



## FOLIC ACID;

### IS SIMPLE FOLIC ACID EFFECTIVE TO REPLENISH RBC FOLATE STORES AMONG PAKISTANI FEMALE PATIENTS? SMALL CASE.

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**ABSTRACT... Objectives:** The objective of our study was to assess the effectiveness of folic acid in optimizing the red blood cells folate levels and to observe the frequency of folate resistance among Pakistani female patients. **Setting:** Outpatient Department of Obstetrics & Gynaecology, Jinnah Post graduate Medical Centre (JPMC), Karachi. **Period:** January–July 2016. **Methodology:** Participants fulfilling the inclusion criteria were included after informed consent. Detail history and physical examination was done in each participant. All study participants received 5mg (400 µg) folic acid as a daily supplement for 24 weeks. Red blood cell folate concentrations were measured at baseline and after 24 weeks of therapy. Paired sample t-test was used to find out significant difference between folate levels. **Results:** A total of 44 women (23 pregnant while 21 non pregnant) were included in the study. Mean age of the participants was 27.6 ± 5.9 years and mean BMI was 23.9 ± 4.1 kg/m<sup>2</sup> respectively. The mean values of Red blood cells folate at baseline and at 24 weeks were 623.6 ± 406.6 and 861.9 ± 432.4 respectively. Paired sample t-test results showed that there was no significant difference. Thirty-two (70.4%) women showed an increase in RBC folate status while 13 (29.6%) women had steady or decreased levels of folate after taking folic acid for 24 weeks which may be due to RBS enzyme methylenetetrahydrofolate reductase (MTHFR) deficiency. **Conclusion:** Simple folic acid supplementation is not very helpful in improving folate status in female Pakistani patients. Resistant to improvement may be due to MTHFR deficiency in our study subjects.

**Key words:** Folic Acid, Red Blood Cell Folate, [6S]-5 Methyl-tetrahydrofolate, Neural Tube Defect.

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## INTRODUCTION

Vitamin B<sub>9</sub> (folic acid and folate) is essential for various metabolic functions in our body. Our body needs folate to synthesize, repair, and methylate DNA as well as to act as a cofactor in certain biological reactions. It is also required during active phases of growth, such as in infancy and pregnancy therefore pregnant women are prone to folate and cobalamin deficiency because of high physiological requirement during pregnancy.<sup>1</sup>

Folate deficiency during pregnancy has been implicated as a risk factor for various complications including neural tube defects (NTDS), low birth weight of newborns, anemia during pregnancy and cervical dysplasia.<sup>2</sup> It has been proven through research that proper supplementation of folic acid intake before and during pregnancy reduce the incidence of NTDS by 72-100%.<sup>2,3</sup>

Recommendations from health care authorities states that women should take 400µg folic acid/day ≥ 4 week before conceiving and at least during the first three months of pregnancy.<sup>4,5</sup> Infact some countries have started the food fortification with folic acid because unplanned pregnancies are common and only 18-45% women were using recommended peri-conceptual Folic acid.<sup>6-11</sup>

There have been some concerns regarding overuse of folic acid supplementation which may mask the signs and symptoms of vitamin B-12 deficiency during reproductive years.<sup>2</sup> Undiagnosed and untreated B 12 deficiency may result in neurological damage, which can be irreversible.<sup>11</sup> Furthermore it has been observed that synthetic folic acid is directly transported into blood and lifelong exposure of fetal cells to these synthetic forms may have some health

risks.<sup>12</sup> The methylenetetrahydrofolate reductase (MTHFR) enzyme plays a central role in folate metabolism. The MTHFR enzyme irreversibly converts 5,10 methylenetetrahydrofolates to 5-methyltetrahydrofolate, which is the main form of folate in circulation. MTHFR gene mutations are a significant health issues affecting around 10% of the world's population.<sup>13</sup> Recently, a stable supplemental form of [6S]-5 methyltetrahydrofolate ([6S]-5 methyl THF) as calcium salt has become available in Pakistan. Efficacy of this compound has been assessed in clinical practice in pregnant, non-pregnant, lactating and non-lactating women and concluded that it is as effective as folic acid in improving blood folate indexes and lowering plasma levels of homocysteine.<sup>13-15</sup>

The objective of our study was to assess the effectiveness of folic acid in improving the red blood cells folate levels and to observe the frequency of folate resistance among Pakistani women in pregnant and non-pregnant state.

## PATIENTS & METHODS

This clinical study was conducted in the out patients department of Gynaecology & Obstetrics Jinnah Postgraduate Medical Centre (JPMC), a tertiary care hospital of Karachi, Pakistan from January to July 2016. Twenty-three pregnant women in first trimester of pregnancy and twenty-one non pregnant women were included in the study after taking informed consent. Participants were divided in two groups (Group A and Group B). A detailed history and physical examination were performed in all participants. Women who were anemic at baseline were excluded from the study. Women who became pregnant after taking treatment for infertility, women already taking iron and folic acid supplements and women with a

history of B6 or B12 deficiency anemia were also excluded from the study. Physical examination including weight, height, and Body mass index (BMI) were obtained. Laboratory tests included complete blood count (CBC), Red Blood Cell Folate and Plasma Folate levels. Hematocrit was measured with the use of a hematology analyzer in freshly collected blood. Red Cell Folate was calculated from whole-blood folate by subtracting plasma folate and correcting for hematocrit. Five mg (400  $\mu$ g) of folic acid was given to every participant for 24 weeks and blood samples were again drawn for RBC Folate.

SPSS version 17 was used for data analyses. Data is presented in the form of mean and percentages. The difference between groups was assessed using Paired t test.

## RESULTS

A total of 44 women (23 pregnant in first trimester, while 21 non pregnant) were enrolled in the study. Mean age of the participants was  $27.6 \pm 5.9$  years and mean BMI was  $23.9 \pm 4.1$  kg/m<sup>2</sup> respectively. The mean values of Red blood cells folate at baseline and at 24 weeks were  $623.6 \pm 406.6$  and  $861.9 \pm 432.4$  respectively (Table-I). Mean values of RBC Folate concentration after 24 weeks of 5 mg folic acid supplementation was  $880.4 \pm 532.1$  in group A and  $856 \pm 404.2$  in group B respectively. Thirty-two (70.4%) women showed an increase in RBC folate status while 13 (29.6%) women had steady or decreased levels of folate after taking folic acid for 24 weeks which may be due to RBS enzyme methylenetetrahydrofolate reductase (MTHFR) deficiency.(Table-I). Statistical analyses showed that there was non significant difference in group A (pregnant female) while there is statistical significance in group B (non pregnant female) (Table-II).

Parameters	Overall	Group-A (pregnant)	Group-B (non-pregnant)
No of participants	44	23	21
Mean age (year)	$27.6 \pm 5.9$	$28.5 \pm 4.3$	$27.3 \pm 6.4$
Mean BMI (kg/m <sup>2</sup> )	$23.9 \pm 4.1$	$25.6 \pm 5.2$	$22.6 \pm 2.3$
Mean RBC folate (at baseline)	$623.6 \pm 406.6$	$745.9 \pm 637.1$	$584 \pm 301.4$
Mean RBC folate (at 24 <sup>th</sup> week)	$861.9 \pm 432.4$	$880.4 \pm 532.1$	$856 \pm 404.2$
Increase in RBC folate (after 5mg folic acid)	31 (70.4%)	17 (73.9%)	15 (71.4%)
Decrease in RBC folate (after 5mg folic acid)	13 (29.6%)	6 (26.1%)	6 (28.6%)

Table-I. Demographic profile and variable of the study subjects

RBC Folate concentration (ng/ml)	n	Baseline Mean $\pm$ SD	At 24 <sup>th</sup> week Mean $\pm$ SD	P-value
Group-A (pregnant)	23	745.9 $\pm$ 637.1	880.4 $\pm$ 532.1	0.462 <sup>a</sup> 0.285 <sup>b</sup>
Group-B (non-pregnant)	21	584.0 $\pm$ 301.4	856.0 $\pm$ 404.2	0.002 <sup>a</sup> 0.003 <sup>b</sup>

**Table-II. Pre and post folate concentration comparison**

*P-value < 0.05 was considered statistically significant*

<sup>a</sup> Paired t-test used for p-value

<sup>b</sup> Wilcoxon test used for p-value

Data presented as mean  $\pm$  S.D. or frequency (percentage)

## DISCUSSION

The present study describes the folate status of pregnant and non-pregnant female and role of folic acid intake at increasing levels of red blood cell folate in these women. The red blood cell folate concentration was used as an indicator of folat status in body. The folate level is shown to be a risk factor for NTDs.<sup>16,17</sup>

Studies have shown that the proper intake of folic acid during peri-gestational period can decrease the chances of having NTDs in newborns. It has also been observed that administration of 5-MTHF is more effective than simple folic acid intake at improving folate status<sup>23</sup>, thus providing a protective effect in preventing neural tube defects in early pregnancy.<sup>24,25</sup> In addition, 5-MTHF was observed to be an equally effective alternative to folic acid in decreasing total homocysteine concentrations (tHcy) in women.<sup>15</sup> Daily use of 400  $\mu$ g folic acid per day has been recommended by public health authorities worldwide before and during early week of gestation to reduce the risk of NTDs.<sup>4,5</sup>

Despite proven efficacy, the use of iron and folic acid (IFA) supplements during pregnancy is low with only 38% of women reporting regular intake during their last pregnancy.<sup>3,17,18</sup> A study regarding Knowledge and practices regarding Folic acid in Pakistan concluded that knowledge regarding folic acid use and its importance is quite deficient especially in women of reproductive age group which is the most vulnerable group.<sup>17</sup>

In our study the baseline value of red cell folate was 629 which is quite low hence there is a need

to adequately use folic acid specially during the reproductive years and more specifically during pregnancy. This finding is of importance as recent observations stated that prevention of most folate related NTDs is possible through increase in folic acid intake.

Thirty-one (68.8%) women in our study showed an increase in folate status while 14(31.2%) women had steady or decreased levels of folate after taking folic acid for 24 weeks. This trend might be due to MTHFR gene mutations which is a significant health issues affecting around 10% of the world's population. MTHFR gene polymorphisms have been linked to a variety of conditions ranging from autism to cancer, from hypertension to recurrent pregnancy loss, as well as peripheral artery disease, migraine, and various neuropsychiatric diseases.<sup>19,20</sup>

Folic acid, is a synthetic vitamin which is not found in large amounts in food. Folic acid is stable while the natural forms of folate are unstable.<sup>21-23</sup> The folate that is most commonly found in foods cannot be absorbed at the brush border of the intestine until it is converted to simpler form of monoglutamate from naturally occurring polyglutamates. Synthetic folic acid is already in the monoglutamate form so absorption is easy and quick. Folic acid must be converted first into tetrahydrofolate (THF) by an enzyme named dihydrofolate reductase and then finally converted into the active form of 5-MTHF by the enzyme MTHFR through a series of steps .5-MTHF is the most common form found in the bloodstream.<sup>21-23</sup> It is still unclear whether supplementation of folic acid or 5-MTHF is preferred and the best form of supplemental folate is unresolved; as both seems to be good choices.<sup>24</sup> Various studies are in progress to understand any differences

in absorption and metabolism of folic acid vs 5-MTHF. Our findings provide important evidence for health care providers dealing with public health, nutrition and supplementation programs specially in developing countries. Health education of general population through health care professionals, symposiums and workshops, print and electronic media, can help in increasing awareness regarding folic acid supplementation in women during pregnancy. This in turn may help in reducing the incidence of neural tube defects and other abnormalities in newborn. However there are certain limitations to our study such as small sample size and absence of control group. We are planning a randomized control trial (RCT) comparing results of new generation of folic acid vs simple folic acid.

## CONCLUSION

Simple folic acid supplementation is not very helpful in improving folate status in Pakistani pregnant women. Resistant to improvement may be due to MTHFR deficiency in our study subjects. Further large scale studies are needed to validate our findings.

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*“Doing what you like is freedom.  
Liking what you do is happiness.”*

**Unknown**

**AUTHORSHIP AND CONTRIBUTION DECLARATION**

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2	Shireen Bhutta	Statistical expertise, Critical revision of the article for important intellectual content.	
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