ZKTB ORIGINAL RESEARCH

Evaluation of Thrombophilic Gene Mutation and Hyperhomocysteinemia in Children with Ischemic Stroke

İskemik İnmeli Çocuklarda Trombofilik Gen Mutasyonu ve Hiperhomosisteinemi'nin Araştırılması

Aslı KIBRIS [1], Eda SÜNNETÇİ [1], Betül Biner ORHANER [2]

1. Clinics of Paediatrics, İstanbul Education and Research Hospital, İstanbul, Turkiye
2. Sect. of Pediatric Haematology and Oncology, Dept. of Paediatrics, Yüksek İhtisas Educ. and Res. Hosp., Unv. of Med. Sciences, Bursa, Turkiye

ABSTRACT

Objective: *Although a variety of potential inherited and acquired aetiologies have been defined as a risk factor for ischemic stroke (IS) in paediatric patients, we aimed to revisit the influence of prothrombin G20210A (PT), methylenetetrahydrofolate reductase C677T (MTHFR-C677T) and hyperhomocysteinemia on the initial stroke episode.*

Material and Methods: *This retrospective cross-sectional survey was conducted between 2003-2004. Paediatric patients who had been admitted and/or followed up with the diagnosis of IS constituted the patient group (Group I). Nineteen children who were followed up in the healthy children policlinics were elected for control group (Group II). Thrombophilic gene mutation analysis was performed through enzymatic polymerase chain reaction. The homocysteine level was quantified through a chemical immunoassay method.*

Results: *There was no significant difference between the groups in terms of age [10 (1-18), p=0.98], gender (p=1.0), and ethnicity (p=0.27). The family history of IS that suggested hereditary thrombophilia was significantly higher in Group I (p<0.001). Additionally, it showed a 2,38 times greater risk of ischemic stroke. The rate of neither PT (p=1.0) nor MTHFR-C677T (p=0.19) were considerably higher in group I. While homocysteine level was higher in group I (12,6 versus 7.5 µmol/L, p=0.014), the rate of hyperhomocysteinemia was near-significant (p=0.09). In multi-variate analysis, none of the variables revealed a significant impact on the İS.*

Conclusion: *Limited number of patient count was the major limitation of the current study. The co-existence of clinical and genetic factors seems to be more determinant than that of a genetic mutation per se.*

Keywords: *methylenetetrahydrofolate reductase, prothrombin G20210A thrombophilia, hyperhomocysteinemia, cerebral stroke, hereditary thrombophilia*

ÖZET

Amaç: *Pediatrik hastalarda iskemik inme (İİ) için birçok kalıtımsal ve kazanılmış sebepler potansiyel risk faktörü olarak tanımlanmış olsa da biz, protrombin G20210A (PT) ve metilentetrahidrofolat redüktaz C677T (MTHFR-C677T) ve hiperhomosisteinemi'nin ilk İİ atağına etkisini tekrar değerlendirmeyi amaçladık.*

Gereç ve Yöntemler: *Bu geriye-dönük kesitsel araştırma 2003-2004 yılları arasında gerçekleştirildi. İİ tanısı ile başvuran veya takip altında olan pediatrik hastalar çalışma topluluğunu (Grup I) oluşturmaktadır. Sağlıklı Çocuk Polikliniği takibi altında olan 19 çocuk kontrol grubu (Grup II) için seçildi. Trombofilik gen mutasyon analizi enzimatik polimeraz zincir reaksiyonu ile gerçekleştirildi. Homosistein düzeyi kimyasal immünoesey metodu ile ölçüldü.*

Bulgular: *Gruplar arasında yaş [10 (1-18), p= 0.98], cinsiyet (p=1.0) ve etnik köken (p=0.27) olarak anlamlı bir fark yoktu. Herediter trombofili'yi işaret eden İİ için aile öyküsü grup I'de anlamlı olarak daha yüksek (p<0.001) olmasına ek olarak İİ için 2,38 kat risk artışını göstermekteydi. Ne PT (p=1.0) ne de MTHFR-C677T oranı grup I'de anlamlı olarak daha yüksekti. Homosistein düzeyi grup I'de daha yüksek iken (12,6 ila 7.5 µmol/L, p=0.014), hiperhomosisteinemi oranı yakın-anlamlı (p=0.009) idi. Çok-değişkenli analizde hiçbir değişken İİ üzerine belirgin etki göstermedi.*

Sonuç: *Sınırlı hasta sayısı mevcut araştırmanın başlıca kısıtlılığıydı. Klinik ve genetik faktörlerin eş-zamanlı birlikte bulunmaları, tek başına genetik mutasyon varlığından daha belirleyici görünmektedir.*

Anahtar Kelimeler: *metilentetrahidrofolat redüktaz, protrombin G20210A trombofili, hiperhomosisteinemi, serebral stroke, herediter trombofili*

INTRODUCTION

Stroke is defined as a clinical condition characterized by a rapidly developing focal or global cerebral dysfunction that lasts more than 24 hours which may be lethal without any reason, except vascular pathologies (1). It is an important cause of severe morbidity and mortality affecting 4,3 – 13 cases per 100,000 children per annum (2, 3). With an almost 20% risk of recurrent episode, and a 10% risk of mortality despite appropriate treatment, 65% of the patients with an initial stroke suffer life-long complications including neurologic deficit and seizure (4).

Although a variety of potential inherited and acquired aetiologies have been defined (2, 4-7) as a risk factor for ischemic stroke in paediatric patients, the leading cause of it in the young is unknown in more than one third of patients (6). Based on the role of thrombophilia and hyperhomocysteinemia in paediatric ischemic stroke, which were poorly characterised, we aimed to revisit the influence of prothrombin G20210A, methylenetetrahydrofolate reductase C677T and hyperhomocysteinemia on the initial paediatric ischemic stroke.

MATERIAL AND METHOD

Design

This retrospective cross-sectional survey was conducted in the Departments of Paediatric Neurology and Haematology, CENTRE between

Contact:
Corresponding Author: Aslı KIBRIS, MD.
Adress: Clinics of Paediatrics, İstanbul Education and Research Hospital, Kasap İlyas Dst., Org. A. N. Gürman St., 34098, Fatih, İstanbul, Turkiye
e-Mail: aslikibris@gmail.com
Phone: +90 (212) 459 6000
Submitted: 13.04.2019
Accepted: 22.07.2019
DOI: http://dx.doi.org/10.16948/zktipb.553407

2003 and 2004. The ethical approval was obtained from Ethical Committee of Non-invasive Clinical Research at Trakya University Faculty of Medicine on 16 December 2004 (Protocol Nr: TUTFEK-2004/163). Paediatric patients who had been admitted and/or followed up with the diagnosis of ischemic stroke constituted the patient group (Group I). Along with the patients who have had a history of cardiac surgery, the patients in whom the ischemic stroke episode developed in the neonatal period were excluded from the study. Additionally, neonates born with neonatal asphyxia or spastic palsy were excluded. The cerebral arterial infarct area was confirmed by means of a computed tomography and/or a magnetic resonance imaging in all patients in group I. On the other side, 19 children who were followed up in the healthy children policlinics were elected for group II. The election of group II was mainly based on a one-to-one correspondence fashion. The informed consent was taken either from the parents or the legal guardian. The predesignated demographic and clinical data were obtained from the medical achieve. The patients in both groups were compared in term of demographics, ethnicity, family history of ischemic stroke attributable to predisposition to thrombosis, PT G20210A mutation, MTHFR C677T mutation, and homocysteine level.

Blood sampling and analysis method

After venous blood sampling through an antecubital superficial vein, the serum was separated by a centrifuge. The obtained serum was collected in a 2 ml of Eppendorf tubes under -80 °C. The homocysteine level was quantified through a chemical immunoassay method using a homocysteine kit Immulite One (BIO-DPC, USA). For Immulite One kit, the designated reference value of homocysteine in adults was given as 5-15 µmol/L, and levels above 15 µmol/L was considered as hyperhomocysteinemia. In contrary, because these reference values are designated for adults, the reference values in the current study was based on the study of Akar et al.(8) in which hyperhomocysteinemia as for the age of 1 – 6 years, 7 – 11 years and 12 – 17 years were determined as 3,87±1,44 µmol/L, 8,70±1,40 µmol/L and 13,54±1,49 µmol/L, respectively. Based upon these data, the threshold level of homocysteine in the current study for patients who were 1 – 6 years, 7 – 11 years and 12 – 17 years were determined as 6,75 µmol/L, 11,5 µmol/L, and 16,52 µmol/L, respectively. The obtained venous blood was collected in tubes with acid citrate under +4 °C. These bloods were analysed for thrombophilic gene mutation through enzymatic polymerase chain reaction; using the Pronto Diagnostics 9909-01M Prothrombin 20210 kit and Pronto Diagnostics 9910-01M MTHFR C677T kit.

STATISTICAL ANALYSIS

The statistical analysis was performed using a licensed Microsoft Excel 2018, version 16.20. The continuous variables were presented as frequency and percent. Because none of the variables revealed normal distribution, the continuous variables were compared using Mann-Whitney U test. For categorical variables, the comparisons were made through Pearson's chi-square test or Fischer's exact test with continuity correction. A forward logistic regression analysis was performed to evaluate the risk factors associated with ischemic stroke. A p value of less than 0.05 was considered significant.

RESULTS

The demographic and clinical characteristics of the patients were presented in Table 1.

Table 1: The demographic and clinical characteristics of the patients.

Characteristics	Overall (n=38)	Group I (n=19)	Group II (n=19)	p value Group I vs. II
Age, years	10 (1 – 18)	10 (1 – 18)	10 (1 – 18)	0.98[a]
Male	18 (47,4)	9 (47,4)	9 (47,4)	1.0[b]
Ethnicity				0.27[c]
Thracian	28 (73,7)	12 (63,2)	16 (84,2)	
Balkans	7 (18,4)	7 (36,8)	–	
Anatolian	3 (7,9)	–	3 (15,8)	
Family history	11 (28,9)	11 (57,9)	–	<0.001[c]
PT-G20210A	1 (2,6)	1 (5,3)	–	1.0[c]
MTHFR C677T	22 (57,9)	9 (47,4)	13 (68,4)	0.19[b]
Homozygous	3 (7,9)	2 (10,5)	1 (5,3)	1.0[c]
Heterozygous	19 (50)	7 (36,8)	12 (63,2)	0.11[b]
Homocysteine, µmol/L	10,3 (2 – 50)	12,6 (2 – 50)	7,5 (4,3 – 24,2)	0.014[a]
Hyperhomocysteinemia	14 (36,8)	10 (52,6)	4 (21,1)	0.04[c]
TGM + Hyperhomocysteinemia	14 (36.8)	10 (52.6)	4 (21.1)	0.04[c]

Abbreviations: *MTHFR: methylenetetrahydrofolate reductase, PT: prothrombin. a Mann-Whitney U test, b Pearson's chi-square test, c Fischer's exact test with continuity correction.*

The median age of the cohort was 10 years (1 – 18 years). No significant difference was detected between the groups in terms of age (p = 0.98). The age of the patients in group I at the time of initial stroke episode was one year (6 months – 11 years). In overall, 18 (47.4%) patients were male, and 20 (52,6%) patients were female. There was no difference between the groups in regard to the distribution of gender (p = 1.0). Most of the patients (73,3%) were Thracian. When the patients whose origin were Balkan and Anatolian and migrated to Thrace (n=7) were united and compared with the native Thracian patients, there was no significant difference between the groups in regard to ethnicity (p = 0.27). Eleven (28,9%) patients had a family history of stroke of which all were within group-I. In comparison to group II, the rate of patients with a family history of ischemic stroke was considerably higher in group-I [χ^2 = 15,48, p<0.001]. Additionally, in univariate analysis, patients with a family history of thrombosis that suggested hereditary thrombophilia showed a 2,38 times greater risk of ischemic stroke (Odds ratio 2,38, 95% confidence interval 1,4 – 4,0).

Overall, there was one (2,6%) patient with PT-G20210A mutation, who was in group I. While 22 (57,9%) patients had MTHFR mutation in overall, two (10,5%) patients in group I and one (5,3%) patient in group II was homozygous for MTHFR mutation. Neither PT-G20210A (p = 1.0) nor MTHFR mutation (p = 0.19) revealed significant difference between the groups. While the median serum homocysteine level was 10,3 μmol/L (2 – 50 μmol/L), the homocysteine level in group I was significantly higher than that of group-II [12,6 μmol/L (2 – 50 μmol/L) versus 7,5 μmol/L (4,3 – 24,2 μmol/L); z= -2,45, p = 0.014]. Additionally, the rate of the patients with hyperhomocysteinemia was higher in group-I, and the difference was significant (52,6% vs. 21,1%, p = 0.04). In further subgroup analysis between the age groups (Table 2), the homocysteine level was higher in group 1 but could not reach to a significant level.

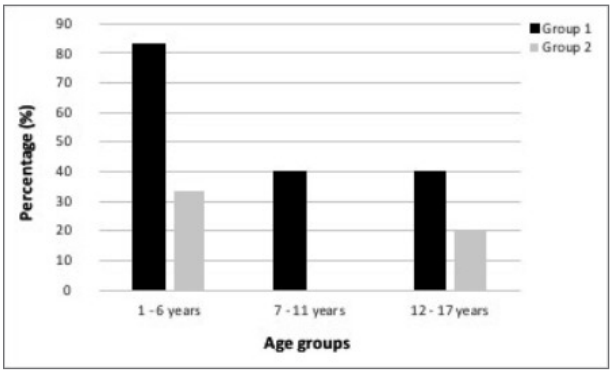
Table 2: The subgroup analysis of homocysteine levels (μmol/L) between the age groups.

Age groups	Group 1	Group 2	p value[a]
1 – 6 years (n = 12)	10,5 (2 – 50)	6,4 (5 – 23,9)	0.485
7 – 11 years (n = 10)	10,3 (4,4 – 19,2)	8,3 (4,5 – 11)	0.421
12 – 17 years (n = 10)	13 (12,3 – 31,8)	7,5 (4,3 – 24,2)	0.056

a *Mann-Whitney U test.*

As revealed in Figure 1, the distribution of hyperhomocysteinemia did not reveal significant difference between the groups in patients who were 1 – 6 years old (83,3% vs. 33,3%, p = 0.242), 7 – 11 years old (40% vs. 0%, p= 0.429) and 12 – 17 years old (40% vs. 20%, p = 1.0). The median homocysteine level between the groups was no significantly different [Group I: 9,45 (2,0 – 31,8) vs Group-II= 11,1 (4,3 – 50); p= 0.584)]. The rate of hyperhomocysteinemia in patients with and without MTHFR mutation (31,8% versus 43,8%) did not reveal a significant difference (χ^2 = 0,58, p= 0.452).

Figure 1: The distribution of hyperhomocysteinemia between the age groups.



In subgroup analysis based on ethnicity (Table 3), none of the TGMs distribution between the ethnicities revealed significant difference. Additionally, hyperhomocysteinemia was not considerably differed (p= 0.89) between the ethnicities. In logistic regression analysis (Table 4), although being Thracian was almost approaching to a level of significance (Odds ratio 3,76, p= 0.052), none of the variables was found as a significant risk factor for ischemic stroke.

Tablo 3: Subgroup analysis results of the distribution of genetic mutation and hyperhomocysteinemia based on ethnicity.

Variable	Thrace (n=28)	Others* (n= 10)	p value
Family history	8 (28,6)	3 (30)	1.0[c]
PT G20210A	1 (3,6)	0 (0)	1.0[c]
MTHFR	16 (57,1)	6 (60)	1.0[c]
Homozygous	2 (7,1)	1 (10)	1.0[c]
Heterozygous	14 (50)	5(50)	1.0[c]
Hyperhomocysteinemia	11 (39,3)	3 (30)	0.89[c]

** Others implies the patients whose ethnicity is Balkans and Anatolian.*
c Fischer's exact test with continuity correction

Tablo 4: Multivariate logistic regression analysis for ischemic stroke.

Variable	Beta	Exp (B)	Odds ratio	p value
Thracian ethnicity	2,18	0,113	3,76	0.052
Family history	21,95	<0,001	<0,001	0.999
PT-G20210A	1,66	0,189	<0,001	1.0
MTHFR C677T	0,84	2,306	0,62	0.433
Hyperhomocysteinemia	1,59	0,205	2,27	0.132
TGM + Hyperhomocysteinemia	1.48	0.24	3.86	**0.049**

Abbreviations: CI: confidence interval.

DISCUSSION

The prevalence of PT G20210A mutation differs in various regions of the world. While it is reported to be between 0 – 2,9% in the healthy population in the Northern Europe, the rate in Southern Europe is 0,7 – 8% (9). It is around 2,7% in Turkish population (10). Akar et al. (11) reported in their study among 32 paediatric patients with cerebral infarction that the rate of PT G20210A was 21,8% and suggested PT G20210A as an important risk factor for cerebral infarction with a hazard ratio of 8,2 in the paediatric age range. Barreirinho et al. (12) showed that the relative risk of ischemic stroke in patients with PT G20210A was 11,8%. In all these studies, the prevalence was shown to be higher in patients who experienced ischemic stroke (11-13). In contrary, in the prospective case-control study of Physician's Health Study involving 259 patients with stroke, no association was found between PT G20210A and stroke (14). Prothrombin G20210A may be more prevalent among paediatric stroke patients than in control subjects, but the data are conflicting and prospective studies are lacking (11-14). In contrary to the literature (11-13), the PT G20210A was not found to be a significant risk factor for ischemic stroke in the current study. On the other hand, it should be taken into account that PT G20210A was detected only one (2,6 %) patient. In our opinion, this expected result was in great part due to the limited number of patients in the study population which weakens the critics made herein. At this point, we believe that a significant level of difference would have been reached if the total number of patients in group 1 was higher.

The existence of MTHFR C677T mutation alone as a risk factor for thrombosis is under debate. Akar et al. (15) did not found a correlation between MTHFR C677T and cerebral infarction in children. In the study of Barreirinho et al. (12), the rate of thermolabile variant of the homozygous MTFHR variant was almost equal between the groups. Other than being a risk factor per se, the coexistence of it with Factor V Leiden or prothrombin G2021A mutation is reported to be an accelerator for the development of thrombosis (7, 16, 17). Furthermore, there is no prospective study that linked MTHFR C677T and stroke, either in paediatric or adult patients (7). In the current series, more than half of the study population (57,9%) had MTHFR C677T mutation in which neither homozygous nor heterozygous trace of the mutation was found to be considerably higher in group 1. Although this outcome supports the literature in terms of having an insignificant effect on ischemic stroke (16), again the insufficient patient count would have an influence on it. From this point of view, supporting the literature, the association of clinical risk factors seem to be more determinant than TGM alone (7, 12, 16).

The literature includes a conflicting evidence regarding a genetic predisposition to hyperhomocysteinemia and ischemic stroke, in which the homogenous form of MTHFR C677T was highlighted (7, 18-21). In their cohort of 64 pediatric patients with stroke, Konanki et al. (20), showed that 11 (17%) patients had hyperhomocysteinemia. Eltayeb et al. (19) supported that the homocysteine level was considerably higher in patients in whom ischemic stroke developed. In the current series, the homocysteine level was significantly higher in group I ($p= 0.09$), which finding supported the literature. From this point of view, this study highlights the importance of identification of the risk factors, including homocysteine level in order to determine the prognosis, the recurrent risk of ischemic stroke, and secondary prophylactic measures (19). Another point of view that should be considered was homocysteine level and the rate of hyperhomocysteinemia in patients with MTHFR mutation. Supporting the literature (7, 20), neither the homocysteine level nor the rate of hyperhomocysteinemia was significantly higher in patients with MTHFR mutation in the current series. On the other hand, Prengler et al. (21) showed the association of ischemic stroke and raised homocysteine levels with MTHFR homozygosity.

The prevalence of PT G20210A in Southern Europe including Serbia, Greece, and Turkey is 0,7 – 8% (9). Thrace region is located in the southern-east of Europe, and it is a region in the Balkans. The result that no significant difference was found between the groups, may reflect the nested ethnic structure of the region of Thrace. The region of Thrace is a cross-continental zone of transition, and when this nested ethnic structure was considered, it would be very difficult to find a significant association between the TGM rates that's because determination of the precise ethnic source in patients living in the Thrace would be so difficult, and furthermore needs to much scrutinising.

Being a retrospective survey and constitution of a small number of patients were the major limitation of the current study. In addition, thrombophilic gene analysis for Factor V Leiden mutation could not be performed due to the funding limitations which we view it as another major limitation. From this point of view, this cannot exclude these mutations as a potential variable. In conclusion, as well as a family history of thrombosis, the presence of different frequency of thrombophilia risk factors in different ethnic groups is essential for demonstra-

ting the presence of hereditary thrombophilia as a risk factor of ischemic stroke in childhood. Further evaluation seems to be beneficial for thrombophilic genetic mutation and hyperhomocysteinemia in not all but selected patients who have influential risk factors, such as family history for ischemic stroke. Additionally, the co-existence of clinical and genetic factors seems to be more determinant than that of a genetic mutation per se.

REFERENCES

1. Ciccone S, Cappella M, Borgna-Pignatti C. Ischemic stroke in infants and children: practical management in emergency. Stroke Res Treat. 2011;2011:736965.

2. Zahuranec DB, Brown DL, Lisabeth LD, Morgenstern LB. Is it time for a large, collaborative study of pediatric stroke? Stroke. 2005;36:1825-9.

3. Giroud M, Lemesle M, Gouyon JB, Nivelon JL, Milan C, Dumas R. Cerebrovascular disease in children under 16 years of age in the city of Dijon, France: a study of incidence and clinical features from 1985 to 1993. J Clin Epidemiol. 1995;48:1343-8.

4. Brankovic-Sreckovic V, Milic-Rasic V, Jovic N, Milic N, Todorovic S. The recurrence risk of ischemic stroke in childhood. Med Princ Pract. 2004;13:153-8.

5. Kenet G, Lutkhoff LK, Albisetti M, Bernard T, Bonduel M, Brandao L, et al. Impact of thrombophilia on risk of arterial ischemic stroke or cerebral sinovenous thrombosis in neonates and children: a systematic review and meta-analysis of observational studies. Circulation. 2010;121:1838-47.

6. Lippi G, Franchini M, Montagnana M, Salvagno GL, Targher G, Guidi GC. Inherited and acquired risk factors for arterial ischemic stroke in childhood. J Thromb Thrombolysis. 2009;27:239-48.

7. Van Cott EM, Laposata M, Prins MH. Laboratory evaluation of hypercoagulability with venous or arterial thrombosis. Arch Pathol Lab Med. 2002;126:1281-95.

8. Altuntas N, Soylu K, Suskan E, Akar N. Homocysteine levels in Turkish children. Turk J Haematol. 2004;21:79-82.

9. Jadaon MM. Epidemiology of Prothrombin G20210A Mutation in the Mediterranean Region. Mediterr J Hematol Infect Dis. 2011;3:e2011054.

10. Akar N, Misirlioglu M, Akar E, Avcu F, Yalcin A, Sozuoz A. Prothrombin gene 20210 G-A mutation in the Turkish population. Am J Hematol. 1998;58:249.

11. Akar N, Akar E, Deda G, Sipahi T, Ezer U. Coexistence of two prothrombotic mutations, factor V 1691 G-A and prothrombin gene 20210 G-A, and the risk of cerebral infarct in pediatric patients. Pediatr Hematol Oncol. 1999;16:565-6.

12. Barreirinho S, Ferro A, Santos M, Costa E, Pinto-Basto J, Sousa A, et al. Inherited and acquired risk factors and their combined effects in pediatric stroke. Pediatr Neurol. 2003;28:134-8

13. Akar N, Akar E, Deda G, Sipahi T, Orsal A. Factor V1691 G-A, prothrombin 20210 G-A, and methylenetetrahydrofolate reductase 677 C-T variants in Turkish children with cerebral infarct. J Child Neurol. 1999;14:749-51.

14. Ridker PM, Hennekens CH, Miletich JP. G20210A mutation in prothrombin gene and risk of myocardial infarction, stroke, and venous thrombosis in a large cohort of US men. Circulation. 1999;99:999-1004.

15. Akar N, Akar E. Methylenetetrahydrofolate-dehydrogenase 1958 G-A (R653 Q) polymorphism in Turkish patients with venous thromboembolism. Acta Haematol. 1999;102:199-200.

16. Şişli E, Oto O. The Influence of Thrombophilic Gene Mutation on Recurrence of Venous Thromboembolism: A Retrospective Cross-Sectional Study. Osmangazi Journal of Medicine. 2019;41:72-80.

17. Ozmen F, Ozmen MM, Ozalp N, Akar N. The prevalence of factor V (G1691A), MTHFR (C677T) and PT (G20210A) gene mutations in arterial thrombosis. Ulus Travma Acil Cerrahi Derg. 2009;15:113-9.

18. Komitopoulou A, Platokouki H, Kapsimali Z, Pergantou H, Adamtziki E, Aronis S. Mutations and polymorphisms in genes affecting hemostasis proteins and homocysteine metabolism in children with arterial ischemic stroke. Cerebrovasc Dis. 2006;22:13-20.

19. Eltayeb AA, Askar GA, Abu Faddan NH, Kamal TM. Prothrombotic risk factors and antithrombotic therapy in children with ischemic stroke. Ther Adv Neurol Disord. 2015;8:71-81

20. Konanki R, Gulati S, Saxena R, Gupta AK, Seith A, Kumar A, et al. Profile of prothrombotic factors in Indian children with ischemic stroke. J Clin Neurosci. 2014;21:1315-8.

21. Prengler M, Sturt N, Krywawych S, Surtees R, Liesner R, Kirkham F. Homozygous thermolabile variant of the methylenetetrahydrofolate reductase gene: a potential risk factor for hyperhomocysteinaemia, CVD, and stroke in childhood. Dev Med Child Neurol. 2001;43:220-5.