Study the Effect of Folic Acid as A supplement on Selected Oxidative Stress and Biochemical Parameters in First Trimester of Pregnancy

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Abstract

Folic acid modulates several disorders in humans, pregnancy mostly because of the mitochondria-rich placenta, is a condition that favors oxidative stress.Transitional metals, especially iron, which are particularly abundant in the placenta, are important in the production of free radicals. Adenosine deaminase (ADA), an enzyme essential for the differentiation of lymphoid cells, has been used for monitoring diseases with altered immunity. In the present study we investigated the effect of regular and irregular administered folic acid in the first and second trimester of pregnancy to be compared with non-pregnant women ,and also the effect of regular and irregular using of folic acid supplement on oxidative stress parameters and several biochemical features in first trimester pregnant women. Methods :The Hemoglobin ,Random plasma glucose, transferrin, iron, adenosine deaminase in addition to antioxidants(Total antioxidant capacity (TAC), Glutathione (GSH), Glutathione peroxidase (GSH-Px), Glutathione reductase (GSH-R) and Superoxide dismutase (SOD)), had been measured in studied groups[non pregnant women],group B-I[first trimester], group B-II[second trimester who used folic acid supplementation regularly 5 mg daily] and group B-III [second trimester who used folic acid supplementation irregularly].Results: The level of transferrin, adenosine deaminase (ADA), malondialdehyde (MDA) and uric acid showed significant increase by comparing group B-II and group B-III] ,also when compared to [non pregnant women],group B-I.Iron, Total antioxidant capacity (TAC), Glutathione (GSH), Glutathione peroxidase (GSH-Px), Glutathione reductase (GSH-R) and Superoxide dismutase (SOD) showed significant decrease in group B-II and group B-III when compared to [non pregnant women and group B-I. In conclusion irregular supplementation of folic acid increased indicators of lipid peroxidation in first trimester of pregnancy. Therefore, this regimen of supplemental folic acid may provide excessive amounts of folic acid and appears to increase the risk of oxidative stress.

Key words: Folic acid, lipid peroxidation, adenosine deaminase ,antioxidants, transferrin, Iron and pregnancy .

دراسة تأثير حامض الفوليك كمكمل على الحالة التاكسدية وبعض العوامل الكيموحيوية في الثلث الاول من الحمل

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الخلاصة

ينظم حمض الفوليك العديد من الاضطرابات في البشر، خصوصا عند الحوامل بسبب وجود المشيمة الغنية، بالمايتوكوندريا والتي تحدث فيها العديد من عمليات الأكسدة .إن المعادن الانتقالية، وخاصة الحديد، متوفرة بكثرة في المشيمة إضافة لدورها في إنتاج الجذور الحرة.إن إنزيم ADA ضروري لتمايز الخلايا اللمفاوية، . لقد بينت الدراسة تأثير الاستخدام المنتظم وغير المنتظم لحامض الفوليك كمكمل في الأشهر الثلاثة الأولى والثانية وبالمقارنة مع النساء غير الحوامل، إضافة لتأثيره على الأكسدة . تم قياس الهيموغلوبين , مستوى كلوكوز، transferrin والحديد وإنزيم ADA، بالإضافة إلى مضادات الأكسدة في مجاميع عدة (النساء غير الحوامل)، مجموعة BI (حوامل في الأشهر الثلاثة الأولى)، مجموعة B-II (حوامل في الأشهر الثلاثة الثانية الذين استخدموا مكملات حمض الفوليك بانتظام 5ملغم يوميا) ومجموعة B-III (حوامل في الأشهر الثلاثة الثانية الذين استخدموا مكملات حمض الفوليك بشكل غير منتظم). لقد أظهرت الدراسة حصول زيادة في مستوى transferrin، ADA، MDA وحمض اليوريك بين مجموعة (B-II ومجموعةB-III)، وأيضا بالمقارنة مع (النساء غير الحوامل) 0اظهرت النتائج انخفاض ملحوظ في مستوى الحديد، TAC، GSH، GSH-Px GSH-R وSOD بين مجموعة B-II ومجموعة B-III ، أيضا بالمقارنة مع (النساء غير الحوامل)، مجموعة BI..نستنتج من الدراسة ان الاستعمال غير المنتظم لحامض الفوليك قد زادت من مستوى MDA في الأشهر الثلاثة الأولى من الحمل, لذلك فأن الاستعمال غير المنتظم لحمض الفوليك قد تزيد من خطر الأكسدة.

الكلمات المفتاحية : حامض الفولك ، اكسدة الدهون ، ادينوسين دي امينيز ، مضادات الاكسدة ، ترانسفيرين ، الحديد ، الحمل .

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Introduction

Folic acid is a water-soluble vitamin essential for cell replication and DNA synthesis, repair and methylation [1]. It plays an important role in the pathophysiology of several disorders in humans including macrocytic anaemia, cardiovascular diseases [2], thromboembolitic processes [3], neural tube and congenital defects [4], adverse pregnancy outcomes [5], and neuropsychiatric disorders [6].Folic acid supplementation is believed to be safe and free of toxicity [7], it may demonstrate some undesirable effects especially in some population not targeted for the dietary fortification [8]. For instance, clinical and experimental studies suggest that folic acid possesses dual modulator effects on carcinogenesis, depending on timing and intervention dose [9].

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent [10]. At the present time lipid peroxidation has become an acceptable trend in medicine to consider at molecular level. Vascular endothelial dysfunction may be caused by uncontrolled lipid peroxidation [11]. Pregnancy is characterized by dynamic changes in multiple body systems resulting in increased basal oxygen consumption and in changes in energy substrate use by different organs including the feto-placental unit. The human placenta influences maternal homeostasis; it is rich in mitochondria and when fully developed it consumes about 1% of the basal metabolic rate of the pregnant woman [12]. It is also highly vascular and is exposed to high maternal oxygen partial pressure. These characteristics of the placenta explain, in part, the generation of superoxide anion [12]

The body protects itself from oxygen free radical toxicity by enzymatic antioxidant mechanisms (eg, Glutathione peroxidase (GSH-Px), Glutathione reductase (GSH-R) and Superoxide dismutase (SOD) and by non-enzymatic antioxidants (eg, vitamins, uric acid, albumin, bilirubin, and many others). Antioxidant enzymes reduce the levels of lipid peroxides as well as hydrogen peroxide and are important in preventing lipid peroxidation and maintaining the structure and function of biologic membranes. Superoxide dismutase (SOD) catalyzes the dismutation of peroxide to hydrogen peroxide and GSH-Px catalyzes the oxidation of glutathione[13].Adenosine deaminase **(**ADA) catalyzes the hydrolytic deamination of adenosine to inosine and 2- deoxyadenosine to 2-deoxyinosine [14]. Adenosine deaminase is widely distributed in human tissues, especially in the lymphoid tissues, and it is essential for the maturation and function of human blood monocytes and macrophages [15].

The aim of the present study is to assess the effect of folic acid supplementation on Adenosine deaminase,lipid peroxidation and enzymatic antioxidant activities in the normal pregnant women (First trimester)and second trimesters who used folic acid supplementation regularly(5mg daily) or irregularly as compared to non pregnant women.

Material and Methods

The present study comprises 65 normal pregnant women ranging in age mean± SD (32.24±5.25) years from antenatal care centers located in Baghdad during their first trimester and assessed in the second trimester were selected from those attending for antenatal checkup to the hospital, in addition to 25 healthy non-pregnant women as control ranging in age mean ±SD (31.01±4.25) years. Women with obesity, diabetes mellitus under medication and untreated diabetes, Severely anemic (<7.0gm% of Hb) and those suffering from any other systemic disorder were excluded from the study. Analysis of studied parameters was performed by taking 10 ml of blood was drawn by venipuncture and collected in a heparinized tube (10 units/ml of blood). Women were divided into the following groups: group A- 25 healthy non-pregnant women, group B- healthy pregnant women, further sub-divided into three sub-groups: group B-I (1st trimester) in the early days of pregnancy and before the use of folic acid, group B-II (2nd trimester who used folic acid supplementation regularly-5mg daily) and group B-III (2nd trimester who used folic acid supplementation irregularly). The following parameters were analyzed within 10-15 minutes of collecting blood sample. Random plasma glucose(RPG), Hemoglobin (Hb) and uric acid levels were measured by spectrophotometeric methods supplied by Giesse Diagnostic .Plasma iron level was measured by spectrophotometeric methods supplied by Biolabo, France. Total antioxidant capacity (TAC) in plasma samples was carried out according to Rice -Evans and Miller [16]. Plasma malondialdehyde(MDA) was determined according to the modified method of Satoh [17]. Glutathione (GSH) was estimated by the method of Beutler's method[18]. Glutathione peroxidase (GSH-Px) activity were determined

according to Pleban's method [19]. Glutathione reductase(GSH-Px) activity was determined according to a modified method of Lee. K and et al[20]. Superoxide dismutase(SOD) activity was determined according to the method of Misra HP and Fridovich I [21]. Adenosine deaminase (ADA) activity was measured by using Giusti's method [22]Transferrin was measured by using Mancini method [23].

All statistical analyses in studies were performed using SPSS version 17.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability $P< 0.05$ = significant, $P> 0.05$ = non-significant . Correlation analysis was used to test the linear relationship between parameters. Analysis of variance (ANOVA) test was used to show the differences between variables of differentiated groups.

Results

Demographic distribution of groups of women according to certain characteristics were shown in table 1.

Table 1: The mean and standard deviation of Age, weight and Gestation age in group A group A(nonpregnant women),group B-I[first trimester), group B-II[second trimester who used folic acid supplementation regularly] and group B-III)second trimester who used folic acid supplementation irregularly).

Characteristic	Group A [n=25]	Group B-I [n=65]	Group B-II [n=35]	Group B-III [n=30]
Age[year] Mean ±SD Range	31.01±4.25 23.90-40.90	32.24±5.24 24.80-42.80	31.24±4.54 25.00-40.80	32.00±3.54 25.20-42.90
Weight[Kg] Mean ±SD Range	68.15±10.84 55.30-79.98	69.07±11.94 52.00-78.60	70.07±10.86 54.00-79.76	70.80±11.66 53.90-81.00
Gestation age [weak] Mean ±SD Range	---------	4.60±0.57 4.50-6.00	13.71±0.92 12.5-14.50	13.82±0.92 12.4-14.50

There were a significant different in mean value of Hb, RPG, .transferrin and iron in groupA[non pregnant women],group B-I[first trimester],group B-II[second trimester who used folic acid supplementation regularly]and group B-III [second trimester who used folic acid supplementation irregularly] as shown in table 2

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Table 2: The mean and standard deviation of Hb, RPG, transferrin and iron in group A group A[(non pregnant women),group B-I[first trimester), group B-II[second trimester who used folic acid supplementation regularly] and group B-III (second trimester who used folic acid supplementation irregularly)

Characteristic	Group A [n=25]	Group B-I [n=65]	Group B-II [n=35]	Group B-III [n=30]
Hb[g/dl]	11.98 ± 1.2	11.47 ± 1.1	11.10 ± 1.57[a]	10.4 ± 1.23[b, c]
RPG [mg/dl]	102.78±5.57	103.71±7.62	103.95±6.51	106.78±5.83[a]
Transferrin [mg/dl]	264.34±18.23	269.34±27.28	278.94±29.84[a,b]	293.62±30.32 [c,d]
Iron [µg/dl]	102.65±12.71	96.04±18.67	89.99±19.33[a]	84.23±21.59[b,c]

Results were expressed as the mean±SD.

a P <0.01 compared with control group.

b P <0.01 compared with group B-I.

c P <0.001 compared with control group

d P <0.001 compared with group B-I

Table 3 showed mean and standard deviation of ADA,TAC, MDA, uric acid, glutathione, GSH-R, GSH-Px and SOD in group A [non pregnant women], group B-I[first trimester], group B-II[second trimester who used folic acid supplementation regularly] and group B-III [second trimester who used folic acid supplementation irregularly].

Table 3: The mean and standard deviation of TAC,MDA,ADA,GSH,GSH-R ,GSH-Px and SOD in group A(non pregnant women),group B-I[first trimester), group B-II[second trimester who used folic acid supplementation regularly] and group B-III (second trimester who used folic acid supplementation irregularly)

Characteristic	Group A [n=25]	Group B-I [n=65]	Group B-II [n=35]	Group B-III [n=30]
ADA [U/L]	16.45 ± 3.13	21.78 ± 8.45[a]	22.55 ±7.04[a]	24.86 ±8.34[c,b]
TAC [µmol/L]	435.48±26.42	368.57±25.32 [c]	298.57±23.56[c,d]	288.49±20.32 [c,d]
Uric acid [mg/dl/]	3.51±1.09	3.92±0.90	4.76±1.40[a,b]	5.08±1.50[c,d]
MDA [µ mol/L]	1.24+0.10	1.44+0.14[a]	1.69+0.16[a,b]	1.80+0.18 [c,b]
GSH [mg/dl]	57.81 ± 7.46	46.61 ± 8.98[c]	39.79±8.44 [c,d]	34.35±9.32 [c,d]
GSH-R [IU/gm Hb]	12.32+4.37	11.17+2.20	10.39+3.07 [a,b]	7.89±2.83 [c,d]
GSH-Px [IU/gm Hb]	36.98+4.84	32.27+3.26 [a]	27.34+3.98 [c,d]	23.14+5.17 [c,d]
SOD [IU/gm Hb]	709.79+138.25	657.40+76.28 [c]	601.64+105.92 [c,d]	570.20+110.33 [c,d]

Results were expressed as the mean±SD.
a P <0.01 compared with control group.
b P <0.01 compared with group B-I.
c P <0.001 compared with control group
d P <0.001 compared with group B-I

Discussion

Iron deficiency anemia is considered the most widespread complication of pregnancy. Severe anemia (hemoglobin (Hb) less than 80 g/l) in the first half of pregnancy is proved to be associated with preterm delivery and for small gestational age fetus [24-27]. In contrast, the values of borderline anemia (95–105 g/l of Hb) appear to be related to the minimum incidence level of preterm delivery [28]. This is attributed to the fact that high (over 120 g/l) values of hemoglobin might indicate the inadequacy of adaptational plasma volume expansion in the third trimester rather than an iron-replete state of the woman [29]. From the result obtained from the current study ,demonstrated that increase in transferrin levels during the second trimester of pregnancy. It is possible that plasma transferrin rises earlier during pregnancy in those with concomitant iron deficiency [30].The administration of supplemental folic acid in this series has probably contributed to the later rise in plasma transferrin.

Adenosine deaminase is a necessary enzyme for the differentiation of lymphoid cells, and changes in ADA activity reflect alteration in immunity[15].Karabulet *et al* have opinion that increased ADA as a marker of immunological disorder may be connected to the pathogenesis of the disease [31]. Normal pregnancy has been associated with low cell immunity [32], and thus, plasma ADA activity may play a significant role [33]. There have been contradictory information about the changes in the activity of plasma ADA in normal pregnancy compared to the age-matched non-pregnant women [34,35].

However, to our knowledge, only few studies have been performed investigating the changes in plasma ADA activity according to gestational age, and the clinical significance of changes in ADA activity throughout normal pregnancy has not been elucidated [34] and no other study referred to the effect of folic acid supplement on ADA level.

The oxidative stress is the principal causal factor, is reflected by increase in MDA and decrease in TAC activity. Significant decrease in TAC is observed in normal pregnant women. This finding is consistent with the report of Davidge *et al* [36]and Harma *et al* [37].In the present study there a significant decrease in TAC level in group B-II and group B-III when compared to group B-I and also to control group . If concentrations of uric acid were subtracted from TAC, this give an signal of the possible change in other contributors to the overall antioxidant capacity (e.g. vitamins C, E and thiols), since uric acid is considered to be one of the major determinants of TAC [38].

The results of this study clearly show that the levels of MDA, were statistically elevated in groups of B-I,B-II and B-III compared to the control group. A significant changes were observed in the second trimester [B-II,B-III]of pregnancy compared to the first trimester of pregnancy MDA , these result may be due to the effect of regularly and irregularly using folic acid . The body counteracts the oxidative stress in normal pregnancy through the induction of some enzymes such as, catalase, SOD, glutathione peroxidase, as well as through non-enzymatic free radicals scavengers i.e., vitamins C and E, and uric acid. Pregnancy is a physiological condition in which, women are more prone to oxidative stress, due to an imbalance between the prooxidant-antioxidant factors [39]. A proper balance between oxidative stress and antioxidant systems during pregnancy is important. The involvement of hypoxia/oxidative stress in the path physiology of a variety of pregnancy complications including preterm labor miscarriage, fetal growth restriction and preeclampsia was reported [40].

Glutathione is a naturally occurring tripeptide whose nucleophilic and reducing properties play a central role in the metabolic pathways of most aerobic cells [41]. The roles of GSH in biological systems include protection against reactive oxygen compounds, other toxic compounds of endogenous and exogenous origin and free radicals. In addition to its role in the maintenance of the redox potential within cells, it is also a key component of the antioxidant system, since GSH is the substrate for glutathione peroxidase, these result showed who the regularly using of folic acid supplement to prevent decrease in level of these factors.

The present study findings in normal pregnancy of decreased GSH , GSH-R, GSH-Px and SOD activities allow us to confirm observations made previously that oxidative damage is higher in healthy pregnant women than in non-pregnant women.

The marked increase in plasma ADA during the study may reflect, in part, an immunity response due to free radical generation consequent to repeated loading of the intestine with unabsorbed iron or the oxidative stress may be due to a more generalized nature in the whole organism. We conclude that plasma ADA activity can be used as a marker of altered cell-mediated immunity in pregnancy, and the significance of the regulatory mechanisms that alter the activity of plasma ADA may be affected by taking folic acid supplements regularly and irregularly. Further studies of folic acid supplementation and markers of inflammation are needed to differentiate the effects of iron storage and
inflammation on circulating levels of ADA and the level of antioxidants in plasma of pregnant women.

In conclusion folic acid supplementation irregularly increased indicators of lipid peroxidation in first trimester of pregnancy. Therefore, this regimen of supplemental folic acid may provide excessive amounts of folic acid and appears to increase the risk of oxidative stress. Larger and longer term studies with different doses and modalities of folic acid of supplementation on lipid peroxidation and oxidative stress in women of childbearing age, pregnant or not, and in children, need to be undertaken to expand the results of this study.

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