Prospective study of relationship between deep vein thrombosis and homocysteine related group B vitamins

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Abstract
Introduction: Hyper homocysteinemia has been considered as a potential risk factor for deep venous thrombosis (DVT) but it is still controversy. This study was aimed to assess the prevalence of hyper homocysteinemia in our population with DVT. Our second objective was to document the prevalence of folate, Vitamin B6, and Vitamin B12 level in this patient population.

Results: Mean serum homocysteine levels among DVT patients was 15.12±5.47 μmol/L. Most of the patients of DVT were from 41-60 years of age group with mean age being 50.11±13.66 years. Of all the patients, 15 patients had homocysteine level above the 15 µmol/L, 22 had folic acid level below 3 ng/ml, 14 had vitamin B12 level below 150 pmol/L, and 3 had vitamin B6 level below 30 nmol/L. In the hyper homocysteinemic group, eleven patients had low folic acid level, ten had low vitamin B12 level and two had low vitamin B6 level.

Conclusions: Hyper homocysteinemia and related Vitamin B group deficiency are risk factors for Deep vein thrombosis. Hyper homocysteinemia and risk of DVT, this correlation is high among older age group and female patients.

Keywords: Deep venous thrombosis, hyper homocysteinemia, B group vitamins

Introduction
Venous thromboembolism (VTE), a disease entity comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), is a frequent and potentially life-threatening event. Venous thromboembolism (VTE) is the third most common vascular disease after myocardial infarction and ischemic stroke [1]. The annual incidence of symptomatic and objectively confirmed VTE, the collective term generally used for deep venous thrombosis, pulmonary embolism or both, is 2 to 3 per 1000 inhabitants [2]. VTE is not only disabling but also prolongs hospital stay and increases the cost of treatment.

The pathophysiology of venous thrombosis is considered to be multicausal [3], with many inherited and acquired risk factors and their interaction playing a role in the etiopathogenesis. Risk factors for venous thrombosis can be divided into acquired and genetic risk factors. Knowledge of these factors is important for prevention purposes.

Among venous thrombosis risk factors, hyper homocysteinemia has been considered as a potential factor by most studies. Hyper homocysteinaemia is a disorder of methionine metabolism [4]. Hyper homocysteinaemia is proposed to be a modifiable risk factor of atherosclerosis [5,6], myocardial infarction [7,8] peripheral arterial thrombosis [9] as well as venous thromboembolism [9,10].

Hyper homocysteinemia due to reduced B-group vitamins (folic acid, vitamin B12, and vitamin B6) levels is thought to be a part of the pathophysiologic link between B-group vitamins and venous thrombosis [11]. This study is aimed to assess the prevalence of hyper homocysteinemia in our population with DVT.

Aims and Objectives
1. To know the prevalence of hyper homocysteinemia in our urban and rural population with Deep vein thrombosis.
2. To document the homocysteine and Vitamin B12, Folic Acid and Vitamin B6 levels in this population affected by Deep Vein Thrombosis.
Materials & Methods
It is a prospective and observational study. Study was conducted after approval from institute’s ethical and research committee. 60 patients of both genders with age 18 and above, who were admitted or referred to department of general surgery, DR. V.M. Govt medical college Solapur, Maharashtra between December 2017 to August 2019 with diagnosis of DVT were included after obtaining written informed consent from each patient. Exclusion criteria were conditions like drug usage (metformin, cholestyramine, hypothyroidism, pernicious anemia, respiratory insufficiency, pregnant females, a history of antiphospholipid syndrome & sepsis may interfere with homocysteine levels, will be excluded from our study. Patient on Folic Acid, Vit B6, Vit B12 therapy were also excluded from the study. Diagnosis of DVT was based on clinical history and thorough physical examination, Well’s scoring system and Venous doppler & duplex scan of limb. Laboratory investigations (lipid profile, coagulation profile) was done in All patients with established diagnosis of DVT. The total Serum homocysteine levels, Vitamin B6, Vitamin B12 levels and folic acid levels were measured on heparinized blood samples collected after 12 hours of fasting.
Normal reference values are as follows-
- Normal homocysteine level <15 μmol/L. Results were quantified as mild (15-30 μmol/L), moderate (30-100 μmol/L) and severe hyper homocysteinemia. (>100 μmol/L)
- Normal Serum vitamin B12 levels (200-900 pg/ml)
- Normal Serum vitamin B6 levels (5.0-50 μg/L)
- Normal Serum folic acid levels (2-20 ng/ml)

Data collected, prevalence of hyper homocysteinemia, prevalence of Vitamin B12, Vitamin B6 and folic acid deficiency were calculated among DVT cases.

Observations and Results
Age and sex wise distribution
Most of patient were from 41-60 years age group. (Figure 1) Mean age of patients of DVT in our study was 50.11±13.64 years.

![Age wise distribution of cases](image1.png)

Out of total 60 patients of DVT 34 (57%) were males and 26 (43%) were females (figure 2). Male to female ratio was 1.30:1.

![Sex wise distribution of cases](image2.png)

Serum homocysteine levels
Out of 60 patients 45 had normal homocysteine levels and 15 patients had hyper homocysteinemia. Out of these 15 patients, 13 had mild hyper homocysteinemia, 2 had moderate hyper homocysteinemia & none of the patients had severe hyper homocysteinemia.

| Serum homocysteine levels (μmol/L) | No. of patients | Percentage |
|-----------------------------------|----------------|------------|
| Normal homocysteine level (<15 μmol/L) | 45 | 75 |
| Mild hyper homocysteinemia (15-30 μmol/L) | 13 | 21.67 |
| Moderate hyper homocysteinemia (30-100 μmol/L) | 2 | 3.33 |
| Severe hyper homocysteinemia (>100 μmol/L) | 0 | 0 |
| Total | 60 | 100 |

Serum vitamin B12, vitamin B6 and folic Acid levels
14 out of total 60 DVT patients (23.33%) were having Vitamin B12 deficiency. (fig. 3) Mean vitamin B12 level in our 60 DVT patients was 295.88±112.8 pg/ml.
3 out of total 60 DVT patients (5%) were having Vitamin B6 deficiency. (figure 4) Mean vitamin B6 level in our 60 DVT patients was 15.57±7.64 μg/L.

22 out of total 60 DVT patients (38.33%) were having folic acid deficiency. (figure 5) Out of these 22 patients 16 were female and 8 were males. Mean Serum folic acid level in our 60 DVT patients was 5.64±4.2 ng/ml.

Table 2: Clinical and Laboratory characteristics of Patients of DVT with hyperhomocysteinemia.

| Sr. No. | Age | Sex | Homocysteine level (μmol/L) | Vitamin B12 levels (ng/ml) | Vitamin B6 levels (μg/L) | Folic acid levels (ng/L) |
|---------|-----|-----|----------------------------|---------------------------|--------------------------|--------------------------|
| 1       | 62  | Male| 21.3                       | 211                       | 19.9                     | 1.9                      |
| 2       | 43  | Female| 35.6                      | 178                       | 4.2                      | 3.7                      |
| 3       | 55  | Male| 19.70                      | 189                       | 27.1                     | 1.7                      |
| 4       | 45  | Male| 15.24                      | 256                       | 19.7                     | 1.9                      |
| 5       | 54  | Female| 29.9                      | 195                       | 4.3                      | 4.7                      |
| 6       | 45  | Female| 32.8                      | 148                       | 5.1                      | 1.8                      |
| 7       | 44  | Female| 21.5                      | 106                       | 5.5                      | 3.4                      |
| 8       | 69  | Female| 16.9                      | 244                       | 11.9                     | 1.8                      |
| 9       | 60  | Male| 22.4                       | 159                       | 6.54                     | 4.9                      |
| 10      | 50  | Female| 17.8                      | 323                       | 8.8                      | 1.8                      |
| 11      | 37  | Female| 26                        | 159                       | 3.9                      | 2.1                      |
| 12      | 62  | Female| 15.7                      | 163                       | 6.4                      | 1.9                      |
| 13      | 55  | Male| 15.8                       | 378                       | 17.1                     | 1.9                      |
| 14      | 54  | Female| 22.8                      | 166                       | 5.7                      | 1.8                      |
| 15      | 65  | Male| 21.6                       | 192                       | 6.3                      | 1.7                      |

Out of total 15 patients of DVT with hyperhomocysteinemia 9 patients were females and 6 were males. Among these 15 patients 13 had mild hyperhomocysteinemia and 2 had moderate hyperhomocysteinemia. Out of these 15 patients with hyperhomocysteinemia 10 patients had vitamin B12 deficiency, two patients had Vitamin B6 deficiency 11 patients had folic acid deficiency also. Mean serum homocysteine level among these 15 patients is 22.336 μmol/L. Mean age of patients with hyperhomocysteinemia was 53.33 years.

Discussion

Mean Age of DVT patients: In our study most of the patients of DVT were from 41-60 years of age group with mean age being 50.11±13.64 years. Study by Sharma V et al. 2019 found mean age of patients being 48.62±10.91 years, study by Ekim M et al. 2015 found mean age of patients 50.5 years, study by Kokturk N et al. 2011 found mean age of patients 55±17 years, study by Unlu Y et al. 2005 found mean age of patients 52 years, study by Cattaneo M et al. 2001 had mean
age of 42 years and study conducted by Den Heijer M et al. 1996 [17] had mean age of patients 44 years. In contrast study by Oger E et al. 2006 [18] having mean age of patients 67.4 ± 17.9 years.

**Sex ratio of DVT patients**

In our study out of total 60 DVT patients 34 were males and 26 were females and male to female ratio was 1.307:1, study by Sharma V et al. 2019 [12] found male to female ratio 1.50:1, Ekim M et al. 2015 [13] found male to female ratio 1.06:1 and Unlu Y et al. 2005 [15] found with male to female ratio of 2.42:1. In contrast study by Kokturk N et al. 2011 [14] had male to female ratio of 1:1.58 and study by Den Heijer M et al. 1996 [17] having male to female ratio of 1:1.13.

**Prevalence of Hyper homocysteinemia**

In our study 15 out of 60 DVT patients (25%) were having hyper homocysteinemia, study by Simioni P et al. 1996 [19] found 25% DVT patients were having hyper homocysteinemia, study by Ekim M et al. 2015 [13] found 15% of DVT patients were having hyper homocysteinemia. In contrast Our study doesn’t confirm with study by N.kokturk et al. 2011 [14] with 63% of DVT patients were having hyper homocysteinemia, study by Oger M et al. 2006 [18] with 66% of DVT patients were having hyper homocysteinemia and study conducted by Cattaneo M et al. 2001 [16] with 10% of DVT patients were having hyper homocysteinemia. Possible explanation for this is study conducted by Oger M et al. 2006 [18] was having relatively older population mean age (67.4 ± 17.9 years) and study conducted by Cattaneo M et al. 2001 [16] was relatively younger population (mean age 42 years). In comparison to our study. (mean age 50.11±13.64 years)

**Mean homocysteine levels**

In our study mean serum homocysteine levels among DVT patients was 15.12±5.47 μmol/L. study by conducted by Sharma V et al. [12] 2019 found mean homocysteine levels 24.62±3.02 μmol/L, study by Kokturk N et al. 2011 [14] found mean homocysteine levels 21.1±12.5 μmol/L, study by Oger E et al. 2006 [18] found mean homocysteine levels 17.5 μmol/L and study by Unlu Y et al. 2005 [15] found mean homocysteine levels 17.1±5.3 μmol/L.

In contrast Our study doesn’t confirm with study conducted by Cattaneo M et al. 2001 [16] having mean homocysteine levels of 9.5 μmol/L. There are two possible reason for this, study conducted by Cattaneo M et al. 2001 [16] was having relatively lesser number of DVT patients with hyper homocysteinemia (38 out of 397, prevalence 10%) in comparison to our study (15 out of 60, prevalence 25%) and study conducted by Cattaneo M et al. 2001 [16] was having younger DVT population (mean age 42 years) in comparison to our study (mean age 50.11 years) and other above mentioned studies.

**Clinical characteristic of DVT patients with hyper homocysteinemia with various studies**

In our study 9 out of 15 (60%) patients with hyper homocysteinemia were females. study by Ekim M et al. 2015 [13] found 6 out of 9 (66.66%) patients with hyper homocysteinemia were females and study by Kokturk N et al. 2011 [14] found 35 out of 59 (59%) patients with hyper homocysteinemia were females.

Mean age of 15 patients of DVT with hyper homocysteinemia in our study was 53.33 years, which is comparable to study by Ekim M et al. 2015 [13] having mean age of patients of DVT with hyper homocysteinemia 53.3 years and study by Kokturk N et al. 2011 [14] having mean age of patients of DVT with hyper homocysteinemia 53.3 years.

**Serum Vit B12, Vit B6 and folic acid**

In our study 14 out of 60 DVT patients (23.33%) were vitamin b12 deficiency, study by Oger E et al. 2006 [18] found 30.17% of DVT patients were having vitamin B12 deficiency; study by Cattaneo M et al. 2001 [16] found 26.47% DVT patients were having vitamin b12 deficiency. In contrast Our study doesn’t confirm with study by Ekim M et al. 2015 [13] with only 1.67% (1 out of 60) patients having vitamin B12 deficiency.

In our study 3 out of 60 DVT patients (5%) were vitamin B6 deficiency. study conducted by Ekim M et al. 2015 [13] found 2 out of 60 patients (3.33%).

In our study 22 out of 60 DVT patients (36.66%) were having folic acid deficiency. study by Ekim M et al. 2015 [13] found 43.33% patients having folic acid deficiency, study by Cattaneo M et al. 2001 [16] found 33.24% DVT patients were having folic acid deficiency. in contrast Our study doesn’t confirm with study conducted by Oger E et al. 2006 [18] with 6.8% of DVT patients were having folic acid deficiency. Possible reason of for this high prevalence of folic acid deficiency that in our population majority are having vegetarian diet and prolonged cooking of vegetable dishes may result in diminished folate content.

**Conclusions**

Hyper homocysteinemia and related Vitamin B group deficiency are risk factors for Deep vein thrombosis. Hyper homocysteinemia and risk of DVT, this correlation is high among older age group and female patients. All at risk patients should receive vitamin B12, vitamin B6 and folic acid supplementation.

**References**

1. Naess IA, Christiansen SC, Romundstad P, Canegiecer ST, Rosendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. Journal of thrombosis and haemostasis. 2007; 5(4):692-9.
2. Reitsma PH, Versteeg HH, Middeldorp S. Mechanistic view of risk factors for venous thromboembolism. Arteriosclerosis, thrombosis, and vascular biology. 2012; 32(3):563-8.
3. Rosendaal FR. Venous thrombosis: a multicausal disease. The Lancet. 1999; 353(9159):1167-73.
4. Wilcken DE, Wilcken B. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. The Journal of clinical investigation. 1976; 57(4):1079-82.
5. Cattaneo M. Hyperhomocysteinemia, atherosclerosis and thrombosis. Thrombosis and haemostasis. 1999; 81(02):165-76.
6. Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland et al. Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. Jama, 1997; 277(22):1775-81.
7. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. New England Journal of Medicine. 1997; 337(4):230-7.
8. Whincup PH, Refsum H, Perry JJ, Morris R, Walker M, Lennon L et al. Serum total homocysteine and coronary heart disease: prospective study in middle aged men. Heart. 1999; 82(4):448-54.
9. Den Heijer M, Koster T, Blom HJ, Bos GM, Briët E, Reitsma PH et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. New England journal of medicine. 1996; 334(12):759-62.
10. Den Heijer M, Rosendaal FR, Blom HJ, Gerrits WB, Bos GM. Hyperhomocysteinemia and venous thrombosis: a meta-analysis. Thrombosis and haemostasis. 1998; 80(12):874-7.
11. Zhou K, Zhao R, Geng Z, Jiang L, Cao Y, Xu D et al. Association between B-group vitamins and venous thrombosis: systematic review and meta-analysis of epidemiological studies. Journal of thrombosis and thrombolysis. 2012; 34(4):459-67.
12. Sharma V, Bendre M, Chavan S. Comparative study to establish significance of D-dimer, lipid profile and homocysteine level in cases of deep vein thrombosis. International Surgery Journal. 2019; 6(5):1733-9.
13. Ekim M, Ekim H, Yılmaz YK, Kulah B, Polat MF, Gocmen AY. Study on relationships among deep vein thrombosis, homocysteine & related B group vitamins. Pakistan journal of medical sciences. 2015; 31(2):398.
14. Köktürk N, Kanbay A, Aydoğan M, Özyılmaz E, Bukan N, Ekim N. Hyperhomocysteinemia prevalence among patients with venous thromboembolism. Clinical and applied thrombosis/hemostasis. 2011; 17(5):487-93.
15. Ünlü Y, Keleş S, Becit N, Koçoğulları CU, Koçak H, Bakan E. Hyperhomocysteinaemia as a risk factor for deep-vein thrombosis. European journal of vascular and endovascular surgery. 2005; 30(3):315-8.
16. Cattaneo M, Lombardi R, Lecchi A, Bucciarelli P, Mannucci PM. Low plasma levels of vitamin B6 are independently associated with a heightened risk of deep-vein thrombosis. Circulation. 2001; 104(20):2442-6.
17. Den Heijer M, Koster T, Blom HJ, Bos GM, Briët E, Reitsma PH et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. New England journal of medicine. 1996; 334(12):759-62
18. Oger E, Lacut K, Le Gal G, Couturaud F, Guenet D, Abalain JH et al. EDITH Collaborative Study Group. Hyperhomocysteinemia and low B vitamin levels are independently associated with venous thromboembolism: results from the EDITH study: a hospital-based case–control study. Journal of Thrombosis and Haemostasis. 2006; 4(4):793-9.
19. Simioni P, Prandoni P, Burlina A, Tormene D, Sardella C, Ferrari V et al. Hyperhomocysteinemia and Deep-Vein Thrombosis A Case-Control Study. Thrombosis and haemostasis. 1996; 76(06):0883-6.