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Premarital screening of 466 Mediterranean women for serum ferritin, vitamin B12, and folate concentrations

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Background/aim: Iron, folate, and vitamin B12 serum levels are closely related with dietary habits and have an essential role in the healthy development of a fetus. We aimed to investigate hemoglobin, ferritin, folate, and vitamin B12 levels in preconceptional women in an area where a plant-based diet referred to as Mediterranean cuisine is commonly used.

Materials and methods: The study population included 466 women between the ages of 18 and 45 years admitted for thalassemia screening. Sociodemographic variables and history of menometrorrhagia, pica, and dietary habits were collected. Serum vitamin B12, folate, ferritin, and hemoglobin levels were measured. Ferritin of <12 µg/L, vitamin B12 of <200 pg/mL, and folate of <4 ng/mL were accepted as deficiencies. Hemoglobin level of <12 g/dL was classified as anemia.

Results: Polymenorrhea was present in 11.7% and hypermenorrhea in 24.8% of women. Anemia was detected in 24.9% and thalassemia trait in 3.0% of women. Low ferritin levels were observed in 46.1%, vitamin B12 in 21.6%, and folate in 3.4% of women. In the group with low vitamin B12, decreased meat consumption was more prevalent (27.5% vs. 16.9%; P = 0.019).

Conclusion: Vitamin B12 and iron are the main micronutrients depleted in our community. This necessitates implementing a public health program for women consuming a Mediterranean diet.

Key words: Vitamin B12, folate, ferritin, anemia

1. Introduction

Iron, folic acid, and vitamin B12 have essential roles for the healthy development of a fetus during pregnancy. Worldwide, iron deficiency is the most common form of microelement deficiency among women, and it is related to preterm delivery and adverse pregnancy outcomes (1). Anemia can affect all age groups and the whole population; however, the most important health impact is seen during pregnancy. According to the estimations of the World Health Organization (WHO), the prevalence of anemia in Turkey is 32% in preschool girls, 40% in pregnant women, and 26% in women of reproductive age (2). Therefore, iron deficiency and related anemia is a significant health issue in Turkey, like in other developing countries.

Pregnancy is a period of rapid growth and cell differentiation that requires a high rate of DNA synthesis for both the mother and the fetus (3). This increased DNA synthesis is associated with a great need for vitamin B12 and folate. The vitamin status of the embryo and fetus depends on the maternal vitamin status and materno-fetal transfer of vitamins (4). Folate acts as an important cofactor during the metabolism of one-carbon groups and has essential roles on regulation of gene expression, protein synthesis, cell proliferation, and bone marrow function (5). Deficient preconceptional folate level is responsible for neural tube defects (NTDs), and a decrease was obtained in countries with folic acid fortification programs (6). Main dietary folate sources are animal products and leafy vegetables (7). Vitamin B12 shows close metabolic association with folate. It functions in the conversion of propionyl-CoA to succinyl-CoA (independent of folic acid) and transfer of a methyl group to homocysteine to form methionine (folic acid-dependent) (8). This step is important in myelin formation, fatty acid degradation, and protein and DNA synthesis (8,9). Vitamin B12 deficiency can be related to abnormal neural development and behavior (10). There has been an ongoing debate about vitamin B12 deficiency as a risk factor for fetal malformations and especially

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NTDs. Up to the beginning of the new millennium, the protective role of vitamin B12 fortification in reduction of NTD incidence remained uncertain. It was shown that deficiency of vitamin B12 may be an independent risk factor almost tripling the risk of NTDs (11,12), and several recent publications suggested that combination of vitamin B12 with a folic acid fortification program may reduce NTDs more efficiently than folic acid fortification alone (9,13).

All the vitamin B12 in nature is produced by microorganisms. Therefore, meat and dairy products are the only dietary sources of vitamin B12. Vegetarian diet, gastric disease, and pernicious anemia are the most common causes of vitamin B12 deficiency (7). Vegetable-based diets may lead to vitamin B12 deficiency. The Mediterranean diet is a traditional food pattern in the olive oil-producing areas of the Mediterranean basin. The diet is rich in vegetables, fruits, legumes, nuts, and olive oil, with a limited consumption of meat and poultry (14). The southwest (Aegean region) of Turkey is located in the Mediterranean area and traditionally people consume a Mediterranean diet. Vitamin and mineral deficiency in pregnant women is quite common in Turkey and we supposed that the Mediterranean diet caused extra risk for this vulnerable population (1). In this study, we primarily aimed to investigate the hemoglobin, folate, and vitamin B12 levels in women just before marriage in the southwest region of Turkey where a Mediterranean-style diet is commonly used. Our secondary aim was to evaluate the effects of sociodemographic, nutritional, and menstrual properties of these women on their vitamin, hemoglobin, and ferritin levels.

2. Materials and methods

This prospective study was carried out between January 2013 and December 2013. The study population was composed of women attending the Thalassemia Screening Laboratory of the Local Public Health Directorate for mandatory thalassemia trait screening before civil marriage. Because of the high rate of thalassemia trait in our region it is mandatory to give blood samples before marriage, and premarital consultation is given in the presence of thalassemia trait. The study population was composed of women between the ages of 18 and 45 years. Patients on vegetarian diets; with previous history of anemia, renal disease, or alcohol consumption; with evidence of malabsorption; with BMI below 18.5 kg/m^2; or taking vitamin or iron supplementation were excluded from the study. A structured questionnaire was administered to gather information including age, educational status, income level, heavy or frequent menstruation, and dietary habits. A menstruation frequency of less than 21 days apart was accepted as polymenorrhea. Menstruation requiring more than 3 pads in a day and subjectively described as heavy by the women was categorized as hypermenorrhea. Consumption of animal-source food (especially red meat), fresh fruits and vegetables, any materials consumed except ordinary food, and tea was recorded. Pica is a frequent problem in this region and was enquired about accordingly. Weight and height of the subjects were obtained to calculate BMI.

The procedures were explained to all subjects and written informed consent was obtained. The study was approved by the regional ethics committee, and the study protocol conforms to the ethical guidelines of the Declaration of Helsinki as reflected in prior approval by the institution's human research committee.

Blood specimens from all subjects were obtained with a standard venipuncture technique after 8 to 10 h of fasting. Blood samples were collected using sterile tubes without any anticoagulant. Samples were refrigerated at 4 °C for a maximum of 8 h; serum and red blood cell pellet were separated and frozen until the analysis. Hemoglobin measurement was performed via photometric assay (Abbott Cell-Dyn 3700, Abbott Laboratories, Abbott Park, IL, USA) on the same day. Ferritin, vitamin B12, and folate measurements were performed using a chemiluminescence assay (Siemens ADVIA Centaur immunoassay, Tarry Town, NY, USA).

Serum vitamin B12 level below 200 pg/mL (15) and folate level below 4 ng/mL (16) were accepted as deficiencies. Hemoglobin level lower than 12 g/dL was considered as anemia according to WHO criteria (17,18), and for the ferritin level 12 µg/L was taken as a cut-off for iron deficiency (2,19).

Continuous variables are expressed as mean ± standard deviation (SD). To analyze the differences between groups, the independent Student t-test was used for continuous variables, and the chi-square test was used for nominal variables. Statistical analysis was performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

3. Results

Overall, 466 women were evaluated after exclusion of the ineligible ones. The sociodemographic characteristics of the participants are summarized in Table 1. The mean age of the women was 25.2 ± 3.7 years (range: 20–41). BMI of the population was 22.1 ± 2.5 kg/m^2. Polymenorrhea and hypermenorrhea were reported in 11.7% and 24.8% of the respondents, respectively. Results of the standard blood count and folic acid, vitamin B12, ferritin, and HbA2 levels are presented in Table 2. None of the evaluated samples were found to have an increased MCV level (megaloblastic anemia). Overall, anemia was detected in 24.9% of cases (<12 g/dL) according to WHO criteria (2). Elevated HbA2

Table 1. Demographic characteristics and dietary habits of the study population.

Characteristics	Total number evaluated[1]	n (%)
Women with polymenorrhea	402	47 (11.7)
Women with hypermenorrhea	408	101 (24.8)
Education level		
University level		190 (40.8)
High grade level	466	193 (41.4)
Primary level		78 (16.7)
No education		5 (1.1)
Low income*	418	249 (77.1)
Current smokers	463	106 (22.9)
Dietary intake of		
Meat and fish, below 5 portions per month	448	86 (19.2)
Vegetables or fruit, below 1 portions per day	458	181 (39.5)
Consuming more than 5 tea glasses of tea per day	457	90 (19.7)

[1]Sample size varies due to missing values, *income level below 500 US dollars.

Table 2. Laboratory results of the study population and percentage of women with abnormal values.

Variable	Mean ± SD	Minimum	Maximum	Women with abnormally low result*
Hemoglobin (g/dL)	12.7 ± 1.5	7.3	17.0	24.9%
Hematocrit	37.9 ± 3.9	25.6	50.8	35.8%
MCV	81.8 ± 6.9	59.0	96.0	26.0%
MCH	27.5 ± 3.1	17.1	38.7	30.4%
MCHC	33.4 ± 2.0	23.1	41.6	10.2%
RDW	13.5 ± 0.7	10.6	17.1	5.3%
Ferritin (µg/L)	20.4 ± 20.3	1.2	161.0	46.1%
HbA2	2.6 ± 0.5	0.9	5.6	3.1%
Folic acid (ng/mL)	7.0 ± 3.3	1.0	31.0	3.0%
Vitamin B12(pg/mL)	283.4 ± 108.3	82.0	996.0	21.6%

*Cut-off was taken as <12 g/dL for hemoglobin, <12 µg/L for ferritin, <4 ng/mL for folate, and <200 pg/mL for vitamin B12.

levels were noted in 14 (3.0%) women. Abnormally low ferritin levels were observed in 214 patients (46.1%), of which 57.0% had normal hemoglobin levels despite low serum ferritin. Vitamin B12 deficiency with a cut-off value of 200 pg/mL was detected in 21.6% of the study population and was much more prevalent than folic acid deficiency (3.0%). Dietary intake was evaluated according to the intake of specific nutrients. Eighteen women (3.4%) reported a habit of pica, and 22.0% of the women were consuming less than 2–3 portions of meat in a month. In the group with low vitamin B12 level, 27.5% of women were consuming less than 2–3 portions of meat in a month, whereas this was only 16.9% in group with normal vitamin B12 levels (P = 0.019).

Hemoglobin, ferritin, folic acid, and vitamin B12 levels were evaluated with respect to the demographics, menstrual disorders, and characteristics of the women (Table 3). Univariate analysis revealed that hemoglobin and ferritin levels were significantly related to the presence of hypermenorrhea (P = 0.04) and pica (P = 0.05), respectively. On the other hand, higher monthly income was associated with better vitamin B12 levels (P = 0.03).

4. Discussion

The current study showed that vitamin B12 and iron deficiency is an important problem in women of reproductive age. On the other hand, probably due to dietary habits, folate deficiency was very scarce in this

Table 3. Univariate comparisons of the results with demographics and characteristics of the women.

		Hemoglobin	Ferritin	Folic acid	Vitamin B12
Age:	<25 years	12.6 ± 1.5	20.8 ± 20.4	7.3 ± 3.1	283 ± 110
	≥25 years	12.7 ± 1.4	19.9 ± 20.3	6.7 ± 3.4	284 ± 107
	P-value	>0.05	>0.05	>0.05	>0.05
BMI:	<20	12.6 ± 1.5	18.1 ± 19.1	6.9 ± 2.8	294 ± 111
	≥20	12.7 ± 1.5	21.2 ± 20.8	7.0 ± 3.4	279 ± 107
	P-value	>0.05	>0.05	>0.05	>0.05
Polymenorrhea:	Present	12.7 ± 1.5	19.3 ± 19.3	7.0 ± 3.3	291 ± 112
	Absent	12.6 ± 1.5	19.8 ± 16.0	6.4 ± 2.4	288 ± 94
	P-value	>0.05	>0.05	>0.05	>0.05
Hypermenorrhea:	Present	12.3 ± 1.5	20.6 ± 19.9	6.9 ± 3.1	287 ± 106
	Absent	12.7 ± 1.5	17.5 ± 19.8	7.0 ± 3.4	289 ± 120
	P-value	0.04	>0.05	>0.05	>0.05
Education:	Less than university	12.6 ± 1.5	21.1 ± 22.3	7.2 ± 3.1	279 ± 113
	University or above	12.8 ± 1.4	19.2 ± 17.1	6.6 ± 3.4	288 ± 101
	P-value	>0.05	>0.05	>0.05	>0.05
Income:	<500 dollars	12.7 ± 1.4	21.1 ± 20.3	7.0 ± 3.1	272 ± 107
	≥500 dollars	12.6 ± 1.6	19.0 ± 19.9	6.5 ± 3.3	295 ± 108
	P-value	>0.05	>0.05	>0.05	0.03
Pica:	Present	12.2 ± 1.3	20.6 ± 20.5	6.9 ± 3.3	282 ± 109
	Absent	12.7 ± 1.5	10.9 ± 10.4	6.7 ± 2.4	275 ± 86
	P-value	>0.05	0.05	>0.05	>0.05
Smoking status:	Smoker	12.5 ± 1.3	23.0 ± 20.7	6.8 ± 2.5	275 ± 115
	Nonsmoker	12.2 ± 1.5	19.5 ± 20.2	7.2 ± 3.4	286 ± 106
	P-value	>0.05	>0.05	>0.05	>0.05
Daily tea consumption:	≥5 tea glass	12.7 ± 1.5	19.7 ± 21.5	6.9 ± 2.8	283 ± 106
	<5 tea glass	12.7 ± 1.5	20.4 ± 20.1	7.0 ± 3.3	282 ± 110
	P-value	>0.05	>0.05	>0.05	>0.05
Meat or fish consumption:	≤2-3 por./month	12.7 ± 1.5	21.8 ± 23.2	7.0 ± 3.3	273 ± 127
	> 3 por./month	12.6 ± 1.5	19.8 ± 19.6	6.9 ± 3.2	285 ± 103
	P-value	>0.05	>0.05	>0.05	>0.05
Vegetable/fruit consumption:	<1 por./day	12.6 ± 1.5	21.2 ± 22.8	7.1 ± 3.1	274 ± 91
	≥1 por./day	12.7 ± 1.4	19.6 ± 18.7	6.8 ± 3.3	291 ± 118
	P-value	>0.05	>0.05	>0.05	>0.05

geographic region. In this study, 21.6% of women had low vitamin B12, 46.1% had depleted iron stores, and 25% were anemic. These women were examined just before marriage and they are candidates for becoming pregnant. We assumed that measurements obtained in this study would give important data regarding the micronutrient levels of women before conception. Since Turkish women do not generally seek preconceptional medical care, many women will enter pregnancy with severely compromised micronutrient stores, which will further deteriorate with the physiologic demands of the pregnancy.

Dietary habit is an important factor determining the micronutrient status of our bodies and it shows great variation according to cultural factors and geographical regions. Nutrition-related iron deficiency is the main cause of anemia throughout the world (2). There is no other report from Turkey that fits to our study population. However, in a hospital-based study that we conducted in women in early pregnancy in the same geographical region, iron, vitamin B12, and folate deficiencies were detected in 40.3%, 29.8%, and 0.5% of the women, respectively (1). Similarly, folate deficiency was very minute, and iron and vitamin B12 deficiency were more remarkable in both studies.

Although we detected iron deficiency in 46.1% of the women, iron deficiency anemia was detected in 24.9% of the women. Our results emphasize that anemia was affecting about one-quarter of the study population, whereas iron deficiency was a more common problem affecting almost half of the population, and the women with depleted stores will probably develop iron deficiency anemia with increasing demands during pregnancy.

Several previous reports documented the variable prevalence of anemia in women of reproductive age. In a study from the northwest of Turkey, anemia prevalence was found to be 32.8% among nonpregnant women of reproductive age (20), and it was found at 40.08% in Southeast Anatolia (21). We detected less anemia compared to previous studies; however, our result is very similar to WHO estimations in 2005 (25.0% vs. 26.2%) (2). Turkey was accepted as an area with moderate prevalence of anemia (20.0%–39.0%) according to WHO data (2). Therefore, the difference from previous studies in different parts of the country probably originated from the variance of socioeconomic factors depending on time and place and reflecting different sides of an extreme.

In a previous study investigating folate level in adolescent girls, prevalence of folate deficiency was reported as 16.3% in the northwest of Turkey (22). Eating habits of that study region were completely different from ours. It was a more meat-based diet compared to the Mediterranean region, which probably create the difference. In a study that we performed previously for first trimester pregnancies, we detected a low level of folate deficiency (0.5%) (1). The relatively low prevalence in our study could be explained by the high vegetable intake in this region of Anatolia.

Folate deficiency is recognized as the main cause of the formation of NTDs. It was shown to be associated with high prevalence of NTDs in folate-deficient areas of the world (23). However, the potential prophylactic value of folic acid fortification for reduction of NTDs is questionable in such a region consuming a folic acid-rich diet. It was also shown that folic acid fortification programs were not successful to diminish NTDs. Vitamin B12 is the second main cause of NTDs and functions in the same methyl donation pathway (23). Ray et al. compared NTD cases with control cases and reported an increased risk of NTDs in the presence of low maternal vitamin B12 levels (9). They concluded that there is a need to consider vitamin B12 supplementation along with folic acid fortification for the prevention of NTDs (9). In another study in Canada, Thompson et al. also highlighted the importance of vitamin B12 fortification along with folic acid to reduce NTDs more effectively (13). Vitamin B12 crosses the placenta during pregnancy and is present in breast milk. Pregnant and lactating women with depleted vitamin B12 stores and their breastfed infants may have very limited reserves of vitamin B12 and such infants can develop vitamin B12 deficiency within months of birth (24,25). Undetected and untreated vitamin B12 deficiency not only increases the risk of NTDs, but it may also result in severe and permanent neurological damage in infants (26). Furthermore, an Irısh study among pregnant women documented that a maternal vitamin B12 level of less than 250 ng/L was associated with the highest risk of NTDs (27). This cut-off was higher than the commonly accepted one. Therefore, vitamin B12 supplementation in the preconceptional period or in early pregnancy would be especially beneficial in populations with relatively high vitamin B12 deficiency like our community.

The decreased vitamin B12 level in our population most likely originated from dietary habits and decreased meat consumption. A previous study among Greek and Albanian immigrants similarly showed low levels of serum vitamin B12 concentration compared to 25 different countries (28). Another study from Spain detected vitamin B12 deficiency of 11% among an adult Mediterranean population including both men and nonpregnant women (29). Therefore, dietary habits of the region in question are important in tailoring fortification programs and micronutrient supplementation. Apart from dietary habits, hypermenorrhea and pica were shown to be associated with low hemoglobin and ferritin levels, and increased monthly income was related with better vitamin B12 levels, which probably originated from better nourishment of this group. Therefore, extra attention needs to be given to women with low income, hypermenorrhea, and a habit of pica.

Our study group was composed of women taking no vitamin or iron supplementation, and this is the main strength of this study to show the micronutrient status in this population. Another important point is that we evaluated women just before their marriage from different socioeconomic classes and therefore our sample is a good representation of the whole population. The limitation is the change in eating habits of people depending on seasonal variances, which was disregarded in our study. As was shown in an earlier study (30), it may cause some oscillations in serum micronutrient levels.

In conclusion, our study demonstrated that vitamin B12 and iron deficiency may be a public health problem in women of reproductive age in Turkey. A Mediterranean diet may be the aggravating factor for vitamin B12 and iron deficiency. This necessitates implementing a public health program for prevention and early diagnosis of iron and vitamin B12 deficiency in women of child-bearing age.

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