World Journal of Nutrition and Health, 2019, Vol. 7, No. 2, 35-41
Available online at http://pubs.sciepub.com/jnh/7/2/2
Published by Science and Education Publishing
DOI:10.12691/jnh-7-2-2

Folate and Homocysteine Status among the Egyptian Adults

Samar Abd-El Mohsen Ali, Maged Ossama Aly*

Nutrition Department, High Institute of Public Health, Alexandria University, Egypt
*Corresponding author: dr.magedossama@gmail.com

Received October 05, 2019; Revised November 06, 2019; Accepted November 11, 2019

Abstract **Background:** Folate is a key nutrient that plays a major role in promoting health, prevention of neural tube defects in infants, and reducing the risk of cardiovascular diseases among adults and the elderly. **Objective:** The aim was to assess the dietary folate status among the Egyptian adults and its relation to some laboratory parameters. **Subjects:** A cross-sectional study was conducted on 100 apparently healthy Alexandria University employees of both sexes. Data was collected for socio-economic characteristics and dietary intake was assessed by a 24-h recall method. Blood samples were collected from participants and serum folate was analyzed using a microbiological assay, while High-Performance Liquid Chromatography (HPLC) was used for the determination of homocysteine. **Results:** The mean dietary folate intake of participants was 257.6 ± 126.9 µg/day with higher intake among males than females. Overall mean serum folate level was 9.7 ± 5.7 nmol/l and folate deficiency identified among 21% of participants. The mean homocysteine level of the participants was 12.8 ± 4.8 µmol/l and hyperhomocysteinemia was present in 54% of the whole sample. Women in childbearing age had a higher mean dietary folate intake than that in older females (252.9± 135.6 vs. 234.5± 91.3µg/day respectively) and only 15.1% of them had adequate folate intake. **Conclusions:** The highest percentage of participants had folate intake lower than RDA. Favorable serum folate was detected in most of the participants while more than half of participants had hyperhomocysteinemia.

Keywords: *folate, homocysteine, adults, childbearing age women, HPLC, Egypt*

Cite This Article: Samar Abd-El Mohsen Ali, and Maged Ossama Aly, “Folate and Homocysteine Status among the Egyptian Adults.” *World Journal of Nutrition and Health*, vol. 7, no. 2 (2019): 35-41. doi: 10.12691/jnh-7-2-2.

1. Introduction

Folate is an essential member of water-soluble B vitamins that are present in human cells in many interchangeable forms [1]. It is naturally found in various food sources such as leafy vegetables, asparagus, broccoli, beets, legumes, egg yolks, liver, some citrus fruits, bananas, nuts , seeds, wheat germ, and fortified and enriched food products, like bread, and some cereals [2]. Folate cannot be stored in the body so, it is mandatory to consume folate-rich foods daily. The Recommended Nutrient Intakes (RNI) for folate differ among countries and range from 300 to 400 µg/d [3,4]. Folate has a crucial role in the cell division process for many reasons such as being a vital component for amino acids interconversions, and biosynthesis of the building blocks of DNA function, integrity, and repair. Folate as methyl group donor is required to convert homocysteine into methionine [3,5].

During the conversion of methionine to cysteine, homocysteine is formed. It is catabolized either to methionine by remethylation reaction or to cystathionine by trans-sulfuration reaction. Remethylation reaction of homocysteine is stimulated by methionine synthase (folate and vitamin B12 dependent) and betaine-homocysteine methyltransferase. The methyl group donor for the methylation reaction is 5-methyl-tetrahydrofolate (a derivative of folic acid), and betaine [6].

Inadequate dietary intake makes many people are vulnerable to folate deficiency. Folate and vitamin B12 deficiencies are the most common causes of macrocytic megaloblastic anemia [7,8]. Folate deficiency during the first weeks of pregnancy has some unpleasant outcomes. On one hand, it increases the risk of neural tube birth defects such as spina bifida or anencephaly, and the incidence of abruptio placentae, preeclampsia, spontaneous abortion, stillbirth, preterm delivery, and low birth weight [9,10,11]. On the other hand, low folate status causes hyperhomocysteinemia. A high plasma homocysteine level is a major risk factor for a variety of diseases such as cardiovascular, mental disorders, and possibly cancer such as colon cancer [10,12,13,14].

Many studies reported dietary folate intake among the population in different countries [15,16,17,18,19]. Yvonne Martiniak and colleagues reported that median intake of dietary folate of almost 14 thousand participants of the German National Nutrition Survey II (NVS II), ranged from 191 µg/d (men) and 168 µg/d (women) without fortification. Thus, only 12.4% of men and 5.9 % of women met the 300 µg/day folate intake recommendation of the nutrition societies of Germany, Austria, and Switzerland [20]. In Egypt, folate status of 1900 participants was assessed by determining serum folate but without dietary folate intake evaluation. The mean serum folate level was 13.4 nmol/l and 13.7% had folate deficiency. 14.7% of women of reproductive age and 12.6% of adolescents were folate deficient [21].

Due to insufficient data about dietary folate intake in Egypt, it was valuable to investigate the dietary folate status among the Egyptian adults and its relation to some laboratory parameters.

2. Materials and Methods

2.1. Study Design and Sampling

A cross sectional study was carried out on 100 apparently healthy staff members, clerks and workers attending High Institute of Public Health and Medical Researches Institute, Alexandria university from June 2018 to August 2018. All participants were selected randomly from the target institutions. Subjects were informed about the purpose of the study and their verbal consent was obtained. Pregnant and breast-feeding women, those taking oral contraceptives, those taking medication for treatment of cardiac or respiratory conditions, those taking antipsychotic medications, those undergoing chemotherapy, and those taking any dietary supplements were excluded from the study.

2.2. Data Collection and Study Questionnaire

A pre structured interviewing questionnaire was used to collect data about socio-economic characteristics as age, gender, occupation, marital status, income, and educational level. Education was categorized into low (illiterate, read and write, and primary education), middle (preparatory, and secondary education), and high education (university education or above). Also, current smoking status of participants was reported.

Body weight and height were measured according to the method described by Gibson [22], body mass index (BMI) was calculated using the following equation: weight(kg)/height (m^2). BMI was classified into underweight (BMI <18.5 kg/m^2), normal body weight (BMI=18.5-24.9 kg/m^2), overweight (BMI=25-29.9 kg/m^2), and obese (BMI ≥30 kg/m^2) [23].

Dietary intake was assessed by a 24-h recall method at the time of interview. The participants were asked to recall and report all foods and beverages consumed by him/her during the previous day, for 3 consecutive days. This was aided using a food and plates model to determine the actual amount of food consumed. Dietary intake data for a 24 h diet recall was analyzed to obtain the mean daily intake of energy and folic acid using the Egyptian food composition tables provided by the National Nutrition Institute [24]. To assess the nutritional adequacy of folic acid, it was expressed as a percentage of the dietary reference intake (DRI) [25].

Laboratory investigation, all subjects were required to fast from 10-12h, then 10ml of blood was drawn in order to determine serum folate and homocysteine level. The obtained serum was stored at -20ºc until the analysis time in Central laboratory of High Institute of Public Health. A commercial kit (Chromosystems, GhbH) for the High-Performance Liquid Chromatography (HPLC) was used to determine total homocysteine level. Homocysteine level was categorized into normal (<14 µmol/l) and elevated (≥14 µmol/l) [26]. Serum folate was determined using Elecsys Folate III kit then classified into normal (≥10 nmol/l) and deficient (<10 nmol/l) [27].

2.3. Statistical Analysis

Data management was conducted using the Statistical Package for Social Science (SPSS) version "21" software (Chicago, Illinois, US) [28]. Data was presented tabular, graphically and mathematically using the mean and standard deviation (SD). For all analyses P value < 0.05 was used to detect statistically significant difference. Data were analyzed using Chi squared test for analysis of categorical data and Fisher exact (FEp) techniques were used as a correction for Chi-square test when more than 20% of the cells have expected count less than 5 for comparing between the two groups. Mann Whitney test for abnormally distributed quantitative variables, to compare between two studied groups. Pearson coefficient to correlate between two quantitative variables.

2.4. Ethical Considerations

This study was conducted according to the guidelines laid down for medical research involving human subjects and was approved by Ethics Committee of High Institute of Public Health, Alexandria University, Egypt. Weight measurement was taken following all privacy procedures and all collected data were kept confidential. Verbal consents were obtained from all subjects after informing them about the study purpose and they had the right to participate or not in the study. There was no conflict of interest.

3. Results

3.1. Socio-economic Characteristics and Current Smoking Status

Table 1 shows that mean age of participants was 36.5 ± 12.5years. Number of females was higher than males. More than half of subjects (55%) were highly educated. The highest percent of participants were clerks and 52% of participants had enough income. Mean BMI of the subjects was 26.9 ± 5.1 kg/m^2. Only 9% of participants were smokers at the time of study.

Table 1. Socio-economic characteristics and current smoking status of participants

Variables	No. (%)
Gender	
Male	29 (29%)
Female	71 (71%)
Age (year)	
20 – 29	39 (39%)
30 -39	25 (25%)
40 – 49	15 (15%)
50- 59	17 (17%)
≥60	4 (4%)
Mean ± SD.	36.5 ± 12.5
Education	
Low	5 (5%)
Middle	40 (10%)
High	55 (55%)
Occupation	
staff members	12 (12%)
Clerk	76 (76%)
Worker	12 (12%)
Marital status	
Married	62 (62%)
Single	32 (32%)
Widow	4 (4%)
Divorced	2 (2%)
Income	
Not enough	47 (47%)
Enough	52 (52%)
Enough and saving	1 (1%)
BMI (kg/m2)	
Underweight	3 (3%)
Normal weight	37 (37%)
Overweight	34 (34%)
Obese	26 (26%)
Mean ± SD.	26.9 ± 5.1
Current smoking status	
No	91 (91%)
Yes	9 (9%)

3.2. Mean Energy and Dietary Folate Intake and Laboratory Investigation

Table 2 shows that mean energy intake was1740.8 ± 728.5 kcal/day with a higher intake among females than males. Dietary folate intake of participants was 257.6 ± 126.9 µg/day with higher intake among males than females (280.6 ± 129.7 and 248.3 ± 125.5 µg/day respectively). Only 15% of participants had dietary folate intake ≥400 µg/day with a higher percent among females than males (60% and 40% respectively). Less than half of participants (42%) had had dietary folate intake ≥267 µg/day.

Regarding laboratory investigation, mean serum folate of participants was 9.7 ± 5.7 nmol/l. The highest percent of them (79%) had normal serum folate with a higher percent among males than females. While 21% of participants had deficient serum folate. A higher percent of females (25.4%) had deficient serum folate in comparison to 10.3% of males. Mean homocysteine level of the participants was 12.8 ± 4.8 µmol/l. More than half of subjects (54%) had elevated homocysteine level with a higher percent in females than males (57.7% and 44.8% respectively).

3.3. Dietary Folate Intake and Laboratory Investigations of Childbearing Age Females

Table 3 shows that mean dietary folate intake of women in childbearing age was higher than that in older females (252.9± 135.6 and 234.5± 91.3 µg/day respectively). The majority of women in childbearing age (84.9%) had inadequate dietary folate intake (<400 µg/day). Almost three quarter of childbearing age women (75.5%) had normal serum folate and only 24.5% had serum folate deficiency. A higher percent of older women (61.1%) had elevated homocysteine level than childbearing women (56.6%).

Table 2. Mean dietary folate intake and its adequacy and laboratory investigations of participants

Variables	Female No. (%)	Male No. (%)	Total No. (%)	P value
Energy Mean ± SD.	1758.4 ± 733	1697.8 ± 728.4	1740.8 ± 728.5	*P* =0.646
Dietary folate intake (µg/day) Mean ± SD.	248.3 ± 125.5	280.6 ± 129.7	257.6 ± 126.9	*P*= 0.203
Folate adequacy (%)				
≥100% of RDA (≥400 µg/day)	9 (60%)	6 (40%)	15 (15%)	^{FE}p=0.359
<100% of RDA (<400 µg/day)	62 (72.9%)	23 (27.1%)	85 (85%)	
≥ 2/3 of RDA (≥267 µg/day)	28(39.4%)	14(48.3%)	42(42%)	*P*=0.416
<2/3 of RDA (<267 µg/day)	43(60.6%)	15(51.7%)	58(58%)	
Laboratory investigations				
Serum folate (nmol/l)				
<10 (deficient)	18 (25.4%)	3 (10.3%)	21 (21%)	*P*=0.095
≥10 (normal)	53 (74.6%)	26 (89.7%)	79 (79%)	
Mean ± SD.	9.3 ± 5.6	10.9 ± 5.9	9.7 ± 5.7	P= 0.143
Homocysteine level (µmol/l)				
<14 (normal)	30 (42.3%)	16 (55.2%)	46 (46%)	*P*=0.240
≥14 (elevated)	41 (57.7%)	13 (44.8%)	54 (54%)	
Mean ± SD.	13.3 ± 4.9	11.6 ± 4.6	12.8 ± 4.8	*P*= 0.119

SD: standard deviation, P value: based on Chi Squared test, P value based on Mann Whitney test, FEp value based on Fisher exact test.

Table 3. Distribution of females according to their age with dietary folate intake and laboratory investigations

Variables	Age of females (years) 20 – 49 (females of childbearing age) (n=53)	Age of females (years) ≥50 (older females) (n=18)	P value
Dietary folate intake (µg/day)			
Adequate (≥400 µg)	8 (15.1%)	1 (5.6%)	^{FE}p= 0.432
Inadequate (<400 µg)	45 (84.9%)	17 (94.4%)	
Mean ± SD.	252.9± 135.6	234.5± 91.3	P=0.905
Serum folate (nmol/l)			
<10(deficient)	13(24.5%)	5(27.8%)	^{FE}p= 0.763
≥10 (normal)	40(75.5%)	13(72.2%)	
Mean ± SD.	9.5± 6.0	8.6± 4.4	P=0.900
Homocysteine level (µmol/l)			
<14 (Normal)	23(43.4%)	7(38.9%)	P=0.738
≥14 (Elevated)	30(56.6%)	11(61.1%)	
Mean ± SD.	13.1± 5.1	13.7± 4.1	P=0.812

SD: standard deviation, P value: based on Chi Squared test, P value based on Mann Whitney test, FEp value based on Fisher exact test.

3.4. Correlation between Dietary Folate Intake and Laboratory Investigations

Table 4 revealed that there was a significant direct positive correlation between dietary folate intake and serum folate. In contrast, the increase in dietary folate intake was significantly associated with the decrease in homocysteine level. Also, there was a significant inverse relationship between serum folate and homocysteine level.

Table 4. Correlation between dietary folate intake and serum folate and homocysteine

Variables	R	P value
Dietary folate intake Vs serum folate	0.921*	<0.001*
Dietary folate intake Vs homocysteine	-0.911*	<0.001*
Serum folate Vs homocysteine	-0.996*	<0.001*

r: Pearson coefficient, *P<0.05: significant.

3.5. Effect of AGE on Serum Folate and Homocysteine Level

Regarding to serum folate in Figure 1, it was the highest in males aged 40-49 years, followed by that in males aged 30- 39 years and the lowest level obtained in males aged ≥60 years. While the highest homocysteine level was prevalent in males aged ≥60yeasr followed by those who aged 50-59 years. Figure 2 shows that females aged ≥60yeas had the highest serum folate level and the lowest homocysteine level.

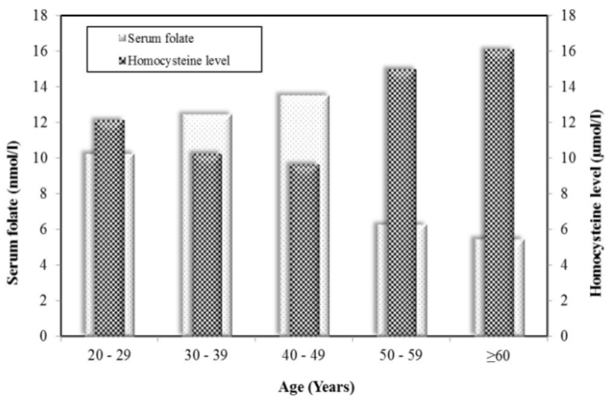

Figure 1. Serum folate and homocysteine levels of different age categories in males

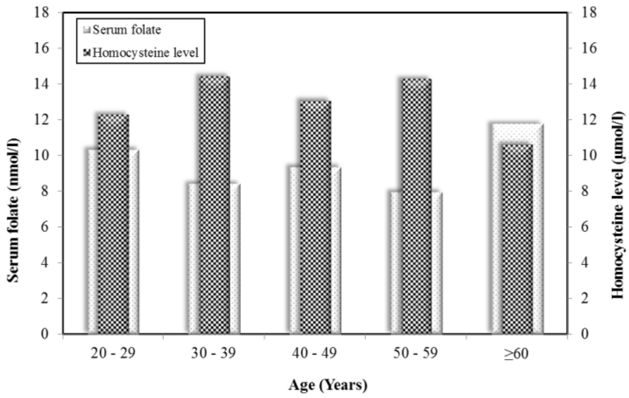

Figure 2. Serum folate and homocysteine levels of different age categories in females

4. Discussion

The level of folate, to be considered as an adequate intake, is when the consumption is above 2/3 of the recommended dietary allowance (400µg/day), which is 267µg/day [29]. In the current study the dietary folate intake of the subjects was lower than the adequate intake (267µg/day) which may be due to firstly, the use of 24h recall method that can lead to under-reporting since it relies heavily on the memory and honesty of participants as well as the difference in the tools used in it (pictures, household measurements) [3]. Secondly, due to the physical characteristics of folate, which is heat-labile where prolonged cooking at high temperatures causes up to 90% destruction of folate content of the food [30].

A study, done by Hefni et al., 2010, revealed that the dietary folate intake per capita in Egypt was 220µg/day which is lower than the current study result (257.6 ± 126.9 µg/day). This underestimation in Hefni study because (1) only 19 food types were used, (2) calculation was based on the sum of some folate forms analysed by HPLC, total folate content was not analysed by microbiological assay, and (3) data for cooking losses were not available for all foods [31].

Folate intake is widely variable among the different world populations which was 440 µg/day in Canadian [32], 380 µg/day in Brazilian [18], 250 µg/day in Swedish and

German and 123 μg/day in Omani population [15,33,34]. This variability is explained by the difference of ethnicity, dietary habits, cooking method and the food fortification strategies in these countries.

Regarding the adequacy of folate intake, only 15% of participants had the RDA equal to 400 μg/day and only 42% had 2/3 of RDA (≥267 μg/day). According to the IMMIDIET project, that investigated folate status in two European populations, found that 83% of Italian and 96% of English participants reached the lowest recommended intake of 200 mg/day because dietary patterns of these two groups included high consumption of food groups common with those included in the traditional Mediterranean diet, a pattern recently associated to a better folate nutritional adequacy [35].

In the present study folate intake of males was higher than females which was approved by others [8,17,36,37]. On the other hand, Malaysians males had lower folate intake than females (260 μg/day and 320 μg/day respectively) that was due to the importance of folate during the pregnancy because it reduces the incidence of neural tube defects, besides their better consumption-ability enable them to access higher folate diet including fruit and vegetables [3].

In the current study, serum folate was normal in 79% of participants that may be due to the fortification program in Egypt that began in 2008. This program aimed to fortify Baladi bread consumed by most of the Egyptians with iron and folic acid which provided a daily intake of approximately 12 mg of iron and 600 μg of folic acid to control iron and folate deficiencies [38].

In the present study, mean serum folate was 9.7 ± 5.7 nmol/l and males had a higher serum folate level than females due to the positive correlation between dietary folate intake and serum folate [17,39,40]. On the other hand, mean plasma concentration of folate was 29.5 nmol/l among Brazilians which was higher than the current study [18] because of the differences in dietary folate intake and fortification strategies. Contrariwise, the median plasma folate concentration was 14 nmol/l among the Swedish population [33]. More than half of our participants (54%) had an elevated homocysteine level while mild hyper-homocysteinemia (>14 nmol/l) was found in 17% of Finnish men and 6% of Finnish women [41].

Consistent with our result, many studies reported a positive relationship between folate intake (excluding folic acid supplements) and plasma folate concentrations [17,39,40]. Taiwanese study found that dietary folate intake is positively correlated with plasma folate levels [28]. Also, among Lebanese women, a significant positive correlation was observed between folate intake and its corresponding plasma levels. On the other hand, a study on the Finnish population cannot reveal this relation which may be due to methodological bias, as food consumption was from a single previous day, which on an individual basis is not accurate enough for quantitative assessment of vitamin intake [41].

The current study found an inverse association between dietary folate intake and homocysteine level which was previously proved in many studies [44-49]. Owusu and her colleagues reported that there were no significant correlations between dietary folate intake in Ghanaian populations and serum folate in either London or Accra but there were negative correlations between serum folate and plasma homocysteine [19].

In the current study dietary folate intake of women of childbearing age was higher than older age women (252.9± 135.6 and 234.5± 91.3 μg/day respectively) due to the importance of folate during pregnancy and its protective role against neural tube defects (NTD) [15]. On the other hand, other studies reported that dietary folate intake among older women was higher than females of childbearing age [33,50].

Folic acid deficiency can lead to NTD, which is a major cause of serious disabilities and mortality among infants. Related disabilities include anencephaly, spina bifida, and encephalocele [51]. World Health Organization (WHO) recommends that all women planning to become pregnant take at least 400 mcg of supplemental synthetic folic acid daily, in addition to eating a folate-rich diet, from 3 months before conception up to 12 weeks of gestation [52,53]. Only 15.1% of the present study women of childbearing age reached the recommended levels of dietary folate intake (400 μg/day). On the other hand, only 8.1% and 41.4% of premenopausal women in Italy and the United Kingdom reached this level [50].

Our dietary folate intake of women of childbearing age (252.9± 135.6 μg/day) in the present study seems low comparing with Brazilian women (315μg/day) [18], German women (300μg/day) [20], and women from the US (389 μg/day) that may be due to use of supplements and fortified foods [54]. Contradictory, low intake of dietary folate was observed in Malaysian women (202 μg/day) [55] and in Chinese women of childbearing age was 211 μg/day in the South and 189 μg/day in the North of China [56]. Korean women of childbearing age were found to be consuming insufficient quantities of folate (207 μg/day), largely due to their diets with low folate density and insufficient energy intakes [57].

Folate deficiency was prevalent in 24.5% of women of childbearing age with mean serum folate <9.5± 6.0 nmol/l. A study done in Egypt revealed that 14.7% of mothers had folate deficiency (<10 nmol/L) based on the estimation of serum folate [21]. Our result was consistent with Lebanese women of childbearing age where folate deficiency was prevalent in 25.1% and the mean folate level was 8.4 ng/ml (19.03 nmol/l) [43].

Elevated homocysteine level was observed in more than half (56.6%) of women of childbearing age with mean equal to 13.1± 5.1 µmol/l. The variance in homocysteine level does not depend only on serum folate but also on serum creatinine and vitamin B_{12} that were not assessed in the current study [58]. Korean study stated that plasma homocysteine concentration in women of childbearing age was 13 µmol/l. and 11% of the subjects evidenced hyper-homocysteinemia (>15 µmol/l) [57].

5. Conclusion and Recommendations

Mean dietary folate intake of participants was 257.6 ± 126.9 μg/day with a higher intake among males than females. Only 15% of participants had 100% of folate RDA while 42% had 2/3 of folate RDA. Most of subjects had favourable serum folate and more than half of them had hyperhomocyteinemia. Mean dietary folate intake of women in childbearing age was 252.9± 135.6 µg/day and only 15% had adequate folate intake. The highest percent of women in childbearing age had normal serum folate level but 56.6% had elevated homocysteine level.

Regular monitoring on the fortification program and folate status of vulnerable groups is required. Nutrition education is essential to increase the consumption of folate rich and fortified foods and to increase the awareness of community about benefits and deficiency consequences of folate. folate supplementation program for women in childbearing age is recommended.

Limitations of the Present Study

Assessment of dietary intake using the 24-h recall method led to underestimation of dietary folate intake because the majority of the participants had a favorable level of serum folate which reflects an adequate intake from the diet.

Acknowledgements

All authors approved the final version of the paper and would like to express the appreciation to participants who accepted to be a part of the research.

Conflict of Interest

There is no conflict of interest.

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