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Effects of long-term folate supplementation on metabolic status and regression of cervical intraepithelial neoplasia: A randomized, double-blind, placebo-controlled trial

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ABSTRACT

Objective: This study was conducted to determine the effects of long-term folate supplementation on regression and metabolic status of patients with cervical intraepithelial neoplasia grade 1 (CIN1).
Methods: This randomized, double-blind, placebo-controlled trial was performed among 58 women diagnosed with CIN1, ages 18 to 55 y old. Participants were randomly divided into two groups to receive 5 mg/d folate supplements (n = 29) or placebo (n = 29) for 6 mo. Fasting blood samples were taken at baseline and 6 mo after intervention to quantify related markers.
Results: A greater percentage of women in the folate group had regressed CIN1 (83.3 versus 52.0%, $P = 0.019$) than those in the placebo group. Long-term folate supplementation resulted in a significant decrease in serum insulin levels (-1.6 ± 6.2 versus $+2.6 \pm 6.9$ μIU/mL, $P = 0.018$) and homeostatic model assessment-beta cell function (HOMA-B) (-13.0 ± 39.0 versus $+11.2 \pm 42.3$, $P = 0.028$) compared with the placebo. Additionally, plasma glutathione (GSH) levels were significantly increased ($+81.5 \pm 264.1$ versus -220.9 ± 342.5 μmol/L, $P < 0.001$) and malondialdehyde (MDA) levels were significantly reduced (-1.0 ± 1.1 versus $+0.1 \pm 1.6$ μmol/L, $P = 0.004$) in the folate group compared to the placebo.
Conclusions: Taken together, folate supplementation (5 mg/d) for 6 mo among women with CIN1 resulted in its regression as well as led to decreased serum insulin, HOMA-B, plasma MDA and increased plasma GSH levels; however, it did not affect other metabolic profiles.

Introduction

Cervical intraepithelial neoplasia (CIN) is the potentially premalignant transformation and abnormal growth (dysplasia) of squamous cells on the surface of the cervix [1]. Due to lack of a screening program, the prevalence of CIN is not clear, however, the prevalence of human papillomavirus (HPV), a major risk factor for CIN, in the Iranian general population is 7.8%. The major cause of CIN is chronic infection of the cervix with the sexually transmitted HPV [2]. Recently, some studies have suggested that polymorphisms in genes related to folate metabolism might be involved in cervical neoplasia [3]. Piyathilake et al. [4] have

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indicated that folic acid fortification for 2 y in US has resulted in a lower risk of CIN, especially when vitamin B12 was sufficient. Others [5] found that women with CIN had significantly lower serum folate and vitamin B12 concentrations compared with controls. However, a meta-analysis revealed that folate supplementation had no significant effect on total cancer incidence [6].

A lower degree of long interspersed nucleotide element 1 (LINE-1) methylation, which results from folate and vitamin B12 deficiency, might explain the risk of HPV-associated cancer [4,7]. Folate deficiency may affect cell-mediated immune functions [8–10] and therefore, may alter cancer risk. In addition, previous studies have shown a link between metabolic profiles, inflammatory factors, biomarkers of oxidative stress, and CIN. Increased levels of unsaturated lipids and very low density lipoprotein (VLDL-C) in patients with CIN supports the abnormal lipid metabolism in response to tissue injury in CIN [11]. Clinical studies have demonstrated that the genital tract concentrations of inflammatory markers were associated with higher cervicovaginal HPV-1 RNA concentrations in CIN [12]. In addition, carcinogenesis is closely associated with increased oxidative stress. Folate deficiency has earlier been indicated to correlate with increased risk of coronary syndromes [13], elevated inflammation [14] and increased levels of oxidative stress [15]. Few studies have examined the effect of folate supplementation on CIN regression; however, indicators of glucose homeostasis, lipid concentrations, inflammatory factors, and biomarkers of oxidative stress in patients with CIN1 have not been assessed. The present study was, therefore, performed to investigate the effects of folate supplementation on regression and metabolic status in women with CIN1.

Materials and methods

Participants

This randomized double-blind clinical trial was performed in Kashan, Iran, from January to July 2014. Based on the suggested formula for clinical trials and assuming possible dropouts, 29 subjects in each group were required for the whole study. Women ages 18 to 55 y with CIN1 based on specific diagnostic procedures (colposcopy, biopsy, and pathologic diagnosis) were included [16]. Patients who had abnormal Pap smear test, abnormal cervical cytology, postcoital bleeding, intermenstrual bleeding, and chronic vaginal discharge were invited to do a colposcopy. Women who had a history of cervical or other cancers of the lower genital tract, hysterectomy or destructive therapy of the cervix as well as pregnant women, and those using antifolate medications were not included. Written informed consent was obtained from all patients. The study was approved by the ethics committee of Kashan University of Medical Sciences and has been registered in the Iranian Registry of Clinical Trial (www.irct.ir: IRCT201403155623 N17).

Study design

At study baseline and after stratification for BMI (<30 and ≥30 kg/m^2) and age (<35 and ≥35 y), subjects were randomly assigned to receive 5 mg per day folate supplements (n = 29) or placebo (n = 29) by a trained midwife at maternity clinic. The folate supplements were provided by Iran Daru Co., Tehran, Iran and the placebos by Barij Essence Co, Kashan, Iran. The intervention was done for 6 mo. Women were advised to not change their diet and physical activity during the study. Compliance to the folate supplementation was assessed through quantification of plasma homocysteine (Hcy) levels. All participants provided three dietary records and three physical activity records to make sure that they maintained their usual diet and physical activity during intervention. Both dietary and physical activity records were taken at mo 2, 4, and 6 of intervention. Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods used to obtain nutrient intakes of participants based on these three-day food diaries [17,18].

Assessment of anthropometric measures

Weight (Seca, Hamburg, Germany) was measured at study baseline and after 6 mo of intervention at gynecology clinics by trained midwifes. Height was measured using a non-stretched tape measure (Seca, Hamburg, Germany). BMI was calculated using the height and weight measurements (weight in kg/[height in meters]^2).

Assessment of outcomes

In the present study, the primary outcome was histology-proven CIN1. All patients had a specimen sent for histology. The primary study outcome was determined through colposcopy, cervical biopsy and pathologic diagnosis. Colposcopy (Siemens AG, Berlin, Germany) was performed with the woman lying back, legs in stirrups, and buttocks at the lower edge of the table (a position known as the dorsal lithotomy position). Specimens were embedded in formalin solution and then sent for pathologic diagnosis. Assessment of the primary outcome was the same at baseline and 6 mo after the intervention.

The secondary outcomes were glucose homeostasis parameters, lipid profiles, inflammatory factors, and biomarkers of oxidative stress. Fasting blood samples (10 mL) were taken at baseline and 6 mo after intervention at Kashan reference laboratory in an early morning after an overnight fast. Commercial kits were used to measure fasting plasma glucose (FPG), serum cholesterol, triacylglycerols, VLDL-C, low density lipoprotein-cholesterol (LDL-C), and high density lipoprotein-cholesterol (HDL-C) concentrations (Pars Azmun, Tehran, Iran). All inter- and intraassay coefficient of variations for lipid profile measurements were less than 5%. Serum insulin levels were assayed by ELISA kit (Monobind, Lake Forest, California, USA). The homeostatic model of assessment for insulin resistance (HOMA-IR), homeostatic model assessment for β-cell function (HOMA-B) and the quantitative insulin sensitivity check index (QUICKI) were calculated based on suggested formulas [19]. Serum high sensitivity C-reactive protein (hs-CRP) was quantified using ELISA kit (LDN, Nordhorn, Germany) with intra- and interassay coefficient of variations of 2.5 and 4.2%, respectively. Plasma nitrite/nitrate, taken as an index of nitric oxide (NO) concentrations, was determined using the Giess method modified by Tatsh et al. [20]. Plasma total antioxidant capacity (TAC) was assessed by the use of ferric reducing antioxidant power method developed by Benzie and Strain [21]. Plasma total glutathione (GSH) was examined using the method of Beutler et al. [22]. Plasma MDA levels was determined by the thiobarbituric acid reactive substance spectrophotometric test [23]. Plasma Hcy was determined using an enzyme immunoassay method by Hcy kit (Axis-Shield Diagnostics, Scotland, UK).

Statistical methods

The normality of the variables was examined by the Kolmogorov-Smirnov test. The analyses were done based on intention-to-treat approach. Within-group comparisons (endpoint versus baseline) were done based on paired samples *t* test. To detect differences between the two groups (folate versus placebo), independent samples Student's *t* test used to compare changes occurred in variables. Pearson chi-square test was used for comparison of categorical variables. To control for several confounders, analysis of covariance applied in which the confounding effect of these variables (baseline values, age, and BMI at baseline) were taken into account. *P* values were considered statistically significant at $P < 0.05$. The statistical analyses were carried out using the statistical packages for SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Among individuals in the folate group, 4 women did not complete the trial. The exclusions in the placebo group were 5 persons as well. Finally, 49 participants (folate [n = 25] and placebo [n = 24]) completed the trial (Fig. 1). However, as the analysis was done based on intention-to-treat approach, all 58 women (29 in each group) were included in the final analysis. On average, the rate of compliance in the present study was high, such that >90% of tablets were taken throughout the study in both groups. No side effects were reported following the consumption of folate supplements in patients with CIN1 throughout the study.

Mean age (36.8 ± 8.8 versus 39.1 ± 9.1 y, $P = 0.0331$), baseline BMI (28.2 ± 3.5 versus 29.8 ± 6.4 kg/m^2, $P = 0.230$) and end-of-trial BMI (27.9 ± 3.3 versus 29.6 ± 6.4 kg/m^2, $P = 0.219$) were not significantly different between folate and placebo groups. Based on the three-day dietary records obtained throughout the intervention, no statistically significant difference was seen between the two groups in terms of dietary intakes of energy,