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Folate and vitamin B_{12} status and their relation to hematological indices in healthy adults of Iranians: Azar cohort study

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Abstract

Background: Folate and vitamin B_{12} are essential micronutrients, the deficiency of which can be associated with public health problems worldwide. **Aim:** The aims of this study were to assess the folate and vitamin B_{12} status of healthy adults and the effect of gender differences on their deficiency using serum folate, vitamin B_{12} and red blood cell (RBC) folate as biomarkers and their relation to hematological indices. **Methods:** This study was a part of the Azar cohort study, which is designed as a noncommunicable disease survey in the population of Shabestar, East Azerbaijan, Iran. A total of 95 healthy adults (35 men, 60 women) were chosen according to exclusion criteria and assessed using demographic characteristics and blood sampling. **Results:** Low concentrations of serum folate, vitamin B_{12} and RBC folate were detected in 16.8%, 61.1% and 40% of all subjects, respectively. Prevalence of low serum folate and vitamin B_{12} was higher in men than women (25.7% versus 11.7% for serum folate, 77.1% versus 51.7% for serum vitamin B_{12}).A significant positive correlation was found between hemoglobin and serum folate, vitamin B_{12} and RBC folate concentrations ($r = 0.279$, 0.335, 0.228, respectively). No statistically significant correlation between mean corpuscular volume and serum folate, vitamin B_{12} and RBC folate was seen ($r = -0.049$, –0.030, –0.016, respectively). **Conclusions:** There was a high prevalence of low folate and vitamin B_{12} concentrations in our community. Regarding the impact of these two vitamins deficiency on overall health, we suggest further investigations with more participants. In addition, monitoring hematological indices could be useful in people with folate and vitamin B_{12} deficiency.

Keywords

Serum folate, serum vitamin B_{12}, red blood cell folate, deficiency, healthy adults, hematological indices

Introduction

Folate and vitamin B_{12} are essential micronutrients, with shared functions. They both participate in one-carbon metabolism, including the remethylation of homocysteine to methionine and take part in the synthesis of DNA (Brito et al., 2015; Mahan et al., 2012; Selhub, 1999; Shane, 2001; Voutilainen et al., 2001; Wagner, 2001). Thus, their deficiency can lead to impairments in one-carbon metabolism and elevated plasma homocysteine, which can be associated with public health problems such as cardiovascular diseases, cancers and cognitive decline. Low concentrations of these vitamins are also associated with complications such as birth defects and neurological disorders (Bailey and Gregory, 1999; Boushey et al., 1995; de Benoist, 2008; Green and Jacobsen, 1995; Marinou et al., 2005; McLean et al., 2008; O'Leary and Samman, 2010). More severe deficiencies of these two vitamins are associated with hematological disorders, such as anemia, leukopenia and thrombocytopenia (Aktas et al., 2014b).

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Vitamin B_{12} deficiency is more frequent in populations with a low dietary intake of animal sources and in the gastrointestinal conditions leading to vitamin B_{12} malabsorption (such as atrophic gastritis in the elderly). The main causes of folate deficiency include low dietary intake, diseases that affect absorption in the gastrointestinal tract (e.g. Crohn's disease, celiac disease), medication side effects (e.g. methotrexate, anticonvulsants, etc), excessive alcohol intake, increased demand without increased intake (e.g. during pregnancy and breast-feeding) and vitamin B_{12} deficiency (Allen, 2008).

Considering the importance of these vitamins and their role in human health, assessment of folate and vitamin B_{12} deficiency in different population groups is crucial. The most useful measurements for assessing folate and vitamin B_{12} status at the population level are serum/plasma concentrations of these vitamins. However, due to the lack of sensitivity or specificity of these measurements, using other laboratory tests, such as red blood cell (RBC) concentrations of folate or metabolic markers such as homocysteine or methylmalonic acid (MMA) for an accurate diagnosis of folate or vitamin B_{12} deficiency are required. In addition, changes in hematological indices, such as mean corpuscular volume (MCV), red cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW), RBC, hemoglobin (Hb) and hematocrit (HCT), in folate and vitamin B_{12} deficiency may be helpful in diagnosis. However, there is little data about these parameters (Green, 2008; Snow, 1999). In addition, studies that have examined these parameters were in places where the prevalence of folate and vitamin B_{12} deficiency were low. However, due to the racial differences in the prevalence of folate and vitamin B_{12} deficiency (Stabler et al., 1999) and considering the previous studies in Iran that showed high prevalence of folate and vitamin B_{12} deficiency, and also since gender plays an important role in folate and vitamin B_{12} metabolism (Dawson et al., 1994), we aimed in this cross-sectional study to assess the folate and vitamin B_{12} status of healthy adults and the effect of gender differences on their deficiency using serum folate, vitamin B_{12} and RBC folate as biomarkers and their relation to hematological indices, such as MCV, RDW, MPV and PDW.

Materials and methods

Study population

A subsample of 95 healthy adults (35 men, 60 women), aged 35–65 years old, were chosen by simple random sampling of people in the enrollment phase of the Azar cohort study (part of the Persian cohort study), which is designed as a noncommunicable disease (NCD) survey in the population of Shabestar, East Azerbaijan, Iran. Since this study was part of the validity study, sample size was calculated using the following formula

$$N = \left(\frac{z_{1-\alpha} + z_{1-\beta}}{C_r}\right)^2 + 3$$

$$C_r = \frac{1}{2}\ln\frac{1+r}{1-r}$$

$1-\alpha = 0.95$ $1-\beta = 0.9$ $r = 0.34$
$Z_{1-\alpha} = 1.64$ $Z_{1-\beta} = 1.28$ $N = 95$

Exclusion criteria included current pregnancy and breast-feeding, any use of vitamins or other supplements, alcoholism, smoking, special diets such as vegetarian, use of drugs known to interfere with folate and vitamin B_{12} metabolism, diseases known to alter folate and vitamin B_{12} metabolism and any gastric or intestinal surgery. Information regarding age, gender, height, weight and medical history were collected. All participants provided an informed written consent and the study protocol was approved by the Ethics committee of Tabriz University of Medical Sciences (TBZMED.REC.1394.116).

Biomarkers

Venus blood samples were taken after an overnight fast of 12–14 hours. Blood was collected in a Vacutainer serum separator tube and in an EDTA (ethylenediaminetetraacetic acid) tube for the determination of serum folate and vitamin B_{12}, and of RBC folate concentrations, respectively, using the electrochemiluminescence immunoassay method (Siemens vitamin B_{12} and folate dual kit, Immulite 2000 systems analyzers, Germany). Directly after blood sampling, the hemolysate was prepared by diluting 1 cc full blood in 1 cc distilled water. A complete blood count was performed on a NIHON KOHDEN cell counter (Celltac model, Japan). All samples were stored at –80°C up to the moment of measurement.

Anthropometric measurement

At the onset of study, the height was measured using a mounted tape, with the participant's arms hanging freely by their sides, and recorded to the nearest 0.5 cm. After ensuring that participants were barefoot and wore light clothing, their weight was recorded to the nearest 0.1 kg with a Secascale. Their body mass index was calculated by dividing weight (in kilograms) by the square of height (in meters).

Statistical analyses

Data normality was checked using the Kolmogorov–Smirnov test. Mean $\pm$ SD was used to describe normal distributed data. Non-normally distributed data was described as Median(IQ). The mean values of the demographic, biochemical and hematological measurements were compared between men and women using the Student's *t*-test for normally distributed continuous variables and the Mann–Whitney U-test for non-normally distributed continuous variables. Also, a χ^2 test was used to compare proportions between men and women. Cut off values served as the criterion for defining low vitamin levels according to the reference article (de Benoist, 2008). Due to

Table 1. Summary of demographic characteristics and the hematological and biochemical profile of participants.

Variable (unit)	All ($n = 95$)	Male ($n = 35$)	Female ($n = 60$)	p-value
Age (yr)	48.09 ± 7.79*	49.97 ± 7.47	47 ± 7.83	0.07
Height (cm)	162.79 ± 9.75	171.86 ± 7.37	157.51 ± 6.56	<0.001
Weight (kg)	73.85 ± 11.45	76.90 ± 10.89	72.07 ± 11.48	0.04
Body mass index (kg/m^2)	27.91 ± 4.30	25.91 ± 2.93	29.07 ± 4.55	<0.001
Serum folate (ng/ml)	6.76(5.73)**	6.71(5.50)	7.08(5.81)	0.26
Serum vitamin B_{12} (pg/ml)	176(106)	152(39)	189(132)	0.002
RBC folate (ng/ml)	190.70 ± 101.34	183.41 ± 87.25	194.95 ± 109.21	0.83
WBC (10*3/μl)	6.29 ± 1.21	6.19 ± 1.06	6.35 ± 1.29	0.51
RBC^3 (10*6/μl)	4.81(00.50)	5.10(0.41)	4.71(0.44)	<0.001
Hb (g/dl)	14.27 ± 1.31	15.46 ± 0.85	13.58 ± 1.008	<0.001
HCT (%)	42.23 ± 3.68	45.51 ± 2.47	40.33 ± 2.83	<0.001
MCV (fl)	87.59 ± 4.56	89.50 ± 4.46	86.48 ± 4.28	0.002
MCH (pg)	29.60 ± 1.75	30.39 ± 1.62	29.14 ± 1.66	0.001
MCHC (g/dl)	33.80(0.70)	34(0.50)	33.80(0.70)	0.001
PLATELET (10*3/μl)	233.21 ± 48.25	204.34 ± 37.27	250.05 ± 46.11	<0.001
RDW (%)	12.80(0.70)	12.80(0.70)	12.75(0.60)	0.88
MPV (fl)	8.29 ± 0.60	8.17 ± 0.65	8.36 ± 0.56	0.15
PDW (%)	16.80(0.05)	17.10(0.90)	16.75(0.50)	0.003
PCT	019(0.05)	0.17(0.04)	0.21(0.05)	<0.001

*Normally distributed variables have been described as Mean ± SD and compared using an independent t-test between males and females.
**Non-normally distributed variables have been described as Median(IQ) and compared using the Mann–Whitney U-test between males and females.
WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; MPV: mean platelet volume; PDW: platelet distribution width; PCT: procalcitonin.

Table 2. Biomarker status of folate and vitamin B_{12} according to cut off values.

Biomarker (unit)	Cut off values	All ($n = 95$) N[a] (%)	Male ($n = 35$) N (%)	Female ($n = 60$) N (%)	p-value[b]
Serum folate (ng/ml)	<4	16(16.8)	9(25.7)	7(11.7)	
	≥4	79(83.2)	26(74.3)	53(88.3)	0.07
Red blood cell folate (ng/ml)	<151	38(40)	14(40)	24(40)	
	≥151	57(60)	21(60)	36(60)	1
Serum vitamin B_{12} (pg/ml)	<203	58(61.1)	27(77.1)	31(51.7)	
	≥203	37(38.9)	8(22.9)	29(48.3)	0.01

[a]Number.
[b]The χ^2 test was used to compare proportions between men and women.

non-normality of the variables, the Spearman correlation was used to measure associations between biochemical and hematological measurements. We used the SPSS software package (version 23; SPSS Inc., Chicago, IL, USA) for data analyses, and p-values <0.05 were considered to indicate statistical significance.

Results

A total of 95 healthy adults (35 men, 60 female), aged between 35 and 65 years old, participated in the study. The mean age of participants was 48.09 ± 7.79 years. Median(IQ) serum folate and vitamin B_{12} were 6.76(5.73) (ng/ml), 176(106)(pg/ml), respectively. No significant difference was found between males and females for serum folate (p-value = 0.26). However, for serum vitamin B_{12}, there was a statistically significant difference between males and females (p-value = 0.002). The mean concentrations of MCV were 87.59 ± 4.56, with a significant difference between males and females (p-value = 0.002). The comprehensive demographic characteristics, hematological and biochemical profile of the studied population are summarized in Table 1.

The participants' status of serum folate, vitamin B_{12} and RBC folate are shown in Table 2. According to the cut off values (4 ng/ml for serum folate, 151 ng/ml for RBC folate and 203 pg/ml for serum vitamin B_{12}), low concentrations of serum vitamin B_{12} (pg/ml) were detected in 61.1% of all participants, which was significantly higher in men (77.1%) than in women (51.7%) (p-value = 0.07). Low

concentrations of RBC folate (ng/ml) were shown in 40% of all participants, which was the same for men and women. Only 16.8% of the participants had low concentrations of serum folate (ng/ml), which was higher in men (25.7%) than in women (11.7%) (p-value = 0.01).

Mean ± SD and Median(IQ) concentrations of hematological indices according to two subgroups (deficiency, sufficiency) of serum folate, vitamin B_{12} and RBC folate are shown in Table 3. There was a significant difference between two subgroups of serum folate, vitamin B_{12} and RBC folate for RBC concentrations (p-values = 0.003, 0.002 and 0.030, respectively). The mean concentrations of Hb (g/dl) in the deficiency group of serum folate were lower than for the sufficiency group (14.11 ± 1.24 versus 15.07 ± 1.41, p-value= 0.007). Also, a similar pattern was seen for vitamin B_{12} and RBC folate, such that the mean concentrations of Hb in the deficiency groups of vitamin B_{12} and RBC folate were lower than those for the sufficiency groups (for vitamin B_{12}: 13.86 ± 1.25 versus 14.50 ± 1.29, p-value = 0.021; for RBC folate: 14.07 ± 1.29 versus 14.58 ± 1.29, p-value = .06). No significant difference was found for white blood cell (WBC), MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), PLATELET, RDW OR PDW between subgroups of serum folate, vitamin B_{12} and RBC folate.

There was a significant positive correlation between serum folate, vitamin B_{12} and RBC folate concentrations with Hb (r = 0.279, 0.335, 0.228, respectively). There was no statistically significant correlation between MCV (fl) and serum folate, vitamin B_{12} and RBC folate (r = –0.049, –0.030, –0.016, respectively). A significant positive correlation was found between serum folate, vitamin B_{12} and RBC folate with RBC (10*6/μl)(r = 0.271, 0.351 and 0.231, respectively). Table 4 shows the spearman correlation between biochemical and hematological measurements.

Discussion

The present study describes the folate and vitamin B_{12} status of 95 healthy adults, aged 35–65 years old in the representative population of Shabestar, East Azerbaijan, Iran, using serum folate, vitamin B_{12} and RBC folate. We also examined the association between biochemical and hematological measurements, such as MCV, RDW, MPV and PDW. Apparently healthy adults were chosen for adjusting the effects of confounding factors on serum folate, vitamin B_{12} and RBC folate concentrations. According to the results of this study, a prevalence of low serum vitamin B_{12} and folate concentrations were seen in 61.1% and 16.8% of all participants, respectively. A study performed with different subgroups of the Brazilian population reported that folate deficiency was practically nonexistent (0.3%), whereas vitamin B_{12} deficiency was present in 4.9% of the studied group (Barnabé et al., 2015). Similar results have been reported in other studies (Alfthan et al., 2003; Fayet-Moore et al., 2014; Lim and Heo, 2002; MacFarlane et al., 2011; Selhub et al., 1999; Shams et al., 2009; Xavier et al., 2010). In a published study that has reported serum folate and vitamin B_{12} in healthy Iranian adults, a high prevalence of low folate and vitamin B_{12} levels was detected in 98.2% and 27.1% of the total participants, respectively (Fakhrzadeh et al., 2006). In developed countries, folate and vitamin B_{12} deficiency is not common; however, this deficiency can be seen in some developing countries (Metz, 2008). In addition, the prevalence of low folate and vitamin B_{12} levels varies between studies due to the variability of the cut offs used to describe deficiency, geographical, racial and ethnic differences between study populations (Dawson and Waters, 1994; Golbahar et al., 2004). The high prevalence of low folate and vitamin B_{12} levels in our study could be due to inadequate dietary intake of folate and vitamin B_{12}, inaccurate cooking of vegetables and no fortification of grain products with folic acid in our country (Golbahar et al., 2004). In addition, considering the relatively high average age of the participants in our study, low serum vitamin B_{12} levels could be associated with chronic atrophic gastritis, which strongly increases with age and reduces the secretion of intrinsic factors and may lead to malabsorption of vitamin B_{12} in the intestine (Weck et al., 2007). Our results showed that the prevalence of low serum folate and vitamin B_{12} concentrations was higher in men than in women, which is consistent with previous studies (Carmel et al., 1999; Clarke et al., 2003; Lim and Heo, 2002; Selhub et al., 1993; Shams et al., 2009).This can be explained by differences in sex hormones. Estrogen, which increases during pregnancy and decreases in postmenopausal women, has a significant impact on plasma total homocysteine (tHcy) levels (Verhoef et al., 1999). Several studies have shown a reduction in plasma tHcy levels during pregnancy (Andersson et al., 1992). There are other reports that plasma tHcy levels are lower in premenopausal women compared with postmenopausal women (Brattström et al., 1994). Also, in some studies estrogen replacement therapy resulted in lower plasma tHcy levels (van Baal et al., 1999). A study conducted by Lim and Heo (2002) showed that there was a significant sex difference in plasma tHcy, folate and vitamin B_{12} concentrations. Males tend to have higher tHcy but lower folate and vitamin B_{12} levels than women.

Since the measurement of serum folate is considered an indicator of recent folate intake and it cannot be used to reflect chronic deficiency status, we also measured folate concentrations in RBCs, which shows constancy from day to day and accurately reflects the longer term body folate status (Klee, 2000). In this study, 40% of all participants had low RBC folate concentrations. The percentage of men and women who had low RBC folate concentrations was similar (40% for both sexes). In a study by Wartanowicz et al. (2001), which assessed the folate status of 78 healthy women of childbearing age, insufficient folate body stores were seen in almost all women. In another study, which assessed folate status in female university students using both serum and RBC folate concentrations, only one

Table 3. Concentrations of hematological indices according to two subgroups of serum folate, vitamin B_{12} and red blood cell (RBC) folate.

	Serum folate (ng/ml)			Serum vitamin B_{12} (pg/ml)			RBC folate (ng/ml)		
Hematological variables (unit)	<4 ($n = 16$)	≥4 ($n = 79$)	*p*-value	<203 ($n = 61$)	≥203 ($n = 34$)	*p*-value	<151 ($n = 38$)	≥151 ($n = 57$)	*p*-value
WBC (10*3/μl)	6.28 ± 1.26[a]	6.35 ± 0.95	0.829	6.21 ± 1.08	6.33 ± 1.28	0.642	6.24 ± 0.92	6.32 ± 1.38	0.740
RBC (10*6/μl)	4.76(0.47)[b]	4.99(0.50)	0.003	4.69(0.46)	4.92(0.48)	0.002	4.76(0.46)	4.92(0.49)	0.030
Hb (g/dl)	14.11 ± 1.24	15.07 ± 1.41	0.007	13.86 ± 1.25	14.50 ± 1.29	0.021	14.07 ± 1.29	14.58 ± 1.29	0.060
HCT (%)	41.74 ± 3.45	44.70 ± 3.91	0.003	41.04 ± 3.45	42.90 ± 3.66	0.017	41.60 ± 3.65	43.19 ± 3.56	0.038
MCV (fl)	87.96 ± 4.92	87.52 ± 4.52	0.727	87.37 ± 5.11	88 ± 3.39	0.523	87.53 ± 4.42	87.63 ± 4.69	0.912
MCH (pg)	29.60 ± 1.74	29.60 ± 1.76	0.990	29.53 ± 1.95	29.72 ± 1.33	0.626	29.52 ± 1.59	29.65 ± 1.86	0.722
MCHC (g/dl)	33.75(0.98)	33.90(0.60)	0.780	33.90(0.60)	33.80(0.65)	0.317	33.80(0.73)	33.90(0.65)	0.373
PLATELET (10*3/μl)	213 ± 48.17	237.30 ± 47.53	0.066	227.37 ± 51.11	243.67 ± 41.30	0.115	227.47 ± 48.59	237.03 ± 48.07	0.347
RDW (%)	12.80(0.57)	12.70(0.70)	0.675	12.80(0.75)	12.80(0.60)	0.732	12.80(0.65)	12.80(0.75)	0.547
MPV (fl)	8.33 ± 0.64	8.28 ± 0.59	0.746	8.19 ± 0.62	8.46 ± 0.52	0.041	8.24 ± 0.54	8.32 ± 0.64	0.553
PDW (%)	16.80(0.83)	16.80(0.70)	0.336	16.90(0.75)	16.80(0.42)	0.162	16.70(0.85)	16.90(0.60)	0.278
PCT	0.18(0.04)	0.19(0.05)	0.042	0.19(0.06)	0.19(0.04)	0.034	0.18(0.05)	0.19(0.05)	0.260

[a]Normally distributed variables have been described as Mean ± SD and compared using an independent *t*-test between two groups.

[b]Non-normally distributed variables have been described as Median(IQ) and compared using a Mann–Whitney *U*-test between two groups.

WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; MPV: mean platelet volume; PDW: platelet distribution width; PCT: procalcitonin.

Table 4. The spearman correlation between hematological and biochemical measurements.

	RBC folate	RBC	WBC	Hb	HCT	MCH	MCHC	MCV	RDW	MPV	PDW	PLATELET	PCT
Serum folate	0.512**	0.271**	0.100	0.279**	0.316**	–0.046	0.016	–0.049	–0.010	–0.044	0.123	0.148	0.179
p-value	<0.001	0.008	0.335	0.006	0.002	0.659	0.877	0.634	0.926	0.671	0.234	0.154	0.083
Serum vitamin B_{12}	0.079	0.351**	0.046	0.335**	0.340**	–0.046	–0.117	–0.030	–0.057	0.158	–0.222*	0.185	0.223*
p-value	0.444	0.001	0.656	0.001	0.001	0.656	0.260	0.774	0.586	0.126	0.030	0.072	0.030
RBC folate	-	0.231*	0.061	0.228*	0.249*	–0.015	0.024	–0.016	0.082	–0.011	0.034	0.142	0.150
p-value	-	0.024	0.559	0.026	0.015	0.885	0.820	0.879	0.427	0.912	0.745	0.171	0.148

*Correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level.

WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; MPV: mean platelet volume; PDW: platelet distribution width; PCT: procalcitonin.

participant had low RBC folate concentrations, which shows that folate fortification in Australia has been successful in increasing the folate status of young women (Fayet-Moore et al., 2014). Also we demonstrated a significant positive correlation between serum and RBC folate ($r = 0.512$, p-value < 0.001).

In our study, serum folate, vitamin B_{12} and RBC folate had a significant positive correlation with RBC ($r = 0.271$, 0.351, 0.231, respectively), Hb($r = 0.279$, 0.335, 0.228, respectively) and HCT ($r = 0.316$, 0.340, 0.249, respectively) concentrations, but not with MCV. This shows that MCV is not a reliable marker for folate or vitamin B_{12} deficiency. Kwok et al. (2002) compared the hematological parameters of patients with vitamin B_{12} deficiency to those of healthy controls and reported that MCV was no different between study groups. In addition, vitamin B_{12} deficiency was associated with a significant decrease in Hb concentrations. De Bruyn et al. (2014) reported that for serum folate concentrations $\leq$5 lg/L or 11.4 nM, a significant negative impact was noted on the RBC count, hematocrit and hemoglobin.

Serum folate, vitamin B_{12} and RBC folate were reversely correlated with RDW and MPV, but this was not statistically significant. PDW had a significant negative correlation with serum vitamin B_{12} ($r = –0.222$), but not with serum and RBC folate. Aktas et al. (2014a) compared hematological parameters in patients with and without vitamin B_{12} deficiency and reported that red cell distribution (RDW) was significantly higher in patients with vitamin B_{12} deficiency and MPV was significantly lower in patients with vitamin B_{12} deficiency. In the present study, serum vitamin B_{12} had negative and positive correlations with RDW and MPV, respectively. However, it was not statistically significant ($r = –0.057$, $r = 0.158$). DNA synthesis defection in vitamin B_{12} deficiency, which leads to the impaired growth and division of hematopoietic cells, and thus the production of larger erythrocytes in bone marrow, might be a possible explanation for an increased RDW. Another explanation could be the association between vitamin B_{12} deficiency and inflammation. Vitamin B_{12} deficiency is associated with increased serum levels of homocysteine, which is considered as an inflammatory factor. In addition, RDW has been found to be related to inflammatory conditions. According to these findings, we can explain a possible linkage between vitamin B_{12} deficiency and an elevated RDW. Two possible reasons may interfere with the MPV reduction in vitamin B_{12} deficiency. Firstly, inflammation caused by increased homocysteine levels in vitamin B_{12} deficiency may lead to the production of smaller platelets in bone marrow. Another explanation could be that activated platelets in inflammatory conditions tend to be larger in size. Moreover, after utilization of active platelets in inflammatory processes, the remaining inactive smaller platelets may cause a reduction in MPV (Aktas et al., 2013a, 2013b; Bhatia et al., 2012; Kapsoritakis et al., 2001; Pongstaporn and Bunyaratavej, 1999; Sit et al., 2013; Song et al., 2012).

Strengths of the present study include the measurement of RBC folate along with serum folate, which allows us a better interpretation of body folate status. Furthermore, healthy adults were chosen by applying strict exclusion criteria for adjusting the effects of any significant health confounders and to better the assessment of biomarker status. Limitations of the present study are the small sample size and the lack of analysis of other variables that reflect the tissue deficiency of folate and vitamin B_{12}, such as MMA and tHcy. Further, this study did not collect any data about the iron status of the population, as this would change the associations between iron status and the erythrocyte indexes. Also, we did not evaluate the dietary intake of folate to better assess the folate status of participants.

To get better and more accurate results, further studies are needed related to the effect of other variables, such as tHcy and MMA, on serum folate and vitamin B_{12} concentrations. In addition, future studies could reveal the impact of folate and vitamin B_{12} supplementation on the concentrations of the mentioned biomarkers.

Conclusion

In conclusion, the prevalence of low folate and vitamin B_{12} concentrations is high in our community, especially in men compared to women. Regarding the impact of the deficiency of these two vitamins on overall health, further investigations with more participants are required. We suggest an adequate intake of foods from animal sources and regular use of green leafy vegetables, education about correct methods of cooking and checking the implementation of a folic acid fortification program in our community is required. In addition, monitoring hematological indices, such as RBC, Hb, HCT, PDW, RDW, MCV and MPV, could be useful in people with folate and vitamin B_{12} deficiency.

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