

Short Communication

Effect of metformin and folic acid on plasma homocysteine level in type 2 diabetic patients

Mohammad Mohammadi^{1*}, Masoud Amini¹, Ashraf Aminiroyay², Hasan Rezvanian², Ali Kachuei², Mansour Siyavash², Soodabeh- Rahimi Saghand¹ and Mohammad Afkhami-Ardekani¹

¹Yazd Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

²Isfahan Endocrine and Metabolism Research Center, Isfahan University of Medical Science, Isfahan, Iran.

Accepted 12 February, 2022

Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease in diabetic patients. The aim of this study was to evaluate effects of metformin and folic acid on plasma homocysteine level in diabetic patients. In this clinical trial 47 new cases of type 2 diabetes were randomized to two groups. We treated patients in trial group with metformin and 1 mg folic acid, whereas patients in control group treated with metformin and placebo for 16 weeks. There was no significant difference in plasma homocysteine level and glycosylated hemoglobin (HbA1c) between two groups at the start of study. After 16 weeks plasma homocysteine level in the trial group did not change. In contrast plasma homocysteine level increased in the placebo group. There was significant difference in mean plasma homocysteine level between two groups (P -value < 0.01). This difference was highly significant in males (P -value < 0.0001). Correlation between glycosylated hemoglobin and plasma homocysteine level at start and end of study was not significant. This finding indicated that metformin increases plasma homocysteine level in diabetic patients especially males, whereas administration of folic acid with metformin can prevent this process.

Key words: Type 2 diabetes, homocysteine, metformin, folic acid, glycosylated hemoglobin, sex.

INTRODUCTION

A number of cardiovascular risk factors such as hypertension, dyslipidemia and obesity are more common in type 2 diabetic patients (Wilson et al., 1999).

It also appears that diabetes interact with other risk factors to increase more risk of cardiovascular disease (Stamler et al., 1993). Hyper homocysteinemia is an independent cardiovascular risk factor especially in diabetic patients (Boushey et al., 1995). For prevention of cardiovascular events in patients with type 2 diabetes in addition to control of type 2, identification and treatment of risk factors such as hyperhomocysteinemia is critical (Wilson et al., 1999; Hoogeveen et al., 1998). It is well known that metformin by decreasing plasma folate and vitamin B12 levels can increase plasma homocysteine levels (Wulfele et al., 2003).

The aim of this study was to evaluate effect of metformin, folic acid and plasma homocysteine levels in type 2

diabetic patients

MATERIALS AND METHODS

This clinical trial included 47 patients with type 2 diabetes mellitus. These patients have been referred to endocrine and metabolism research center of Medical university of Isfahan. All patients gave informed voluntary consent to participate in the study. The study was approved by the ethics committee of Medical university of Isfahan.

Inclusion criteria were non usage of oral hypoglycemic agents or insulin, baseline creatinine lower than 1.1 mg/dl, fasting plasma glucose lower than 250 mg/dl, and no history of treatment with the drugs that affect plasma homocysteine.

There were various levels such as cholestyramine, metformin, fibric acids, folic acid, vitamin B12 and vitamin B6 from 3 months before the start of trial. Exclusion criteria were intolerance of metformin, so there was need to treat patients with other hyperglycemic agent or drugs which affect plasma homocysteine levels in duration of study. Patients were randomized to two groups, trial and control. Each group treated initially with 1000 mg metformin daily. Dosage of metformin based on results of monthly plasma glucose, as need be increased to 2000 mg daily. The patients in trial group in addition to metformin took 1 mg folic acid per day whereas in control

*Corresponding author. E-mail: afkhamiam@yahoo.com. Tel.: +983515223999 or +989131510803. Fax: +985258354.

Table 1. Variables of the subjects studied.

Variables	Group 1		Group 2	P-value
	Metformin without folic acid		Metformin with folic acid	
Age(years)	47±11.2		47.5±7.8	0.
Daily dose of metformin (mg)	1180±350		1378±422	0.07
Duration (day)	121.5±4.9		121±5	0.7
Baseline homocysteine(micromole/L)	12±3.5		11.5±4	0.6
Homocysteine at end of study	14.3±3.5		11±2.7	0.002

Table 2. Plasma homocysteine levels in two groups based on sex.

Sex	Male			Female		
	Metformin without folic acid (n = 8)	Metformin with folic acid (n = 7)	P-value	Metformin without folic acid (n = 12)	Metformin with folic acid (n = 13)	P-value
Base line homocysteine (µmole/l)	13.2 ± 2	13.6 ± 4	0.8	11.4 ± 4	10.3 ± 3.5	0.3
Homocysteine at end of study	15.4 ± 1.5	11.5 ± 1.1	0.001	10.8 ± 3.2	13.6 ± 4.2	0.07

group patients took metformin and placebo. All blood specimens were drawn at 8:00 am after 12 h fast. Fasting plasma glucose, triglyceride, total cholesterol and HDL cholesterol were determined by the enzymatic method. LDL cholesterol was calculated based on Fried wald formula. Glycosylated hemoglobin percent was determined by tonic exchange chromatography. Plasma homocysteine level was determined by Eliza (Axis. Homocysteine – ELA).

Normal levels of plasma homocysteine are between 5 - 15 µmol/l and average level is 9 - 10 micro mol/l in healthy people.

Plasma homocysteine level of 15 µmol/l or more is considered to be hyperhomocysteinemia (Duell et al., 1997). The data were analysed by using spss version 11 and p-value < 0.05 were considered to be statistically significant. Difference between mean plasma homocysteine levels in groups were assessed by student t test. In each group, mean plasma homocysteine levels at start and end of the study was assessed by paired t-test. Relationship between HbA1c and homocysteine was calculated by Pearson's correlation coefficient.

RESULTS

Of 47 participants, we excluded seven persons. Overall this study was carried out in 40 patients (15 males, 25 females) in two groups of twenty persons (Table 1). This was no statistically significant difference in mean plasma homocysteine levels and HbA1c between the two groups at start of the study ($p = 0.6$).

After 16 weeks, administration of metformin mean plasma homocysteine levels in trial group (group 2) did not change. In contrast, mean plasma homocysteine levels increased in placebo group (group 1) ($p < 0.01$).

After 16 weeks this was statistically significant difference in mean plasma homocysteine levels between two group ($p < 0.01$) that was highly significant in male (Table 2) ($p < 0.0001$).

Correlation between HbA1c and plasma homocysteine levels at start of the study ($r = 0.06$, $p = 0.7$) and end of the study ($r = 0.02$, $P = 0.9$) was not significant.

DISCUSSION

This study demonstrated that metformin can increase plasma homocysteine levels in type 2 diabetic patients. Our findings are similar to those obtained by Wulffele et al. (2003) that concluded in patients with type 2 diabetes, 16 weeks of treatment with metformin lead to modest increase in plasma homocysteine levels (Wulffele et al., 2003). This study shows that plasma homocysteine levels can be increased by metformin in placebo groups whereas plasma homocysteine levels did not change in trial group that took folic acid in addition to metformin. The study demonstrated that metformin by itself increases plasma homocysteine levels in diabetic patients whereas administration of folic acid with metformin can prevent this processes plasma.

Plasma homocysteine level was higher in males than females. That was corresponded by other studies (Alan et al., 2005; Passaro et al., 2000). Furthermore the study showed that metformin can increase more plasma homocysteine levels in males and females. In addition, effect of folic acid in prevention of rise homocysteine in males was highly significant.

This study did not show relationship between plasma homocysteine levels and glycosylated hemoglobin, a well known marker of prolonged impaired glucose control that confirmed some study.

Whereas some studies suggest plasma homocysteine levels decrease in response of glucose control, other studies rule out this correlation (Agullo-Ortuno et al., 2002; Russo et al., 2004; Passaro et al., 2000; Van Guldener et al., 2002).

Conclusion

The findings demonstrated that, metformin increases

plasma homocysteine levels in type 2 diabetic patients specially males; whereas administration of folic acid with metformin can prevent this process.

Also there is no correlation between homocysteine levels and severity of hyperglycemia. We say that folic acid should be administrated together with metformin in type 2 diabetes.

ACKNOWLEDGMENT

We thank all employees of Endocrine and Metabolism Research Center of Medical University of Isfahan.

REFERENCES

- Agullo-Ortuno MT, Albaladejo MD, Parra S, Rodriguez-Manotas M, Fenollar M, Ruiz-Espejo F, Tebar J, Martinez P (2000). Plasmatic homocysteine concentration and its relationship with complications associated to diabetes mellitus. *Clin. Chim. Acta.* doi:10.1016/S0009-8981(02)00287-5. 326 (1-2):105-112
- Alan N, Elias, Steven Eng (2005). Homocysteine concentrations in patients with diabetes mellitus-relationship to microvascular and macrovascular disease. *Diabetes Obes. Metab.* doi:10.1111/j.1463-1326.2004.00376.x. 7(2):117-121
- Boushey CJ, Beresford SA, Omenn GS, Motulsky AG (1995). A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA.* doi:10.1001/jama.274.13.1049 274(13):1049-1057
- Duell PB, Malinow MR (1997). Homocyst (e) ine: an important risk factor for atherosclerotic vascular disease. *Curr. Opin. Lipidol.* PMID: 9127708 [PubMed - indexed for MEDLINE]. 8(1):28-34
- Hoogveen EK, Kostense PJ, Beks PJ, Mackaay AJ (1998). Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: a population-based study. *Arterioscler. Thromb. Vasc. Biol.* <http://atvb.ahajournals.org/cgi/content/full/18/1/133> 18(1):133-138
- Passaro A, D'Elia K, Pareschi PL, Calzoni F, Carantoni M, Fellin R, Solini A (2000). Factors influencing plasma homocysteine levels in type 2 diabetes. *Diabetes Care.* doi:10.2337/diacare.23.3.420. 23(3):420-421
- Russo GT, Di Benedetto A, Giorda C, Alessi E, Crisafulli G, Ientile R, Di Cesare E, Jacques PF, Raimondo G, Cucinotta D (2004). Correlates of total homocysteine plasma concentration in type 2 diabetes. *Eur. J. Clin. Invest.* doi:10.1111/j.1365-2362.2004.01319.x. 34(3):197-204.
- Stamler J, Vaccaro O, Neaton JD, Wentworth D (1993). Diabetes, other risk factors, and 12 year cardiovascular mortality for men screened in multiple risk factor intervention Trial. *Diabetes care.* 16: 434-444. doi:10.2337/diacare.16.2.434.
- Van Guldener C, Stehouwer CD (2002). Diabetes mellitus and hyperhomocysteinemia. *Semin. Vasc. Med.* doi:10.1055/s-2002-23099 2(1):87-95
- Wilson PW, Kannel WB, Silbershatz H, D'Agostino RB (1999). Clustering of metabolic factors and coronary heart disease. *Arch. Intern. Med.* <http://archinte.ama-assn.org/cgi/content/full/159/10/1104> 159(10):1104-1109.
- Wulffele MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger (2003). Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. *J. Int. Med.* doi:10.1046/j.1365-2796.2003.01213.x. 254(5):455-463.