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Evaluation of Antithrombin-III Deficiency and Factor V Leiden Mutation in Patients with Stroke in Young Patients

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ABSTRACT

Objective: To evaluate Antithrombin III and Factor V Leiden mutation in stroke among young patients and its association with various socio-demographic aspects.
Study Design: Cross-sectional study.
Place and Duration of Study: Pak-Emirates Military Hospital, Rawalpindi Pakistan, from Jan to Dec 2020.
Methodology: A total of 103 individuals, aged 18-65 years, with acute ischemic stroke were included. The diagnosis was confirmed using computed tomography/Magnetic Resonance Imaging Brain scan. Plasma AT III activity and Factor V Leiden mutation were analyzed using standard techniques. Various socio demographic features like age, gender, body mass index, smoking, deployment at High altitude (>10,000 feet), family history of stroke and hyperlipidemia were recorded and statistically analyzed.
Results: Only two (1.9%) patients had AT III deficiency while one (0.9%) patient showed Factor V Leiden mutation. All patients were male. Working at high altitude, family history of stroke and tobacco smoking were related with the presence of low AT III levels (p-value <0.05).
Conclusion: Testing for hypercoagulable states in the setting of stroke in young may be useful if there is family history of stroke, smoking and high-altitude deployment. Investigations to search for the cause should be tailored as per individual patients.

Keywords: Antithrombin III, Comorbid, Factor V leiden, Ischemic stroke.

How to Cite This Article: *Khan H, Nawaz KH, Nizami MA, Mukhtar H, Sheikh FS, Anjum M. Evaluation of Antithrombin-III Deficiency and Factor V Leiden Mutation in Patients with Stroke in Young Patients. Pak Armed Forces Med J 2024; 74(3): 698-702. DOI: https://doi.org/10.51253/pafmj.v74i3. 6629*

INTRODUCTION

Stroke and its sequelae account for a considerable percentage of human morbidity and mortality across the world.[1] The situation is no different in Pakistan, where such patients constitute a major portion of inpatient admissions, thus draining the already overburdened health resources.[2] Various biochemical anomalies are said to be linked to the development of stroke include hyperlipidemia, hyperglycemia and altered homocysteine and vitamin B-12 levels etc.[3] Stroke in young is associated with high mortality and morbidity. It may constitute 10-15% of all stroke cases4. Despite this, majority of the studies have focused on elderly population with regard to genetic association of this disease. There is clearly a far more conspicuous role of genetic factors in early-onset stroke compared to late-onset.[5] The etiology of most of ischemic strokes in young age group, at hospital admission, remains unknown. Antithrombin III (AT III) deficiency and Factor V Leiden (FVL) mutation are amongst these important factors. The former is a proteases inhibitor which brings about the inhibition of thrombin activation. Its deficiency could be acquired secondary to nephrotic syndrome or colitis or may be as a result of an autosomal dominant deficiency.[6] Factor V forms a complex with factor X, responsible for significantly raising the production of thrombin. Thrombin also activates protein C, which degrades factor V to prevent the overproduction of the clot. With factor V Leiden mutation, the altered protein structure arrests its inactivation by activated protein C, causing a hypercoagulable state and potentially leading to stroke. The factor V Leiden mutation, prompted by replacement of arginine, with glutamine residue at position 506,[7] is one of the most commonly reported prothrombotic genetic element in stroke in young. The incidence varies across different ethnicities with a 5.2% in white population and a far less rate in other ethnic groups Asian, African etc.[8] Except for a few recent systemic reviews,[9] the association of factor V with

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Received: 23 Apr 2021, revision received: 14 Jun 2021; accepted: 07 Jul 2021

ischemic stroke in young adults is said to be ambivalent. Studies in Pakistan that relate inherited deficiencies and various socio demographic features to stroke in the young are even more limited. Recognition of biochemical and coagulation disturbances and other primary and secondary preventive aspects of stroke in young, can limit its debilitating sequelae. Our research aims to record the prevalence of Antithrombin III deficiency and heterozygous factor V Leiden mutation among the patients of stroke in young and analyze the socio-demographic elements linked with the presence of such abnormalities.

METHODOLOGY

The cross-sectional study was conducted at Department of Neurology, Pak-Emirates Military Hospital, Rawalpindi Pakistan, from January to December 2020 after approval by Institutional Ethical Committee (Certificate number A/28/EC/222/2020). The sample size was calculated by the WHO calculator by using population prevalence proportion of Factor V Leiden deficiency as 5.2%.[8] Non-probability purposive sampling was employed for patient selection.

Inclusion Criteria: All patients presenting with signs and symptoms of acute stroke and age ranging from 18 to 65 years were included.

Exclusion Criteria: Patients with a history of trauma, any chronic illness such as diabetes, chronic renal, liver, thyroid or cardiac diseases, malignant tumors, psoriasis, previous stroke, pregnancy/recent childbirth, were excluded.

A total of 103 individuals meeting the inclusion criteria were included in the study. The diagnosis of such patients was confirmed using CT/MRI Brain scan, within 24 hours of presentation. Five milliliters of venous blood was withdrawn. The plasma AT III activity was determined by a chromogenic assay (Spectrolyse AT III, Biopool). Thrombin was mixed to diluted plasma containing AT III in the presence of excess heparin. Following an initial incubation period, the residual thrombin was determined using a thrombin-specific chromogenic substrate. The residual thrombin activity is inversely proportional to the antithrombin III concentration. The functional percentage values of 80-120% for AT III levels were interpreted as normal range.[10]

Detection of heterozygous factor V Leiden mutation was done by employing polymerase chain reaction (PCR) following DNA extraction from peripheral blood lymphocytes. The genes being studied were amplified by PCR in thermocycler Proflex. The products of PCR were electrophoresed on 6% Polyacrylamide gel (PAGE), following which, visualization was done by utilizing silver nitrate staining.

The lipid profile and coagulation workup were done that included prothrombin time (PT) and activated partial thromboplastin time (APTT), protein C, protein S. Vasculitis workup including rheumatoid arthritis (RA) factor, anti-nuclear antibody (ANA), and Anti-neutrophil cytoplasmic antibodies (ANCA) was also done.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Various risk factors and socio demographic features of the patients were recorded using structured proforma. This included age, gender, body mass index, smoking, deployment at High altitude (>10,000 feet), Intake, family history of stroke and hyperlipidemia. Descriptive statistics were used to describe the risk factors and the frequency of AT III and factor V Leiden. Pearson chi-square test and Fischer exact test were done to evaluate factors.

RESULTS

Following application of the exclusion criteria, a total of 103 young patients with stroke were included in the final analysis. The age of the participants ranged from 22-43 years. The mean age was 35.44±5.59. Only two (1.9%) patients exhibited AT III deficiency, only one patient (0.9%) showed heterozygous factor V Leiden mutation. All these patients were male.

Table-I shows that low AT III levels were seen statistically significantly in patients working at high altitude of >10, 000 feet, positive family history of stroke and tobacco smoking (p-value<0.05). No such associations were observed in case of heterozygous Factor V Leiden mutation as seen in Table-II.

DISCUSSION

Stroke can emanate from a host of biochemical as well as coagulation anomalies in susceptible individuals. Our study was purposed at highlighting the significant contributory elements of thrombophilia with regard to stroke in young, in a military tertiary healthcare setup. The number of young male individuals with stroke, surpassed their female counterparts in our sample. This contrasted with previous local studies where female patients were recorded to have a higher incidence of stroke in young.[4,11] The findings however are consistent with a study by George *et al*. in 2020, where men between ages 45-49 years outnumbered

Table-I: Various Socio-Demographic Variables Observed in Stroke in Young Patients and their Association with the Presence of Low Antithrombin III Levels (n=103)

Socio-demographic Variables	Patients with Antithrombin III deficiency n(%) (n=2)	Patients with normal Antithrombin III n(%) (n=101)	*p*-value
Age			
<32 years	1(50%)	40(39.7%)	0.769
>32 years	1(50%)	61(60.3%)	
Gender			
Male	2(100%)	58(57.4%)	0.139
Female	Nil	43(42.6%)	
Body Mass index			
<25	1(50%)	76(75.2%)	0.448
>25	1(50%)	25(24.8%)	
Smoking			
No	Nil	86(85.1%)	0.006
Yes	2(100%)	15(14.9%)	
Deployment at High altitude (>10,000 feet)			
No	Nil	90 (89.1%)	0.003
Yes	2(100%)	11(10.9%)	
Family History of Stroke			
No	Nil	83(82.1%)	
Yes	2(100%)	18(17.9%)	0.01
Hyperlipidemia			
No	1(50%)	8 (82.1%)	
Yes	1(50%)	18(17.9%)	0.307

Table-II Socio-demographic factors relating to Heterozygous Factor V Leiden in Stroke in Young Patients (n=103)

Socio-demographic variables	Patients with Heterozygous Factor V Leiden Mutation n(%) (n=1)	Patients with no Factor V Leiden Mutation n(%) (n=102)	*p*-value
Age			
<32 years	1(100%)	40 (39.2%)	0.173
>32 years	Nil	62 (60.8%)	
Gender			
Male	1 (100%)	59 (57.8%)	0.297
Female	Nil	43 (42.2%)	
Body Mass index			
<25	1 (100%)	76 (74.5%)	0.444
>25	Nil	26 (25.5%)	
Smoking			
No	1 (100%)	85 (83.3%)	0.547
Yes	Nil	17 (16.3%)	
Deployment at High altitude (>10,000 feet)			
No	1 (100%)	89 (87.2%)	0.602
Yes	Nil	13 (12.8%)	

women.[12] The incidence of Factor V Leiden (FVL) mutation in stroke in young is highly variable when the earlier studies in the eastern and western parts of the world are compared. Its incidence ranges from 5-8% in European samples,[13] while in Asian studies,[14] it is found to be ranging from 1.2-4%. The lowest incidence is reported from studies conducted on East Asian, African, and indigenous Australian cohorts.[8] A local study that followed stroke in young age group, did not detect any FVL mutation in its sample.[11]

Although statistically insignificant, our study found that Heterozygous Factor V Leiden mutations could be associated with stroke in a young male population with a positive family history of stroke. Similar findings were observed by an Indian study which reported a relatively higher yet statistically insignificant prevalence of FVL compared to its controls. The study noted a causal relationship of FVL in young Indian patients afflicted with large vessel ischemic strokes.[15] The observation of lower incidence of FVL could partly be ascribed to the relatively smaller size of our cohort. As noted above, various studies in the past have reported conflicting results on association of factor V Leiden and ischemic stroke in young. Similar to our study, the small sample size of the previous studies was considered a limiting factor for detection of such associations to modest level.[16] Having said that, the presentation of a young male with stroke bearing a positive family history of CVA should prompt the clinicians to consider and evaluate the presence of FVL mutation, especially where resources allow. FVL was not found to be significantly associated with body mass index, smoking, deployment at High altitude (>10,000 ft), history of Oral Contraceptive (OCP) Intake and hyperlipidemia in our sample. It, however, has been associated with smoking and high BMI in other thromboembolic events, particularly in pregnant women,[17] and European population.[12] The frequency of AT III deficiency though was higher than that of Heterozygous Factor V Leiden in our sample, the data however was not statistically significant in our cohort. This finding is consistent with a study by Hossmann *et al*. in 1983 where 12(33%) patients showed AT III deficiency though the statistical association was not significant.[18] Another study reported a negative association of the inherited AT III deficiency with ischemic stroke in young patients and even those with a patent foramen ovale.[19] The acute phase of stroke is characterized by a higher tendency of intravascular coagulation as well as the formation of inactive AT III-thrombin complexes. A fall in the levels of AT III is also recorded during this phase.[18] This whole process has to have a nexus with multiple environmental factors that may precipitate

the ultimate coagulation event. Thus, stroke in young is more likely to be associated with more than one risk factor.[20]

In coherence with an Indian research,[21] our study reported a higher frequency of smoking as a risk factor of stroke in young soldiers deployed at higher altitude. The same study reported a positive association of ischemic stroke in young soldiers who are deployed at high altitude for a prolonged duration than those at lower altitudes. It should be noted that prolonged immobilization, decreased physical activity[22] and polycythemia associated with high altitude deployment in soldiers may combine with inherent tendencies of hypercoagulability in susceptible individuals, ultimately leading to stroke. As reported by earlier studies we found a positive association of hyperlipidemia and family history with stroke in young.[23] However, according to our results, young stroke patients with hyperlipidemia and a positive family history of stroke were more likely to exhibit the presence of low AT III levels. Such association was not found by other studies, to the best of our knowledge. The findings may be corroborated by further large scale well-designed studies.

Our study found that an increasing age and male gender are more likely to present with stroke among the young age group. A study by Putaala *et al*. in 2009, on young population reported that although females were more likely to suffer from stroke when <30 years of age, the male preponderance was significantly raised when they crossed the age of 44.[24] Multiple studies in the past have correlated various biochemical anomalies as risk factor for stroke in young individuals, an example being a study by Arif *et al*. in 2019 which reported that 63.2% patients with high body mass index and 54.4% patients with tobacco smoking were associated with the presence of raised homocysteine levels such population (*p*-value0.003 and 0.005 respectively).[25]

LIMITATIONS OF STUDY

However, given the absence of randomized sampling technique, our results may not be generalized to whole young population with stroke from various healthcare centers of the country. Our study also lacked a control group, due to which comparison of coagulopathies could not be done. Further large-scale case-control studies with randomized sampling may be carried out to determine the association of Factor V Leiden mutation and AT III deficiency with various socio-demographic factors in stroke in young patients.

CONCLUSION

The appropriateness of testing for hypercoagulable states in the settings of stroke in young may be equivocal but with the presence of smoking, family history of stroke and high-altitude deployment as possible risk factors, clinicians should be prompted to check for the presence of Factor V Leiden and AT III levels, where resources at hand might allow. A case to case based tailored evaluation of the patients would help appropriate management and prevention of long-term sequelae.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

HK & KHN: Conception, study design, drafting the manuscript, approval of the final version to be published.

MAN & HM: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

FSS & MA: Data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Feigin VL, Norrving B, Mensah GA. Global burden of stroke. Circ Res 2017; 120(3): 439-448. https://doi.org/10.1161/CIRCRESAHA.116.308413
2. Khan MI, Khan JI, Ahmed SI, Ali S. The epidemiology of stroke in a developing country (Pakistan). Pak J Neurol Sci 2019; 13(3): 30-44.
3. Bhosale VV, Singh S, Srivastava M, Pathak P, Prakash S, Sonkar S, et al. A case control study of clinical and biochemical parameters of metabolic syndrome with special attention among young and middle aged population. Diabetes Metab Syndr 2019; 13(4): 2653-2659. https://doi.org/10.1016/j.dsx.2019.07.012
4. Ekker MS, Verhoeven JI, Vaartjes I, van Nieuwenhuizen KM, Klijn CJM, de Leeuw FE. Stroke incidence in young adults according to age, subtype, sex, and time trends. Neurology 2019; 92(21): e2444-2454. https://doi.org/10.1212/WNL.0000000000007523
5. Markus HS, editor. Stroke genetics. Oxford: Oxford Medical Publications; 2003.
6. Corral J, de la Morena-Barrio ME, Vicente V. The genetics of antithrombin. Thromb Res 2018; 169 Suppl 1: S23-29. https://doi.org/10.1016/S0049-3848(18)30078-1
7. Mann KG, Kalafatis M. Factor V: A combination of Dr. Jekyll and Mr. Hyde. Blood 2003; 101(1): 20-30. https://doi.org/10.1182/blood-2002-05-1404
8. Ridker PM, Miletich JP, Hennekens CH, Buring JE. Ethnic distribution of factor V Leiden in 4047 men and women: implications for venous thromboembolism screening. JAMA 1997; 277(16): 1305-1307. https://doi.org/10.1001/jama.1997.03540400045035

9. Chiasakul T, Jesus ED, Tong J, Chen Y, Crowther M, Garcia D, et al. Inherited thrombophilia and the risk of arterial ischemic stroke: A systematic review and meta-analysis. J Am Heart Assoc 2019; 8(19): e012877. https://doi.org/10.1161/JAHA.119.012877
10. Pagana K, Pagana T, Pagana T. Mosby's Diagnostic & Laboratory Test Reference. 14th ed. St. Louis, Mo: Elsevier; 2019.
11. Khalid A, Khan SA, Alamgir W, Anwar R. Thrombophilia profile in young patients of cerebrovascular accident-an institution based study. Pak Armed Forces Med J 2018; 68(4): 701-705.
12. George MG. Risk factors for ischemic stroke in younger adults. Stroke 2020; 51(3): 729-735. https://doi.org/10.1161/STROKEAHA.119.024156
13. Alakbarzade V, Taylor A, Scully M, Simister R, Chandratheva A. Utility of current thrombophilia screening in young patients with stroke and TIA. Stroke Vasc Neurol 2018; 3(4): 231-236. https://doi.org/10.1136/svn-2018-000163
14. Lim MY, Deal AM, Kim S, Musty MD, Conard J, Simioni P, et al. Thrombophilic risk of individuals with rare compound factor V Leiden and prothrombin G20210A polymorphisms: an international case series of 100 individuals. Eur J Haematol 2016; 97(4): 353-360. https://doi.org/10.1111/ejh.12721
15. Anadure R, Christopher R, Nagaraja D, Narayanan C. A genetic study of factor V Leiden (G1691A) mutation in young ischemic strokes with large vessel disease in a South Indian population. J Clin Neurosci 2017; 43: 346-352. https://doi.org/10.1016/j.jocn.2017.05.012
16. Kim RJ, Becker RC. Association between factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations and events of the arterial circulatory system: a meta-analysis of published studies. Am Heart J 2003; 146(6): 948-957. https://doi.org/10.1016/S0002-8703(03)00426-0
17. Kohli R, Moliterno AR, Streiff MB, DeBaun MR, Kickler TS, Blakemore K, et al. Risk factors for pregnancy-associated venous thromboembolism: the role of maternal age, obesity, smoking and other modifiable risk factors. Blood 2018; 132(Suppl 1): 3804. https://doi.org/10.1182/blood-2018-99-116789
18. Hossmann V, Heiss WD, Bewermeyer H. Antithrombin III deficiency in ischaemic stroke. Klin Wochenschr. 1983; 61(12): 617-620. https://doi.org/10.1007/BF01724844
19. Morris JG, Singh S, Fisher M. Testing for inherited thrombophilias in arterial stroke. Stroke 2010; 41(12): 2985-2990. https://doi.org/10.1161/STROKEAHA.110.592873
20. Lasek-Bal A, Kopyta I, Warsz-Wianecka A, Puz P, Łabuz-Roszak B, Zaręba K. Risk factor profile in patients with stroke at a young age. J Neurol Res 2018; 40(7): 595-601. https://doi.org/10.1007/s00415-018-8970-6
21. Jha SK, Anand AC, Sharma V, Kumar N, Adya CM. Stroke at high altitude. High Alt Med Biol 2002; 3(1): 21-27. https://doi.org/10.1089/152702902753639485
22. Subha PP, Geethakumari SMP, Athira M, Nujum ZT. Pattern and risk factors of stroke in the young among stroke patients admitted in medical college hospital, Thiruvananthapuram. Ann Indian Acad Neurol 2015; 18(1): 20. https://doi.org/10.4103/0972-2327.144286
23. Soliman RH, Oraby MI, Fathy M, Essam AM. Risk factors of acute ischemic stroke in patients presented to Beni-Suef University Hospital: prevalence and relation to stroke severity at presentation. Egypt J Neurol Psychiatr Neurosurg 2018; 54(1): 8. https://doi.org/10.1186/s41983-018-0011-4
24. Putaala J, Metso AJ, Metso TM, Konkola N, Kraemer Y, Haapaniemi E, et al. Analysis of 1008 consecutive patients aged 15 to 49 with first-ever ischemic stroke. Stroke 2009; 40(4): 1195-1203. https://doi.org/10.1161/STROKEAHA.108.529883
25. Arif S, Hashmat A. Assessment of homocysteine levels in patients with stroke in young. Pak Armed Forces Med J 2019; 69(6): 1179-1183. https://www.pafmj.org/PAFMJ/article/view/3629