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ORIGINAL PAPER

The Effects of Folic Acid on Markers of Endothelial Function in Patients with Type 1 Diabetes Mellitus

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Background: Endothelial dysfunction is considered as a fundamental and also preventable factor in the progression of vascular complications among type 1 diabetic patient. It occurs before the clinical manifestation of the mentioned complications. The aim of this study was to evaluate the effects of folic acid on endothelial function by measurements of adhesion molecules and von Willebrand factor (vWF) in patients with type 1 diabetes in Isfahan, Iran. **Methods:** This double blind, placebo-controlled crossover trial included type 1 diabetic patients aged 5-20 years old. Selected patients were randomized into two groups of A and B to receive folic acid 5 mg daily or placebo for 8 weeks. After a 2-week washout period, patients in the two groups were swapped to receive placebo or folic acid, respectively, for another 8 weeks. Blood and urine samples were taken to evaluate glycosylated hemoglobin (HbA1c), folic acid, vWF, intracellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), and microalbuminuria levels. Results of these measurements were compared in the two groups before and after folic acid and placebo administrations. **Results:** Fifty five type 1 diabetic patients aged 12.1 ± 3.4 years with diabetes duration of 3.9 ± 2.1 years were enrolled. Mean of folic acid level in the two studied groups was increased significantly (10.1 ± 3.8 vs. 21.2 ± 1.1in group A and 15.5 ± 1.9 vs. 19.9 ± 2.8 in group B, $p < 0.05$). Mean of VCAM and microalbuminuria was decreased significantly after folic acid administration in the two groups ($p < 0.05$). Mean of HbA1c, ICAM and vWF did not significantly change after folic acid administration in the two groups ($p > 0.05$). **Conclusion:** Folic acid administration decreased the level of endothelial dysfunction measured by adhesion molecules, especially VCAM and microalbuminuria. However, it did not significantly affect vWF. Further studies with larger sample size and long-term administration of folic acid are necessary for making precise decisions in this field. **Key words: endothelial function, Diabetes Mellitus, folic acid.**

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1. INTRODUCTION

Type 1 diabetes is considered as the second most common chronic disease in children worldwide (1). It is a multifactorial disease with high rate of mortality and morbidity mainly due to vascular complications (2). Many experimental studies on the mechanisms of microvascular and macrovascular complications of the disease have suggested endothelial dysfunction as a fundamental factor in the progression of vascular complications which occur before the clinical manifestation of the mentioned complications (3, 4).

Endothelium believed to be the key regulator of vascular tone and structure. It balances the release of contracting and endothelial-derived relaxing factors in blood vessels. This balance is delicate during the progression of diabetes, resulting in the development of vascular complications (5, 6).

Factors such as poor glycemic control are associated with endothelial dysfunction in children with type 1 diabetes. On the other hand, lifestyle modification, dietary changes or supplementations such as folic acid, and pharmacologic interventions (statins and angiotensin-converting enzyme (ACE) inhibitors) have protective effects in this field (7, 8, 9). Increasing evidence during the last decades suggests that folic acid supplementation improves endothelial dysfunction in type 1 diabetic patients, even in those with higher folic acid levels (10, 11).

There are different invasive (coronary catheterization) and non-invasive (ultrasound) methods for measurement of abnormal endothelial function (12). It could also be evaluated by mea-

surement of blood levels of some compounds such as endothelin, von Willebrand factor (vWF), thrombomodulin, selectin, intracellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), and tissue plasminogen activator (t-PA) and its inhibitor, and plasminogen activator inhibitor (PAI-1) (13, 14, 15).

In order to prevent vascular complications of diabetes, proper strategies need to be implemented in the initial phase of the disease. Endothelial dysfunction is considered as a critical early target for achieving such goals.

Development of vascular complications in diabetes is associated with increased blood levels of inflammatory factors. In addition, adhesion molecules such as ICAM and VCAM have a regulatory role in the inflammatory reaction (16). Therefore, the current study was conducted to evaluate the effects of folic acid on endothelial function of type 1 diabetic patients in Isfahan, Iran, by measurement of adhesion molecules and vWF.

2. METHODS

This double blind, placebo-controlled crossover trial included type 1 diabetic patients aged 5-20 years old, with at least a 1-year history of the disease. Using convenience sampling method, subjects were selected from diabetic patients who referred to diabetes clinics of Isfahan Endocrine and Metabolism Research Center and Alzahra Hospital.

Patients with more than 10 years of type 1 diabetes, history of systemic disorders such as celiac or hypothyroidism, history of taking medications other than insulin, and history of ketoacidosis or hypoglycemia 48 hours before blood sampling were excluded. Smokers, pregnant women, and patients unwilling to participate were also excluded from the study.

Voluntary informed consents were obtained from all participants before the study was initiated. The protocol of the study was approved by the Institutional Review Board and Medical Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran.

The selected patients were randomized into two groups of A and B to respectively receive folic acid 5 mg daily or placebo (both supplied by Amin Pharmaceuticals, Tehran, Iran) for 8 weeks. After a 2-week washout period, patients in the two groups were swapped for another 8 weeks. Therefore, Group A received folic acid for the first period of 8 weeks and Group B received folic acid for the second period of 8 weeks. Subjects were recommended not to take any folic acid or additional vitamin supplements and not to change their insulin dose or routine diet during the study period. Baseline characteristics of patients including age, sex, and duration of the disease were recorded using a questionnaire. Peripheral blood samples and urine samples were obtained from the studied patients after overnight fasting for 4 times (at baseline, after the first 8 weeks, after the washout period, and after the second 8 weeks). Blood samples were tested for glycosylated hemoglobin (HbA1c), folic acid, vWF, ICAM, and VCAM levels. In order to measure vWF, ICAM, and VCAM levels, 2 cc of plasma was obtained by centrifuging the blood sample. It was then stored at -70°C until assayed. Urine samples were taken to assess microalbuminuria. Results of these measurements were compared in the two groups before and after folic acid and placebo administration.

Plasma vWF was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Diagnostica Stago, France). Plasma levels of folic acid, ICAM, and VCAM were measured by ELISA method using a Bender Med Systems GmbH kit. Serum folic acid was measured by ELISA method using a DRG Diagnostics GmbH kit. HbA1c was measured by ion exchange chromatography with a DS5 set. Urine micro albumin concentration was measured by radioimmunoassay method. Microalbuminuria was defined as urinary albumin of 30-300 mg/24 hours or 20-200 g/min in 2 out of 3 consecutive tests taken within 2-3 months (17).

The obtained data was analyzed using $SPSS_{13}$ (SPSS Inc., Chicago, IL, U.S.A.). Descriptive data was expressed as mean ± standard deviation (SD). Quantitative data of the 2 groups was compared using the student's t- test or chi-square test. P values less than 0.05 were considered as statistically significant.

3. RESULTS

Fifty five type 1 diabetic patients aged 12.1 ± 3.4 years with diabetes duration of 3.9 ± 2.1 years were enrolled in this study. Baseline characteristics of the studied population in groups A and B are presented in Table 1. There were no significant differences in the baseline characteristics of the two groups. The compliance to folic acid and placebo was 91.7% and there were not any reports of adverse effects.

Serum folic acid changes during treatment with folic acid and placebo in groups A and B at baseline and after treatment are presented in Figure 1. The effects of folic acid administration on endothelial function indicators, microalbuminuria and HbA1c in two the studied groups are presented in Table 2.

4. DISCUSSION

In this study, we aimed to investigate the effects of folic acid on the markers of endothelial function. We thus assessed the adhesion molecules and vWF levels in patients with type 1 diabetes in Isfahan. The findings of the study indicated that folic acid administration decreased the level of endothelial dysfunction measured by adhesion molecules, especially VCAM. However, it did not significantly affect vWF in the studied type 1 diabetic patients. Folic acid also decreased the level of microalbuminuria among the participants.

Type 1 diabetes is associated with higher risk of micro- and macrovascu-

	Group A	Group B	P value
Age(years)	11.5 ± 3.3	13.2 ± 3.5	NS
Female/Male	21/13	10/11	NS
Diabetes duration (years)	4.4 ± 2.1	4.2 ± 1.9	NS
Fasting Blood Sugar (mg/dL)	182 ± 92.9	148.1 ± 70.8	NS
Weight (kg)			NS
Height (cm)			NS
HbA1c (%)	8.2 ± 0.3	8.1 ± 0.3	NS
Serum folic acid(ng/mL)	10.1 ± 3.8	10.9 ± 4.4	NS
vWF (%)	4.0 ± 0.1	3.5 ± 0.1	NS
ICAM	42.7 ± 4.2	43.3 ± 8.8	NS
VCAM	5.6 ± 5.2	9.1 ± 1.3	NS

Table 1. Baseline characteristics of the studied population in groups A and B

	Baseline	After folic acid	P value	Baseline	After placebo	P value
HbA1c						
Group A	8.2 ± 0.3	8.1 ± 0.2	NS	8.1 ± 0.3	8.2 ± 0.4	NS
Group B	7.9 ± 0.3	7.8 ± 0.7	NS	8.1 ± 0.3	8.4 ± 1.2	NS
Serum folic acid						
Group A	10.1 ± 3.8	21.2 ± 1.1*	< 0.05	15.0 ± 0.1	10.1 ± 1.2	< 0.05
Group B	15.5 ± 1.9	19.9 ± 2.8	< 0.05	10.9 ± 4.4	15.4 ± 1.4*	< 0.05
vWF						
Group A	4.0 ± 0.1	4.1 ± 0.1	NS	3.8 ± 0.03	2.7 ± 0.04	< 0.05
Group B	3.9 ± 0.3	4.0 ± 0.3	NS	3.5 ± 0.1	3.6 ± 0.2	NS
ICAM						
Group A	42.7 ± 4.2	31.3 ± 3.3	0.05	35.4 ± 5.9	48.5 ± 5.4	P<0.05
Group B	41.3 ± 3.8	41.4 ± 8.1	NS	43.3 ± 8.8	40.3 ± 4.9	NS
VCAM						
Group A	5.6 ± 5.2	4.5 ± 5.0	< 0.05	3.9 ± 1.0	3.5 ± 0.9	NS
Group B	3.5 ± 0.9	3.5 ± 0.9	< 0.05	9.1 ± 1.3	9.5 ± 1.9	NS
Albuminuria						
Group A	18.5 ± 2.1	14.2 ± 1.3	< 0.05	14.6 ± 1.4	17.2 ± 2.5	NS
Group B	19.6 ± 2.6	16.9 ± 2.3	< 0.05	22.5 ± 4.1	20.2 ± 2.5	NS

Table 2. The effects of folic acid administration on endothelial function indicators, microalbuminuria, and HbA1c in the two studied groups *Serum folic acid was increased significantly both after folic acid and placebo, but increases in folic acid group was significantly higher than the placebo group (21.2 ± 1.1 vs. 15.4 ± 1.4, p = 0.01).

lar complications which cannot be explained only by impaired metabolic factors. Many studies have demonstrated endothelial dysfunction as the main factor in the pathogeneses of these complications. Moreover, many biochemical mechanisms have been identified for the explanation of endothelial dysfunction (4, 18). Type 1 diabetes also seems to be associated with increased oxidative stress which consequently stimulates the release of proinflammatory biomarkers such as ICAM and VCAM. A proinflammatory state has been detected among diabetic patients partly independent of clinical manifestation of its vascular complications (19, 20). With the recognition of the relation between type 1 diabetes and endothelial dysfunction, new researches should focus on interventions, such as folic acid supplementation, to improve endothelial dysfunction.

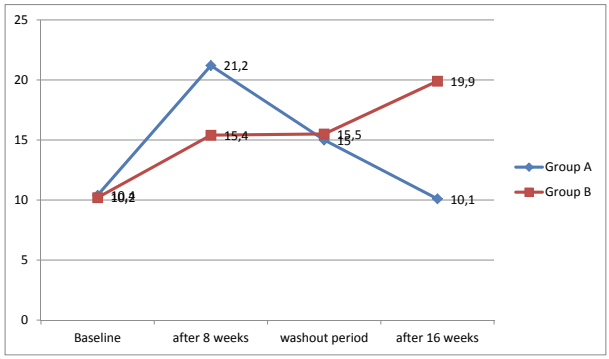

Figure 1. Serum folic acid changes during treatment with folic acid and placebo at baseline and after treatment in groups A and B

The effects of folic acid on the endothelial function have been assessed by different methods and reported by many studies. Most studies have indicated several known and unknown mechanisms to be able to enhance the endothelial function in type 1 diabetes (10, 11, 21). However, other studies could not establish the same effects (22, 23). Therefore, controversy exists about the effects of folic acid on endothelial function of type 1 diabetic patients.

During this study the mean level of folic acid increased significantly after supplementation. The levels in group B also increased by placebo. Although the increase in group A was significantly higher than group B, it seems that participants of group B used a high folic acid diet.

Pena et al. conducted a similar study in Australia to investigate the effects of folic acid supplementation on endothelial function of type 1 diabetic patients. They concluded that short-term high-dose folic acid improved endothelial function in type 1 diabetes patients. Moreover, in line with our results, folic acid did not have significant effects on vWF and HbA1c levels (11). In Australia again, Wiltshire et al. measured flow-mediated dilatation and serum vWF levels and indicated that folic acid may improve endothelial function or delay its dysfunction in type 1 diabetic patients (10). The findings of MacKenzie et al. were also in accordance with the mentioned studies (21). However, in the UK, Wotherspoon et al. investigated the effects of oral folic acid supplementation on plasma endothelial function among patients with type 1 diabetes. They reported folic acid not to have any protective effects on endothelial function (23).

Endothelial function in the mentioned studies was evaluated by different methods. In this study, we measured the adhesion molecules and vWF which are not invasive tests. These factors increase during the early phase of disease and simultaneous with the initiation of micro- and macrovascular complications. Moreover, expression of these factors in children is greater than in adults (24).

Although many studies have reported an elevated level of adhesion molecules among type 1 diabetic patients and their usefulness in the study of the dynamics of micro and macroangiopathy development among type 1 diabetic patients (24), no similar study among type 1 diabetic patients has evaluated the effects of folic acid on endothelial function using adhesion molecules. According to recent studies, ICAM and VCAM, known as inflammatory factors, are associated with atherosclerotic process in diabetic patients (25, 26). Several studies have shown the association between these adhesion molecules and endothelial dysfunction in type 2 diabetic patient. They also indicated that VCAM significantly increased in type 1 diabetic patient with retinopathy than those with nephropathy (6, 27). In addition, ICAM did not significantly change even in patients with microvascular complication (27). Similarly, in this study, folic acid decreased the level of VCAM but did not have a significant effect on ICAM levels. In group A, after 8 weeks of folic acid administration and the washout period, the level of ICAM increased significantly after using placebo. Therefore, it can be concluded that although

folic acid could not decrease the level of ICAM, discontinuing the supplementation increased inflammatory reaction. However, more studies with larger sample sizes are needed for more definite conclusions in this field.

Previous studies have considered vWF as an early marker of microvascular disease especially in children. It has also found to be negatively correlated with serum folic acid levels (10,28). In the current study, serum vWF did not significantly change after folic acid supplementation. Our results were in accordance with the study of Pena et al. in Australia (11). In the study of Wiltshire et al. vWF was not significantly different in diabetic and control groups after folic acid administration (10).

There are different reports regarding the association between endothelial dysfunction and HbA1c. Johnston et al. did not find any correlations between endothelium-dependent vasodilation and HbA1c (29). Similarly, Pena et al. indicated that HbA1c was not affected by folic acid administration and endothelial function (21). In our study, there was not a significant change in HbA1c levels after folic acid administration. However, longer administration of folic acid may have significant effects on HbA1c levels, i.e. folic acid may improve the endothelial function before it influences the HbA1c levels. Further long-term studies are thus needed in this field. The association between microalbuminuria and cardiovascular disease and endothelial dysfunction among type 1 diabetic patients has been reported by many studies (30). Microalbuminuria has been reported to predict the development of diabetic nephropathy as well as other micro and macrovascular complications (31).

Recent evidence has indicated that inflammation contributes to the development of diabetic nephropathy. Many studied have demonstrated the association between microalbuminuria and diabetic nephropathy with elevated levels of inflammatory biomarker such as ICAM and VCAM (16). Sahakyan et al. have reported the relationship of inflammation and endothelial dysfunction with the development of proteinuria in children with type 1 diabetes (32). Wotherspoon et al. examined the effects of oral folic acid supplementation on endothelial function in patients with type 1 diabetes and microalbuminuria. According to their findings, there were no associations between folic acid administration and improvements in endothelial function or markers of oxidant stress (23). In this study however, folic acid administration significantly decreased the mean microalbuminuria in the studied population.

The limitations of our study included small sample size, lack of normal non-diabetic subjects as the control group, and poor glycemic control of the studied patients reflected by suboptimal HbA1c levels. Therefore, studying a larger sample size of non-diabetic children and type 1 diabetic patients with optimal glycemic control will provide us with more conclusive information in this field. In addition, although many studies have demonstrated folic acid to improve endothelial function independent of homocysteine levels (33), other studies indicated that the protective effect of folic acid was enhanced through lowering serum homocysteine levels (34). Further studies with this consideration are thus recommended.

In conclusion, the findings of this study indicated folic acid to improve endothelial dysfunction. However, the sustained increase in serum folic acid level can be achieved by long-term administration of folic acid supplements. Therefore, further studies with larger sample sizes and long-term administration of folic acid are recommended.

Conflict of interest: none declared.

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