worthwhile to stimulate people to consume foods naturally rich in folate especially in countries like UK, Sweden and Ireland where folate intake is below 200 ug/d. Fortification can provide answer to achieve increased intake of folic acid in population.

References

1. Baird PA. Neural tube defects in the Sikhs. American Journal of Medicinal Genetics,1983:16:49-56.
2. Berry RJ, Erickson JD. Prevention of neural tube defects with folic acid in China. New England Journal of Medicine,1999:341(20):1485-90.
3. Campbell RL, Dayton DH, Sohal GS. Neural tube defects: a review of human and animal studies on the etiology of neural tube defects. Teratology,1986:34:171-187.

4. Canada Health and Welfare. Food Consumption Patter. Report. Otttawa: Canadian Government Publishing Centre, 1997.
5. Chanarin I. The Megealoblastic Anaemics. 3rd Ed. Oxford. Blackwell Scientific Publications, 1997.
6. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by preconceptual vitamin supplementation. New England Journal of Medicine,1992:327:1832-1835.
7. Department of Health. Folic Acid and the Prevention of Neural Tube Defects. Report from an Expert Advisory Group. London: Department of Health, 1992.
8. Desposito F, Cunniff C, Frias JJ, Panny SR. Folic acid for the prevention of neural tube defects (RE 9834). Pediatrics,1999:104(2):325-327.
9. Dickinson, C.J. Does folic acid harm people with vitamin BI2 deficiency? Quarterly Journal of Medicine,1995:88:357-364.
10. EAG (Export Advisory Group). Folic Acid and the Prevention of Neural Tube Defects. London: Department of Health, 1992.
11. Elwood JH. Major central nervous system malformations notified in Northern Ireland 1964-1968. Development of Medicinal Child Neurology,1972:4:731-739.
12. Feldkamp M, Carey JC. Clinical teratology counselling and consultation case report; low dose methohrexate exposure in early weeks of pregnancy. Teratology,1993:47:533-539.
13. Food and Nutrition Council. Report on the relationship between folic acid intake and neural-tube defects. The Hague: Voorlichtingsbureau voor de voiding, 1992.
14. Gregory J, Fosler K, Tyler H, Wiseman M. The Dietary and Nutritional survey of British Adult. London: H. M. Stationery Office, 1990.
15. Halsted CH. Alcohol and folate interactions: clinical implications. In Folate in Health and Disease, pp. 313-327. [L. B. Bailey, editor]. New York: Marcel Dekker Inc, 1990.
16. Herbert, V. Recommended dietary intakes (RDI) of folate in humans. American Journal of Clinical Nutrition,1987:45:661-670.
17. Herbert V. Development of human folate deficiency. In Folic Acid Metabolism in Health and Disease, 1990, 195-210 [M. F. Picciano, E. L. R. Stockstad, and J. F. Gregory, editors]. New York: Wiley-Liss.
18. Hibbard ED, Smilhells RW. Folic acid melabolism and human embryopathy. Lancet,1965:1:1254-1256.
19. Irish Nutrition and Dietetic Institute. Irish National Nutrition Survey. Dublin: Irish Nutrition and Dietetic Institute, 1990.
20. Jan D, Herbert H, Hildegard L, Luc MS. Folic acid and prevention of neural tube defects: A review. Reproductive Toxicology,2018:80:73-84.
21. Janerich DT. Anencephaly and maternal age. Americal Journal of Epidemiology,1972:95:319-326.
22. Kirke PN, Daly LE, Elwood JH. A randomized trial of low dose folic acid to prevent neural tube defects. The Irish Vitamin Study Group, Arch, Dis, Child,1992:67:1442-1446.
23. Knox EG. Anencephalus and dietary intakes. British Journal of Preventive Medicine,1972:26:219-233.
24. Lammer EJ, Sever LE, Oakley GP. Teratogon update; Valproic acid. Teratology,1987:35:465-473.
25. Laurence KM, James N, Miller MH, Tennant GB, Campbell H. Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. British Medical Journal,1981:282:1509-1511.
26. Leck I. Epidemiological clues to the causation of neural tube defects. In: Dobbing J, ed. Prevention of spina bifida and other neural tube defects. London: Academic Press, Inc, 1983, 155-95.
27. Lindenbaum J, Allen, RH. Clinical spectrum and diagnosis of folate deficiency. In: Bailey LB, ed Folate in Health and Disease. New York, NY: Marcel Dekker, 1995, 43-74.
28. McNulty H. Folate requirements for health in different population groups. British Journal of Biomedical Science,1997:52:110-119.
29. McNulty H, McPartlin JM, Weir DG, Scott JM. Folate catabolism is related to growth rate in weanling rats. Journal of Nutrition, Volume 125, 99-103.
30. Myatt L, Miodovnik M. Prediction of pre-eclampsia. Semin Perinatology; Volume 23, Issue 1, 199, Pages 45-57.
31. Rampersaud GC, Kauwell GPA, Huston AD. Genomic DNA methyllation decrease in response to moderate folate depletion in elderly women. Americal Journal of Clinical Nutrition,2000:72(4):998-1003.
32. Reisenauer AM, Halsted CH. Human folate requirements. Journal of Nutrition,1878:117:600-602.
33. Roeper PI, Harris JA, Croen LA *et al*. Congenital malformation prevalence and racial variation. (Abstract). Teratology,1988:37:485.
34. Rosenquist TH, Ratashak A, Selhub J. Homocysteine induces congenital defects of the heart and neural tube: effect of folic acid. Proceedings National Academic Science., USA,1996:93:15227-15232.
35. Sauberlich, H. E. Folate status of U. S. population groups. In Folate in Health and Disease, 1995, 171-194. [L. B. Bailey, editor). New York: Marcel Dekker Inc.
36. Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL, Taylor PC. Folate requirement and metabolism in nonpregnant women. American Journal of Clinical Nutrition,1987:46:1016-1028.
37. Scott JM, Weir DG, Kirke PN. Folate and neural tube defect. In Bailey L; ed Folate in Health and Disease. New York: Marcel Dekker, 1995, 329-60.

38. Seller MJ. The cause of neural tube defects: some experiments and a hypothesis. Journal of Medical Genetics,1983:20:164-168.
39. Senti FR, Pilch SM. Analysis of folate data from the Second National Health and Nutrition Examination Survey (NHANES). Journal of Nutrition,1985:115:1398-1402.
40. Shane B. Folate chemistry and metabolism. In Folale in Health and Disease [L. B. Bailey, editor], New York: Marcel Dekker Inc, 1995, 1-22.
41. Slattery ML, Janerich DT. The Epidemiology of Neural Tube Defects: A Review of Dietary Intake and Related Factors as Etiologic Agents. American Journal of Epidemiology,1991:133(6):526-540.
42. Steegers-Theunissen RPM, Boers GHJ, Trijbels FJM, Eskes TKAB. Neural tube defects and dearangement of homocysteine metabolism (letter). New England Journal of Medicine,1991:324:199-200.
43. Stephens JD, Golbus MS, Miller TR. Multiple congenital anomalies in a fetus exposed to 5-fluorouracil during the first trimester. America Journal of Obstetrics & Gynecology,1980:137:747-749.
44. Strassburg MA, Greenland S, Portigal LD *et al*. A population-based case-control study of anence- phalus and spina bifida in a low-risk area. Development of Medicinal Child Neurology,1983:25:632-641.
45. Subar AF, Block G, James LD. Food intake and food sources in the US population. American Journal of Clinical Nutrition,1989:50:508-516.
46. Whitehead AS, Gallagher P, Mills JL, Kirk PN, Burke H, Molloy AM *et al*. A genetic defect in 5, 10 methylene tetrahydrofolate reductase in neural tube defects. Quarterly Journal of Medicine,1995:88:763-766.
47. Whitehead N, Reyner F, Lindenbaum J. Megaloblastic changes in the cervical epithelium. Association with oral contraceptive therapy and reversal with folic acid. Journal of the American Medical Association,1973:226:1421-1424.
48. Wills L. Treatment of 'pernicious anaemia of pregnancy' and 'tropical anaemia' with special reference to yeast extract as a curative agent. British Medical Journal,1931:1:1059-1064.
49. Williams J, Mai CT, Mulinare J, Isenburg J, Flood TJ, Ethen M *et al*. Updated estimates of neural tube defects prevented by mandatory folic acidfortification-United States, 1995-2011. MMWR Morbidity and Mortality Weekly Report,2015:64:1:1-5.
50. Yi P, Melnyk S, Pogribna M *et al*. Increase in plasma homocysteine associated with parallel increases in plasma S-adenosyl homocysteine and lymphocyte DNA hypomethylation. Journal of Biological Chemistry,2000:275(38):29318-29323.