Methylenetetrahydrofolate Reductase (MTHFR C677T) Polymorphism in Sudanese Patients with Deep Vein Thrombosis

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Abstract
Deep vein thrombosis (DVT) is a common cause of morbidity and mortality, particularly in older people. Many genetic polymorphisms are considered as a risk factor of Hypercoagulability in DVT. MTHFR C677T polymorphism is reported to be associated with hyperhomocysteinemia through its inability to convert homocystein to methionnine which lead to hypercoagulable state that may promote DVT. The aim of this study was to examine the association of MTHFR (C677T) polymorphism with the risk of DVT in Sudan. The study included 50 DVT patients, their MTHFR C677T genotype frequencies (detected by PCR/RFLP) and haematological characteristics (measured by Sysmex KX-21N) were determined and compared with 50 age and sex matched normal subjects as control. Low frequency of mutant MTHFR C677T genotype with 0% TT (homozygote) & 12% CT (heterozygote) was observed. Our study showed a statistically insignificant association between MTHFR C677T polymorphisms and the risk of DVT with 1.5 fold increased risk. In conclusion, our results indicated low frequency of MTHFR C677T mutant genotypes with low impact in the risk of DVT among the study group.

Keywords: Deep vein thrombosis; MTHFR C677T; Sudan.

1. Introduction
Deep vein thrombosis or deep venous thrombosis (DVT) is the formation of a blood clot (thrombus) in a deep vein, predominantly in the legs[1]. It is a disease of aging, with a low rate of about 1 per 10,000 annually before the fourth decade of life, rising rapidly after age 45 years, and approaching 5–6 per 1000 annually by age 80[2]. DVT is the first and more common clinical form of venous thromboembolism (VTE). VTE is potentially lethal clinical form that carries high mortality risk[3]. The processes that trigger venous thrombosis are not obvious. However, it is clear that the mechanisms initiating the venous thrombosis are very different from those initiating the arterial thrombosis[4]. Thrombus formation and propagation depend on the presence of abnormalities of blood flow, blood vessel wall, and blood clotting components, known collectively as Virchow triad[5]. Endothelial imbalance may possibly play an important role[6]. Vascular endothelial surface normally creates a non-thrombogenic structure. When endothelial injury occurs related to factors such as anoxia, mechanical stress, free radicals, cytokines, and thrombin, it may lead to platelet activation and coagulation[7].

Venous thrombosis (VT) is multifactorial, and its exact pathogenesis has not been fully elucidated. Most cases of venous thrombosis arise due to prolonged immobilization, major surgery, trauma, or cancer, but genetic or acquired hemostatic abnormalities, including elevated plasma homocysteine (Hcy) levels, have also been implicated[8]. Many genetic polymorphisms such as methylenetetrahydrofolate reductase (MTHFR), a clotting Factor V Leiden and prothrombin are considered as a risk factor of Hypercoagulaability in DVT[9].

The MTHFR gene is located on the short (p) arm of chromosome 1 at position 36.3; it spans approximately 2.2Kb and consists of 11 exons. This gene encoded for MTHFR enzyme[10]. The enzyme
plays a central role in folate metabolism by irreversibly converting 5,10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulating form of folate. 5-Methyltetrahydrofolate donates a methyl group to homocysteine to be converted to methionine[11].

Two common single nucleotide polymorphisms in MTHFR have been reported, a C→T transition at nucleotide 677 in exon 4 and an A→C transversion in exon 7 at position 1298. In C677T, the polymorphism occurs at nucleotide 677 (C- T) in the human MTHFR gene results in an alanine to a valine substitution at position 222 in the amino acid structure of the MTHFR protein. Individual with two copies of 677C (677CC) have the "normal" or "wildtype" genotype. 677TT individuals (homozygous) are said to have mild MTHFR deficiency. 677CT individuals (heterozygotes) are almost the same as normal individuals with normal enzymatic activity [12].

MTHFR C677T polymorphism is reported to be associated with hyperhomocysteinemia through its inability to convert homocysteine to methionine which lead to hypercoagulable state that may promote DVT[13]. The aim of this study was to examine the association of MTHFR (C677T) polymorphism with the risk of DVT in Sudan.

2. Materials and methods

Following informed consent, one hundred subjects were enrolled: fifty known DVT patients, whose attending Military hospital; and fifty age and sex matched apparently healthy subjects as controls. Subjects with trauma, known history of cardiopulmonary disease, autoimmune disease, malignancy and pregnant women were excluded from the study.

Two ml of EDTA anticoagulated blood was collected from each subject. Laboratory investigations were performed at the department of haematology, faculty of medical laboratory sciences, Alneilain University, Sudan. DNA was extracted by salting out method. MTHFR C677T fragment was amplified using the forward primer: 5’-TGAGAGGAGGACGTCTGCAGGGA-3’ and reverse primer: 5’-AGGAGCCGCTGTGACAGG3’. The amplification was carried out in thermo-cycler (Techne TC-412, UK) with initial denaturation step for 5 minutes at 94°C Followed by 40 Cycles consisting of 3 steps: Denaturation step at 94°C for 30 second, annealing step at 59°C for 1 minute and extension step at 72°C for 1 minute, with final Extension step at 72°C for 7 minutes. The PCR reactions was performed in a final volume of 20 μl containing (4 μl premixed ready to use 5x FIREPolmaster mix (Solis BioDyne, Russian), 11.0μl DNAase free DW, 3μl genomic DNA and 1.0 μl from each primer). The amplified fragment was digested with 10 U Hinf1endonuclease(New England Biolab, UK) over night and was visualized on agarose gel electrophoresis. Blood cell count was performed by automated cell counter (Sysmex KX-21N).

Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient’s data was performed using the t-test. Comparison of frequency distribution between groups was made by means of the X² test. All tests are two-sided and P-value less than 0.05 have been considered as statistically significant. Crude odds ratios (OR) were also calculated and given with 95% confidence intervals (CI).

3. Result

The study included 50 patients with DVT, their median age was 35 year, with minimum age of 14 and maximum of 80 years. All patients were tested for the blood cell counts and MTHFR C677T Polymorphism. 82%(n 41/50) has one attack of DVT, 10% (n 5/50) with 2 attacks, 6%(n 3/50) with 3 and 2%(n 1/50) with 4 times attacks of DVT. The results of blood count for DVT cases were as follows: Mean haemoglobin (Hb) level 11.3±1.5g/dL; mean packed cell volume (PCV) 33.8±4.1%; mean red blood cell (RBC) count 3.9±0.6×10¹²/L; mean total white cells (TWBC) count 7.0±3.1×10⁹/L; mean platelet count 322±299.6×10⁹/L. While for the control group: Mean Hb concentration 14.0±1.0g/dL; mean PCV 40.2±2.8%; mean RBC count 4.7±0.4×10¹²/L; mean TWBC count 6.0±1.6×10⁹/L; mean platelet count 261.6±71.7×10⁹/L.

Table.1 shows the distribution of MTHFR C677T genotype frequencies between DVT patients and control group. When the MTHFR 677CC-genotype was defined as the reference, the OR for the CT genotype was 1.568 (95%CI: 0.414-5.935, P=0.508).

| Genotype | DVT patients (n%) | Controls (n%) | OR       | 95%CI       | P value |
|----------|------------------|--------------|----------|-------------|---------|
| CC       | 88               | 92           | Referent |             |         |
| CT       | 12               | 8            | 1.568    | 0.414-5.935 | 0.508   |

Haematological values, including Hb level, PCV, RBC count, TWBC count and platelets count showed no significant differences between DVT patients with MTHFR C677T CC genotype and those with CT genotype (Table 2).
Table 2: Comparison of haematological characteristic between DVT patients with wild type and those with mutant types

| Parameter               | 677CC genotype | 677CT genotype | P value |
|-------------------------|----------------|----------------|---------|
| Hbmean±SD (g/dl)        | 11.3±1.5/g/dl  | 11.2±1.9/g/dl  | 0.902   |
| PCVmean±SD (%)          | 33.7±3.9%      | 34.5±5.5%      | 0.752   |
| RBCmean±SD x10^12/L     | 3.8±0.4 x10^12/L | 4.3±1.2 x10^12/L | 0.506 |
| TWBCmean±SD x10^9/L     | 6.7±2.8 x10^9/L | 9.1±4.0 x10^9/L | 0.317   |
| Plateletsmean±SD x10^9/L| 265.3±117.5 x10^9/L | 633.8±705.9 x10^9/L | 0.374 |

4. Discussion

Several studies identified MTHFR C677T polymorphism as an independent risk factor for DVT[14,15], whereas others failed to prove any association with thrombosis[16,17]. We examined the association between MTHFR C677T polymorphism and the risk of DVT. This study included 50 DVT patients, their MTHFR C677T genotype frequencies and haematological characteristics were determined and compared with 50 age and sex matched normal subjects as control.

The frequency of the C677T mutation differs among ethnic populations. The T allele frequency ranges from 0.06 to 0.59 and the frequency of the T/T genotype (homozygosity for the C677T mutation) ranges from 0.00 to 0.35 among ethnic populations[18]. Our study showed low frequency of mutant MTHFR C677T genotype with 0% TT genotype (homozygote) & 12% CT (heterozygote) our finding is similar with previous reports among different study population in Africa[19,20].

Our study showed a statistically insignificant association between MTHFR C677T polymorphisms and the risk of DVT with 1.5 fold increased risk. Low impact of MTHFR C677T polymorphism as a risk factor in DVT, in the study population, may be attributed to the low frequency of the mutant genotypes, in particular 677 TT, rather than the actual contribution in the pathogenesis of DVT. The present study included a relative small sample of patients. The assessment of homocysteine, protein C and S, and the other genetic risk factors for DVT has not been performed, representing another limitation of our study. Future studies, with large sample size are required to confirm our finding.

5. Conclusion

In conclusion, we examined the association of MTHFR C677T polymorphism and the risk of DVT. Our results indicated low frequency of MTHFR C677T mutant genotypes with low impact in the risk of DVT among the study group.

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