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Correlation of Iron, Folic acid and Vitamin B_{12} in Anemic Cases in India

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Abstract

The relationship between iron deficiency and vitamin B12 and folate was recognized several decades ago. Combined deficiency is important in clinical practice owing to its relationship with malabsorption syndromes. By contrast, iron deficiency and low levels of serum vitamin B12 with normal metabolic markers were often found mostly in young adults. In this work, vitamin B12/folate changes were investigated during treatment of iron deficiency anaemia (IDA) with pharmacological iron in young adult women. A study of 135 young adult women with IDA was treated with oral iron. An haematological response was obtained in 97.2% at 4-month follow-up. Changes in serum vitamin B12, serum folate and other biochemical parameters were monitored. Results: Treatment with iron increased significantly serum folate and vitamin B12 from baseline. This increase was also observed in vitamin B12 levels ≤200 pmol/L (six patients, 17.1%), in

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whom serum vitamin B12 was above 200 pmol/L at the end of the study in all cases. Other biochemical parameters also changed. Significant increases were seen for glucose (P = 0.012), uric acid (P < 0.001), total cholesterol (P = 0.023), HDL cholesterol (P = 0.026) and bilirubin (P < 0.001). Urea decreased significantly (P = 0.036). Data from our work suggest that iron deficiency could affect many metabolic pathways, including vitamin B12, folate and lipids. These changes normalize after iron therapy, even in women with baseline low levels of serum vitamin B12. Healthcare practitioners should be aware of these changes in IDA management.

Keywords: Folate, Vitamin B12, Homocysteine, Folic acid fortification, Reduced folate carrier, Glutamate carboxypeptidase II, Methylene tetrahydrofolate reductase.

Introduction

Vitamin B9, also called folate or folic acid, is one of 8 B vitamins. All B vitamins help the body convert food (carbohydrates) into fuel (glucose), which is used to produce energy. These B vitamins, often referred to as B complex vitamins, also help the body use fats and protein. B complex vitamins are needed for healthy skin, hair, eyes, and liver. They also help the nervous system function properly. Folic acid is the synthetic form of B9, found in supplements and fortified foods, while folate occurs naturally in foods. All the B vitamins are water-soluble, meaning that the body does not store them. Folic acid is crucial for proper brain function and plays an important role in mental and emotional health. It aids in the production of DNA and RNA, the body's genetic material, and is especially important when cells and tissues are growing rapidly, such as in infancy, adolescence, and pregnancy. Folic acid also works closely with vitamin B12 to help make red blood cells and help iron work properly in the body. Vitamin B9 works with vitamins B6 and B12 and other nutrients to control blood levels of the amino acid homocysteine. High levels of homocysteine are associated with heart disease. Alcoholism, inflammatory bowel disease, and celiac disease can cause folic acid deficiency.

Vitamin B12 deficiency is relatively common, but the majority of subjects in epidemiological studies have subclinical vitamin B12 deficiency and do not present the classic signs of clinical deficiency [1-5]. However, patients with subnormal vitamin B12 levels and

normal vitamin B12-related metabolites (homocysteine and methylmalonic acid) are not uncommon and are often found in pregnancy, iron deficiency, HIV infection, etc. Such patients must be differentiated from subjects with iron deficiency and low serum cobalamin levels, but a real cobalamin deficiency. Moreover, low to normal or borderline values of cobalamin (150–200 or 250 p mol/L) are far more common (for instance, 15–20% of Western population), especially in the elderly . .Among the elderly, combined iron deficiency and vitamin B12 deficiency have been found in some of these subjects using vitamin metabolic markers . By contrast, iron deficiency and low levels of serum vitamin B12 with normal metabolic markers were often found mostly in young adults [6-10] . Combined deficiency is important in clinical practice owing to its relationship with malabsorption related syndromes, such as pernicious anaemia, Helicobacter pylori infection, coeliac disease and gastrectomy/ gastroplasty . These disorders induce refractory or unexplained iron eficiency, a frequent cause for consultation in haematology. The aetiologic approach to these patients with combined deficiency is different, and the aforementioned causes should be rapidly confirmed or discarded [11-15]. As iron deficiency anaemia is the most frequent cause of The aims of this study were to assess folate, vitamin B12 and homocysteine status in Indian patients after the initiation of folic acid fortification .

Methods

135 young adult women with IDA was treated with oral iron. Laboratory assays

Serum levels of folate,vitamin B12 and Hcy in plasma were determined using chemiluminescence immunoassays We separated folate, vitamin B12 and Hcy levels into categories, based on the reference values used in our service. For folate levels, categories were: <4 ng/mL, ≥4 ng/mL; for vitamin B12: <200 pg/mL, 200 – 300 pg/mL, >300 pg/mL; and for Hcy levels, these categories were: <15 μmol/L, ≥15 μmol/L. Individuals with levels of folate below 4 ng/mL and vitamin B12 below 200 pg/mL were categorized as having deficiency. Vitamin B12 levels between 200 – 300 pg/mL were considered as marginal status . Levels of Hcy ≥15 μmol/L were considered elevated . It is noteworthy that the reference values used to

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describe folate and vitamin B12 deficiencies are similar to those cited by WHO. Although the major purpose of folic acid fortification is to reduce the occurrence of neural tube defects (NTD) during pregnancy, an additional benefit is a potential protection against chronic diseases, through its association with lower Hcy levels . In this context, the aims of this study were to assess folate and vitamin B12 status, as well as the frequency of elevated Hcy levels

Background and aims

More than 60% people in India suffer from anemia. Supplementation of iron as a state policy for decades has not brought about significant decline in its incidence. India also harbors high malnutrition rates hence it is imperative that additional nutritional components as risk factors for anemia are to be examined in Indian population. This study explores the possible association of vit-B12 and folate deficiency with the prevalence of anemia in an Indian Hematological profile of 135 individuals from North Indian states were correlated with their Homocysteine, vitamin-B12 and folate levels.

Results: Vitamin-B12 and folate deficiency were significantly associated with anemia in the studied cases, suggesting them as vital risk factors in Indian population. Most of the macrocytic and greater than 50% of subjects with microcytic and normocytic anemia showed vit-B12 deficiency, indicating the plausible coexistence of iron and vit-B12 deficiency in the system. Conclusions: Vitamin B12 deficiency is a compounding factor in the metabolic etiology of anemia along with folate, and this may be one of the plausible reasons for ineffectiveness of iron supplementation in reducing the incidence of anemia in Indian population.

Study population

The study groups were composed of individuals consecutively attended to at GGS Medical College & Hospital, Faridkot Thus, all eligible participants, who were in accordance

with the study protocol and who agreed to participate in the study, provided a written informed consent. For children, the written informed consent was obtained from mothers or guardians. The study protocol was approved by the Ethics Committee.

Blood collection

Fasting blood samples were obtained from all participants in tubes without anticoagulant, for measurement of folate and vitamin B12, and in tubes containing EDTA to measure Hcy levels.

Discussion

Our results represent the profile of these vitamins in the investigated group, as the measurements were performed in 135 individuals of various ages under several clinical conditions using laboratory methods considered suitable for analysis. Furthermore, the subgroup-specific evaluation is also important, enabling the identification of individuals at an increased risk of developing vitamin deficiencies. These individuals should be considered for specific prophylactic measures, as a problem in clinical practice is that sometimes the deficiencies are identified only when complications, such as anemia, NTD, and neurological disorders, have already occurred. Thus, prevention of folate and vitamin B12 deficiencies becomes a major challenge for health worldwide.

As described previously, folate deficiency may occur at any age, mostly in individuals ingesting a poor diet or suffering from intestinal malabsorption. Moreover, vitamin B12 levels frequently decrease with age due to malabsorption of vitamins from food, which is more common in the elderly. Approximately 10% of the elderly are estimated to present reduced levels of vitamin B12, with this prevalence increasing approximately 5% at the age of 65 years and to 20% at the age of 85 years . In our study, we identified 0.4% and 4.2% of the elderly with folate

and vitamin B12 deficiencies respectively, whereas 14.5% showed marginal levels of vitamin B12. 545 Brazilian elderly individuals were evaluated 0.5% came with folate deficiency, 5.5% with vitamin B12 deficiency, while 23.3% had marginal levels of vitamin B12. In another study it was shown that 7.2% of the elderly had a vitamin B12 deficiency [7,8].

Our results show a situation that can often go unnoticed in the elderly, which is a vitamin B12 deficiency. Atrophy of the gastric mucosa, the presence of auto-antibodies against intrinsic factors (often undiagnosed), or the presence of *H. pylori* may play a role as an etiological factor of vitamin B12 deficiency in this age group, because it results in malabsorption of vitamin B12.

Although almost all of these individuals in our study showed no anemia (data not shown), it should be emphasized that symptoms such as depression, dementia and impaired cognitive function, which have been associated with vitamin B12 deficiency, may be misinterpreted as aging-related co-morbidities rather than vitamin B12 dependent co-morbidities]. In this sense, our findings have great relevance in clinical practice, suggesting that the measurement of this vitamin should be part of routine diagnosis in patients over 60 years, even in the absence of hematological symptoms. The high frequency of elderly individuals with marginal levels of vitamin B12 is another important fact, although the clinical significance of these levels on their health are not clear [16-20] .

Children aged from 0.5 – 6 years, included in our study, did not exhibit folate and vitamin B12 deficiencies. Several studies conducted in developing countries, such as Colombia, have described a very low prevalence of deficiency of these vitamins . In Brazil, a study that included 1111 Amazonian children reported a folate and vitamin B12 deficiency of 2.5 and 3.7%, respectively. Another study of 164 Brazilian children showed that deficiency of folate and vitamin B12 were present in 2.2 and 11.7%, respectively. It is important to note that this study included children under 2 years of age, who continued to receive cow’s milk and porridge or did not consume vegetables, fruits, and animal products until over the age of one; a fact that might

have contributed to the high prevalence of vitamin B12 deficiency . It has been found out that after food fortification with folic acid, folate deficiency has been reduced to practically 0% in children aged 1 – 14 years in Canada [12-14]. These results suggest that there is apparently no additional advantage of supplementation with folic acid in countries where food products are folic acid-fortified .

Our results suggest that fortification was adequate to prevent folate deficiency in these children, and that diet was capable of meeting vitamin B12 requirements. Vitamin B12 deficiency in children is exceptional as daily requirements are very small, and children normally consume food that contains this nutrient.

During pregnancy, folate and vitamin B12 are essential for normal fetal development. Furthermore, pregnant women have an increased physiological need for these nutrients, and their inadequate intake increases the risk of developmental abnormalities, including NTD]. The results of the present study showed that the median of folate (10.7 ng/mL) and vitamin B12 (325.0 pg/mL) in pregnant women were higher than those reported by Guerra-Shinohara *et al.* (5.6 ng/mL and 181.1 pg/mL, respectively) before folic acid fortification in Brazil .

According to some studies, the mandatory fortification of flour with folic acid resulted not only in an improvement in input and levels of folate in the blood, but also in a reduction in the occurrence of NTD [9-11] . Despite the lack of data on the outcome of these pregnancies, our results corroborate the effectiveness of this program, since normal folate levels were present in all pregnant women, irrespective of folate supplementations. However, we cannot exclude that supplementation may have contributed to achieving adequate folate levels in some of these pregnant women.

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Vitamin B12 deficiency (7.9%) or marginal levels (33.7%) were frequent in the group ofpregnant women. A high prevalence of this deficiency in this group has also been described in other populations . It was suggested that 1 in 20 Canadian women may be vitamin B12 deficient during the critical period of closing the embryonic neural tube . Increased risk of NTD has also been associated with vitamin B12 deficiency, especially after the fortification of flour with folic acid [20-22]. In the current study, practically all pregnant women with vitamin B12 deficiency were in their second and third trimester of pregnancy. It is known that vitamin B12 decreases through gestation due to an increase in fetal requirements and therefore, the deficiency in this group should be interpreted with caution. In addition, we cannot rule out the effect of hemodilution upon the levels of vitamin B12 in pregnant women. Moreover, the cut-off used to identify the vitamin B12 deficiency in the general population may not apply during pregnancy.In lactating women, a higher folate intake is also required. Folate concentration in human milk is strongly regulated and not affected by maternal folate status, except in clinically folate-deficient mothers . Maternal vitamin B12 levels have also been correlated with milk vitamin B12, and infant urinary methylmalonic acid levels, inversely related to milk vitamin B12 levels. However, in breast-fed infants the deficiency may become clear due to the low milk concentration of vitamin B12. In this study, we did not observe folate deficiency in lactating women; however 1.9% showed vitamin B12 deficiency.Although the main purpose of fortifying flour with folic acid is the reduction of NTD, the potential benefit of reducing the risk of cardiovascular disease by reducing the levels of Hcy is also relevant . Hyperhomocysteinemia has been considered a risk factor for vascular diseases and its increase is related to folate and vitamin B12 deficiencies, and genetic polymorphisms.

Some population-based studies suggest that a decline in mortality related to strokes coincided with the introduction of folic acid fortification in the United States and Canada [10] . However, some meta-analysis studies that evaluated the risk of cardiovascular disease or death in patients with or without previous disease, failed to show any beneficial effect of this strategy.

Apparently, for some subgroups of patients, such as those with kidney disease, supplementation may have a beneficial effect .Our results showed a higher frequency of hyperhomocysteinemia, mainly in the elderly. Hyperhomocysteinemia may be a consequence of ageing, oxidative stress, hypertension, diabetes and dyslipidemia. These factors were common in this group and could justify the high frequency of this condition. Moreover, our results corroborate with studies that consider the reduction of folate and vitamin B12 and age as factors related to hyperhomocysteinemia .On the other hand, hyperhomocysteinemia was nonexistent in all the children included, and multivariate analysis demonstrated the effect of vitamin B12 levels. A study performed in 207 children from the region of Campinas demonstrated that acquired factors, vitamin B12 and folate, were the most important factor in defining the levels of plasma Hcy [17] .We have also observed that Hcy levels were normal in most of the pregnant women (99.3%). Hcy levels are known to be lower in pregnant women than in non-pregnant women . However, studies have reported that elevated Hcy levels (>15.33 μmol/L) were observed in mothers of infants with NTD . Furthermore, several studies have also associated high levels of Hcy to a variety of adverse effects during pregnancy .In lactating women, hyperhomocysteinemia was present in 5%. Ramlau-Hansen *et al.* demonstrated that breastfeeding mothers who did not take folic acid supplements had a higher prevalence of elevated Hcy, compared to breastfeeding mothers taking folic acid supplements, and to a control population . Despite this relationship between folate and hyperhomocysteinemia in lactating women, the multivariate analysis showed no factor that might interfere with Hcy levels, and this fact can be attributed to the number of participants included.Finally, we were able to demonstrate that Hcy levels are influenced mainly by folate and vitamin B12. Plasma Hcy may serve as an indicator of status and perhaps of the intake of vitamins such as folate and vitamin B12 . These results also corroborate with previous findings where acquired factors contributed more to hyperhomocysteinemia than genetic factors .

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Results

During the enrollment period, all individuals who assisted at a public health care center were invited to participate in the study. A total of 135 individuals were included in our study. Among them, 40.5% were pregnant women, 36.5% were elderly, 14.7% were children and 8.3% were lactating women. The demographic characteristics of the studied population are summarized in

Table 1: Demographic characteristics of the study population

	Elderly	Children	Pregnant women	Lactating women
Age (y)	67 (60 – 91)	3 (0.5 - 6)	26 (14–43)	26.5 (14–40)
Gender (male)	114	24	-	-
BMI (mean ± SD)	26.7 (23.9, 29.9)	15.9 (14.8, 17.9)	26.4 (23.4, 30.7)	23.4 (21.5, 26.4)
Supplementation [1] [n (%)]	43 (16.4)	22 (16.6)	138 (47.4)	10 (16.6)
Smoking [n (%)]	20 (7.6)	-	27 (9.3)	8 (13.3)
Hypertension [2] [n (%)]	158 (60.3)	-	42 (14.4)	5 (8.3)
Diabetes [n (%)]	65 (24.8)	3 (2.8)	12 (4.1)	1 (1.6)
Dyslipidemia [n (%)]	65 (24.8)	-	2 (0.7)	-
Gestational stage [3] n (%)]				
First trimester	-	-	73 (27.8)	-
Second trimester	-	-	125 (47.5)	-
Third trimester	-	-	65 (24.7)	-

Age is expressed as median and range in parentheses. Body Mass Index (BMI) is expressed as median and percentiles (25 and 75) in parentheses. Gender is expressed as number of individuals.

Folate, vitamin B12 and homocysteine levels

Among the distinct study groups, children had higher vitamin B12 levels, the elderly had higher Hcy levels and lactating women had lower folate levels (Table 2).

Table 2: Folate, vitamin B12, and Hcy levels in different study groups

	Folate(ng/mL)	Vitamin B12(pg/mL)	Hcy [1] (µmol/L)
Elderly	11.2(8.7, 13.6)	443(333, 620.2)	13.5(11.1, 17.1)
	N = 262	N = 262	N = 262
Groups	12.4(9.4, 14.6)	853.0(611, 1188)	6.2(5.2, 7.3)
	N = 103	N = 103	N = 105
Pregnant women	10.7(8.3, 14.1)	325.0(257, 424)	6.4(5.3, 7.5)
	N = 291	N = 291	N = 291
Lactating women	9.8(7.6, 12.2)	523.0(415.7, 641.5)	9.2(7.6, 10.8)
	N =21	N = 54	N = 60
P [2]	0.003	p < 0.001	p < 0.001

The overall frequency of folate and vitamin B12 deficiencies were estimated to be 0.3% and 4.9% respectively (Table 3). Interestingly, we observed no folate or vitamin B12 deficiencies in the children included in our study, and only 1% presented marginal status of vitamin B12. Folate deficiency could be identified in 0.4% of elderly and 0.3% of pregnant women. None of the lactating women showed folate deficiency. The pregnant women group was the one which presented a higher frequency of vitamin B12 deficiency (7.9%), followed by the elderly (4.2%) and lactating women (1.9%). Marginal status of vitamin B12 was observed in 14.5% of the elderly, 33.7% of pregnant women and 7.4% of lactating women. The frequency of elevated Hcy levels was observed mainly in the elderly (33.6%). Among pregnant and lactating women, the frequencies of elevated Hcy levels were 0.7 and 5.0%, respectively.

Table 3: Folate, vitamin B12 and Hcy status according to cut-off values

	Cut-off values	All	Elderly	Children	Pregnant women	Lactating women
		N, %	N, %	N, %	N, %	N, %
Folate						
	<4 ng/mL	2, 0.3	1, 0.4	-	1, 0.3	-
	≥4 ng/mL	708, 99.7	261, 99.6	103, 100	290, 99.7	54, 100
Vitamin B12						
	<200 pg/mL	35, 4.9	11, 4.2	-	23, 7.9	1, 1.9
	200 - 300 pg/mL	141, 19.9	38, 14.5	1, 1.0	98, 33.7	4, 7.4
	>300 pg/mL	534, 75.2	213, 81.3	102, 99.0	170, 58.4	49, 90.7
Homocysteine						
	<15 µmol/L	625, 87.0	174, 66.4	105, 100	289, 99.3	57, 95.0
	≥15 µmol/L	93, 13.0	88, 33.6	-	2, 0.7	3, 5.0

Overall genotype frequencies of RFC1 A80G, GCPII C1561T and MTHFR C677T polymorphisms

We next evaluated the impact of clinical and laboratorial parameters on Hcy levels (Table 6). Using a multiple linear regression analysis with stepwise criteria, the variables independently associated with Hcy levels were: folate, vitamin B12, gender, age and RFC1 A80G polymorphism (genotype AA) in the elderly; vitamin B12 in children; and folate in pregnant women. None of the variables evaluated showed any impact on Hcy levels in lactating women.

Table 4: Folate, vitamin B12 and Hcy levels according to different polymorphisms in the study groups

	GROUPS	RFC1 A80G [3]			*P* [1]	GCPII C1651T [4]		*P* [2]	*P* [1]	
		AA	AG	GG		CC	CT		TT	
Folate (ng/mL)	Elderly	11.2 (8.4,13.2)	11.2 (8.8, 13.9)	11 (8.6, 13.2)	0.840	11 (8.5, 13.7)	12.2 (11.1, 13)	0.252	10.2 (7.3, 12.4)	0.158
		N = 95	N = 125	N = 26		N = 241	N = 14		N = 36	
	Children	12.5 (8.2,16.1)	12.5 (9.4, 15.1)	12.5 (10.2, 14.1)	0.863	12 (9.4, 15.1)	11.5 (8.2, 18.3)	0.932	13.8 (9.6, 15.6)	0.441
		N = 20	N = 60	N = 20		N = 79	N = 6		N = 11	
	Pregnant women	9.8 (8.0,13.6)	11 (8.4, 15)	10.9 (8.5, 14.2)	0.346	10.8 (8.2, 14.3)	11.5 (8.5, 13.3)	0.927	8.8 (7.3, 11.6)	
		N = 74	N = 129	N = 83		N = 250	N = 21		N = 26	
	Lactating women	10.6 (9.4,12.7)	9.5 (7, 12.1)	9.5 (7.3, 11.7)	0.300	9.8 (7.7, 12)	12.6 (7.5, 14.3)	0.429	9.7 (6.8, 10.8)	
		N = 12	N = 28	N = 14		N = 49	N = 3		N = 4	
Vitamin B_{12} (pg/mL)	Elderly	418 (310, 687.5)	446 (341, 623)	442 (337.5, 584.7)	0.650	444 (333, 616.5)	373.5 (288.2, 647.7)	0.317	391.5 (276.2, 577.5)	
		N = 49	N = 125	N = 86		N = 241	N = 14		N = 36	
	Children	827 (638.5, 1047.5)	874 (650.2, 1200)	770.5 (513, 1207.2)	0.840	901 (611, 1204)	772.5 (518.7, 1000.5)	0.359	756 (553, 1049)	
		N = 20	N = 60	N = 20		N = 79	N = 6		N = 11	

	GROUPS	RFC1 A80G [3]			*P* [1]	GCPII C1651T [4]		*P* [2]	*P* [1]
		AA	AG	GG		CC	CT		TT
	Pregnant women 748	341.5 (267.7, 462)	324 (253, 419)	319 (240, 412)	0.218	322 (253.7, 424)	324 (263.5, 399.5)	0.738	308.5 (236.2, 441.7)
		N = 74	N = 129	N = 83		N = 250	N = 21		N = 26
	Lactating women	563 (470, 648)	467 (400.2, 540.7)	624 (408.7, 682)	0.114	488 (414, 622.5)	627 (576, 699)	0.094	617 (520, 712)
		N = 12	N = 28	N = 14		N = 49	N = 3		N = 4
Homocysteine (μmol/L)	Elderly	14.1 (11.7, 17.9)	12.9 (10.7, 16.4)	13.8 (11.4, 17.2)	0.170	13.5 (11.2, 17.1)	13.3 (10.7, 18.4)	0.896	14.8 (12.1, 18.4)
		N = 49	N = 125	N = 86		N = 241	N = 14		N = 36
	Children	6.0 (5.3, 6.7)	6.3 (5.1, 7.4)	6.7 (5.2, 7.4)	0.582	6.2 (5.2, 7.1)	5.9 (5.2, 8.4)	0.876	6.8 (6.5, 8)
		N = 20	N = 60	N = 20		N = 78	N = 6		N = 11
	Pregnant women	5.9 (5.1, 7.6)	6.4 (5.4, 7.6)	6.3 (5.5, 7.5)	0.151	6.4 (5.4, 7.5)	6.2 (5.1, 7.8)	0.710	7.8 (6.3, 9.2)
		N = 74	N = 129	N = 83		N = 250	N = 21		N = 26
	Lactating	9.4 (8.2, 10.4)	9.7 (7.4,	8.7 (7.8,	0.929	9.4 (7.5,	12.6 (8.9,	0.1	7.8

	GROUPS	RFC1 A80G [3]			*P* [1]	GCPII C1651T [4]		*P* [2]	*P* [1]
		AA	AG	GG		CC	CT		TT
749	women		11.5)	10.9)		10.8)	12.6)	93	(6.8, 11.4)
		N = 12	N = 28	N = 14		N = 49	N = 3		N = 4

Table 5: Impact of clinical and laboratory parameters on Hcy levels

Overall genotype frequencies of RFC1 A80G, GCPII C1561T and MTHFR C677T polymorphisms

We next evaluated the impact of clinical and laboratorial parameters on Hcy levels (Table 6). Using a multiple linear regression analysis with stepwise criteria, the variables independently associated with Hcy levels were: folate, vitamin B12, gender, age and RFC1 A80G polymorphism (genotype AA) in the elderly; vitamin B12 in children; and folate in pregnant women. None of the variables evaluated showed any impact on Hcy levels in lactating women.

Table 6: Predictors of Hcy levels in the elderly, children and pregnant women

Groups	Independent variables	β	R^2	*P*
Elderly	Folate	−4.594	0.0441	p < 0.001
	Vitamin B12	−0.044	0.0324	p < 0.001
	Gender (male)	52.785	0.1622	p < 0.001
	Age	3.219	0.0716	p < 0.001
	RFC1 (AG x AA)	−26.995	0.0192	0.011
	(GG x AA)	−11.736		
Children	Vitamin B12	−0.023	0.0782	0.011
Pregnant women	Folate	−4.556	0.0750	p < 0.001

Conclusion

Folate deficiency is practically nonexistent in the post-folic acid supplementation in the studied population. However, our study suggests that screening for vitamin B12 deficiency may be particularly relevant, especially in the elderly, and the impact of the relatively high frequency of this deficiency on the overall health of our population deserves additional studies. Finally, we confirmed that folate and vitamin B12 are important determinants of Hcy levels.

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