ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 05, pp.16252-16255, May, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR C677T) POLYMORPHISM IN SUDANESE PATIENTS WITH SICKLE CELL ANAEMIA

[1]Ghada M. Merghani, [2]Rashad M. O. Mahmoud, [2]Idris M. M. Hamid and [*,2]Mahdi H.A. Abdalla

[1]Department of Haematology, Faculty of Medical Laboratory Sciences, Alneelain University, Sudan
[2]Department of Haematology, Faculty of Medical Laboratory Sciences, Omdurman Ahlia University, Sudan

ARTICLE INFO

Article History:
Received 05th February, 2015
Received in revised form
23rd March, 2015
Accepted 08th April, 2015
Published online 31st May, 2015

Key words:

Sickle cell anaemia,
Vaso-occlusive crisis,
MTHFR C677T,
Sudan.

ABSTRACT

Sickle cell anemia (SCA) is one of the major types of anemia found in Sudan. Numerous studies provide Hypercoagulable state in SCA. Many genetic polymorphisms are considered as a risk factor for Hypercoagulability in sickle cell anemia. The aim of this study was to examine the association of MTHFR (C677T) polymorphism with the risk of vaso-occlusive crisis (VOC) among sickle cell (SC) patients in Sudan. The study included 34 SC patients with VOC, their fibrinogen level (measured by Clauss modified method), D-Dimer (measured by i-CHROMA^{TM} system), MTHFR C677T genotype frequencies (detected by PCR/RFLP) and haematological charecteristics (Sysmex KX-21N) were determined and compared with 62 SC patients in steady state, without history of previous VOC, as control. Fibrinogen and D-Dimer levels were elevated in all SC patients either in steady state or with VOC, the levels were significantly higher among SC patients with VOC than those in steady state. Our study showed low frequency of mutant MTHFR C677T genotype with 1% TT genotype (homozygote) & 12% CT (heterozygote) with no significant association of MTHFR C677T polymorphisms with the risk of VOC in SCA. In conclusion, our data suggest a low impact of MTHFR C677T polymorphism as risk factors in the pathogenesis of VOC among the study group.

Citation: Ghada M. Merghani, Rashad M. O. Mahmoud,Idris M. M. Hamid. And Mahdi H.A. Abdalla, 2015. "Methylenetetrahydrofolate reductase (MTHFR c677t) polymorphism in Sudanese patients with sickle cell Anaemia", *International Journal of Current Research*, 7, (5), 16252-16255

INTRODUCTION

Sickle cell anemia (SCA) is one of the major types of anemia found in Sudan, especially in western Sudan in which the sickle cell gene is frequent (Osman *et al*., 2010).It is a hereditary blood disorder, characterized by red blood cells that assume an abnormal, rigid, sickle shape. Sickling decreases the cells' flexibility and results in a risk of various life-threatening complications (Platt *et al*., 1994). This property is due to a single nucleotide change in the β-globin gene leading to substitution of valine for glutamic acid at position 6 of the β-globin chain (β6 glu→val). The homozygosity of sickle cell genes (HbSS) results in SCA, while the heterozygosity results in other sickle cell diseases (SCD) which include sickle cell trait with one sickle cell gene and a normal haemoglobin gene (HbAS), and a double heterozygosity of a sickle cell gene with other abnormal haemoglobin variants gene (e.g HbSC) (Pauling *et al*., 1949; Setty *et al*., 2001).

Corresponding author: Mahdi H.A. Abdalla,
Department of Haematology, Faculty of Medical Laboratory Sciences, Omdurman Ahlia University, Sudan.

Pathophysiological studies have shown that the dense, dehydrated red cells play a central role in acute and chronic clinical manifestations of SCA, in which intravascular sickling in capillaries and small vessels leads to vaso-occlusion and impaired blood flow(Solovey *et al*., 2001; Steinberg, 1999). Numerous studies provide Hypercoagulable state in SCD that has been documented by various abnormalities of cytokines, coagulation markers, and increased phosphatidylserine exposure (Ataga& Key, 2007). Genetic heterogeneity is associated not only with the degree of anemia, but also with many other clinical complications in SCA including pain crisis, prevalence of stroke, leg ulcers, pulmonary hypertension, osteonecrosisand hepatobiliary complications (Steinberg &Adewoye, 2006; Steinberg, 2005; Steinberg, 2009). Many genetic polymorphisms such as methylenetetrahydrofolatereductase (MTHFR), a clotting Factor V Leiden and prothrombin 20210A are considered as a risk factor for Hypercoagulability in sickle cell anemia (Bansal *et al*., 2011). MTHFR, anenzyme encoded by the MTHFR gene which is located on the short (p) arm of chromosome 1 at position 36.3 (Goyette *et al*., 1994), catalyzes the conversion of

5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteineremethylation. Genetic variation in this gene may influence susceptibility to occlusive vascular disease (Födinger *et al*., 2000). The MTHFR nucleotide at position 677 in the gene has two possibilities: C (cytosine) or T (thymine). C at position 677 (leading to an alanine at amino acid 222) is the normal allele. The 677T allele (leading to a valine substitution at amino acid 222) encodes an enzyme with reduced activity. 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 (Schneider *et al*., 1998). It is known that patients with SCA present activation of the blood coagulation and fibrinolytic systems, especially during vaso-occlusive crises (VOC); the elevated level of homocysteine increases the risk of Hypercoagulability in sickle cell (SC) patients. It is possible that raised homocysteine levels in SC Anemia predispose to the development of thrombosis through inhibition of protein C anticoagulant pathway (Pandey *et al*., 2012). Several studies have found a relationship between the MTHFR geneand the vascular complications in sickle cell anemia. However these studies also showed differences in the occurrences and frequencies of this relationship. The aim of this study was to examine the association of MTHFR (C677T) polymorphism with the risk of VOC among SC patients in Sudan.

MATERIALS AND METHODS

This is a prospective cross-sectional study included 96 patients with SCA either in steady state(defined as a ≥ 4 weeks from an acute illness and ≥10 weeks post-transfusion) without history of previous VOC or with VOC who have attendedSickle Cell Anaemia Center, Elobidcity, Sudan. Five ml of venous blood was collected from each subject: 2.5 ml in 3.8% trisodium citrate (9:1 vol/vol), kept on ice until centrifugation at 2500g for 30 minutes at 4°C, plasma samples were immediately frozen and stored at - 80°C for subsequent coagulation analysis; and 2.5 ml in EDTA for the blood count and Molecular study. Laboratory analysis was performed at the Department of Haematology, Faculty of Medical Laboratory Sciences, Alneelain University.

Blood cell count was performed by automated cell counter (Sysmex KX-21N). Fibrinogen level was measured by Clauss modified method using a test kit (TECHNOCLONE GMBH, AUSTRIA). The method uses a functional assay based upon the time for fibrin clot formation, in brief, Diluted plasma is clotted with a high concentration of thrombin, and the concentration of fibrinogen is determined by comparing the plasma clotting time to a calibration curve of a reference plasma with a series of dilutions (1:5 –1:40). D- Dimer was measured using i-CHROMA^{TM}system (Boditech–Korea). The test uses the sandwich immunodetection method. D-Dimer is bound with an antibody in buffer and the antigen-antibody complexes are captured by antibodies that have been immobilized on the test strip as sample mixture migrates through nitrocellulose matrix. Signal intensity of fluorescence on detection antibody reflects the amount of the antigen captured and is processed by iCHROMATM Reader to show D-Dimer concentration in the specimen. The working range of i-CHROMA^{TM} D-Dimer test is 50 – 10,000 ng/ml. DNA was extracted by Salting out method. MTHFR C677T fragmentwas amplified using the forward primer: 5`-TGAAGGAGAAGGTGTCTGCGGGA-3`and reverse primer: 5`-AGGACGGTGCGGTGAGAGTG-3`. The amplification was carriedout in thermo-cycler (Techne TC-412, UK) with initial denaturation step for 5 minute at 94 °CFollowed by 40 Cycles consisting of 3 steps: Denaturing step at 94°C for 30 minute, Annealing step at 59°CFor 1 minute and extensionsteps at72°C for 1 minute with final Extensionstep at 72 °Cfor 7 Minutes.The PCR reactions was performed in a final volume of 20 µl containing (4 µl premixed ready to use 5XFIREPol master mix (Solis BioDyne,Russian),11.0µlDNAase free DW, 3µl genomic DNA and 1.0µl from each primer).The amplified fragment was digestedwith 10 U Hinf1endonuclease(New England Biolab, UK)over night and was visualized on agarose gel electrophoresis. 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 X^2 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).

RESULTS

Patients included 36 males and 60 females; their median age was 5 years, with minimum age of 1 year and maximum of 33 years. All patients were tested for the blood cell count, fibrinogen level, D-Dimer leveland MTHFR polymorphism. The results of the blood count for SCA cases were as follows: Mean haemoglobin (Hb) level 6.3±1.1g/dL; mean red blood cell (RBC) count 2.4±0.46 X10^{12}/L; mean packed cell volume (PCV) 20.4±3.4%; mean total white cells (TWBC) count 19.7±9.5X10^9/L and mean platelets count 390.8±164.2X10^9/L; mean D-Dimer level 2254.4±24054ng/ml; mean fibrinogen level 460.2±101.0.

Table 1. Comparison of haematological characteristic between VOC and Steady State patients

Parameter	VOC	Steady state	P value
TWBC mean±SD (X109/L)	18.5±8.6	20.2±10.0	0.381
RBC mean±SD(X1012/L)	2.4±0.42	2.4±0.5	0.667
Hbmean±SD (g/dl)	6.7±1.1	6.4±1.2	0.246
PCV mean±SD (%)	20.9±3.3%	20.3±3.7%	0.333
Platelets mean±SD (X109/L)	424.2±148	363±167.1	0.068
D-Dimer mean±SD(ng/ml)	3930.9±3260.8	1335.1±912.7	0.000
Fibrinogen level	516.8±92.1	429.1±92.7	0.000

Table 2. Comparison of MTHFR C677T Polymorphism frequencies in VOC and Steady state Patients

Genotype	VOC n (%)	Steadystate n (%)	OR	95%CI	P value
CC	29 (85.3)	54 (87.0)	Referent		
CT	5 (14.7)	7(11.2)	1.400	0.388-4.565	0.566
TT	0	1(1.6)	-	-	-

Table 2 shows the distribution of MTHFR C677T genotype frequencies between VOC patients and Steady state patients.

When the MTHFR 677CCgenotype was defined as the reference, the OR for the CT genotype was 1.400(95% CI: 0.388- 4.565, P = 0.566).

DISCUSSION

It is known that patients with SCA present activation of the blood coagulation and fibrinolytic systems, especially during VOC. Our study included 34 SCA patients with VOC, their fibrinogen level, D-Dimer, MTHFR C677T genotype frequencies and haematological charecteristics were determined and compared with 62 SCA patients in steady state as control. We utilized a quantitative measurement for the determination of D- Fibrinogen and D-Dimer levels as markers for hypercoagulability. Fibrinogen and D-Dimer levels were elevated in all SCA patients either in steady state or with VOC,Numerous studies provide laboratory evidence of a hypercoagulable state in patients with SCD (Ataga *et al*., 2007; Stuart and Setty, 2001). The levels were significantly higher among SCA patients with VOC than those in steady state,this finding confirm a hypercoagulable state among SC patients in the steady state with further escalation during VOC. The elevation of homocysteine increases the risk of Hypercoagulability.

MTHFR enzyme catalyzes the conversion of homocysteine to methionine. With reduced activity of mutant MTHFR protein, hyperhomocyteinaemia may contribute a hypercoagulability in SCA. We examined the association of MTHFR C677T polymorphism with the risk of VOC. Our study showed low frequency of mutant MTHFR C677T genotype with 1% TT genotype (homozygote) and 12% CT (heterozygote).Worldwide prevalence of MTHFR C677T polymorphism shows extensive geographic variation, with lowest prevalence in Afarican Blacks (Franco *et al*., 1998; Annichino-Bizzacchi and Costa, 1998). Our study showed a statistically insignificant association between MTHFR C677T polymorphisms and the risk of VOC in SCA with 1.4 fold increased risk. Similar finding was reported in previous studies in different study populations (Al-Saqladi*et al*., 2010; Kutlar *et al*., 2001; Cumming *et al*., 1999). Low impact of MTHFR C677T polymorphism as a risk factor in VOC, 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 SCA.

Conclusion

In conclusion, we examined the association of MTHFR C677T polymorphism and the risk of VOC among Sudanese patients with sickle cell anaemia. Our data suggest a low impact of MTHFR C677T polymorphism asrisk factors in VOC among the study group.

Acknowledgements

Special thanks to the Staff of Haematology Department, Faculty of Medical Laboratory Sciences, Alneelain University and the staff of Sickle Cell Anaemia Center in ElobidCity, Sudan.

REFERENCES

Al-Saqladi, A.M., Harper, G., Delpisheh, A., Fijnvandraat, K., Bin-Gadeem, H.A. and Brabin, B.J. 2010. Frequency of the MTHFR C677T Polymorphism in Yemeni Children with Sickle Cell Disease. *Bernard J. Brabin,* 2,3,5–0. Hemoglobin. 34:67–77

Annichino-Bizzacchi, J.M. and Costa, F.F. 1998. Prevalence of the mutation C677T in the methylenetetrahydrofolateReductase gene among distinct ethnic groups in Brazil. *Am J Med Genet.,*78 :332- 335.

Ataga, K .I. and Key, N.S. 2007. Hypercoagulability in sickle cell disease: new approaches to an old problem. *Am. Soc. Hematol.Educ. Program.,* 91-96.

Ataga, K.I., Cappellini, M.D. and Rachmilewitz, EA. 2007. Beta-thalassaemia and sickle cell anaemia as paradigms of hypercoagulability. *Br J Haematol.,* 139:3–13

Bansal, P. 2014. Factor V Leiden and MTHER mutations as a combind risk factor for hypercoagubility in referred patients population from western India. *Molecular cytogenetics,* 7(Suppl 1): P28.

Cumming, A.M., Olujohungbe, A., Keeney, S., Singh, H., Hay, C.R.M. and Serjeant, G.R. 1999. The methylenetetrahydrofolatereductase gene C677T polymorphism in patients with homozygous sickle cell disease and stroke. *British Journal of Haematology*, 107:569-571

Födinger, M., Hörl, W.H. and Sunder-Plassmann, G. 2000. Molecular biology of 5,10-methylenetetrahydrofolate reductase. *J Nephrol.,* 13: 20–33.

Franco, R.F, Araujo, A.G., Guerreiro J.F., Elian, J. and Zago, M.A. 1998. Analysis of the 677 C→T mutation of the methylenetetrahydrofolateReductase gene in different ethnic groups. *ThrombHaemost.,* 79:119-121.

Goyette, P., Sumner, J.S., Milos, R., Duncan, A.M., Rosenblatt D.S., Matthews R.G. and Rozen R. 1994. Human methylenetetrahydrofolatereductase: isolation of cDNA, mapping and mutation identification. *Nat. Genet.,*7 : 195–200.

Pandey, S., Pandey, H.R., Mishra, R.M., Pandey, S.W. and Saxena, R. 2012. Increased Homocysteine Level in Indian Sickle Cell Anemia Patients.*Indian J ClinBiochem.,* 27: 103–104

Kutlar, A., Kutlar, F., TurkerI, and Tural, C. 2001. The methyletetrahydrofolatereductase (C677T) mutation as a potential risk factor for a vascular nicrosis in sickle cell disease.*USA Hemoglobin,* 25: 213-217

Osman, N.O.M., Alfadni, M.H.I. and Ibrahim, M.H. 2010. The Prevalence of Sickle Cell Anemia in Northern Areas of AlgadarefState,Sudan in *Sudanese Journal of Public Health,* 5: 22-24.

Pandey, S., Pandey, H.R., Mishra, R.M., Pandey, S.W. and Saxena, R. Pauling L, Itano HA, Singer SJ, Wells IC. 1994. Sickle cell anemia. *a molecular disease Science*, 110:543-549.year missing

Platt, O.S., Brambilla, D.J., Rosse W.F, *et al.* 1994. Mortality in sickle cell disease.Life expectancy and risk factors for early death.*N. Engl. J. Med.,* 330: 1639–44.

Schneider, J.A., Rees, D.C., Liu, Y.T. and Clegg, J.B. 1998. Worldwide distribution of a common

methylenetetrahydrofolatereductase mutation. *Am. J. Hum. Genet.,* 62: 1258–60.

Setty, B.N., Rao, A.K. and Stuart, M.J. 2001. Thrombophilia in sickle cell disease: the red cell connection. *Blood,* 98: 3228-3233.

Solovey, A.A., Solovey, A.N., Harkness, J. and Hebbel, R.P. 2001. Modulation of Endothelial Cell Activation in Sickle Cell Disease: A Pilot Study. *Blood*, 97: 1937-1941.

Steinberg, M.H. and Adewoye, A.H. 2006. Modifier genes and sickle cell anemia. *Curr.Opin.Hematol.,*13: 131-136.

Steinberg, M.H. 2009. Genetic etiologies for phenotypic diversity in sickle cell anemia. *Scientific World Journal,* 9: 46-67.

Steinberg, M.H. 1999. Management of Sickle Cell Disease.*N. Engl. J. Med.,*340: 1021–1030.

Steinberg, M.H. 2005. Predicting clinical severity in sickle cell anaemia. *Br. J. Haematol.,* 129: 465-481.

Stuart, M.J. and Setty, B.N. 2001. Hemostatic alterations in sickle cell disease: relationships to disease pathophysiology. *PediatrPatholMol Med.,* 20:27–46.

****