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Original Article

Epidemiological determinants of Folate deficiency among pregnant women of district Dehradun

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ABSTRACT

Background: Folate deficiency has been found to be associated with congenital birth defects like spina bifida, anencephaly, etc. Pregnant women have an increased need for folic acid (600 µg/day) to support the growth of the placenta and foetus and prevent birth defects. **Objectives:** The present study was conducted to assess deficiency of serum folate among pregnant women and its epidemiological determinants.

Methods: A cross-sectional study was conducted in the Doiwala block of Uttarakhand state, a hilly state of the country, and a total of 95 pregnant women participated. Pregnant women were randomly selected from antenatal clinics organized at sub-centres and primary health centres. Blood samples were analyzed for serum folate by competitive immunoassay using direct chemiluminescent technology. Fully automated ADVIA Centaur XP Immunoassay system was used for analysis.

Results: Analysis of data showed that mean serum folate level among pregnant women was 10.1 ± 5.9 ng/ml (95% confidence interval 8.9–11.2). As per World Health Organization cut-off levels, a total of 28 (29.4%), 95% CI (20.2–38.5), women were having folate deficiency (≤ 5.9 ng/ml), and out of them 3 were having folate levels below 3 ng/ml (severe folate deficiency). 9 (9.4%) women had elevated levels (>20 ng/ml). 57 (60%) women were in the normal range (6–20 ng/ml).

Out of various biosocial determinants considered, education and economic status have shown significant association with folate deficiency. A significant difference was observed in the mean serum folate level among pregnant women living in joint and nuclear type of family. Mean serum folate levels showed a slight negative correlation with body mass index (Spearman's $\rho = -0.22$, $p = 0.03900$) and gestational age (correlation coefficient: $r = -0.26$, $p = 0.009$).

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Conclusion: It is concluded that higher level of education, living in joint family during pregnancy and availability of designated APL (Above Poverty Line) or BPL (Below Poverty Line) card are important determinants for better serum folate level and lower chances of folate deficiency.

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1. Introduction

Globally, each year, 3–4 lakh infants are born with neural tube defects (NTDs) like spina bifida, anencephaly, myelomeningocele (open spina bifida), craniorachischisis, spinal dysraphism and encephalocele.¹ The prevalence is approximately 1–5 per 1000 live births at global level.² In India, the overall birth prevalence of neural tube defects is 4.5 per 1000 total births (95% confidence interval (CI) 4.2–4.9), which is higher than global level.³

Folate is one of the components required for neural tube closure.⁴ Neural tube closure is a complex, inadequately understood morphogenetic process, which is accomplished by 28 days of gestation, before the woman realizes she is pregnant.⁴ Folate (the naturally occurring form) or folic acid (the synthetic form) is a member of the water-soluble B complex vitamins. Studies till date have shown decreased maternal folate levels in NTD-affected pregnancies. The decreased levels of serum folate could arise as a consequence of dietary deficiency, a genetic defect in the folate metabolism or both.⁵ A study conducted by Basu et al. in India reported 41.6% folate deficiency by measuring serum folate level among pregnant women,⁶ but this is the status reported 42 years ago. There has been very limited published data available on serum folate level among pregnant women in the recent past from the country.^{7,8}

Various researches have reported that the extent of potential nutrient deficiency during pregnancy may vary by demographic characteristics, such as younger maternal age and lower income and education.^{1,9} One of the underlying causes of dietary deficiency is increased requirement of folate during pregnancy due to changes in physiology and homeostatic control.^{4,10} Although increased requirement of folate should preferably be met through food sources, even within the developed world, it may be unlikely that pregnant and childbearing age women meet their needs for folate and other micronutrients, such as iron and calcium, through foods alone.^{10,11}

As the neural tube closure completes by the first month of gestation, adequate levels of folic acid are important during peri-conceptional period. Supplementation of peri-conceptional folic acid has already shown to decrease both the occurrence and recurrence of NTD, and approximately 75% of spina bifida and anencephaly are preventable by folic acid supplementation; however, many studies have reported¹² that there is very poor awareness and supplementation practices of folic acid among health providers and pregnant women. So even after being a very successful strategy, its effectiveness remains low in many community settings.

So, it becomes important to assess current level of folate deficiency among pregnant women along with its various determinants, so that besides putting emphasis on peri-conceptional folic acid supplementation, other determinants can also be considered to make comprehensive strategy for addressing the problem and reducing the NTDs occurrence.

2. Methods

The present research paper has developed out of the comprehensive intramural research project entitled “A KAP study on folic acid supplementation – Perspective of health providers & pregnant women and assessment of serum folic acid among pregnant women of Block Doiwala, District Dehradun”.

Study location: Uttarakhand is one of the newly formed states of the country (India). District Dehradun is one of the 13 districts of the state with totally six blocks. Out of six blocks, Doiwala block was randomly selected for the study purpose.

Study design, period and population: A cross-sectional study was conducted from August 2014 to August 2015.

Sample: For the overall study, it was estimated that a sample of 400 pregnant women would be required based on presumed prevalence of knowledge of folic acid as 50% (no previous data on the mentioned indicator was available from the region) with allowable error of 5% at 95% confidence level. However, due to limited financial resources, folate estimation was planned for the sub-sample of 100 women (25% of the total sample size). Out of the total 400 pregnant women who participated for the above-mentioned KAP study, as per systematic random sampling, every fourth woman was selected for blood examination after informed consent.

Exclusion criteria: Any woman found seriously ill or having diarrhoea, steatorrhoea and hyperemesis was excluded, as these diseases have been reported to vary the serum folate level.

Site selection and data collection: Pregnant women were recruited in the study from the antenatal clinics organized weekly at sub-centres (at village level) of the block. There were totally 23 sub-centres in the block. As on an average 6–7 pregnant women attend these antenatal clinics, hence all the women attending the clinic for the day were recruited for overall KAP study and from them blood sample was drawn for every fourth woman for the estimation of folate level. Totally, 100 blood samples were drawn.

Data were collected using pre-designed and pre-tested questionnaire, which was administered by the study investigators and field staff after 1 day of orientation. Questionnaire has included information regarding biosocial profile,

obstetrical history, current practice of folic acid intake/history of starting folic acid pre-conceptionally, etc.

Body mass index (BMI) was calculated by using the standard formula, i.e. weight in kilogram and height in metre². Height was measured by stadiometer nearest to 1 cm. Pre-pregnancy weight was noted by asking pregnant women about the record of her weight in a month before she got pregnant. Only 89 women could provide the information about their weight during pre-pregnancy status.

Economic status of the participants was decided as per the type of ration cards available with them. BPL card is provided to the families with income less than 19,884 per year, with average of five members in the family.¹³

Method of folic acid estimation: Blood was drawn from the ante-cubital vein and samples were processed within 4 h of blood collection. Aliquots were stored at -20°C for batch analysis at the end of the study. Blood samples were analyzed for serum folic acid using competitive immunoassay using direct chemiluminescent technology. Fully automated ADVIA Centaur XP Immunoassay System was used for analysis. Out of total 100 samples, five samples were found to be haemolysed and were not analyzed; hence, serum folate could be assessed for 95 pregnant women.

According to the World Health Organization (WHO) folate cut-off points reported in 2015, a serum folate concentration of $>20\text{ ng/ml}$ is considered elevated, $6\text{--}20\text{ ng/ml}$ is normal, $3\text{--}5.9\text{ ng/ml}$ is defined as possible deficiency and concentration of $<3\text{ ng/ml}$ is considered presence of severe folate deficiency.¹⁴

Data analysis: The data were entered into Microsoft-Excel 2007. The statistical analysis was performed using the SPSS (version 17.0). The results were expressed as the mean $\pm$ SD. Frequency tables and cross tables were made. The data were further categorized according to different covariates under study. Pearson's chi-square test was applied in comparisons of independent and dependent proportions and t-test was applied to compare the mean. A p value <0.05 was deemed significant.

Ethical considerations: The study was undertaken after approval from the Institutional Ethics Committee. Informed consent in writing was obtained from all the study participants prior to interview and drawing blood sample.

3. Results

This research paper presents the analysis of 95 pregnant women, for whom serum folate results could be analyzed.

The mean age of the study participants (pregnant women) was 24.6 ± 2.95 years. Majority of them (85) were Hindu by religion and homemaker by occupation (90). Twenty-two (23.15%) women were illiterate and 73 (76.84%) were literate, having varying degree of formal education. Forty-two (44.21%) participants were primigravida while 53 women had at least one pregnancy in the past, which lasted at least for 28 weeks. Only 12 women gave positive history of abortion or still birth in the past, while 83 (87.36%) did not report any event suggestive of bad obstetric history. Unexpectedly, 85 out of 95 pregnant women reported that the current pregnancy under study is their planned pregnancy.

Overall mean serum folate level of the pregnant women was $10.1 \pm 5.9\text{ ng/ml}$ (95% CI $8.9\text{--}11.2$) with minimum value being 2 ng/ml to maximum value 24 ng/ml . Median was 9.01 ng/ml (inter-quartile range 7.5). As per WHO cut-off levels, totally 28 (29.4%) (95% CI $20.2\text{--}38.5$) women were having folate deficiency ($\leq 5.9\text{ ng/ml}$). Out of them, 3 were having folate levels below 3 ng/ml (folate deficiency). 9 (9.4%) women had elevated levels ($>20\text{ ng/ml}$). 57 (60%) were in normal range ($6\text{--}20\text{ ng/ml}$).

Age-wise analysis showed variable serum folate levels at different age groups, lowest being in women more than 30 years of age ($8.32 \pm 4.89\text{ ng/ml}$), and folate deficiency was also highest among them (50%); however, the results were not statistically significant. Increasing level of education showed rise in the level of mean serum folate while decrease in the proportion of folate deficiency and the association was statistically significant ($p = 0.002$). Family type had a distinct effect on serum levels, and those living in nuclear family as compared to the joint families were more likely to have lower folate levels ($p = 0.02$) (Table 1).

When mean serum folate was analyzed against BMI, it was observed that women having BMI in the overweight and obese category were having lower serum folate levels than the women belonging to normal and underweight BMI category, but on applying t-test, difference was not significant ($p = 0.08$); similarly, overweight and obese women were having more folate deficiency in comparison to normal and underweight women, but in this case also association was not significant ($p = 0.33$). However, BMI was found to be negatively correlated with mean folate levels (Spearman's $\rho = -0.218$, $p = 0.039$) (Table 1).

In economic status-wise analysis of serum levels, it was found that those having any kind of card issued from the government (APL and BPL card holders) were having higher serum folate levels and lesser folate deficiency than those not having any card or not aware about the status of the card and the results were statistically significant (Table 1).

Besides the above-discussed variables, some more variables were also analyzed like occupation, religion, bad obstetric history, parity, whether pregnancy was pre-planned or not, pregnant women reporting peri-conceptional folic acid supplementation, etc. These variables did not show any significant association with either mean serum folate level or with folate deficiency.

On examination of the relation between gestational age and mean folic acid level (Table 2), it was found that serum folate was highest during 17–20 weeks ($10.94 \pm 5.24\text{ ng/ml}$) and they showed statistically significant, slightly negative correlation (correlation coefficient = -0.26 , $p = 0.009$).

4. Discussion

This study was a step towards assessing the prevalence of folate deficiency and its associated factors among pregnant women of Uttarakhand, India. This research article focuses on analysis of 95 pregnant women for whom the serum folic acid level could be assessed.

Overall mean serum folate level of the pregnant women was $10.1 \pm 5.9\text{ ng/ml}$ with 28 (29.4%) women having folate

Table 1 – Bio-demographic variable of pregnant women and their mean folic acid levels and prevalence of folic acid deficiency.

Variable	Total number (N = 95)	Serum folate level, mean $\pm$ SD (ng/ml)	Folate deficiency (serum folate $\leq$ 5.9 ng/ml) No. (percentage)
Overall folate level	95	10.1 $\pm$ 5.9 95% CI (8.9–11.2)	28 (29.4%) 95% CI (20.2 –38.5)
Age (years)			
$\leq$ 20	9	11.1 $\pm$ 5.9	1 (11.1%)
21–25	55	9.48 $\pm$ 5.4	15 (27.2%)
26–30	27	11.2 $\pm$ 6.9	10 (37%)
>30	4	8.32 $\pm$ 4.89	2 (50%)
ANOVA (F) = 0.77, p = 0.51; Pearson's correlation (r) = -0.036 , p = 0.72			
Education			
Illiterate	22	7.98 $\pm$ 5.07	11 (50%)
Up to 5th class	9	9.54 $\pm$ 3.96	1 (11.1%)
Up to 8th class	14	6.54 $\pm$ 3.90	8 (57.1%)
Up to 10th class	18	12.98 $\pm$ 6.0	1 (5.5%)
Up to 12th class	16	10.60 $\pm$ 5.67	4 (25%)
Graduate or postgraduate	16	12.70 $\pm$ 7.30	3 (18.7%)
t-test between education $\leq$ up to 8th class and $\geq$ up to 10th class, t = 3.77, df = 93, p = $<.001$; chi-square = 9.2, df = 1, p = 0.002			
Occupation			
House wife	90	10.0 $\pm$ 5.8	26 (28.8%)
Others	5	10.43 $\pm$ 8.42	2 (40%)
t-test, t = 0.12, p = 0.90; Fischer exact test, df = 1, p = 0.62			
Family type			
Nuclear	33	8.19 $\pm$ 4.47	13 (39.3%)
Joint family	62	10.0 $\pm$ 6.40	15 (24.1%)
t-test, t = 2.3, df = 93, p = 0.021, Chi = 2.39, df = 1, p = 0.12			
Economic status			
BPL card holder	29	10.5 $\pm$ 6.24	8 (27.5%)
APL card holder	25	11.95 $\pm$ 6.17	3 (12%)
No card/don't know	41	8.66 $\pm$ 5.29	17 (41.4%)
t-test, t = 2.1, p = 0.037, between APL + BPL and Don't know/no card holder, Chi = 4.99, df = 1, p = 0.025			
Body mass index (BMI) (N = 89)			
<18.5 kg/m ² (underweight)	10	11.7 $\pm$ 5.8	2 (20%)
18.5–22.9 kg/m ² (normal)	48	10.7 $\pm$ 5.8	12 (25%)
23–25 kg/m ² (overweight)	20	7.88 $\pm$ 5.47	9 (45%)
>25 kg/m ² (obese)	11	9.67 $\pm$ 6.14	4 (36.4%)
Spearman's rho = -0.218 , p = 0.039, two-tailed; Kruskal–Wallis chi = 6.5, df = 3, p = 0.08			
Obstetric history			
Previous abortion/still birth	12	12.04 $\pm$ 6.79	2 (16.7%)
No previous abortion/no still birth	83	9.83 $\pm$ 5.79	26 (31.3%)
t-test, t = 1.1, df = 93, p = 0.27, Fischer exact test p = 0.34			
Pre-planned pregnancy			
Yes	85	10.11 $\pm$ 5.93	26 (30.5%)
No	10	10.27 $\pm$ 6.30	2 (20%)
t-test, t = 0.089, df = 93, p = 0.92, Fischer exact test p = 0.71			
Pregnant women taken peri-conceptional folic acid supplementation			
Yes	13	9.2 $\pm$ 5.8	4 (30.7%)
No	82	10.2 $\pm$ 5.9	16 (19.5%)
t-test, t = 0.57, df = 93, p = 0.56, Fischer exact test p = 0.46			
SD, standard deviation; CI, confidence interval; ng/ml, nanogram/millilitre.			

deficiency ($\leq$ 5.9 ng/ml), and out of them 3 were having folate deficiency ($<$ 3 ng/ml).

Variable levels of serum folic acid were observed at different age groups, on applying ANOVA, and findings were not statistically significant (p = 0.51). Similar findings were reported by McArthur et al. that serum folate and erythrocyte folate concentrations did not show any relationships with age.¹⁶

Study findings revealed that with increasing level of education, serum folic acid level increases, while folic acid deficiency decreases and these findings show significant

association. Similar findings have been mirrored by various studies in which it was found that education beyond primary school was associated with higher intakes of iron, folate, vitamin B12 and vitamin A, when compared to those who had not completed primary school.¹⁷ The better dietary pattern gets reflected in better serum folate levels. Also it was found that those who were educated were more likely to take supplementation.¹² Studies in India and elsewhere have confirmed these finding. Both Iron Folic Acid receipt and consumption improved with higher educational attainment and household wealth.¹⁸

Table 2 – Gestational age and mean serum folate level and folate deficiency.

Gestational age (weeks)	Total number	Mean serum folate $\pm$ SD (95% CI)	Percentage of women below 5.9 ng/ml folate level
Up to 8 weeks	4	8.2 $\pm$ 4.0 (4.28–12.1)	1 (25%)
8–12 weeks	29	12.78 $\pm$ 6.97 (10.1–15.2)	6 (20.6%)
13–16 weeks	15	9.86 $\pm$ 5.72 (6.98–12.7)	2 (13.3%)
17–20 weeks	15	10.94 $\pm$ 5.24 (8.27–13.5)	2 (13.3%)
21–24 weeks	14	7.39 $\pm$ 4.10 (5.24–9.54)	7 (50%)
25–28 weeks	11	7.22 $\pm$ 4.94 (4.3–10.1)	7 (63.6%)
29–33 weeks	3	11.59 $\pm$ 5.29 (9.7–13.2)	1 (33.3%)
34–38 weeks	4	6.89 $\pm$ 4.27 (2.7–11)	2 (50%)
Correlation coefficient: $r = -0.2$, $p = 0.009$; Kruskal–Wallis $\chi^2 = 13.3$, $df = 7$, $p = 0.065$			
SD, standard deviation; CI, confidence interval; ng/ml, nanogram/millilitre.			

This relationship has been found in many studies and shown to play a role in a variety of maternal health service utilization patterns as well as pregnancy outcomes.^{19,20,21}

No significant difference was found with occupation or religion but the family type had a distinct effect on serum levels, and those living in nuclear family as compared to the joint families were more likely to have lower folic acid levels ($p = 0.02$). Such a finding has been reflected in many studies.^{22,23} Some underlined the importance of family type over financial background and pointed out that high nutritional status was not just a function of income because even in same income group the joint families had higher levels of consumption than nuclear families, suggesting that types of family influenced nutrient intake. It has been traditionally argued that urbanization and educational growth would lead to a decline in extended families with the complementary rise in nuclear families. It has been suggested that such transitions are good for reproductive age group women because living in nuclear families benefits their health. However, recent studies including ours suggest otherwise. A study conducted by Varadharajan et al. in 2013 reported that young married women living in nuclear families do not have better health than those in patrilocal extended families.²⁴

On comparison of economic status, it was found that those having any kind of card issued from the government and thus receiving specified quantities of selected commodities at subsidized prices (APL and BPL card holders) were having higher serum folate levels and lesser folate deficiency than those not having any card, and thus not being supported by the government. The findings are in concurrence with many studies suggesting that underprivileged women are at increased risk for insufficient micronutrient intakes due to poor diet quality. Malnutrition is high among women in India with multiple reasons; however, one of the usual determinants, namely poverty, is of paramount importance since not only is poverty one of the basic causes of malnutrition, but malnutrition is also considered to be both an outcome and a manifestation of poverty.²⁴

In the study findings, in spite of not having any significant association of BMI and folate deficiency, a slightly negative correlation was observed, which was statistically significant. Our findings have been supported by many previous studies,^{25,26} which state that after controlling for intake of

folate in food and nutritional supplements, increased BMI in childbearing age women was associated with a lower serum folate level. The reason for such a finding may be that the body's distribution of folate is significantly affected by obesity and points towards a possibility of recommending a higher folic acid supplementation in those having higher BMI.

One very interesting finding that has come out from the study is that out of 95 pregnant women, 85 reported that their current pregnancy was pre-planned. Such a high level of planned pregnancy could be attributed for high level of literacy rate among pregnant women (77%), and this has also been pointed out in some other recent study that educated women are more aware about the significance of planned family formation approach.²⁷ This is a very important finding as this indicates towards the opportunity for programme implementers to counsel and educate such women regarding folic acid and other interventions even before conception for healthy outcome of both mother and baby.

5. Conclusion

Study findings reveal that high level of folate deficiency (29.4%) is prevalent among pregnant women. Education, type of family, economic status and BMI came out to be important determinants of folate deficiency. These identified determinants could be considered while planning comprehensive strategy for reduction of folate deficiency. Higher level of education, living in joint family during pregnancy and availability of designated APL or BPL card may be important strategies for reducing the deficiency. The study has also reported very high level of planned pregnancies, indicating an opportunity for providing timely interventions.

The study recommends that health system should take opportunity to counsel and educate pregnant women during their pre-pregnancy period for improving their dietary intake by ensuring availability of APL/BPL card with them, and suggests to stay with joint family or some family member to take care of their diet. Education is the important factor for adopting healthy practices, so higher education for girls should be promoted by coordinating with education department.

5.1. Strengths and weaknesses of the study

The present research took up an important topic linked with maternal and child health and it was conducted at primary care setting to avoid various confounders. Implicit aim of the study was to emphasize importance of already existing solutions for reducing the folic acid deficiency rather than stressing only on pre-conceptional folic acid intake.

The present research being the secondary objective of larger KAP study entitled “A KAP study on folic acid supplementation – Perspective of health providers & pregnant women and assessment of serum folic acid level among pregnant women of Block Doiwala, District Dehradun” attempted to assess the folic acid level in the sub-sample (100) of total sample (400) selected for the above study. The current status of deficiency, analyzed in the study, represents the status only for the women who participated in the study. It is a pilot study for the area as no data on folate deficiency were available from the region. A study with larger sample size covering wider region to include all other possible variability should be conducted for generalization of the findings.

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Conflicts of interest

The authors have none to declare.

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