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Burden of Folic Acid Deficiency in India

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Abstract:- Folic acid is the most vital micronutrient for the survival of human beings, regardless of age and gender. The need for folic acid and its significance is spoken well and addressed in India, particularly for reproductive women during the preconception period and pregnant mothers. The supplementation is taken care of through iron-folic acid under the national programme for other beneficiaries also, i.e., adolescents and under-five children. The current review is done to know the burden of folic acid deficiency across different age groups in India. The prevalence of folic acid deficiency varied from 2% to 79.5%. The higher range of prevalence was observed during adolescence, followed by under-five children. Though folic acid supplementation is ensured during antenatal care, 17.5% to 29.4% of pregnant mothers had folate deficiencies. This indirectly conveys the increased risk for the birth of newborns with neural tube defects. Not only associated with anemia or neural tube defects, folate deficiency results in abnormalities in the cardiovascular system, gastrointestinal system, neuro-cognitive abnormalities, visual defects, developmental delay, and cancers. With such a high burden, folic acid deficiency becomes a significant public health problem. Due to dietary variations, most people in the country still need to meet the average folate requirement. Strategies strengthening the nutritional changes and supplementation need to be revised time-to-time appropriately with the difference in the prevalence.

***Keywords*:-** *Burden, Folic Acid Deficiency, India, Prevalence*

I. INTRODUCTION

Nutrition is defined as the 'Science of food.' Nutrients are classified into 'Macronutrients,' which consist of carbohydrates, proteins, and fats, and 'Micronutrients,' which consist of dietary fibers, minerals, and vitamins. Folate was known initially as 'Folium,' as it was isolated from 'leaf' and first found by Lucy Wills. (1) Folate is a micronutrient and water-soluble vitamin. It is often synonymously called folic acid, vitamin B9, Vitamin M, and pterygoglutamic acid. (2) Mitchell, Snell, and Williams coined "Folic acid" in 1941. (3) Though they are often synonymously, folates are naturally occurring compounds in plants and lack stability in food storage and preparation. In contrast, folic acid is stable and used to denote the synthetic pharmacological compounds used in supplements and food fortification. (2).

➢ *Structure*

Folate consists of a para-amino benzoic acid attached to a pteridine ring through a methylene group, and an L-glutamic acid residue is linked to the same. The pteridine ring of folic acid is wholly oxidized, whereas folates occur naturally as di-hydro or tetra-hydro folates. (3)(Figure 1) Folate takes different forms in the human body. The main circulating form in the blood is 5-methyltetrahydrofolate (5-methylTHF). Apart from the circulating form, there are other forms of folate depending on food fortification and dietary supplementation, viz. unmetabolized folate, tetrahydrofolate (THF), 5-formylTHF, and pteroylglutamic acid. (4,5)

➢ *Sources of Folate*

In this review, the sources of folate have been divided into three categories based on their nutritive value excellent, good, and poor. Excellent sources of folate are food items that help achieve the Recommended Dietary Allowances (RDA) of the sedentary male, good sources help in attaining the Estimated Average Requirements (EAR) of the sedentary male, and poor sources are those who aren't able to meet both. Tables 1 and 2 describe the dietary sources of folate and requirements for the Indian population as per activity and sex. (6,7)

➢ *Functions of Folate*

Folate plays a crucial role in many enzymatic reactions. It takes part in the conversion of methionine to homocysteine. It is a vital vitamin for Deoxyribonucleic Acid (DNA) synthesis and repair. It is required for cell replication and survival. It helps in the prevention of neural tube defects in newborns. (8,9)

➢ *Causes of Folate Deficiency:* (10,11)

Folate deficiency can be caused because of multiple factors. These factors can be broadly classified into due to insufficient intake, insufficient absorption, increased need and drugs.

- Due to insufficient intake- lack of dietary diversity with reduced intake of green leafy vegetables and other rich sources of folate is one of the most common reasons for deficiency.

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- Due to insufficient absorption- folate is absorbed by active and passive transport from the jejunum. Hence, diseases affecting the jejunum can lead to reduced absorption. These diseases include- tropical sprue, gastric bypass, celiac disease, short bowel syndrome. Absorption is hampered in the elderly age group because of villous atrophy.
- Due to increased need: Among populations belonging to certain age groups, there is an increased need of folic acid. Age groups such as adolescence and pregnancy need an increased folic acid consumption.
- Drugs- drugs such as sulfasalazine, trimethoprim, and methotrexate can hamper the folate absorption mechanism and lead to deficiency.

II. DIAGNOSTIC CUT-OFF

The folate levels in the body are measured directly using serum/plasma, red cells, and cerebrospinal fluid (CSF). The recent diet influences the serum level, whereas the red cell level reflects the status over the generation of red blood cells, i.e., around three months. (12,13)Serum homocysteine level is yet another gauge for folate status, but the low specificity and technical difficulty in sampling and analysis make it less useful in routine testing. (13) Serum homocysteine level initially rises in folate deficiency and then returns to normal after supplementing folate externally. (14) The standard range, deficit level, and excess level of serum/plasma folate concentration are 6-20 ng/mL, <3 ng/mL, and > 20 ng/mL, respectively. The possible deficiency is said to be present when the serum/plasma folate ranges between 3 to 5.9 ng/mL. A red cell folate level of <100ng/mL is considered a deficiency. RBC concentration of folate above 400 ng/mL is considered sufficient for the most significant reduction of neural tube defects in pregnant women. (15)

III. METHODS FOR MEASURING FOLATE STATUS

Serum folate and RBC folate were considered to have almost equal accuracy in determining folate status. There is no requirement for routine red cell folate estimation as the serum folate is often regarded as sufficient. (16) Another study established a positive correlation between RBC and serum folate. (14) The high cost of performing red cell folate testing as a routine measure could not be justified compared to serum folate estimation. (17) False normal value of red cell folate can be expected in a deficient patient if a recent blood transfusion has been done or if the reticulocyte count is elevated. (12) Red cell folate levels may also be falsely raised by the decreased oxygen saturation of hemoglobin and decreased hematocrit value. (17) The fasting serum level accurately reflects the tissue levels of folate, as there will be a false elevation in post-prandial status. Also, there is a false elevation of folate levels in vitamin B12 deficiency and hemolysed blood samples.

Total folate in serum (both oxidized and reduced) is measured both by a microbiological assay (MA) and a competitive protein binding assay (CPB). The MA uses the growth of the bacteria Lactobacillus rhamnosus in the presence of folate. (18,19) It is considered reliable for measuring RBC folate levels. (20) In CPB, the affinity of folate to folate binding protein (FBP) is used to estimate the folate concentration using chemiluminescence or fluorescence detection systems. (13,19) Most of these methods are time-intensive and cumbersome due to the cold chain requirement, complex laboratory set-up, and trained personnel. Hence, a point-of-care test was devised for plasma folate estimation, namely, lateral flow assay (LFA), which requires less time, portable machinery, and minimal expertise. The LFA technique was found to be 93% (95% CI: 54.7-100.0) sensitive and 91% (95% CI: 80.0–100.0) specific and highly accurate (by the area under the curve). The disadvantages were the necessity of the heating step as some forms of folate are heat labile (5methylTHF), and there was the risk of plasma getting coagulated. (19)

IV. PREVALENCE OF FOLATE DEFICIENCY IN INDIA

The cross-sectional studies were included in the review to assess the prevalence of folate deficiency in India. The present review comprehensively described the prevalence of folate deficiency across different age groups, i.e., under-five children, adolescents, pregnant mothers, and women of reproductive age. We included hospital-based, school-based, and community-based studies to estimate the prevalence. The overall prevalence of the included studies ranged from 2 to 79.5%, which is more expansive and varied. The adolescent population (79.5%) had the highest prevalence rate, followed by under-five children (63.2%). (21,22) (Table 3)

Very few studies have been conducted among children under five (both sexes). But the prevalence assessed has a considerable margin of difference. (22–24)This could be due to variances in methodology, i.e., hospital vs. community-based setting, diagnostic methods. Kapil U et al. attributed the significant prevalence to the lack of food diversity among the study participants using the food frequency questionnaire. (22)Taneja S et al. studied folate deficiency among infants included in a double-blinded Randomised Controlled Trial (Zinc vs. placebo). They also took a cut of<5nmol/L to be categorized as folate deficiency. (24)

Most school-based (19% - 52.2%) were published for the adolescent population, except for a hospital-based study which estimated a high prevalence (79.5%). (21,25–28) The diagnostic cut-offs and methods were different in these studies. Also, the various dietary intake, socio-demographic differences, i.e., low-income group (52.2%), and food fads may have resulted in the different prevalence rates.

The prevalence among pregnant women ranged from 17.5% to 29.4%, consistent across the studies. (29–32) Adhikari et al. and Bhide P et al. found that their study participants didn't consume pre-conceptional folic acid

tablets. (29,31) Two published studies among women of the reproductive age group found a lower prevalence rate. This may be due to the difference in the study setting, diagnostic cut-offs, or the different cut-off levels used. (33,34)

Despite the differences in the study methodology, the high prevalence rate of folate deficiency was observed in adolescents, followed by under-five children. These two age groups demand micronutrients to meet their RDA during their growth spurt. Though low prevalence is observed during pregnancy, the deficiency increases the risk of neural tube defects in the fetus. Overall, folate deficiency is a significant public health concern irrespective of age group in India.

➢ *Abnormalities Associated with Folate Deficiency*

- During pregnancy: The dietary recommendations are often doubled during pregnancy. Folate deficiency profoundly affects the growing fetus and placenta. The mother's low serum folate level has been associated with miscarriage, low birth weight, preterm birth, and neural tube defects. (8) Neural tube defects are due to the incomplete closure of the neural tube. This is a significant abnormality; hence, for the prevention of NTD in the newborn, folic acid supplementation during the preconception period and the first trimester has been made as a guideline. (35,36) Studies on mice and human placenta have shown that folate deficiency leads to autophagy and abnormal placentation. (37)
- Anemia: The classical and most common feature of folate deficiency is megaloblastic anemia. This is due to the inhibition of the maturation of the RBCs' precursors; hence, large nucleated precursors of RBCs are released into the bloodstream. (36)
- Cardiovascular abnormalities: The primary mechanism by which folate deficiency leads to cardiovascular abnormalities is increased homocysteine levels. Folate is required for the conversion of homocysteine to methionine. And folate deficiency leads to increased serum homocysteine levels, known as hyperhomocysteinemia. (38) Increase in the homocysteine levels leads to abnormal phenotypic plasticity in the vascular smooth muscles, leading to an increased risk of ischemic heart disease, thrombosis, atherosclerosis, and hypertension. (39,40) Reducing serum homocysteine levels to 3 micromol/L reduces the risk of IHD by 16%, DVT by 25%, and stroke by 24%. (39)
- Neurological and cognitive abnormalities: Studies have shown that folate deficiency is linked to increased cerebrovascular accident risk. (39,40). Folate deficiency markers increased in patients with distal symmetric neuropathy and other peripheral neuropathies (40). Reduced folate levels have also been linked with increased chances of neurodegeneration, leading to age-related dementia and Alzheimer's disease. Folate supplementation effectively delays disease progression in such cases. Folate deficiency is associated with numerous psychiatric illnesses like depression and schizophrenia. Such patients are also found to have a poor response to antidepressants (39).
- Abnormalities in vision: Folate is necessary for the normal functioning of the human eye. Folate deficiency has been linked to causing nutritional amblyopia, optic atrophy, maculopathy, open-angle glaucoma, diabetic neuropathy, and cataracts. (41)
- Developmental abnormality: Cerebral folate deficiency syndrome is characterized by reduced folate in the CSF but normal parameters in the serum. This is often due to the defect in the folate transporters present in the blood-brain barrier (BBB). The symptoms start appearing around 4-6 months of life. They have delayed development with decreased head growth, ataxia, and hypotonia. (42)
- Gastrointestinal (GI) abnormalities: GI features such as malabsorption, steatorrhea, and small intestinal villous atrophy are often seen in patients suffering from folate deficiency. But whether this association is, a cause or effect of folate deficiency is still a query (40). A study found that the presence of concurrent alcoholism and folate deficiency accelerated the occurrence of alcoholic liver disease (ALD). In this model, ALD was found to occur within three months, compared to 1 year, wherein alcoholism was present without folate deficiency. (43)
- Cancers: Folate deficiency leads to the misincorporation of uracil in DNA and hypomethylation of DNA. These two factors lead to the expression of genes involved in carcinogenesis and the induction of carcinogenesis. Breast, colorectal, and prostate cancer are associated with reduced folate levels. (36,39,40)

V. CONCLUSION

The prevalence of folate deficiency is considerable leading to a significant public health concern in India. It is proportionate to demand and growth, with adolescent population experiencing the highest level of deficiency. Folate-rich diet and prophylactic medications aid in the reduction of deficiency burden. The existing policies focusing on the supplementation of iron along with folic acid need to be channeled and enforced for the effectual outcome.

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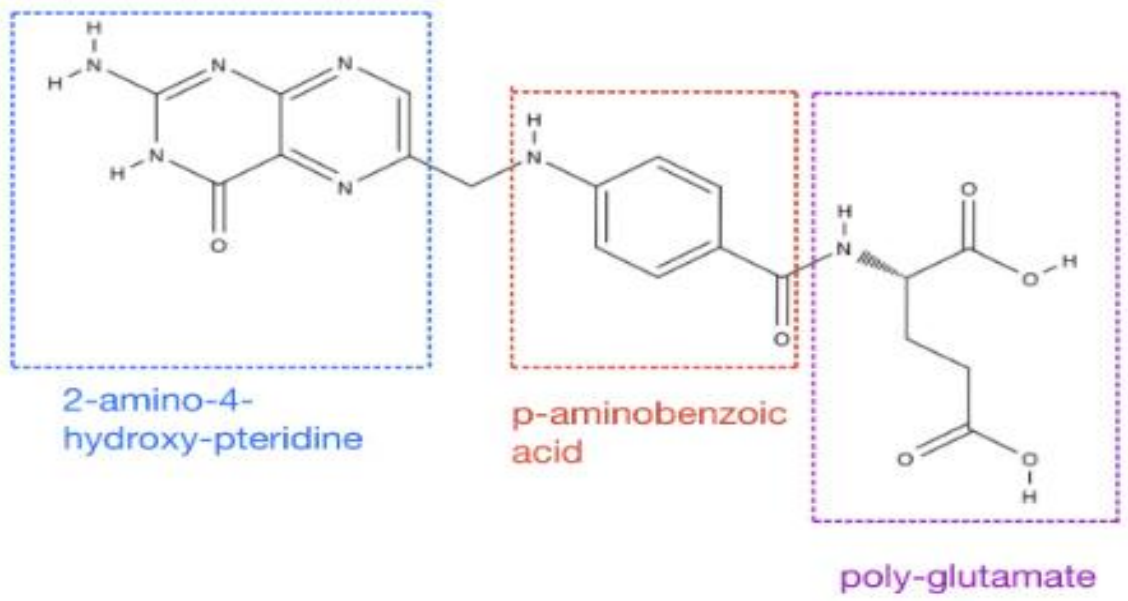

Fig 1 Structure of Folate

Table 1 Dietary Sources of Folate (6)

Excellent sources of folate (>300mcg/100g)		Good sources of folate (150-300mcg/100g)		Poor sources of folate (<150mcg/100g)	
Source	**Value (mcg/100g)**	**Source**	**Value (mcg/100g)**	**Source**	**Value (mcg/100g)**
Fish	Generally >500	Soybean	297	Spinach	142
Liver (beef)	1744	Bengal gram, whole	233	Black gram, whole	134
Calf liver	1473	Brown cowpea	231	Gingelly seeds, white	131
Chicken liver	1032	Red gram whole	229	Agathi leaves	120
Moth bean	349	Whole horse gram	163	Mint leaves	106
Rajma black, brown, red	332	Colocasia leaves, green	159	Green gram dal	92

Table 2 RDA and EAR of Folate for the Indian Population (7)

S.No	Age	Category of work	Body weight	EAR of folate (microgram/day)	RDA of folate (microgram/day)	Tolerable upper limit
1	Men	Sedentary	65	250	300	1000
		Moderate	65	250	300	1000
		Heavy	65	250	300	1000
2	Women	Sedentary	55	180	220	1000
		Moderate	55	180	220	1000
		Heavy	55	180	220	1000
		Pregnancy	55+10	480	570	1000

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		Lactation: 0-6 month	-	280	330	1000
		Lactation: 7-12 month	-	280	330	1000
3	Infants	0-6 month	5.8	-	25	-
		7-12 month	8.5	71	85	-
4	Child	1-3y	11.7	90	110	300
		4-6y	18.3	111	135	300
		7-9y	25.3	142	170	300
5	Boy	10-12y	34.9	180	220	600-800
		13-15y	50.4	238	285	600-800
		16-18y	64.4	283	340	600-800
6	Girl	10-12y	36.4	186	225	600-800
		13-15y	49.6	204	245	600-800
		16-18y	55.7	223	270	600-800

Table 3 Prevalence of Folate Deficiency Across Different Age Groups

S.No	Author, Year	Study setting *, Sample size (N)	Diagnostic method	Cut off level	Prevalence (%, 95% Confidence Interval)
Under-five children					
1.	Gupta S et al., 2022	HB, Haryana (N=420)	Enhanced Chemiluminescence based Immunoassay auto-analyzer	<4ng/ml	10.9 (8.2–14.6)
2.	Kapil U et al., 2015	CB, Delhi (N=470) Urban	Radioimmunoassay method	<4ng/ml	63.2 (58.5-67.7)
3.	Taneja S et al, 2007	CB, Delhi (N=2296) Urban	Microbiological assays using a colistin sulfate–resistant strain of *Lactobacillus leichmannii*	<5nmol/L	Breastfed infants - 6 (5-6.9) Non-breast fed- 33 (31 34.9)
Adolescent (10-19 years)					
4.	Awasthi A et al., 2022	Multi-centric, SB, (N=2276) Urban (6-16 years)	Chemiluminescent Micro particle Immunoassay	<3ng/ml	Overall -22.2 (21-24)
5.	Kumar J et al., 2020	SB, Karnataka (N=100) (14-16 years)	Electro chemiluminescence immunoassay "ECLIA" in fully automated hormone analyzer Cobas E601E4level	<2.7ng/ml	19 (11.3-26.6)
6.	Verma S et al., 2016	SB, Uttar Pradesh (N=373) (11-18 years)	Radioimmunoassay method	<3ng/ml	40.2 (35.2-45.2)
7.	Thomas D et al., 2015	HB, Delhi (N=200) (10-18 years)	Automated immunoassay system using Beckman Coulter Access-2 (Beckman Coulter, Inc. Access Folate Reagent- A14208	<5ng/ml	79.5 (73.9-85.1)
8.	Kapil U et al., 2014	SB, Delhi (N=347) (11-18 years)	Radioimmunoassay method	<3ng/ml	High-income group- 22.5 (14.4-30.6) Middle income group-40.4 (31.2-49.6) Low-income group-52.2 (43.8-60.6)
Pregnant women					
9.	Adhikari et al., 2022	HB, Maharashtra (N=240) 1st trimester	Chemiluminescence immunoassay	<3ng/ml	Primi- 17.5 (10.7-24.3) Multi- 28.3 (20.2-36.3)
10.	Bhide P et al., 2018	HB, Pune (N=584)	ID-Vit® FA microbiological assay kit (Immundiagnostik AG,	<3ng/ml	24 (21-27.9)

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		(11+/-3 weeks)	Germany) Consisting of Lactobacillus rhamnosus-coated microtitre plates.		
11.	Saxena V et al., 2016	HB, Uttarakhand (N=100) No specific trimester	Direct chemiluminescent technology.	<3ng/ml	29.4 (20.2–38.5)
12.	Pathak P et al., 2004	CB, Rural Haryana (N=283) Third trimester	Radioimmunoassay method	<3ng/ml	26.3 (21.2-31.4)
Women of reproductive age group (WRA)					
13.	Finkelstein J L et al, 2021	CB, South India (rural+urban) (N=979) (15-40 years)	Microbiological assay	<7nmol/L	16.5 (16-17)
14.	Menon K C et al, 2010	CB, Nagpur (N=109) (18-30 years) Rural tribal	Radioimmunoassay method	<6.8nmo/L	2 (0-4)

*CB-Community-Based, HB-Hospital-Based, SB-School-Based