Folic Acid Improves Vascular Endothelial Dysfunction in Children with Type-1 Diabetes Mellitus

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Abstract

Background: Diabetic endothelial dysfunction stimulates release of inflammatory factors such as von Willebrand factor (vWF) and vascular cell adhesion molecule-1 (VCAM-1) and also results in microalbuminuria. Folic acid had shown to improve endothelial dysfunction.

Objective: To evaluate the effects of folic acid supplementation on VCAM-1, vWF and microalbuminuria in children with type-1 diabetes mellitus.

Methods: Our study was conducted upon 30 children with type 1 diabetes mellitus (aged between 8-15 years). They received oral folic acid in a dose of 5mg daily for 3 months. Blood samples were taken before and after folic acid supplementation to evaluate glycosated hemoglobin (HbA1c), vWF, VCAM-1, and albumin to creatinine ratio (A/C ratio).

Results: VCAM-1 and A/C ratio levels were decreased significantly after folic acid administration in the two groups (p<0.001 for each). vWF decreased after folic acid supplementation, but this was of statistical insignificance. On the other hand, HbA1c did not significantly change after folic acid administration (p>0.05).

Conclusion: Folic acid improves endothelial function in diabetic children measured by VCAM-1 and A/C ratio. However, it did not significantly decrease vWF which may need longer periods of folic acid administration.

Keywords: Folic acid; VCAM-1; vWF; Microalbuminuria; Endothelial dysfunction; Diabetes; Children

Introduction

Type-1 Diabetes Mellitus is considered as the second most common chronic disease in children. It is a multifactorial disease with high rates of morbidity and mortality due to microvascular or macrovascular complications [1]. The microvascular complications in diabetes encompass long-term complications affecting small blood vessels including diabetic retinopathy, nephropathy and neuropathy. The macrovascular complications include the diseases of large blood vessels throughout the body including coronary and peripheral arteries leading to cardiovascular and cerebrovascular diseases and stroke [2]. Endothelial dysfunction is the main factor in the progression of vascular complications which occur before the clinical manifestations [3].

Endothelial cells produce specific adhesion molecules, such as E-selectin, intracellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM), for the regulation of cell adhesion and permeability [4]. The intact endothelium expresses low levels of these adhesion molecules, and upon activation, over-expression of these molecules has been reported, which play a role in maintaining endothelial barrier integrity [5]. Also, Endothelial cells have major roles in regulating hemostatic balance, preventing the activation of thrombin and inhibiting platelet adhesion, thereby mediating anticoagulant activity [6]. Upon development of endothelial dysfunction, there is increase in the release of endothelial products, such as von Willebrand factor (vWF), angiopoietin-2 and P-selection, which are involved in the modulation of inflammatory response [7].

Microalbuminuria has been considered an expression of endothelial dysfunction. It is a disorder of the capillary wall in the glomerulus with trans-capillary escape of albumin [8]. In diabetes, endothelial dysfunction has been correlated with microalbuminuria [9] and may precede its development [10].

Folic acid supplementation had shown to improve endothelial dysfunction. The mechanism underlying the

improvement in endothelial function remains controversial [11]. As, folic acid increases nitric oxide (NO) bioavailability and decreases oxidative stress, which improves endothelial progenitor cell dysfunction in diabetic patients, through improving endothelial nitric oxide synthase (eNOS) function by a number of suggested mechanisms, such as: 1) Stabilizing tetrahydrobiopterin by preventing its oxidation and regeneration stimulating its from the oxidized dihydrobiopterin 2) Facilitating binding of the tetrahydrobiopterin to eNOS 3) 5-methyltetrahydrofolate may mimic tetrahydrobiopterin at its receptor site on eNOS or may facilitate the electron transfer to produce NO [11].

Our aim was to evaluate the effect of folic acid supplementation on endothelial function in diabetic children. We assessed: urinary albumin/creatinine ratio (A/C ratio) for development of microalbuminuria, regulation of platelet adhesion and aggregation (vWF), leucocyte adhesion (VCAM-1) [12]. The rationale for using these endothelial dysfunction marker proteins is that they reflect endothelial damage.

Material and Methods

Subjects

This prospective cohort study was conducted on 35 diabetic children who had regular follow up in the Pediatric Endocrinology Outpatient Clinic, Minia University Children Hospital. We excluded from our study children who had diabetes for less than 2 years, had any systemic diseases other than diabetes, suffered from DKA or hypoglycemia before blood sampling by 2 weeks or refused to participate in the study.

The study was explained in detail to the parents or legal guardians of the participant children and written consents were taken from them. The study was designed respecting the expected ethical aspects. It was performed according to the Declaration of Helsinki 1975, as revised in 2008 and approved by the Institutional Review Board and Medical Ethics Committee of Minia University Hospital.

All included children were given oral folic acid supplementation in a dose of 5 mg daily for 3 months with regular follow up of the patients checking their compliance. Five patients dropped out due to non-compliance.

Methods

All included children had undergone the following at start of the study and after 3 months of folic acid therapy:

6 ml of morning venous blood after overnight fasting was withdrawn.

2 mL was collected in tubes, left to clot for 30 minutes then the sera were separated from the cells using centrifuge at 1000 xg for 15 minutes and stored at temperature -20°C or less until VCAM-1 assay was done. VCAM-1 measured by Quantikine Human sVCAM-1 immunoassay (ELISA) kit (Minneapolis, United States of America).

2 ml was collected in tube containing sodium citrate as an anticoagulant, centrifuged immediately after collection to separate plasma from cells and then stored at -70°C until von Willebrand facror antigen assay was done. Von Willebrand antigen was measured by (ELISA) kit (Corgenix, United States of America).

2 ml was collected in tubes containing EDTA as anticoagulant for Glycosylated Hemoglobin HbAic assay, which was measured by Stanbio glycohemoglobin procedure for quantitative colorimetric determination of glycohemoglobin (Stanbio Laboratory, Boerne, Texas).

Urine samples were collected in a clean container under complete aseptic conditions to assess albumin to creatinine ratio (A//C ratio) as an indicator of microalbuminuria. Albumin in urine was measured by turbidmetry method using sulfosalicylic acid reagent (3:1) and creatinine was measured by Mindrray BS 300 chemical analyser.

Statistical methods

The collected data were statistically analyzed using statistical package for social sciences (SPSS) program for windows version 20. Quantitative results were presented as mean ± standard deviation (SD) while qualitative data were presented by frequency distribution as percentage (%). Chi square test (X²) was utilized for analysis of qualitative data, and Student (t) test for analysis of quantitative variables. Correlations were performed by using Pearson's and Spearman's correlation coefficient (r). The improvement in the endothelial function markers were calculated by their values before folic acid supplementation-their values after folic acid supplementation. Receiver operating characteristic (ROC) curve analysis was performed using MedCalc version 12.1.4.0. to determine: the optimal cut-off values and the diagnostic performance of the variable, the diagnostic sensitivity and specificity, and comparison of sensitivity and specificity for VCAM-1 and A/C ratio after folic acid supplementation. p-value if less than 0.05 was considered as a cut off for significance.

Results

Thirty diabetic children were included in our study. Their ages ranged between 8 and 15 years with a mean of 12.2 ± 1.9 years, 11 (36.7%) of them were males while 19 (63.3%) were females, their mean duration of diabetes was 6.7 ± 1.9 years. Family history of diabetes was positive in 14 children (46.7%).

We found significant decrease in VCAM-1 and A/C ratio levels after folic acid supplementation (p<0.001). VCAM-1 and A/C ratio levels before folic acid supplementation were 45.6 \pm 9.4 ng/ml and 33.1 \pm 8.2 mg/g respectively. While their levels after folic acid supplementation were 37.9 \pm 7.5 ng/ml and 28.3 \pm 5.8 mg/g respectively. On the other hand, HbA1c and vWF did not show statistically significant changes (p>0.05). As, their levels before folic acid supplementation were 10.3 \pm 1.9% and 65.7 \pm 47.2% respectively. While their levels after folic acid

supplementation were 10.07 \pm 1.9% and 56.9 \pm 38.4% respectively (Figure 1).



Figure 1 HbA1c, endothelial function markers VCAM-1, vWF antigen and A/C ratio levels before start and after 3 months of folic acid supplementation. (n=30); **A:** HbA1c and vWF antigen; **B:** VCAM-1; **C:** A/C ratio, p=0.001 for VCAM-1 and A/C ratio, p=0.07 for HbA1c and p=0.4 for vWF antigen. (HbA1c: Glycosylated Hemoglobin assay; vWF: Von-Willbrand Factor antigen, VCAM: Vascular Cell Adhesion Molecule-1, AC ratio: Albumin to creatinine ratio.* statistical significance <0.05).

Pearson's and Spearman correlation tests were performed to study the association of the improvement in VCAM-1, VWF and A/C ratio after folic acid supplementation with the demographic, clinical and HbA1c level. None of the studied markers showed significant correlations with age, family history, sex, duration of diabetes or HbA1c level (p>0.05).

Validity tests were used to demonstrate the optimal cut-off values of VCAM-1 and A/C ratio. VCAM-1, at a cutoff value of \leq 37.5 ng\ml, was more sensitive (60%) and specific (76.6%) with higher PPV (68.2%) and NPV (63.6%) than A/C ratio to detect the improvement in endothelial function after folic acid supplementation in diabetic children **(Table 1)**.

Table 1 Validity tests of VCAM-1 and A\C ratio in predicting endothelial dysfunction after folic acid supplementation.

Variables	Cut-off	Sensitivity %	Specificity %	PPV %	NPV %
VCAM-1	≤ 37.5 ng/ml	60	76.6	68.2	63.6
A\C ratio	≤ 29 mg/g	50	70	66.7	60.5

VCAM-1: Vascular Cell Adhesion Molecule-1; A/C ratio: Albumin to creatinine ratio; PPV: positive predictive value; NPV: negative predictive value

Discussion

In this study after 3 months of folic acid supplementation, there was significant improvement in endothelial dysfunction represented by significant decrease in VCAM-1 and A/C ratio. The results of the present study may be attributed to the antioxidant properties of folic acid through which it may improve endothelial progenitor cell function [13]. This was in agreement with Alian et al. in 2012 who found that folic acid supplementation improved endothelial dysfunction, as VCAM-1 and microalbuminuria decreased significantly in the studied group [14]. While, a more recent study, by Schneider et al. in 2014, demonstrated that folic acid fails to improve endothelial dysfunction in diabetic nephropathy patients [15]. This contradiction may be attributed to that Schneider's study was carried out on type 2 diabetic patients, and oral antioxidant treatment was found to improve endothelial dysfunction in type 1 more than type 2 diabetic patients [16].

Alain's study in 2012 did not find serum vWF to change significantly after folic acid supplementation in a dose of 5mg daily for 2 months [14]. In our study, we gave the same folic

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acid dose but for 3 months. vWF decreased in our study after folic acid supplementation, but this decrease did not reach statistical significance. Surprisingly, Mierzecki et al. in 2012 found a significant decrease in vWF concentrations in atherosclerotic patients low-dose folic after acid supplementation (0.4 mg daily) for 3 months [17]. This may be attributed to the fact that low dose of folic decrease homocysteine level. Homocysteine inhibits vWF processing and secretion by preventing its transport from the endoplasmic reticulum [18]. High dose of folic acid improves endothelial function, as we mentioned before, through improving eNOS function mainly, and this occurs before changing homocysteine level [19]. So, vWF antigen decreased in our study after folic acid supplementation. Further prolonging the periods of folic acid administration may be necessary to achieve a statistical significant decrease.

HbA1c did not show significant difference before and after folic acid supplementation. This alleviate the role of hyperglycemia on the studied markers. Our results are in accordance with many studies that reported the trend of folic acid supplementation to be associated with better glycemic control, but this control was with insignificant effect on HbA1c levels [14,20]. However, Pena and his study group got two contradicting results regarding the relation of folic acid supplementation and HbA1c level [21,22]. They explained this contradiction by the effects of study participation on patients' motivation which lead to the significant improvement in HbA1c level [21].

Although, a study by Ebbing et al. in 2009 reported an increased cancer incidence and mortality, especially lung cancer, when folate was co-administrated with vitamin B12 supplementation [23]. But, they did not study the effect of family history of cancer, environmental and occupational factors on their results. Besides, other studies have demonstrated no associations between intakes of folate or folic acid and lung cancer risk [24,25].

VCAM-1, at a cutoff value of \leq 37.5 ng/ml, was more sensitive (60%) and specific (76.6%) than microalbuminuria measured by A\C ratio, to detect the improvement in endothelial function after folic acid supplementation in diabetic children. VCAM-1 is known to be a dynamic surrogate marker for the effectiveness of therapeutic interventions in diabetic patients with microalbuminuria [26]. Moreover, it adds additional information on cardiovascular risk [27].

Conclusion

Folic acid supplementation improved endothelial dysfunction in children with type-1 diabetes mellitus, and we recommend that all diabetic children should receive oral folic acid supplementation as a part of their chronic therapy. Our study has several limitations, for example, serum homocysteine and folic acid level were not feasible to be assessed. Longer periods of follow up with larger sample size might have been more informative regarding the effect of folic acid on vWF and HbA1c.

Conflict of Interests

All authors declare that they have no conflicts of interests.

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