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Effect of high dose folic acid supplementation in pregnant women on pre-eclampsia

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قال عز وجل:

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ﴾

[المجادلة:١١]

A knowledge

First we must thank Allah for give us the chance to do and complete this research.

And thank our superviser Rawaa G. Abdullah for teach and help us to do it .

Thanks for the hospital and the lab for help us getting some results.

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ABSTRACT

Objective: To determine the efficacy of high dose folic acid supplementation for prevention of pre-eclampsia in women with at least one risk factor: pre-existing hypertension, pre-pregnancy diabetes (type 1 or 2), twin pregnancy, pre-eclampsia in a previous pregnancy, or body mass index ≥35.

Introduction

Pre-eclampsia and the role of folic acid in it

One of the main goals of prenatal care is control of hypertension disorders and pre-eclampsia.

Pre-eclampsia is one of the unsolved puzzles in medicine with high prevalence and maternal mortality, especially in low- and middle-income countries (2). One of the main hypotheses associated with the cause of pre-eclampsia is placental insufficiency and its vascular discrepancy, which is caused by increasing levels of homocysteine (Hcy)(3). Homocysteine is a sulfur containing amino acid metabolized through remission of methionine, which is one of the two pathways requiring folic acid (Methyl tetra hydro folate pathway) (3).Decreasing folate levels lead to lower erythrocyte folate levels, an increase in Hcy concentration and variations in other fast-growing tissues (4). These problems may cause a vaso-occlusive effect on the placenta ,neural tube defects, recurrent abortion and pre-eclampsia late in pregnancy (5). It has been shown that the risk of pre-eclampsia in pregnant women with hyperhomocysteinemia and low folate status increased several times compared to the controls(6). One study showed that taking high doses of folic acid (3–9 mg daily) reduced the rate of preterm labor and early onset of pre-eclampsia (7). In another study, folic acid intake early in the second trimester reduced the risk of pre-eclampsia (8). There was no difference in the rate of pregnancy complications or pre-eclampsia between taking 200 µg, 400 µg and 5 mg of folic acid per day in another study (9,10). Supplementation of 4 mg of folic acid throughout pregnancy is considered a new prevention strategy for pre-eclampsia. Daily

supplementation with 4mg of folic acid starting in early pregnancy (8 to 16 weeks of gestation) until delivery has been effective in preventing pre-eclampsia(11). Further studies are needed to specify whether a Hcy metabolism disorder is the main cause or increased Hcy levels are a secondary cause of pre-eclampsia/eclampsia during pregnancy and whether it is necessary to maintain the high level of folate or not. There is no definitive known cause of pre-eclampsia and there is no definitive way to identify the individuals at risk. It occurs in women with first or multiple pregnancies and is characterized by new onset hypertension and proteinuria [14].Preeclampsia has several different underlying pathologies and pathologic phenotypes. So identifying women at risk from low-risk individuals is very difficult from clinical characteristics and biochemical markers in first-trimester women that would possibly predict the subsequent development of preeclampsia.

Folic Acid in general:

What is the Folic acid?

Folate and folic acid are forms of vitamin B9 used for deficiency and to prevent pregnancy complications. Many foods contain folate or have folic acid added. (1)

Where can we find it?

Since 1998, folic acid has been added to cold cereals, flour, breads, pasta, bakery items, cookies, and crackers, as required by federal law. Foods that are naturally high in folate include leafy vegetables, okra, asparagus, certain fruits, beans, yeast, mushrooms, animal liver and kidney, orange juice, and tomato juice. Folic acid is also available as a supplement, and is often used in combination with other B vitamins.(1)

What is the use of it?

Folic acid is used for preventing and treating low blood levels of folate (folate deficiency) and high blood levels of homocysteine (hyperhomocysteinemia). People who are pregnant or might become pregnant take folic acid to prevent serious birth defects such as spina bifida. Folic acid is also used for many other conditions including

depression, stroke, decline in memory and thinking skills, and many others.(1)

The usual dose if you're trying to get pregnant and during the first 12 weeks of pregnancy is 400 micrograms, taken once a day.(12)

The recommended daily amount of folate for adults is 400 micrograms (mcg). Adult women who are planning pregnancy or could become pregnant should be advised to get 400 to 1,000 mcg of folic acid a day.

How Much Folic Acid Should You Take?

Category Folate (Folic Acid) Recommended Dietary Allowance (RDA) For children under 1, only an adequate intake (AI) is available4-8 years200 mcg/day9-13 years300 mcg/day14 years and older400 mcg/day Pregnant teens and women (16)

What is Pre-eclampsia ?

Pre-eclampsia, also known as toxemia of pregnancy, “is persistent high blood pressure that develops during pregnancy or the postpartum period and is often associated with high levels of protein in the urine OR the new development of decreased blood platelets, trouble with the kidneys or liver, fluid in the lungs, or signs of brain trouble such as seizures and/or visual disturbances.”(13)

Difference between high BP and preeclampsia?

Pregnancy-induced hypertension is a rise in blood pressure, without proteinuria, during the second half of pregnancy. Pre-eclampsia is a multisystem disorder, unique to pregnancy, that is usually associated with raised blood pressure and proteinuria.

The number one way to treat preeclampsia is to deliver the baby.

First line of treatment for preeclampsia?

Hydralazine and labetalol are the two “first line” agents used for hypertension in preeclampsia. Hydralazine is an arteriolar dilator that reduces blood pressure but may cause tachycardia

Common medications used to treat preeclampsia?

For emergency treatment in preeclampsia, IV hydralazine, labetalol and oral nifedipine can be used [1]. The ACOG Practice Bulletins also recommend that methyldopa and labetalol are appropriate first-line agents and beta-blockers and angiotensin-converting enzyme inhibitors are not recommended

Short term problems

For mom, preeclampsia can lead to eclampsia (full-blown seizures or coma), HELLP syndrome (a life-threatening complication), liver, lung, heart, kidney, or eye damage, and/or stroke.(14) For baby, preeclampsia can lead to intrauterine growth restriction (IUGR), leading to low birth weight (meaning that your baby doesn't grow properly due to insufficient oxygen and nutrients because of placental problems), and/or preterm birth.(14)

Long-term problems

A recently released study found that children born to mothers who had preeclampsia during their pregnancy or postpartum period were more likely to develop heart disease and/or suffer a stroke down the road [15]. Mothers who have had preeclampsia are themselves at increased risk for developing heart disease and/or high blood pressure later on.

Method

Study design and settings

The aim of this study was to determine the association between iron-folic acid supplementation and pregnancy-induced hypertension among pregnant women in the public hospital of Babylon . An institution-based, unmatched case–control study was conducted among pregnant women admitted for delivery in obstetrics and gynecology departments.

Case definition

Cases are defined as pregnant women whose blood pressure was greater than or equal to 140/90 mmHg in two separate readings taken 4 h apart [15]. They were diagnosed and confirmed by obstetrics and gynecology physicians. Controls are defined as pregnant women in the same hospital whose blood pressure is less than 140/90 mmHg after 20 weeks of gestation. (During the study period, cases and controls were identified through record review and after physician diagnosis in ANC clinics and obstetrics and gynecology wards)x. The diagnosis includes history-taking, clinical manifestations, a physical examination, and laboratory tests.

Population

The source populations were pregnant women, both cases and controls, who were admitted for delivery in public hospital in the Babylon zone. The study population consisted of pregnant women who fulfilled the eligibility criteria. These were both cases and controls who were admitted for delivery in the selected hospital during the study period. Consecutively chosen pregnant women, both cases and controls, in the selected hospitals during that study period were the sampled populations.

Laboratory measurements and individuals

Blood samples were drawn from the women's veins on the inside of their elbows. Needles were inserted into the veins ,and the blood samples were collected using airtight vials. The blood samples were put into the centrifuge to separate the serum from the blood and then measure the folic acid amount .

What is the Folic Acid ELISA Kit?

The Folic Acid ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of folic acid in serum, cell or tissue samples. The quantity of folic acid in unknown samples is determined by comparing its absorbance with that of a known folic acid standard curve.

Preparation of Reagents:

Folic Acid Conjugate Coated Plate: Dilute the proper amount of 100X Folic Acid Conjugate 1:100 into IX PBS. Add 100 µL of the diluted 1X Folic Acid Conjugate to each well and incubate at 37 °C for two hours or overnight at 4 °C. Remove the coating solution and wash twice with 200 µL of IX PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4 °C and remove the Assay Diluent immediately before use.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each folic acid sample including unknown and standard should be assayed in duplicate.

2. Add 50 µL of unknown sample or Folic Acid standards to the wells of the Folic Acid Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.

3. Add 50 µl of the diluted Anti-Folic Acid antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.

4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell II strips on absorbent pad or paper towel to remove excess IX Wash Buffer.

5. Add 100 µL of the diluted Secondary Antibody-HRP Enzyme all wells.

6. Incubate at room temperature for 1 hour on an orbital shaker.

7. Wash microwell strips 3 times according to step 4 above.

immediately to the next step.

8. Warm Substrate Solution to room temperature. µL of Substrate Solution to each well, including the blank wells. Incubate at room time may vary from

2-30 minutes. an orbital shaker.

Note: Watch plate carefully; if color et to prevent saturation.
rapidly, the reaction may need to be stopped sooner

Stop the enzyme

9. Actual incubation reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

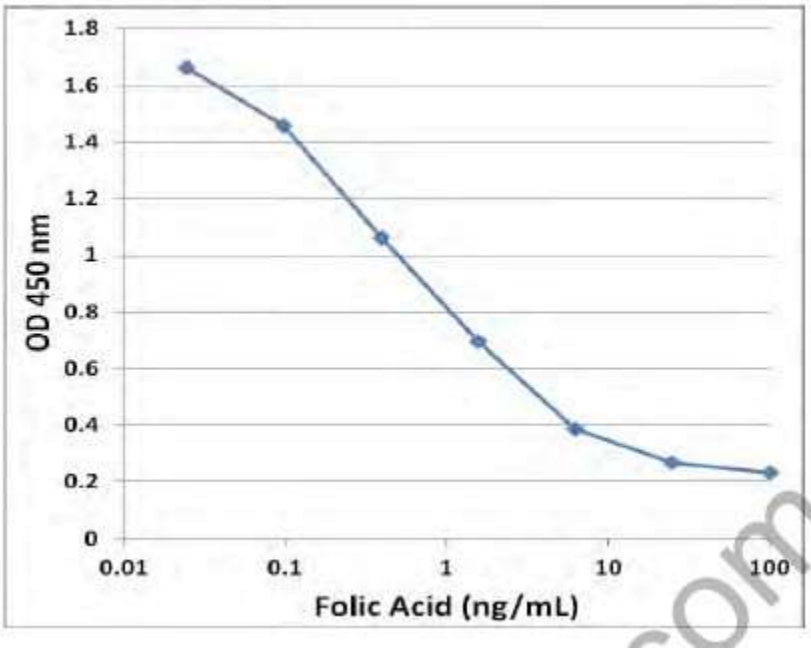


Figure 2: folic acid ELISA standard curve

RESULTS

First we knew that there are a different conditions may lead the folic acid to various range than the normal one,

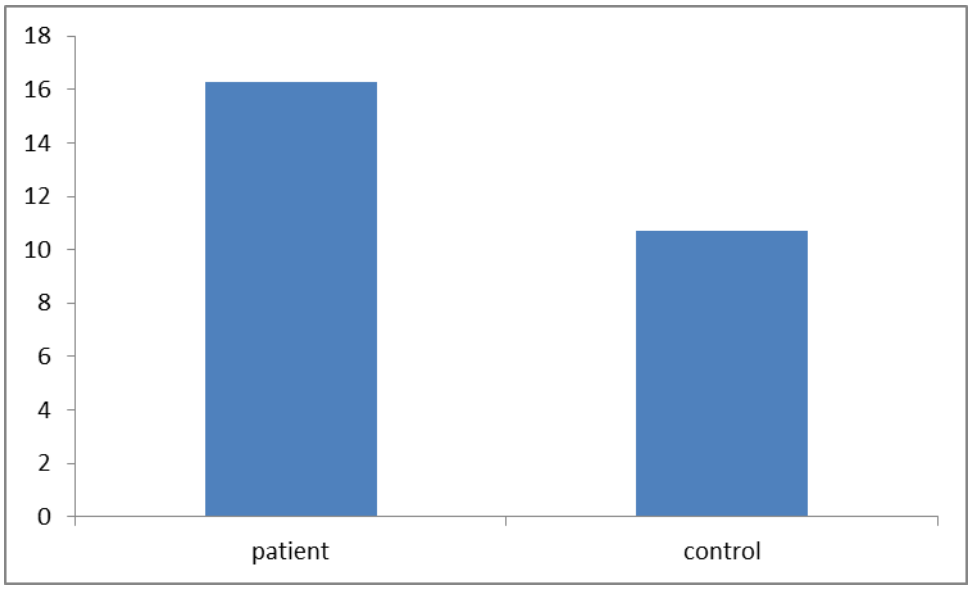
(Knowing that the normal range of folic acid in)

infant is : 14-51 ng/mL

children is: 5-21 ng/mL

adults is: 2-20 ng/mL

	age	Pre eclampcia	control	*P -value*
Experiment 1	38	20	14.5	
Experiment 2	35	5.3	15	
Experiment 3	25	8.2	16.5	
Experiment 4	32	6.5	17.5	
Experiment 5	40	11.5	18.2	
Experiment 6	21	7.5	14.2	
Experiment 7	18	12.4	15.2	
Experiment 8	41	3.2	16	
Experiment 9	27	4.3	18.7	
Experiment 10	20	9.5	17.1	
Experiment 11	24	12.8	17.6	
Experiment 12	33	11.5	13	
Experiment 13	35	17.2	16.8	
Experiment 14	21	14	15.6	
Experiment 15	37	16.5	18.7	
Mean± SD(age)		29.8667± 7.96	29.51 ±7.32	*p >0.05*
Range (age)		19-40	18-41	
Mean± SD(folic acid)		10.69 ± 3.95	16.3 ±1.71	*p <0.05*
Range (folic cid)		5.8 -16.9	13.7 -19.9	



A chart show the SD of folic acid

Discussion

We adjusted analyses for potential confounders by parity, maternal age, and cigarette smoking and confirmed there was no effect of folic acid on the prevention of pre-eclampsia. When we explored the effect of high dose folic acid on risk of pre-eclampsia by country, no difference in effect was observed.

Supplementation with 4.0 mg/day folic acid beyond the first trimester appeared that it does not prevent pre-eclampsia in women at high risk for this condition, while we can give it in the second trimester. However, little research has been conducted to show the effect of folic acid supplement but it do not give such a significant effect.

There are several medical conditions can impact on results which are:

repeated abortion- chronic Diabetes mellitus- gestational diabetes – multiparty- chronic use with overdose folic acid supplement.

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