

ASSOCIATION BETWEEN SERUM FOLATE LEVELS AND EARLY PREGNANCY LOSS AT KNH. A CASE CONTROL STUDY

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ABSTRACT

Background: The World Health Organization (WHO) defines Early Pregnancy Loss (EPL) as termination of pregnancy before 20 weeks gestation or with a fetal weight of below 500grams. EPL occurs in 10% of all clinically recognized pregnancies, 80% of which occurs in the first trimester. About 50% of EPLs are due to chromosomal abnormalities. Other factors implicated include: immunologic; anatomic; endocrine; hematologic and micronutrient factors, including folic acid deficiency. This study aimed at assessing the association between serum folic acid levels and EPL among patients attending Ante natal services at the Kenyatta National Hospital (KNH).

Methodology: This was a case control study where cases were women aged 18 to 40 years presenting with EPL (below 16 weeks of gestation) while the controls were women with viable pregnancy matched for gestation age. Consecutive sampling was used to identify 82 women with EPL and 95 women with normal pregnancy. 2mls of blood was collected from the ante cubital fossa for assessment of folic acid levels.

Results: There was a statistically significant difference in the median folic acid level between the EPL group and the normal pregnancy group (17.3ng/ml and 19.7ng/ml respectively) (p, 0.022). Using the KNH normal reference range for serum folic acid levels in pregnancy of 12.9 – 20ng/ml, there was no significant association between folate levels and EPL. Two-thirds in the EPL group (69.5%) and majority (89.5%) of the pregnant group were married. Pregnancy loss was significantly associated with marital status (p, 0.001), education level (p, 0.042), number of previous miscarriages (p, 0.003) and antenatal clinic attendance (p,0.016).

Conclusion and recommendations: From our study we concluded that normal serum folate levels do not seem to confer protection against EPL in this population. Supplementation is essential to high risk population i.e. those with low education level, single status and those not attending antenatal clinics. It is recommended that WHO levels for normal serum folic acid be used as the normal and then conduct further studies using this standard.

INTRODUCTION

Folate, also referred to as folic acid (vitamin B9), is one of the 13 essential micronutrients (1). It is required for DNA replication and forms a substrate for various enzymatic reactions involved in the synthesis of amino acids and the metabolism of vitamins (1). Folic acid is crucial in maintaining pregnancy as it is essential factor for the rapidly growing products of conception (2). Low serum folate levels have been associated with early

pregnancy loss (3), while some studies found no association between the two (4). It usually results in megaloblastic anemia and neural tube defects (5). Hyperhomo-cysteinemia is caused by inadequate intake of folic acid, vitamins or from physiologic variations in the efficiency of the enzymes taking part in the one-carbon folate metabolism pathway (6). Typical Kenyan diet is monotonous with little variation; consisting mainly of starches like potato, rice, ugali ; legumes like beans, peas, green grams, and vegetables like kale, cabbage and spinach.

Early Pregnancy Loss (EPL) occurs in 10% of all clinically recognized pregnancies, with 80% occurring during the first trimester (7). About 50% of EPLs are thought to be due to chromosomal abnormalities. Other factors implicated include: immunologic; anatomic; endocrine; hematologic and micronutrient factors, including folic acid deficiency (8). Causes of folic acid deficiency include reduced dietary intake, overcooking of food, advanced age, alcohol consumption, malabsorption syndromes, certain medications and increased requirements during pregnancy. However, there is no consensus on the specific role of folic acid deficiency in early pregnancy loss. Existing literature is based on studies done in Caucasians, Hispanics and Asians, hence a hiatus of local data.

The objective of this study was to determine the association between serum folate levels and early pregnancy loss at Kenyatta National Hospital (KNH).

METHODOLOGY

Study Design: This was a case control study in which 95 women with viable pregnancy as controls and 82 women with EPL as cases participated in the study and their serum folate levels measured.

Study setting: This study was carried out at Kenyatta National Hospital (KNH); Kenya's national referral and teaching hospital, located in Nairobi County, from March to May 2019. Cases were found in the acute gynaecology ward, Accident and Emergency consultation room and controls in antenatal outpatient clinic. KNH has an ISO certified Biochemistry Laboratory well equipped to perform serum folate analysis and is subjected to regular internal and external quality control measures. The accident and emergency unit sees between 569 and 863 patient annually, requiring manual vacuum aspiration for early pregnancy loss. Antenatal clinic at KNH serves 300- 400 women per week. Provision of routine and consistent folic supplementation is not practiced in this country including KNH.

Study population: The study population at KNH comprises women of low and low middle class social economic status. The cases in this study comprised women aged 18 – 40 years presenting with loss of an intrauterine pregnancy before 16 weeks gestation.

The control group constituted women with viable pregnancies at similar gestation. We excluded women with ectopic pregnancies and those with viable pregnancies beyond 16 weeks, too sick to give consent and those who did not consent.

Sample size calculation and sampling procedure:

Using Fleiss' formula (9) based on a Z score of 1.96 at 95% confidence level, Z 1- β value of 0.842 at 80% power, an odd ratio of 2.6, and a ratio of cases to controls of 1:1, the calculated sample size was 79 cases and 79 controls. With a 10% contingency, 82 cases and 95 controls. Simple consecutive sampling of consenting eligible participants was performed until the required sample size was achieved. For each case identified the control at or below 16 weeks was identified and selected from the antenatal clinic.

Data Collection and management: Following informed consent, data were collected by trained research assistants using a structured questionnaire. The data were cleaned and entered into excel version 21. Data on socio demographic / reproductive and medical factors were collected. A 2ml blood sample was also collected using a plain vacutainer as shown in Box 1. The sample was then analyzed for serum folic acid levels in the KNH biochemistry laboratory using COBAS machine, quality control measures are elucidated in Box 2. Data collected were then cleaned and analyzed using the STATA 13.

BOX 1: Sample collection procedure

- Drawing of blood: standard SOPs
- Amount: 2mls
- Vacutainer: red topped bottle
- Sample labelling: use of participants unique identifier
- Lab request form: use of participants unique identifier
- Sample taken to KNH lab 16 lab within 30 mins, analysis done within 24 hours
- Result collection by PI and RA

BOX 2: KNH Laboratory 16 Quality Control Measures

- This was done twice a week, every Monday and Wednesday the machine was run to ensure proper functioning using presi control 1 (precision control) for normal values and presi control 2 for abnormal value.
- Reference range given was specific to the reagent used. Calibrant was specific to the analyte (serum folate) and also specific to the batch of reagent used.
- All samples used the same batch of reagent which was calibrated once.

Data analysis: Descriptive statistics included median, interquartile estimates for continuous variables. Percentage and proportions were used for categorical variables. In the univariate analysis, the crude odd ratio and its 95% confidence interval were used as measures of association.

Multivariate analysis was used to control for confounding factors for the association between the folic acid level and early pregnancy loss.

Ethics: The protocol for this study received approval from the KNH and UON Ethics Research Committee (P512/07/2018). Informed consent was sought from each participant individually and only the study team had access to the data collected, which was de-identified before analysis.

RESULTS

A total of 177 women of reproductive age (19-40 years) were screened for plasma folic acid levels; 82 of them had suffered EPL while the rest (95) were still pregnant. The median ages were 28.5 years (IQR=24-32yrs) and 29 years (IQR=26-35yrs) among women that had suffered EPL and the pregnant group, respectively.

Over half of the cases and controls were aged 19-29 years. More than two-thirds in the EPL group (69.5%) and majority (89.5%) of the pregnant group were married. On educational attainment, 65.8% of cases 81.9% of controls had attained at least secondary level. Less than half of cases and controls (46.3% and 36.2% respectively) were unemployed. Among the cases and controls, 47.6% and 43.2% respectively earned a monthly income <Ksh 30,000 (Table 1).

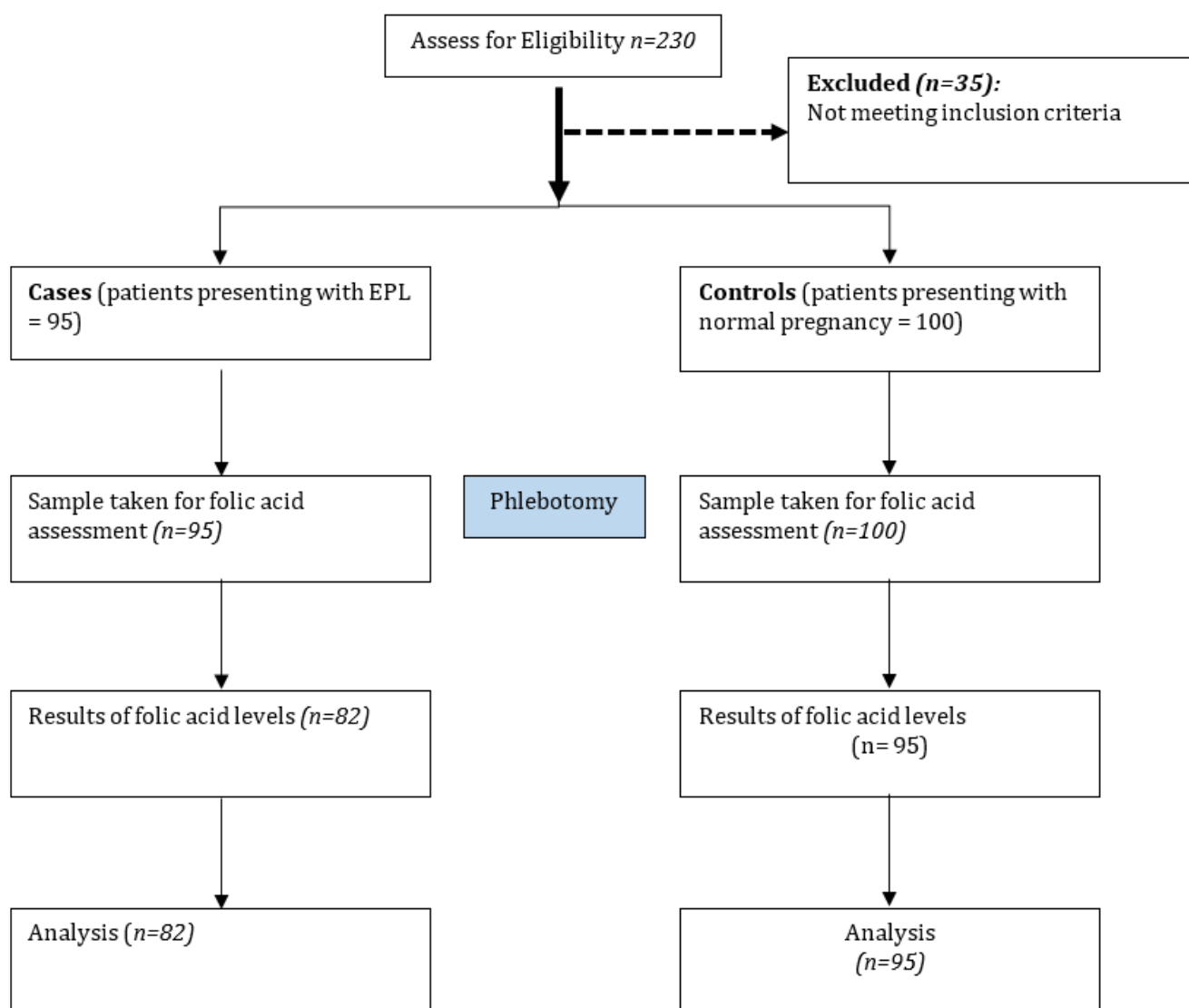


Figure 1: Study Flow Diagram

Table 1: Socio-Demographic Characteristics of women with early pregnancy loss and those with viable pregnancies at 16 weeks and below at Kenyatta National Hospital, March 2019

Characteristic	EPL group	Pregnant group	Chi 2	P-value
Age group (n=154)				
19-29 years	43 (59.7)	43 (52.4)	0.825	0.364
30-40 years	29 (40.3)	39 (47.6)		
Marital status (n=177)				
Single/divorced	25 (30.5)	10 (10.5)	11.055	0.001
Married	57 (69.5)	85 (89.5)		
Education level (n= 176)				
Primary	28 (34.2)	17 (18.1)	6.361	0.042
Secondary	27 (32.9)	34 (36.2)		
Tertiary	27 (32.9)	43 (45.7)		
Employment (n=176)				
Salaried	25 (30.5)	24 (25.5)	4.701	0.095
Self-employed	19 (23.2)	36 (38.3)		
Unemployed	38 (46.3)	34 (36.2)		
Monthly income (Ksh) n=177				
Zero	36 (43.9)	43 (45.3)	0.609	0.894
1-15,000	23 (28.1)	24 (25.3)		
15,001-30,000	16 (19.5)	17 (17.9)		
>30,000	07 (08.5)	11 (11.6)		

The distribution of women in terms of age group, employment and monthly income did not vary significantly between the pregnant women group and those that had suffered EPL. Women of low formal educational status and those who were single/divorced were more likely to experience EPL compared to women with viable pregnancies (table 1).

More than two-thirds of the women in the EPL (71.4%) and pregnant (66.7%) groups had at least one previous live birth. The proportion of women reporting experiencing at least one miscarriage

was higher in the EPL group (67.5%) compared to pregnant group (46.2%). Number of previous live births ($p= 0.026$) and previous miscarriages ($p=0.003$) were found to be significantly associated with a woman experiencing EPL. In table 2, women who had no history of a miscarriage were less likely to experience early pregnancy loss compared to those with a history of at least one miscarriage ($p.val.0.003$). At the same time, women who had not attended ANC were 2.5 times likely to experience EPL compared to those who had attended (95% C I 1.2-5.7; $p val. 0.016$).

Table 2: Reproductive Health Factors and EPL among women at 16 weeks and below and those with early miscarriage at KNH, March 2019

Characteristic	E P L group	Pregnant group	OR (CI)	P-value
Previous live births(n=170)				
Zero	22 (28.6)	31 (33.3)	0.80	0.50
More than 1	55 (71.4)	62 (66.7)	(0.41-1.54)	
Previous miscarriages(n=170)				
Zero	25 (32.5)	50 (53.8)	0.41	0.005
One	52 (67.5)	43 (46.2)	(0.22-0.77)	
ANC (n=177)				
Yes	11	27	OR 2.5	0.016
No	71	68	2.1 – 5.7	

The distribution of folic acid level among pregnant women and those that had an EPL was notably different as shown in Figure 4. Whereas half of the current pregnant women had at least 19.7ng/ml (median), 50% of those that had suffered EPL had less than 17.3ng/ml. This difference in the distribution was found to be statistically significant using the two-sample Wilcoxon rank-sum test (Z-statistic=-2.288; p value – 0.022).

Using the KNH normal reference range for serum folic acid levels in pregnancy, folic acid was disaggregated into two categories as shown in the Figure 5 in which serum folic acid was considered low when less than 12.9ng/ml. Approximately 20.0% of the women in each group had serum folic acid level less than the cut off at 12.9ng/ml.

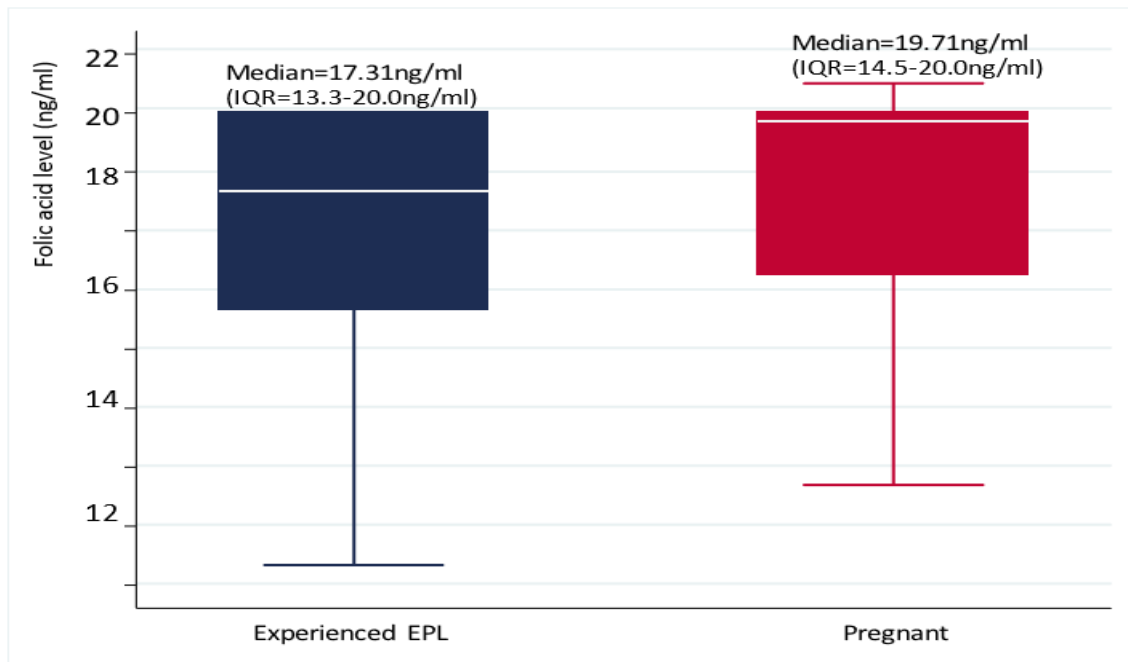


Figure 4: Distribution of folic acid among women that have experienced EPL and those currently pregnant women

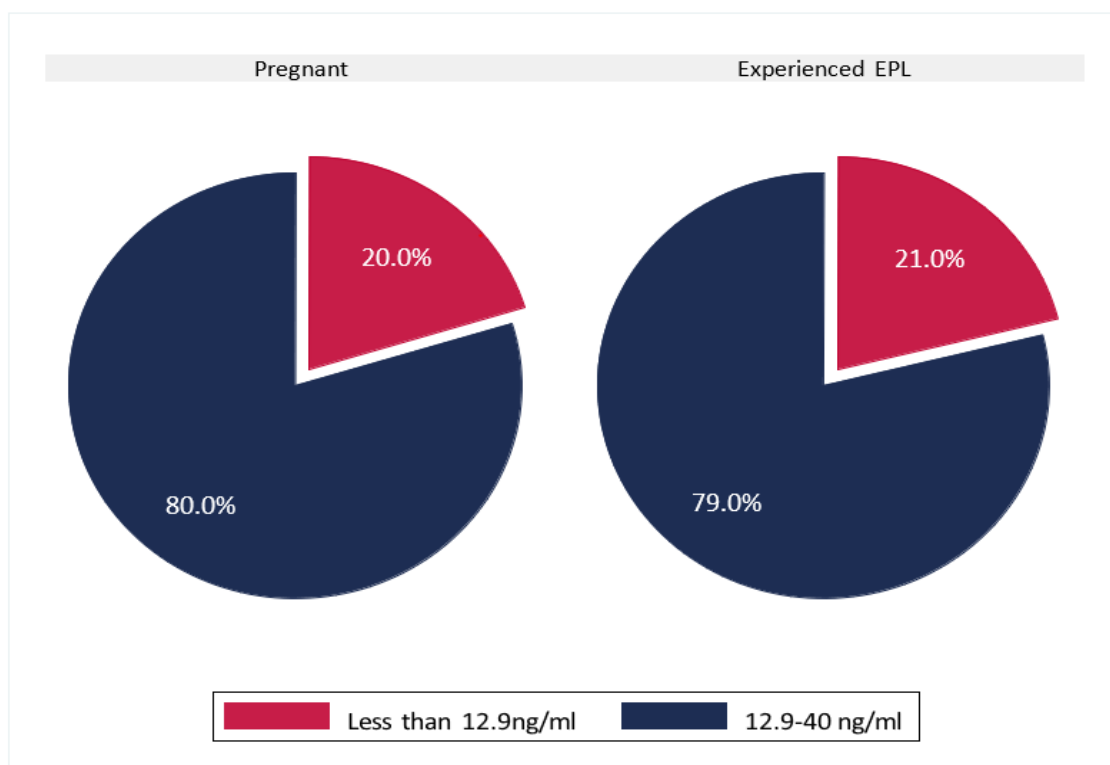


Figure 5: Folate levels classification by study group

Association between women's Sociodemographic, Nutritional, Obstetric Characteristics and Serum Folate Levels in Pregnancy.

Chi-square test of association was done to assess the relationship between women's sociodemographic, nutritional, obstetric characteristics and serum folate levels. As shown in Table 3, none of the factors was significantly associated with the serum folate level in pregnancy.

DISCUSSION

In this study the median serum folic acid level among the cases that experienced EPL was statistically lower than that among the control of women with continuing pregnancy, although four out of every 5 in both groups had serum levels within the normal ranges. EPL was more likely among women who were single or divorced, had attained low education level and had a history of previous

miscarriage. History of antenatal clinic attendance in the incident pregnancy was associated with less likelihood of early pregnancy loss.

This study found that majority of the cases and controls had normal serum folate levels, despite not being on antenatal folic supplementation. This may imply diet rich in folic acid although this aspect was not captured in this study. Family/ spousal support may explain why single/divorced women have a higher risk of EPL. The participants with high education may have more awareness about and means to get proper folate supplementation, diet and also attend Antenatal clinic early, thus reducing the risk of EPL.

In this study, marital status and education were associated with risk of EPL while the other factors like age, income were not. This compares with the findings by George et al in Sweden who found that women with low plasma folate (≤ 2.19 ng/ml) at

Table 3: Relationship between women characteristics and serum folate levels in pregnancy

Characteristic	<12.9ng/ml	12.9-40.0ng/ml	Odds Ratio (CI)	P-value
Age group (n=131)				
19-29 years	11	64	1.94	0.137
30-40 years	14	42	(0.80-4.68)	
Marital status (n=150)				
Single/divorced	3	23	2.24	0.29
Married	28	96	(0.63-7.80)	
Education level (n=149)				
Primary	7	34	1.39	0.48
Higher than primary	15	36	(0.55-3.52)	
Employment (n=150)				
Salaried	7	33	1.31	0.57
Self-employed	24	86	(0.52-3.34)	
Monthly income (Ksh) (n=150)				
Up to 15000	24	85	0.93	0.89
More than 15000	9	34	(0.39-2.22)	
No of live births (n= 143)				
Zero	6	39	2.1	0.13
More than one	24	74	(0.79-5.58)	
No of miscarriages (n= 143)				
Zero	12	52	1.28	0.55
More than one	18	61	(0.56-2.89)	
Two or more	5	21		

increased risk of spontaneous abortion. Patients with higher level equal to or above 6.17 ng/ml showed no increased risk of spontaneous abortion. Ronnenberg in China also found an increase in risk of 'spontaneous abortion with decreasing plasma vitamin B6 and folate. Strength further reduced in logistic models that included age, BMI, both vitamins, risk of spontaneous abortion was four fold higher among women with suboptimal plasma concentration of folate and vitamin B6 cut off 2.64 ng/ml (8.4 nmol/l). Deficiency of folate and vitamin B6 tended to be about 50% more common among women with clinical spontaneous abortion than among those with live births. Ronnenberg et al found folate deficiency < 21.3 ng/ml (68 nmol/L) tended to be more common among women with spontaneous abortion than those with live births. Nelen et al used a cut off of 8.4, suggested suboptimal folate status may play a role in both first and recurrent spontaneous abortion but larger prospective studies are needed to confirm possible association. Hoffman et al found that abnormal folate metabolism was not an apparent risk factor for 1st trimester loss. Audrey et al found that risk of spontaneous abortion was 20% lower among women in the highest category of supplemental folate compared to that in the lowest category. Yamada Takashi et al had different findings; low serum folate levels during 1st trimester were not associated with risk of late abortion, preterm birth or fetal growth restriction.

The weaknesses of this study were that chromosomal causes of EPL could not be ruled out, and that the specific dietary practices of the study population were not assessed. The strength is that this study followed the STROBE guidelines, was the first of its kind in our set up and will therefore form a baseline for other prospective studies. This study also provides baseline data in the Kenyan population, which may be used to inform other studies and policy formulation i.e. early antenatal visits, diet and folate fortification of food.

CONCLUSION

From our study we concluded that normal serum folate levels do not seem to confer protection against EPL in this population. It is recommended that

WHO levels for normal serum folic acid be used as the normal and then conduct further studies using this standard.

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