Research Article

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Role of Nutritional Supplementation with Vitamin B12 and Folic Acid on Serum Homocysteine Levels of Deep Vein Thrombosis

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Abstract

Deep vein thrombosis is a common vascular disorder and association of Homocysteine with DVT is known. Evidence of decrease B12 and folic acid levels in DVT with Hyperhomocysteinemia has also been reported. Scanty data is available regarding role of folate and vitamin B12 on Homocysteine levels in DVT patients. The present study was undertaken in 100 patients. Study group comprised of 50 patients who had clinical features suggestive of DVT and 50 patients, who did not have clinical features suggestive of any venous or arterial disorder, were labelled as control group. All the patients of study group were given vitamin B12 and folic acid treatment along with standard treatment of DVT. Serum Homocysteine, folate, vitamin B12 were measured in all the patients in both the groups and estimated by chemiluminescence technology. After 12 weeks of vitamin B12 and folic acid therapy, serum Homocysteine levels, folic acid levels and vitamin B12 levels of study group were again estimated. The collected records and data was analysed statistically by Student t-test and Chi-square test. Mean Homocysteine level in study group was significantly higher as compared to control group ($19.63 \pm 19.14 \mu\text{mol/mL}$ vs. $7.0 \pm 4.94 \mu\text{mol/mL}$). Mean Homocysteine levels before treatment were $19.63 \pm 19.14 \mu\text{mol/mL}$ and after treatment they were significantly reduced. Hyperhomocysteinemia is a risk factor for deep vein thrombosis. Folic acid and B12 therapy reduced the level of Homocysteine suggesting that this may decrease the chances of recurrence of DVT.

Keywords: Nutritional supplementation, Vitamin B12, Folic acid, Serum homocysteine, Deep vein thrombosis

Introduction

Deep Vein Thrombosis (DVT) has an incidence of 5, 00,000 cases per year worldwide [1][2][3]. The factors predisposing to venous thromboembolism include a triad of hyper coagulability, endothelial damage or intimal changes and reduced blood flow or stasis [1][2]. Various thrombophilic factors known to cause DVT are namely, deficiency of anti-thrombin III, protein C or protein S, antiphospholipid antibody or lupus anticoagulant, factor V Leiden gene defect or activated protein C resistance, dysfibrinogenaemias and homocysteinemia [1][4][5]. Hyperhomocysteinemia acts via various mechanisms to cause increased potential for venous thrombosis [6][7][8]. 10% to 20% of women and men with DVT have been

reported to show higher Homocysteine levels as compared to those who have not suffered from DVT [9]. One analysis has reported that 50% increase in Homocysteine raises the risk of DVT or pulmonary embolism by 60% [10]. D'Angelo et al. observed the prevalence of hyper Homocysteinemia in DVT group to be 10.4% as compared to 4.8% in control group with a calculated odds ratio of 2.40 and concluded a positive relationship between DVT and Hyperhomocysteinemia [11]. Souheil et al. in their case control study found that plasma total Homocysteine concentrations were significantly higher in patients with DVT and in patients with idiopathic DVT as compared to controls. They concluded that Hyperhomocysteinemia is independently associated with DVT and Homocysteine levels should be assessed in patients with DVT and effect

of vitamin B6, B12 and folic acid supplementation should be tested among them [12]. Dietary intake of B12 and folic acid affect the Homocysteine levels as they are required in its metabolism [13]. Increased Homocysteine level is an independent risk factor for atherosclerosis, venous thromboembolic disorders like DVT and pregnancy related complications [1][14]. Relationships between coronary artery disease, vascular diseases and Homocysteine have been known for a long time. Homocysteine levels can be affected by dietary or supplemental vitamin B update. Therefore, Homocysteine levels should be tested in patients with or at risk for blood clots, atherosclerosis or pregnancy complications [15]. Hence the present study was planned to explore the role of folate and vitamin B12 on Homocysteine levels in DVT patients and the possible benefits of folate and vitamin B12 supplementation.

Materials and Methods

The present prospective study was conducted in the Department of Biochemistry in collaboration with Department of Surgery and Venous Clinic, Pt. B.D. Sharma Postgraduate Institute of Medical Sciences, Rohtak. A total of 100 patients were studied. The patients were divided into two groups. Group A included 50 patients who had clinical features suggestive of deep vein thrombosis and diagnosis was confirmed on colour doppler. Group B included 50 patients who did not have clinical features suggestive of any venous or arterial disorder and they served as control. The informed consent was obtained from all the participants. Patients having chronic co-morbid conditions like obesity, diabetes mellitus, and hypertension were excluded from group B. Physical examination was done to rule out any venous or arterial disorder in group B patients. All the patients of group A were given vitamin B12, 1500 µg once a day and folic acid 5 mg once a day along with standard treatment of DVT.

Serum Homocysteine, folate, vitamin B12 were measured in all the patients in both the groups. After overnight fasting for 12 hours, 2 ml of venous blood sample was taken in EDTA and heparinised lithium tubes and serum Homocysteine, folic acid and vitamin B were estimated by chemiluminescence technology [5]. After 12 weeks of vitamin B12 and folic acid therapy, serum Homocysteine levels, folic acid levels and vitamin B12 levels of group A were again estimated. The collected records and data was analysed statistically by Student *t*-test and Chi-square test.

Results

Mean Homocysteine level in study group was 19.63 ± 19.14 µmol/mL and in control group, it was 7.0 ± 4.94 µmol/mL and it was statistically significant ($p < 0.001$, HS). In the present study, 30 patients before treatment and 46 patients after treatment had serum Homocysteine level < 13.3 µmol/mL. Before treatment, 9 patients had moderately high Homocysteine levels (13.3-30 µmol/mL), 10 patients had intermediately high levels (31-100 µmol/mL) and 1 patient was found to have very high Homocysteine level (> 100 µmol/mL). After treatment, 2 patients had moderately high, 1 patient had intermediately high and none was found to have very high Homocysteine levels. Mean Homocysteine levels before treatment were 19.63 ± 19.14 µmol/mL and after treatment they were significantly reduced. Serum folic acid levels before therapy were low only in 4 patients (< 2 ng/mL) and rest of the patients had levels > 2 ng/mL. Forty six patients who had folic acid levels > 2 ng/mL after therapy levels showed improvement. Before therapy, serum folic acid levels were 5.03 ± 2.21 ng/mL and after treatment they improved to 10.38 ± 5.86 ng/mL, and it was statistically highly significant ($p < 0.001$) (Figure 1).

Figure 1: Effect of vitamin B supplementation on serum hcy, folate and vitamin B12 levels.

Before B12 therapy, 41 patients had low levels (< 200 pg/ml) of vitamin B12 and after therapy the levels improved in all the patients (> 200 pg/ml), it was statistically significant ($p < 0.001$). Before therapy, mean B12 levels were 181.3 ± 63.57 pg/mL, and after therapy it improved to 612.67 ± 194.88 pg/mL, statistically highly significant ($p < 0.001$, Table 1).

Discussion

Falcon et al found higher mean serum Homocysteine levels in patients with DVT to be higher as compared to 8.3 ± 3.4 in control group [16]. A few other studies also shown higher mean values of serum Homocysteine in DVT patients as compared to controls but they found no significant association between serum Homocysteine

Table 1: Homocysteine, folate, vitamin B12 levels in serum of group A patients before and after treatment.

Group A (Mean ± SD)	Before treatment	After treatment	Statistical significance
S. Homocysteine levels (μmol/L)	19.63 ± 19.14	8.86 ± 2.74	< 0.01 Significant
S. Folate levels (ng/ml)	5.03 ± 2.21	10.38 ± 5.86	< 0.001 HS
S. Vitamin B12 levels (pg/ml)	181.3 ± 63.57	612.67 ± 194.88	< 0.001 HS

levels and DVT [17][18]. In a study done by E Kim et al reported that 43.3% patients with low folic acid concentration along with Hyperhomocysteinemia [19]. Souheil et al conducted a study on patients with DVT and concluded that Hyperhomocysteinemia is independently associated with DVT and Homocysteine levels should be assessed in patients with DVT and effect of vitamin B6 and B12 and folic acid supplementation should be tested among them [12]. Vuckovic et al in their study reported that vitamin supplementation yield a 37% lower risk of deep venous thrombosis [20].

In the study done by E Kim et al. patients with Hyperhomocysteinemia, vitamin B12 and folic acid therapy should be recommended which will lower the Homocysteine levels and may help in reducing the recurrence of DVT [19].

In present study, the mean levels of serum Homocysteine levels were 19.63 ± 19.4 μmol/L in patients with DVT as compared to levels of controls ($p < 0.001$). Out of 50 patients, folic acid deficiency was observed in 4 patients while B12 deficiency was seen in 41 patients. Before B12 and folic acid therapy, 20 patients had high levels of the Homocysteine more than 13.3 μmol/l among these 20 patients, 2 patients had low folic acid levels below 2 ng/ml and all 18 patients had low levels (< 200 pg/ml) of vitamin B12. After 12 week therapy with vitamin B12 and folic acid, folic acid and vitamin B12 levels improved in all the patients and they were in normal range (folic acid > 2 ng/ml and vitamin B12 > 200 pg/ml) in all the 50 patients and Homocysteine levels were decreased. The finding of the present study is in agreement with those reported in literature.

Before therapy serum Homocysteine levels were 19.63 ± 9.14 μmol/L while after therapy got lowered to 8.86 ± 2.74 μmol/L. Before therapy mean B12 levels were 181.3 ± 63.57 pg/mL while after therapy they improved to 612.67 ± 194.88 pg/mL. Serum folic acid levels before therapy were 5.03 ± 2.21 ng/mL and after therapy they improved to 10.36 ± 5.88 ng/mL. Hence, after 12 weeks of supplementation with vitamin B12 and folic acid. Serum vitamin B12 and folic acid levels returned to normal in

all the patients and the previously high Homocysteine levels were decreased to normal values in DVT patients (Table1).

The prevalence of Hyperhomocysteinemia in cases of DVT in present study was 40% as compared to 6% in control group which was statistically significant. Present study demonstrated that Hyperhomocysteinemia is a risk factor for deep vein thrombosis and folic acid and B12 therapy reduced the level of Homocysteine suggesting that this may decrease the chances of recurrence of DVT. The finding of the present study and other studies have shown that high Homocysteine, low serum folic acid and vitamin B12 are high risk factors for thromboembolic events and necessary testing of Homocysteine, folic acid and vitamin B12 levels and appropriate treatment for these metabolic disturbances may be of help in reducing incidence, treatment cost and morbidity in these patients. Hence, it is advisable to give folic acid and vitamin B12 supplement to DVT patients with Hyperhomocysteinemia.

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