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Prevalence and factors associated with folate deficiency among Filipino women of child-bearing age

Aiza Kris M. Bernardo[1*], Normahitta P. Gordoncillo[1], Liezl M. Atienza[1], Maria Theresa M. Talavera[1] & Mariam C. Recuenco[2]

[1]*Institute of Human Nutrition and Food, College of Human Ecology, University of the Philippines Los Baños, Philippines 4031;* [2]*Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños, Philippines 4031*

ABSTRACT

Introduction: Folate deficiency is associated with many complications of pregnancy. A cross-sectional survey was conducted to determine the prevalence and factors associated with deficiency in red blood cell (RBC) folate among Filipino women of child-bearing age in the Province of Batangas, Philippines. **Methods:** A total of 184 Filipino women aged 15 to 49 years were interviewed on their socio-economic and demographic profiles. Mean energy and folate intakes were obtained using food recalls over a three-month period. RBC folate was used to measure long-term folate status and analysed through immuno-assay method. **Results:** About two in ten women were folate deficient based on the normal cut-off points (<400 ng/mL) preventive of neural tube defect-affected pregnancies. Respondents have very low intake of folate at 81 and 239 micrograms dietary folate equivalent (µg DFE) from dietary sources and with folic acid supplementation, respectively. Vegetable gardening and livestock raising ($p<0.10$) and use of folic-acid containing supplements showed significant correlations with folate status ($p<0.05$). Further, regression analysis showed that among the significant factors, the non-usage of folic acid-containing supplements showed increased likelihood of RBC folate deficiency by six times compared to users ($OR=6.391$, $p<0.10$). **Conclusion:** The findings of the study suggest a high prevalence of folate deficiency among Filipino women. Folate is an essential nutrient for healthy pregnancy. It is important that women, capable of bearing a child must assure adequate folate intake from foods and folic-acid containing supplements. The study recommends a more aggressive campaign on the importance of folate among women of child-bearing age.

Keywords: Folate, dietary folate equivalent, neural tube defects, red blood cell folate deficiency, women of child-bearing age

INTRODUCTION

Nutrition plays an essential role in maternal and child health, peri-conceptionally. A woman's nutritional status before and during pregnancy and during lactation helps determine the outcome of her pregnancy and the long-term health of herself and her child (Reifsnider & Gill, 2000). Precociously, when a woman enters the stage when she is capable of bearing a child, she needs to be prepared for the demands of pregnancy and subsequent lactation.

Micronutrient deficiency in women of child-bearing age can lead to birth defects (Kraemer & Zimmermann, 2007).

*Corresponding author: Aiza Kris M. Bernardo
Institute of Human Nutrition and Food, College of Human Ecology
University of the Philippines Los Baños, Philippines 4031
Tel: +639495362445; +69776442663; E-mail: ambernardo1@up.edu.ph
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Micronutrient deficiencies that present significant burdens to the totality of the child-bearing age women population are often included in nutrition surveys. Vitamin A, iron, iodine, vitamin C are examples. Folate is among the most missed nutrient in country-wide assessments. In the Philippines, for example, the most recent study on folate among Filipino women was conducted in 2008 as part of the 7th Food and Nutrition Research Institute (FNRI) National Nutrition Survey.

Evidences have been building on the importance of folate as an essential nutrient associated with healthy pregnancy outcomes. Folate is a member of the water-soluble B-vitamins. Folate, a term that denotes folate from foods and folic acid from fortified foods and supplements are expressed using dietary folate equivalents (DFE). DFE accounts for variances in folate bioavailability, of which dietary folate is low while the synthetic folic acid is more bioavailable. Women of child-bearing age within 15 to 49 years need 400 micrograms (µg) DFE/day from dietary folate and folic acid from supplements and fortified foods (Bailey, 1998). Certain conditions affect folate status: inadequate intake, increased needs, absorption and other factors associated with malabsorption and some medications (Allen, 2008).

Folate has been recognised in women of child-bearing age to be necessary for normal foetal development and to reduce the risk of babies born with neural tube defect (NTD). Folate deficiency is associated with several maternal and foetal conditions. These include fatal megaloblastic anaemia, pre-eclampsia, abruption placentae, spontaneous abortion, stillbirths, pre-term delivery, maternal morbidity, low infant birth weight, and developmental and adverse health outcomes such as birth anomalies (Molloy *et al.,* 2008).

The number of registered infant deaths in the Philippines has declined from 34 per 1000 live births in 1993 to 21 in 2017, which accounts for a total of 20,311 infant deaths (PSA, 2019). Congenital defects have been in the top ten leading causes of mortality in the country (IHG-UPM, 2019). In a study by Maceda & Alcausin (2017), one in every 36 infants was born with birth defect from the 2011-2014 data of the Philippine General Hospital, a state-own hospital, which is the largest tertiary and referral hospital in the Philippines. Specifically, NTDs (anencephaly Q00, encephalocoele Q01 and spina bifida Q05) are the 5th most common birth defect with 24 affected infants per 10,000 deliveries.

Folate status among women of child-bearing age is affected by different factors in varying degrees. These include socio-demographic and socio-economic factors such as age, income, education, among others. Genetic predisposition to gene polymorphism of 677C-T allele that encodes methylenetetrahydrofolate reductase (MTHFR) is a significant predictor of folate status. Certain physiological (rapid growth in adolescence, pregnancy and lactation) and pathological conditions (cancer, gastro-intestinal inflammation and disorder etc.) affect folate status through increased demands for folate intake and reduced absorption of folate, thereby reducing the circulating folate in the blood. Use of anti-folate drugs are associated with lower folate status. Anti-folate drugs include anti-cancer drugs, anti-epileptics, some anti-bacterial and anti-malarial drugs and oral contraceptive pills. Use of metformin, an anti-diabetic drug, also poses an issue on folate status (Bailey *et al.*, 2015). More importantly, diet affects folate status through inadequate intakes of folate-rich foods and folic acid supplements, as well as folate interaction with vitamins B_6, B_{12} and zinc. Lifestyle factors like smoking and alcoholism, also affect folate status through its effect on total dietary intake and reduced folate absorption (Bailey *et al.,* 2015; WHO, 2012).

Recent evidence showed that mandatory folic acid fortification in flour significantly reduced cases of NTDs in 13 countries (FFI, 2018), especially in countries with mandatory folic acid fortification act. Philippines already has mandatory wheat flour fortification but only with iron and zinc. It is one of the few countries in the world that has mandatory fortification of wheat flour but does not include folic acid.

The aim of the study is to describe the characteristics of Filipino women of child-bearing age based on selected explanatory variables and to determine the factors associated with deficient RBC folate. To the knowledge of the authors, there has not been a study in the Philippines that targeted the assessment of total folate intake among women of child-bearing age in consideration to its sources and bioavailability. This study attempts to determine the mean intakes of folate in microgram DFE. In addition, this study also measured folate status using the recommended biomarker - erythrocyte or red blood cell folate, which makes the result comparable to similar studies in the global setting.

MATERIALS AND METHODS

Study design

A cross-sectional survey was used in this study.

Study subject and area

The study was conducted in eight randomly selected Barangays in the Province of Batangas, Philippines from April to November 2018. Sample size was computed with 7% margin of error, 0.25 variability (p=0.25), and 90% confidence level. Participants were selected using a two-staged sampling procedure. A total of eight Barangays were randomly selected from where 25 respondents were sampled from a list of women aged 15 to 49 years. A total of 184 healthy women aged 15 to 49 years participated in the survey with 148 women who completed blood collection for folate analysis. Total sample size included 5% allowance (n=14) for non-response due to participants who were never present during visits, those who were not able to return the informed consent form, and those who expressed disinterest to join. Further, sample size allowance was applied for the set exclusion criteria. Preliminary questions were asked to the respondents to exclude factors that may confound the results of their RBC tests. These questions included: 1. Have you had any illnesses in past three months?; 2. Do you have current medical ailments in relation to diseases of the cardiovascular, kidney, abdominal, or respiratory function?; and 3. Do you take medications for high blood pressure, cancer, epilepsy and other drugs for serious medical conditions? Respondents who answered “yes” in any of the questions were excluded from the study.

Data collection procedure and management

The study followed a three-section data collection procedure which consisted of 1. Survey on socio-demographic and socioeconomic, obstetric and lactation history; 2. Dietary recalls and use of supplements; and 3. Anthropometric measurements and blood collection for folate status analysis.

The first section included a face-to-face interview using a structured questionnaire. Age, religion, education, marital status, employment, income, access to market and electricity, house ownership and vegetable gardening and livestock raising were among the socio-demographic / economic information obtained from the respondents. Obstetric and lactation history questions were partly adapted from the University of California, Los Angeles (UCLA) Department of

Obstetrics and Gynaecology Patient History Questionnaire consisting of data on status of pregnancy, lactation experience, number of children, use of oral contraceptive pills (OCP), antenatal care visit (ANC) and history of stillbirths and miscarriages. Use of folic-acid containing supplements was recorded using mean intake in µg DFE, which was computed from the dosage per intake and frequency of intake.

The second section consisted of diet recalls collected either through personal visits or phone calls and smart phone applications (Facebook, messenger app etc.) three times in a month (non-consecutively) for three months. This was done to assure that the blood folate status of women reflected long-term intakes of dietary and supplemental folic acid. Mean energy intakes were analysed using the online MenuEvalPlus developed by the Food and Nutrition Research Institute-Department of Science and Technology (FNRI-DOST). Adequacy of mean dietary intakes was assessed based on local references for energy and nutrient requirements - the Philippine Dietary Reference Intakes (PDRI) (FNRI-DOST, 2015). Mean total folate intake was summed up from total folate in the diet analysed using the 2010 Folate Content of Foods Consumed by Filipinos produced by the FNRI-DOST, and folic acid from fortified foods and folic acid-containing supplements. Total folate intake was expressed as µg DFE using the folate conversion factor where 1 µg DFE is equivalent to 1 µg DFE folate from food, and 0.6 µg DFE folic acid from supplements and fortified foods (Suitor & Bailey, 2000).

The last section composed of anthropometric and biochemical data collection. In this study, anthropometric measurement procedures followed the National Health and Nutrition Examination Survey Anthropometry Procedure Manual (CDC, 2007). Respondent's height was measured using a microtoise (Seca 206 Mechanical Measuring Tape). Weight of the respondents was measured using a battery-operated digital weighing scale (OMRON HN289 Digital Scale). Among the non-pregnant respondents with an age of 15 to 19 years, BMI-for-age was used to interpret nutritional status, while the WHO BMI cut-off points for adults was used for respondents aged ≥20 years. Nutritional status of pregnant subjects was assessed using Magbitang weight-for-height tables.

Blood samples for biochemical analysis were collected at non-fasting state by a registered medical technologist and analysed as a special test in an accredited laboratory (Hi-Precision Diagnostics). Four ethylenediamine tetra-acetic acid (EDTA) tubes were prepared and filled with 4 mL of blood drawn following the venipuncture blood collection protocol. EDTA tubes were labelled with respondent's code, name, time and date of collection. Each batch of data collection was scheduled for utmost 3 or 4 hours to preserve the stability of samples. Samples were transported immediately to the nearest holding or analysing site at 2 to 8^0C in cold packs. Improperly labelled and clotted specimens, overly-filled and under-filled tubes, insufficient quantity of blood samples, and specimens stored and transported outside the required temperature were rejected. Results of RBC folate in ng/mL were interpreted as normal or deficient using the cut-off values sensitive to risk of NTD-affected pregnancy set at >400 ng/mL. RBC folate below this value was interpreted as deficient in folate status (WHO, 2015).

The implementation of the study protocol was approved and was given ethical clearance by the Ethics Review Committee (ERC) at the Research Institute for Health Sciences (RIHS), University of the East Ramon Magsaysay Memorial Medical Center, Inc. (UERMMMCI) with RIHS ERC Code: 0482/E/O/17/141.

Statistical procedure

The data were analysed descriptively and inferentially. Sampling weights were applied in the analysis to maintain representativeness of the samples and to account for non-responses. Descriptive statistics included frequencies and percentages for categorical variables, while mean and standard deviation were used to describe continuous variables. Test of association between a continuous variable and a categorical variable with two levels was carried out using Pearson's Correlation. Rao-Scott Chi-square test was performed to determine a significant association between two categorical variables in a single population. For categorical variables that presented values without zero, Fisher's exact test of association was used. Statistical results were interpreted with a 95% level of significance. Binary logistic regression was used to determine the factors that affected deficient RBC folate at 10% level of confidence. Individual logistic regression analysis was performed to identify the most significant factor that gave the highest variability, measured as R^2, that explains the likelihood of the variable of interest. All statistical analysis were performed using SAS statistical software v.9.4.

RESULTS

Respondents' profile

The mean age of the respondents was 32.9±0.9 years (95% CI: 31.2 years, 34.6 years). Majority of them belonged to the age group of ≥30 years, mostly married or living with a partner and were Catholics. At least one third of respondents reached college or postgraduate level of education and majority of them were also employed. There was a significant association between education and status of employment (p<0.0001). Those who had 12 years of education or more tended to be employed. Male-headed household still dominated. Only 19.0% (n=23) of women were heads of their households. Household headship was significantly associated with the level of education (p=0.0025) and status of employment (p=0.0034). About 42.2% (n=68) of the respondents had difficulty accessing the market. One in every three (37.8%, n=66) women of child-bearing age had a vegetable backyard garden and/or practised livestock raising. Table 1 summarises the characteristics of the respondents in terms of their socio-demographic and socio-economic profiles.

Obstetric and lactation history

The respondents' mean age at menarche was 13.0±0.1 years (95% CI: 12.7 years, 13.3 years). About 12.6% (n=28) of respondents were never pregnant, 78.8% (n=144) were not pregnant but have had previous pregnancies and 8.6% (n=12) were currently pregnant. For pregnant respondents, the mean age of pregnancy was 5.09 months. Majority of women (77.0%, n=124) lactated previously. Exclusive breastfeeding was defined in the study as breastfeeding alone from birth up to 6 months. It was observed that 44.0% of the respondents breastfed their infants exclusively. Table 2 summarizes the obstetric, lactation history and lifestyle-related behaviors of the respondents.

Lifestyle-related behaviours

About 2.8% reported being current smokers while majority (92.1%) have never smoked tobacco. Only about 0.7% of the respondents were regular alcohol drinkers, 29.9% were occasional drinkers and 69.4% never drinks alcoholic beverages.

Use of folic acid-containing supplements

Current use of supplements in general was observed among one third of the respondents (33.5%). Use of supplements

Table 1. Socio-demographic and socio-economic profiles of respondents, *N*=184

Variables	*Mean±SD (95% CI)*	*Frequency distribution n (%)*
Age, years	32.89±0.85 (31.21, 34.57)	
15.0 to 19.9 years		15 (7.0)
20.0 to 29.9 years		56 (26.7)
30.0 to 49.0 years		113 (66.3)
Civil status		
Single		44 (15.0)
Married/Lived-in		134 (79.0)
Separated/Widowed/Divorced		6 (6.0)
Religious affiliation		
Roman Catholic		171 (94.2)
Muslim		1 (0.6)
Iglesia Ni Cristo		4 (1.4)
Evangelicals		4 (2.2)
Others		4 (1.6)
Educational attainment		
No formal education		1 (0.6)
Have reached elementary education		9 (7.9)
Have reached high school education		117 (58.9)
Have reached college education or higher		57 (32.6)
Employment		
Student/unemployed		94 (41.4)
Employed/self-employed		90 (58.6)
Household head		
No		161 (81.0)
Yes		23 (19.0)
Monthly income	13,942±1468 (11044, 16838)	
Below poverty threshold		117 (56.5)
Above poverty threshold		67 (43.5)
House ownership		
Owned		105 (57.1)
Rented		29 (14.9)
Residing for free		23 (12.1)
Living with parents/in-laws		27 (15.9)
Vehicle ownership		
No		108 (56.6)
Yes		76 (43.4)
Access to electricity		
No		2 (2.8)
Yes		182 (97.2)
Access to market		
No		68 (42.2)
Yes		116 (57.8)
Backyard vegetable gardening and/or livestock raising		
No		118 (62.2)
Yes		66 (37.8)

Table 2. Obstetric, lactation history and lifestyle-related behavior of respondents, *N*=184

Variables	*Mean±SD*	*Frequency distribution n (%)*
Age at menarche, years	13.0±0.14	
Menstruation cycle		
Irregular		38 (26.5)
Regular		146 (73.5)
Pregnancy		
Never pregnant		28 (8.6)
Currently pregnant		12 (12.6)
Not pregnant but had previous pregnancy		144 (78.8)
Number of children		
No children		30 (9.5)
1 to 2 children		89 (52.8)
≥3 children		65 (37.7)
Bleeding complications during pregnancy, *n=156*		
No		122 (75.3)
Yes		34 (24.7)
Still birth/ Miscarriage, *n=156*		
No		124 (79.6)
Yes		32 (20.4)
Reproductive health check-up if never been pregnant, *n=28*		
No		25 (89.4)
Yes		3 (10.6)
ANC visit, *n=156*		
No		0 (0.0)
Yes		156 (100.0)
Lactation		
Never lactated		42 (15.9)
Currently lactating		18 (7.0)
Previously lactating		124 (77.1)
OCP use		
Never		91 (48.9)
Former user		58 (32.7)
Current user		35 (18.4)
Tobacco smoking		
Never		174 (92.1)
Former smoker		8 (5.1)
Current smoker		2 (2.8)
Alcohol drinking		
Never		140 (69.4)
Occasional		42 (29.9)
Regular		2 (0.7)

was defined in this study as any nutrient supplements taken during the time of interview. The use of multivitamin tablets was the most common supplement taken by the respondents (55.0%). Not all the supplements recorded contained folic acid. In fact, only about 17.0% of women took folic acid-containing supplements. The mean folic acid dosage from supplements was computed at 337 µg. Intake of folic acid-containing supplements was noted to

Table 3. List of folic acid-containing supplements consumed by the respondents

Brand name	*Manufacturer*	*Dosage*	*Folic acid content (μg)*
†Obimin Plus	UNILAB	One capsule daily	1000
†Hemarate FA	UNILAB	One capsule daily	600
‡Stresstabs	Pfizer Consumer	One tablet daily	400
‡Centrum Advance	Pfizer Consumer	One tablet daily	400

Reference: †unilab.com.ph; ‡mims.com

be intentional, either due to medical reasons or current pregnancy. Among the multivitamin brands that contained folic acid and were consumed by the respondents were Obimin, Hemarate FA, Stresstabs, Centrum and C24/7 as shown in Table 3.

Body mass index (BMI)

In general, mean weight of the population was 59.9 kg (95% CI: 57.3 kg, 62.5 kg) and mean height was 154.5 cm (95% CI: 153.5 cm, 155.4 cm). Mean BMI was also computed at 25.1 kg/m^2 (95% CI: 24.1 kg/m^2, 26.2 kg/m^2). Among the non-pregnant respondents, 55.8% (*n*=96) were at normal nutritional status. About 6.4% (*n*=11) were underweight and about one-third (37.8%) were overweight or obese. Based on BMI-for-age alone, 73.4% (*n*=11) had normal nutritional status while 26.6% (*n*=4) belonged to overweight nutritional status. At the age range of 15.0 to 19.0 years, there was no reported status of underweight. Among non-pregnant respondents aged between 19.1 to 49.0 years, 54.3% (*n*=85) fell under normal nutritional status, 7.1% (*n*=11) were underweight and 38.6% (*n*=61) were either overweight or obese (Table 4). There was no correlation noted between BMI and folate status.

Total energy and folate intake

Mean total energy intake was 1542 kcal (95% CI: 1465 kcal, 1620 kcal). Energy intake was lower compared to the recommended energy intake (REI) of 1870 kcal (for 30-49 years), (FNRI-DOST, 2015). With an additional 300 and 500 calories for pregnant and lactating women, respectively, the mean energy intake was much lower for these population groups. Folate intake was expressed in μg DFE, which measured both dietary folate and folic acid from fortified foods and supplements. Resulting mean folate intake was 239 μg DFE (95% CI: 117 μg DFE, 360 μg DFE). Results showed that 84% (*n*=172) of respondents had inadequate folate intakes, while only 16% (*n*=12) had adequate or above intakes based on the 320 μg/day estimated average requirement (EAR) for females aged 19-49 years. From dietary sources alone, mean folate intake was only 81 μg DFE (95% CI: 67 μg DFE, 95 μg DFE), which contributed to 25% of EAR for folate. The mean total folate intake of women of reproductive age in Batangas was observed as low when compared to the estimated average intake of 320 μg DFE (FNRI-DOST, 2015). There was no folic acid from fortified foods recorded in the study. Moreover, mean intake was much lower when compared to the estimated average requirement for pregnant and lactating women at 520 μg DFE and 450 μg DFE, respectively (FNRI-DOST, 2015). Table 4 shows the respondents' BMI, total energy and total folate intakes.

The respondents were distributed according to the status of their red blood cell folate. About 36 (19.3%) respondents were folate deficient and 112 (80.7%) have normal folate status. According to age group, there were more folate deficient women age 15.0 to 19.9 years than their normal counterparts. For adult respondents age 20.0 to 49.0 years, there were more normal folate

Table 4. BMI, total energy and folate intakes of respondents and red blood cell folate status, *N*=184

Variables	*Mean±SD*	*Frequency distribution n (%)*
BMI, kg/m^2	25.1±0.5	
Normal		96 (55.8)
Underweight		11 (6.4)
Overweight and Obese		77 (37.8)
BMI for age (15.0 to 19.0 years), kg/m^2		
Normal		11 (73.4)
Underweight		0 (0.0)
Overweight and Obese		4 (26.6)
BMI (Non-pregnant, 19.1 to 49.0 years), kg/m^2		
Normal		85 (54.3)
Underweight		11 (7.1)
Overweight and Obese		61 (38.6)
Total energy, kcal	1542±39	
Total folate intake, μg DFE	239.0±61.6	
Below EAR		172 (84.4%)
Above EAR		12 (15.6%)
Folate intake from dietary sources, μg DFE	81.0±7.1	
RBC folate, ng/mL, *n=148*	485.0±15.5	
Normal		112 (80.7)
Deficient		36 (19.3)

status than those with deficient folate. Folate deficient respondents were spread across different level of education. Among pregnant respondents, there was no recorded folate deficiency while those who were lactating at the time of interview, about 22.2% were detected folate deficient. All but one (97.2%) alcoholic drinkers were folate deficient. Conversely, mean total energy intake of folate deficient respondents were higher than those with normal folate status while mean total folate intake was almost two times higher among respondents with normal folate status than those who were folate deficient. In addition, almost all folate deficient respondents were not taking folic acid supplements. Table 5 summarises the distribution of respondents according to their red blood cell folate across selected variables.

Among the explanatory variables included in this study, religious affiliation, education, access to electricity, pregnancy and lactation status, total folate intake and use of folic acid-containing supplements showed significant correlations with deficient folate status. Results showed that Catholicism, high school education and access to electricity increased the likelihood of folate deficiency among women of child-bearing age. However, the results for education, access to electricity and religion were statistically significant due to the homogeneity of the sampled population where 94.2% of respondents were affiliated in Catholicism, 91.5% have at least reached high school education and 97.2% had access to electricity.

Vegetable backyard gardening and alcohol drinking were both significant predictors of deficient folate status ($p<0.1$). Women who reported without vegetable backyard gardening and/

Table 5. Distribution of respondents according to their RBC folate status across selected variables, *n*=148

Variables	*Normal RBC folate (≥400 ng/mL), n=112*	*Deficient RBC folate (<400 ng/mL), n=36*
	Frequency distribution, n (%)	*Frequency distribution, n (%)*
Age, years		
15.0 to 19.9 years	8 (7.1)	6 (16.7)
20.0 to 29.9 years	31 (27.7)	13 (36.1)
30.0 to 49.0 years	73 (65.2)	17 (47.2)
Educational attainment		
No formal education	1 (0.9)	0 (0)
Have reached elementary education	6 (5.4)	1 (2.8)
Have reached high school education	68 (60.7)	27 (75.0)
Have reached college education or higher	37 (33.0)	8 (22.2)
Backyard vegetable gardening and/or livestock raising		
No	65 (58.0)	25 (69.4)
Yes	47 (42.0)	11 (30.6)
Currently pregnant		
Yes	12 (10.7)	0 (0.0)
No	100 (89.3)	36 (100.0)
Currently lactating		
Yes	10 (8.9)	8 (22.2)
No	102 (91.1)	28 (77.8)
Alcohol drinking		
No	2 (1.8)	1 (2.8)
Yes	110 (98.2)	35 (97.2)
Mean total energy intake	1397 kcal	1467 kcal
Mean total folate intake	118.7 µg/day	82.1 µg/day
Taking folic acid-containing supplements		
No	103 (92.0)	34 (94.4)
Yes	9 (8.0)	2 (5.6)

or livestock raising had twice as much likelihood of being red cell folate deficient than those who did (regression coefficient 0.3411; *OR*=1.978, $p<0.10$).

In this study, both pregnancy (regression coefficient -6.2348) and lactation (regression coefficient -0.6773) correlated with deficient folate status among women of child-bearing age at opposing directions. Odds ratio suggested that women who were pregnant were less likely to become folate deficient compared to non-pregnant women (*OR* <0.001, $p<0.05$), and that lactating women were more likely to be folate deficient compared to non-lactating women (*OR*=0.258, $p<0.05$). Table 6 shows the results of regression analysis between selected variables and folate status.

Total folate intake showed a statistically significant correlation to RBC folate status ($p<0.012$). The result only showed that adequate folate intake supports normal blood folate level and that inadequate intake leads to more likelihood of becoming folate deficient (*OR*=-1.088, $p<0.05$). The odds of being RBC deficient was six times more likely for those not using folic acid supplementation than those who used it (regression coefficient 0.9274; *OR*=6.391, $p<0.10$), as shown in Table 7.

DISCUSSION

The mean RBC folate among women of child-bearing age (n=148) was 485±16 ng/mL (95% CI: 454 ng/mL, 515 ng/mL). About 80.7% of respondents had normal RBC of >400 ng/mL. Based on the blood analysis, 19.3% of women suffered from folate deficiency, which may be contributory to NTD-affected pregnancies. More than a decade ago, at the national level, about 20.9% of Filipino women were folate deficient using RBC folate as a biomarker. However, the cut-off used was much lower at 175 ng/mL compared to the present study which used 400 ng/mL (Cheong *et al.,* 2008), which suggests that the prevalence of folate deficiency in the Philippines back in 2008 may be lower than the actual 20.9% prevalence noted using a different cut-off point. The results may suggest that folate deficiency may have increased at present since 2008. Kyrgyzstan and Guatemala, both lower middle income countries like the Philippines, showed prevalences of deficient RBC folate at 9% and 49%, respectively between 2009 and 2010 (Rogers *et al.,* 2018). Apart from risks of NTDs and other pregnancy-related complications, folate as a vitamin can cause megaloblastic anaemia, glossitis, angular stomatitis and mouth ulcers when deficient (Green & Miller, 1999).

Total folate intake was generally low due to low consumption of folate-rich foods as evidenced by the mean total dietary folate intake of 81 μg DFE. Supplementation of folic acid was also low and folic acid from fortified foods was negligible to none. Perhaps awareness on folic acid supplementation is low, thus the consumption, and the lack of policy on mandatory folic acid fortification on foods. Similarly, a study on folate intake and status of Malaysian women of child bearing-age found that total folate intake among the subjects only met 16.5% of the Malaysian recommended nutrient intake without folic acid supplementation (Khor *et al*., 2006). It is notable that without fortification and supplementation of folic acid, intake is observed to be very low.

Majority (69.4%) of respondents without backyard garden and/or livestock production were folate deficient. The study showed evidence of increased folate consumption from food alone, and total folate intake from foods and supplements among women with vegetable backyard gardening and/or livestock production. Smoking and drinking were more common in women <30 years old. Mean dietary folate intake among those women with vegetable backyard gardening and/

Table 6. Regression analysis of factors influencing RBC folate deficiency

Variable	*Category*	*Reference Category*	*Odds Ratio (OR)*	*Coefficient*	*Standard error*	*p-value*	*R-square*
Religion	Catholic	Religion other than catholic, Muslim, INC and Evangelicals	0.239	6.520	0.706	<0.001***	0.022
	Muslim		<0.001	-4.823	0.448	<0.001***	
	INC		<0.001	-4.823	0.889	<0.001***	
	Evangelicals		<0.001	-4.823	0.448	<0.001***	
Education	No formal education	College/Post Grad	<0.001	-0.608	0.774	<0.001***	0.003
	Elementary		0.710	1.578	0.896	0.122	
	Highschool		0.328	3.109	0.509	0.01**	
Access to electricity	No	Yes	<0.001	-6.169	0.531	<0.001***	0.002
Vegetable gardening and/or livestock raising	No	Yes	1.978	0.341	0.166	0.079	0.015
Pregnancy	Pregnant	Non-pregnant	<0.001	-6.234	0.227	<0.001***	0.048
Lactation	Non-lactating	Lactating	0.258	-0.677	0.219	0.017*	0.032
Alcohol drinking	No	Yes	3.067	0.560	0.280	0.085	0.026
Total folate intake, μg DFE			0.997	-0.003	0.001	0.012*	0.041

*Statistically significant at $p<0.05$; **Statistically significant at $p<0.01$; ***Statistically significant at $p<0.001$

Table 7. Result of the regression analysis of the variable ‘intake of folic acid-containing supplements’

Parameter	*Category*	*Odds Ratio*	*Estimate*	*Standard Error*	*Wald Chi²*	*p-value*
Intercept			-2.230	0.659	11.439	0.007**
Folic acid-containing Supplement	No	6.391	0.927	0.556	2.785	0.095

**Statistically significant at $p<0.01$

or livestock raising was 74.8 μg DFE, which was 18% higher compared to the mean intake of women without vegetable backyard gardening and/or livestock raising at 61.5 μg DFE. The same pattern was observed when total folate intake (including folic acid from supplements) was compared from those with and without vegetable backyard gardening and/or livestock raising. In this case, mean intake for total folate was 19% higher among those with vegetable backyard gardening and/or livestock raising compared to those without. To date, no similar study has found direct correlations between vegetable backyard gardening and/or livestock raising with RBC folate deficiency.

All pregnant women who participated in the study had adequate folate intakes and normal RBC status. Majority of them were taking folic acid-containing supplements. Perhaps the physiological drive to increase folate intake from foods and folic acid supplements influenced their intakes and thus, their folate status. The increased need for folate to be transferred to the breastmilk during lactation may be achieved at the expense of maternal folate stores. Lactating women with deficient folate status were also those who had experienced recent child birth. Child birth may lead to loss of blood during delivery and during the course of pregnancy for nourishment of the foetus. In the study of Metz, Zalusky & Herbert (1968), apparently healthy women can become folate depleted in the early postpartum period (Metz *et al.,* 1968).

Finally, the odds of being RBC deficient was higher among those who did not use folic acid supplementation compared to those who did (OR=6.391, p<0.10). Moreover, 5% of the variation in RBC deficiency can be explained by intake of folic acid-containing supplements, the highest variability among all selected variables used in this study. The result of regression analysis was consistent with the results of studies conducted in different regions in the recent years (Cummings *et al.,* 2017; Ma *et al.,* 2017).

CONCLUSION

The high prevalence of folate deficiency reported in this study is alarming. Folate is an essential nutrient, which must be supplied adequately in the diet and through supplementation before pregnancy. It is therefore important to remind those women planning for pregnancy of consumption of foods rich in folate and to take folic acid-containing supplements to avoid complications such as NTDs. Folic acid supplementation is one way to achieve adequate folate levels because of its high bioavailability and folic acid content. With supplementation, women are less at-risk of folate deficiency and the risk of NTD-affected pregnancies can be lowered. Moreover, conducting campaigns should be in place to educate women of child-bearing age on the importance of folic acid supplementation.

The results of the study suggested that women of child-bearing age who did not take folic acid supplementation were six times more likely to become RBC folate deficient compared to those who took folic acid supplementation. This warrants a more detailed assessment of the role of folic acid supplementation on folate status of women. Questions such as intention to supplement, duration, prescription, among others can be surveyed, which may help establish a deeper discussion on the association of folic acid supplementation with RBC folate status. However, further studies may be conducted taking into consideration the composition of population involved since this study was conducted both in pregnant and non-pregnant women of child-bearing age.

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Authors' contribution

AKMB, contributed to the conception, design and protocols of the study; conducted data collection, analysis and interpretation, as well as the write up of draft manuscript; NPG, contributed to the conception, design and protocols of the study and supervised the write up of the draft manuscript; LMA, contributed to the conception, design and protocols of the study, and supervised the write up of the draft manuscript; MTMT, contributed to the conception, design and protocols of the study, and supervised the write up of the draft manuscript; MCR, contributed to the conception, design and protocols of the study, and supervised the write up of the draft manuscript. All of the authors contributed to the final manuscript.

Conflict of interest

There is no conflict of interest in the conduct of the study.

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