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Research Article

The Effect of Vitamin B and Folic Acid Supplementation on Plasma Homocystiene Levels in Women with Polycystic Ovary Syndrome Treated with Metformin. A Randomized Controlled Trial

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Abstract

Objective: To assess the effect of vitamin B-group and folic acid supplementation on serum levels of homocysteine (Hcy) in patients with polycystic ovarian syndrome (PCOS) on short term metformin treatment.

Design: Randomized controlled study.

Methods: PCOS patients were randomly assigned to two groups. Group 1 (n =40) received metformin only (500mg 3 times daily); group 2 (n=40) received metformin (500mg 3 times daily) plus vitamin B-group (twice daily) and folic acid (once daily) for 16 weeks. In groups, plasma Hcy, vitamin B12, folic acid, lipid profile, and HOMA test were recorded at baseline and after 16 weeks.

Results: There were no statistically significant differences with respect to background and hormonal characteristics of the two groups. An 18% increase in Hcy levels was seen after 16 weeks of metformin therapy, while 23% decrease in Hcy levels was detected when B-group vitamin and folic acid were added to metformin. There was a 14.3% decrease in vitamin B12 levels and 5.8% in folic acid levels in Group 1.

Conclusions: Vitamin B-group and folic acid supplementation to PCOS women treated with metformin is essential when one considers the elevated levels of Hcy and the possible increasing effect of metformin on Hcy levels in patients with PCOS.

Keywords: Vitamin B; Homocysteine; Metformin; Polycystic ovarian syndrome

Introduction

Homocysteine (Hcy) is an intermediate formed amino acid during the breakdown of the amino acid methionine. Hyperhomocysteinaemia is an accepted risk factor for premature cardiovascular disease (CVD) and stroke risk in healthy populations [1-3]. Experimental studies have demonstrated that high plasma concentrations of Hcy have been shown to correlate with blood pressure [4], body mass index and insulin resistance [5, 6].

Apart from the well established positive association between Hcy and risk of cardiovascular disease, elevated levels of Hcy in pregnant women have been linked to increased risk of preeclampsia, abruption of the placenta intrauterine growth restriction, and early pregnancy loss [7].

Polycystic ovary syndrome (PCOS) is the most common endocrine disease in women of reproductive age and is estimated to affect 5-10 % of the population [8]. PCOS is not only the most common reproductive disorder but also a plurimetabolic syndrome [9]. Obesity, insulin resistance and resultant hyperinsulinemia are cardinal features of PCOS with a greater risk of developing diabetes mellitus, hypertension and dyslipidemia [10].

Higher serum Hcy concentrations have been found in women with PCOS compared with controls by several authors [11-13], while others have found no association between PCOS and Hcy [14,15].

All current data suggest that PCOS possesses the intrinsic conditions that lead to an increased incidence of factors predisposing to cardiovascular diseases [16-18].

Recognition of insulin resistance as a principal factor in the pathogenesis of PCOS has led to the use of insulin sensitizers for its treatment [19]. The most extensively studied insulin sensitizer is metformin-an oral antihyperglycaemic agent used initially in the treatment of type 2 diabetes mellitus. It has been shown that metformin increases total serum Hcy levels in PCOS women [20] as well as it reduces levels of vitamin B_{12} and folic acid, which results in a modest increase in Hcy levels [21].

In view of these considerations, this study was designed to evaluate the effect of vitamin B and folic acid supplementation on plasma Hcy concentrations in women with PCOS treated with metformin.

Material and Methods

Eighty women with PCOS participated in this randomized

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controlled study. Institutional review board approval was attained before beginning the trial. PCOS women were recruited from the gynaecology & dermatology clinics of Cairo University Hospital during the period from September 2010 to October 2011. Most of the women were complaining from infertility and minority were unmarried complaining from hirsutism, acne and/or oligomenorrhea or amenorrhea. Informed written consent was obtained from all participants after explanation of the nature and purpose of the study. PCOS was defined when at least two of the following three features were present after the exclusion of other etiologies: oligomenorrhea or amenorrhea, signs of hyperandrogenism (e.g. hirsutism, acne and alopecia) or elevated levels of total or free testosterone, and polycystic ovaries on ultrasonography according to the Rotterdam criteria [22]. All women had normal thyroid, renal and hepatic functions. Their prolactin levels were within normal limits. Exclusion criteria were as follow; pregnancy, current or within previous 6 months use of oral contraceptives, vitamins, antiandrogens, anti-diabetics, statins, glucocorticoids or other hormonal drugs, cigarette smoking, chronic alcohol consumption, coffee consumption more than 2 cups/day, blood pressure of ≥ 130/85 mmHg or treated hypertension, known CVD and diabetes mellitus. During the testing period, all participants were asked to keep their normal diet and not perform any sporting activity.

Patients were randomly assigned by a research nurse into two groups the randomization was completed by opening sealed envelopes containing random numbers generated by computer. Group 1(n=40) received metformin (500 mg 3 times daily) (Glucophage; MerckSerono); group 2 (n =40) received metformin (500 mg 3 times daily) and vitamin B (Neurobion ;Merck) [vitamin B1 (100 mg); vitamin B6 (200 mg); vitamin B12 (200mcg) twice daily plus folic acid 500 mcg (Folic acid-Mepaco; Egypt) for 16 weeks.

All women were examined clinically. Weight, height, body mass index (BMI) and waist/hip ratio (WHR) were recorded. Baseline day 3 FSH, LH, E_2 and total testosterone were measured in spontaneous or progestogen-induced menses with specific chemiluminescence assays from the Abbott Architect system (Chicago, USA). The plasma glucose and insulin were measured after 12 hours fasting. Plasma glucose levels were measured using the glucose oxidase method; plasma insulin concentrations were measured by the micro-particle enzyme immunoassay method (AxSYM insulin assay; Abbott, Japan). The lipid profile was measured using an Abbott-Aeroset (Chicago, IL, USA) auto analyser with original kits after an overnight fast.

Homocysteine was measured as total homocysteine from plasma separated and frozen immediately after venipuncture; the plasma homoysteine concentration was measured by chemiluinescent enzyme immunoassay (Immulite 2000 Diagnosic Products, LA, and CA). Plasma folic acid and vitamin B_{12} concentrations were measured using the chemiluminescent method with an E170 immunoassay analyser (Roche Diagnostics Corp., USA).

Insulin resistance (IR) was determined by fasting insulin, the glucose: insulin ratio [glucose (mmol/l)/insulin (mIU/l)], the HOMA (homeostasis model assessment) index [glucose (mmol/l)*insulin (mIU/l)/22.5], [23] and quantitative insulin sensitivity check index (QUICKI) which is derived by calculating the inverse of sum of logarithmically expressed values of fasting insulin and glucose [24].

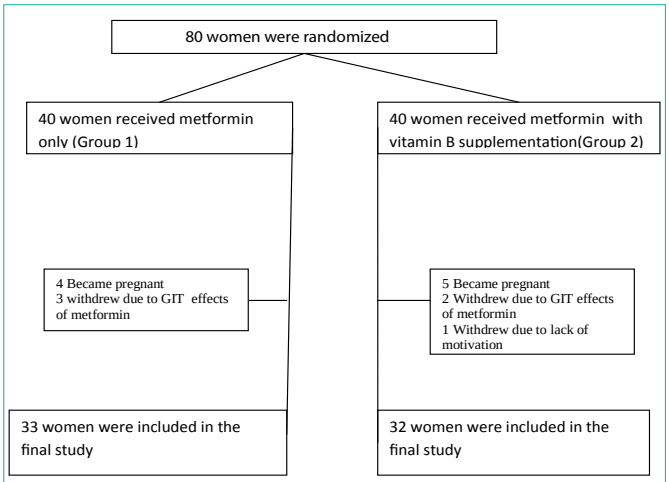

Figure 1: Chart of patients who were randomized.

Plasma Hcy, vitamin B12, folic acid, lipid profile, and HOMA tests were recorded at baseline and after 16 weeks. Safety measures included clinical assessment for adverse events and monitoring of complete blood count and renal and liver function tests.

Statistical Analysis

Data were statistically described in terms of mean ± standard deviation (±SD). Comparison of numerical variables between the study groups was done using Student *t* test for independent samples. Within group comparison between before and after treatment was done using paired *t* test. A *p* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

Eighty patients were included and 65 patients completed the study; 33 patients in the group 1, and 32 patients in group 2 (Figure 1). Nine patients became pregnant (4 in group 1 and 5 in group 2). Five patients withdrew because of gastrointestinal adverse effects of metformin (3 in group 1 and 2 in group 2). One patient withdrew from the study owing to lack of motivation in group 2. There were no statistically significant differences in baseline age, BMI, duration of infertility, waist: hip ratio, FSH, LH, E2, total testosterone, Hcy concentrations, vitamin B_{12} , folic acid ,lipid profile and insulin sensitivity markers between the two groups (Table I).

Hcy levels increased from 13.76± 6.28 to 16.26± 5.25 µmol/l in group 1(P=0.042), while Hcy levels in group 2 significantly decreased from 13.25 ± 7.2 to 10.15± 2.2 µmol/l (P = 0.012). Folic acid levels decreased in group 1, from 10.52 ± 3.14 to 9.90 ± 2.5 ng/ml (P = 0.189) while in group 2 increased from 10.83 ± 3.39 to 13.2 ± 3.1 ng/ml (P = 0.002). Vitamin B12 levels in group 1 decreased from 379.3 ± 108.1 to 324.7 ± 126.4 pg/ml (P = 0.032) while it increased in group 2 from 384.9 ± 118.3 to 496.5 ± 109.7 pg/ml (P = 0.00). No significant differences in T. Cholesterol, Triglcerides (TG), High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C), were found in the two groups before and after treatment. Insulin resistance was improved in the current study as measured by HOMA test which is decreased in both groups (4.78 ±

Table 1: Baseline characteristics of the patients in the two groups.

	P value	Group 1	Group 2
Age (Yrs)	71	23.92 ± 4.64	24.31 ± 4.75
BMI (Kg /m^2)	67	25.82 ± 3.44	26.16 ± 3.75
WHR	1	0.83 ± 0.08	0.83 ± 0.09
Duration of infertility (months)	0.44	18.3±5.1	17.2±7.6
FSH(mIU/ml)	0.77	6.11 ± 2.27	5.96 ± 2.47
LH(mIU/ml)	0.21	18.0±2.2	16.9±5.1
E_2(pg/ml)	0.59	33.8±8.3	34.9 ±9.8
Testosterone(ng/ml)	0.14	1.0 ±0.3	0.9 ± 0.3
Fasting glucose(mg/dl)	0.33	81.7± 7.1	79.9 ± 9.3
Fasting insulin(µU/ml)	0.46	21.4± 5.6	20.5 ± 5.4
GIR (mg/10^{-4} U)	0.79	4.1± 1.7	4.2± 1.65
HOMA	0.78	4.78 ± 1.19	4.86 ± 1.45
QUICKI	0.1	0.313 ± 0.021	0.321 ± 0.023
Total cholesterol (mg/dl)	0.64	170.8 ± 41.5	167.1 ± 29.9
Triglycerides (mg/dl)	0.48	128.6± 28.7	132.9 ± 26.6
HDL cholesterol (mg/dl)	0.5	75.1 ± 19.3	77.8 ± 16.9
LDL cholesterol (mg/dl)	0.71	99.72 ± 26.34	101.83 ± 25.55
Vit B12 (pg/ml)	0.82	379.3 ± 108.1	384.9 ± 118.3
Folic acid (ng/ml)	0.67	10.52 ± 3.14	10.83 ± 3.39
Homocysteine (µmol/L)	0.73	13.76 ± 6.28	13.25 ±7.2

1.19 to 4.07± 1.2) in group 1and (4.86 ± 1.45to 4.13 ±1.1) in group 2 (P = 0.009, 0.013) respectively (Table 2).

Discussion

Metformin has been introduced as a therapeutic option for PCOS, targeting cardio metabolic and reproductive abnormalities on the basis of its action in reduction of glucose levels , attenuation of insulin resistance, as well as its molecular mechanism involving the liver, the muscles, the endothelium, and the ovary [25].

Hcy is an essential amino acid required for the growth of cells and tissues in the human body. The only source of Hcy comes from the methionine in dietary proteins, which are mainly of animal origin. Homocysteine may undergo remethylation to methionine or trans-sulphuration to cystathione and cysteine. Vitamin B_6, B_{12} and folate are essential factors in these processes. Plasma homocysteine levels are influenced by a number of variables including smoking, coffee consumption, renal function, vitB_{12}, folate status and some drugs such as methotrexate, nitrous oxide, metformin or azaribine, that reacts with folic acid, vitamin B12 or B6 respectively can cause hyperhomocysteinemia. Ten per cent of the risk of coronary artery disease in the general population is attributable to an increase in Hcy levels [1] which may be due vascular damage and alteration in the coagulation process.

The present study shows that after 16 weeks of metformin therapy in patients with PCOS, 18% increase in Hcy levels was seen with metformin only therapy (group1), and 23 % decrease in Hcy levels was seen in the B-group vitamin and the folic acid plus metformin (group2) in agreement with others [21, 22].

Although fasting Hcy levels increased significantly in metformin therapy group, (Group 1) there were decrease 14.3% in vitamin B_{12} levels and 0.5% in folic acid levels. Therefore, it seems likely that a rise in Hcy levels may be related to a decrease in vitamin B_{12} and folic acid levels.

One explanation for this is that vitamin B_{12} and vitamin B_6 are two factors that act separately in Hcy metabolism and probably potentiate each other. Metformin does, however, induce vitamin B_{12} malabsorption; also it decreases folate concentration, although the mechanism has not been elucidated. In this study a combination of vitamins B_1, B_6 and B_{12} was used, because the oral preparation in Egypt

Table 2: Homocysteine, folic acid, vitamin B12 levels ,lipid profile and HOMA test before and after treatment in the two groups.

Variable	Group	Before treatment	After treatment	Change from month 0 to 4	*P* value
Hcy (µmol/l)	1	13.76 ±6.28	16.26±5.25	2.50	0.042
	2	13.25±7.20	10.15±2.20	- 3.10	0.012
Folic acid (ng/ml)	1	10.52 ±3.14	9.90 ± 2.5 0	- 0.62	0.189
	2	10.83 ± 3.39	13.20± 3.10	2.73	0.002
Vitamin B12(pg/ml)	1	379.31 ± 108.11	324.70± 126.40	-54.60	0.032
	2	384.9 ± 118.3	496.50± 109.70	111.60	0.000
T.cholesterol (mg/dl)	1	170.81 ± 41.51	166.71 ± 37.71	- 4.10	0.338
	2	167.10 ± 29.90	161.9 1± 41.21	- 5.20	0.283
Triglycerides (mg/dl)	1	128.62 ± 28.72	127.4 2± 27.62	-1.20	0.432
	2	132.90 ±26.60	127.7 1± 26.61	- 5.20	0.219
HDL cholesterol (mg/dl)	1	75.10 ± 18.30	76.71 ± 17.51	1.60	0.359
	2	77.82±16.92	80.22 ± 17.32	2.40	0.288
LDL cholesterol (mg/dl)	1	99.72±26.34	91.36 ±25.12	- 8.36	0.096
	2	101.83 ± 25.55	93.13±27.42	- 8.70	0.097
HOMA	1	4.78 ± 1.19	4.07± 1.20	- 0.71	0.009
	2	4.86 ± 1.45	4.13 ±1.10	- 0.73	0.013

contains only a combination of vitamin B, there is no oral preparation includes vitamin B_{12} or B_6 only.

In the current study vitamin B_{12} and folic acid supplementation significantly decreases Hcy concentration (P=0.012) in agreement with Kilicdag EB 2005 [26] that founded that B-group vitamins and folic acid administration separately decreased Hcy level (P= < 0.001,0.04) respectively. Also previously folic acid administration has been shown to reduce Hcy levels in healthy subjects and in patients with kidney or vascular disease [27]. These findings suggest that B-group vitamins and folic acid administration, especially B-group vitamins, can counteract the Hcy-increasing effects of metformin therapy.

Recently de Jager J and colleagues 2010 [28] showed that long term metformin therapy in patients with type 2 diabetes , decreases vitamin B_{12} (-19%) , which results in raised homocysteine concentrations (5%) and since vitamin B_{12} deficiency is preventable; they suggest regular measurement of vitamin B_{12} during long term metformin therapy. Carlsen et al 2007 [29] founded that homocystiene levels are not affected in nonpregnant and pregnant PCOS women receiving metformin which may be attributed to the vitamin B group and folic acid supplementation to both study groups as well as physical activity & exercise that had been encouraged through the whole study.

Lifestyle measures in treatment of PCOS such as diet and exercise could play an important role. Although the exact mechanism is not known, regular exercise significantly lowers plasma Hcy levels in young, overweight or obese women with PCOS [30].

Insulin resistance was improved in this study in both groups as measured by HOMA test, which may be a beneficial effect of metformin in correction of one of the cardiovascular risk factors.

In conclusion, the results suggest that 16 weeks of metformin therapy in patients with PCOS results in a significant increase in plasma Hcy concentrations as well as a significant decrease in plasma levels of B_{12} vitamin with minor decrease in folic acid concentration, which may be corrected by administration of B-group vitamins and folic acid. Although the sample size in the current study was small, these findings suggest that daily administration of B-group vitamins and folic acid may be effective in reducing elevated Hcy levels in patients with PCOS undergoing short-term metformin therapy. Because of the chronic use of this medication from as early as the teenage years in patients with PCOS, future studies with long-term follow-up should investigate the duration of therapy and the benefit or detrimental effects of the medication on the cardiovascular risk factors.

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