

## THROMBOPHILIA PROFILE IN YOUNG PATIENTS OF CEREBROVASCULAR ACCIDENT- AN INSTITUTION BASED STUDY

Amna Khalid, Saleem Ahmed Khan, Nasirudin, Wasim Alamgir\*, Rabia Anwar\*\*

Army Medical College/ National University of Medical Sciences (NUMS) Rawalpindi Pakistan, \*Military Hospital/ National University of Medical Sciences (NUMS) Rawalpindi Pakistan, \*\*Ayub Medical College Abbotabad Pakistan

### ABSTRACT

**Objective:** To find out frequency of different causes of thrombophilia in young patients of cerebrovascular accidents.

**Study Design:** Cross sectional descriptive study.

**Place and Duration of Study:** This study was carried out at department of haematology, Army Medical College, Military Hospital and Armed Forces Institute of Pathology Rawalpindi, from Nov 2016 to Nov 2017.

**Material and Methods:** Young patients aged 18 to 45 years were included in this study. Relevant history was obtained and general physical examination was done. Base line investigations included, complete blood count, prothrombin time, activated partial thromboplastin time and lipid profile. Tests for inherited thrombophilia (protein C, protein S, antithrombin, factor V leiden and prothrombin gene mutation) were carried out along with anticardiolipin, antiphospholipid antibodies and plasma homocysteine levels were measured to rule out, thrombophilia as a cause of CVA in young patients. The frequencies, of various causes of thrombophilia in CVA patients were analyzed.

**Results:** Out of 30 patients there were 26 (86.7%) males and 04 (13.3%) females. The mean age was 36.6 years. The results of protein C, protein S, prothrombin gene mutation, antiphospholipid anticardiolipin antibodies, antithrombin and factor V leiden mutation were normal in all 30 patients. However, plasma homocysteine levels were raised in 20 patients with mean of 22.90. A statistical significance between the homocysteine levels and male genders was observed in this study.

**Conclusion:** Raised plasma homocysteine levels were most common finding in young patients of CVA. All other laboratory parameters of thrombophilia were negative in young patients of CVA in our study.

**Keywords:** Antithrombin, Anticardiolipin and Antiphospholipid antibodies, Cerebrovascular accidents, Factor v laiden, Homocystein, Protein C, Protein S, Prothrombin gene.

---

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

---

### INTRODUCTION

Thrombophilia is an increased predisposition to thrombosis which may be inherited or acquired<sup>1</sup>. Antithrombin (AT), protein C (PC) and protein S (PS) are natural inhibitors of coagulation. Inherited causes of thrombophilia include, prothrombin G20210A gene mutation (PTM) and factor V leiden mutation (FVL). Frequency of FVL and prothrombin gene mutation mutation was fairly less common in our region<sup>2</sup>. Raised, levels of homocysteine also contribute to thrombosis. It is considered as a warning in young patients and needs to be

evaluated regardless of arterial or venous thrombosis. However precise mechanism by which raised homocysteine levels can result in thrombosis, is not clear. Hyperhomocysteinemia can be related to malnutrition and malabsorption of vitamin B12 and folate<sup>3</sup>. Antiphospholipid antibodies (APLA), are a documented cause of acquired thrombophilia in CVA under 50 years<sup>4</sup>. Other acquired causes of thrombophilia include immobilization, pregnancy / postpartum state, obesity, malignancy, sickle cell disease, lupus anticoagulant, thrombotic thrombocytopenia purpura, heparin induced thrombocytopenia, myeloproliferative disorders, paroxysmal nocturnal haemoglobinuria and chemotherapy<sup>1,4</sup>. The inherited risk factors for thrombosis require synergism with other inherited or acquired risk

---

**Correspondence:** Dr Amna Khalid, Department of Pathology, Army Medical College Rawalpindi Pakistan

Email: [amnakhaldpk@yahoo.com](mailto:amnakhaldpk@yahoo.com)

Received: 08 Jan 2018; revised received: 23 Feb 2018; accepted: 13 Mar 2018

factors. Consequently patients with genetic predisposition are more prone to stroke or cerebrovascular accident (CVA)<sup>5,6</sup>. CVA is associated with increased morbidity and mortality all over the world including Pakistan<sup>1,7</sup>. Several studies have focused on thrombophilia in our region but very little work is available on young adults and middle aged patients<sup>2,8,9</sup>. A patient with genetic predisposition to thrombophilia is more prone to stroke. Therefore early diagnosis and intervention in such patients can reduce the mortality and morbidity, rates<sup>7,10</sup>. The incidence of CVA in young adults is less in our region as compared to other parts of world. But it is more commonly seen in male population<sup>11</sup>. Although thrombophilia testing is performed routinely in western world despite advising not to perform these tests as it cannot alter management<sup>12</sup>. Although CVA is more common in the elderly than in the young but an association between inherited causes of thrombophilia and stroke in the young has been documented<sup>13</sup>. There is no validated recommendation as to how such patients of CVA should be selected for thrombophilia profiling. However guidelines are advised by different committees on regional basis. World Health Organization (WHO) recommends that patient less than 45 years of age and those with a family history of thrombosis should be tested for thrombophilia if they present with CVA<sup>8,14</sup>. Present study was carried out, to find out frequency of different causes of thrombophilia in young adults of CVA admitted to Neurology unit of Military Hospital, Rawalpindi.

## **MATERIAL AND METHODS**

This study was a cross sectional descriptive carried out at Department of Hematology, Army Medical College, Rawalpindi, in collaboration with the Neurology Department of Military Hospital (MH) Rawalpindi and Armed Forces Institute of Pathology (AFIP), Rawalpindi from November 2016 to November 2017 after approval from Institutional ethical review board. Both males and females 18 to 45 years of age, who presented with CVA, were included in this study.

Patients already on anti thrombotic treatment, diabetics and hypertensive were excluded. Patients were selected by non- probability purposive sampling and sample size was calculated by WHO calculator. Relevant history and findings of general physical examination were documented on a proforma. Ten ml of blood was drawn from patients under aseptic condition. Two ml was transferred to ethylene diamine tetraacetic acid (EDTA) tube for complete blood counts which were generated through automated haematology analyser Sysmex KX-21. Prothrombin gene mutation and factor V leiden mutation were detected by polymerase chain reaction (PCR) after extracting DNA from peripheral blood lymphocytes using standard techniques. PCR amplification of genes of interest was done in thermalcycler Proflex. PCR products were electrophoresed on 6% Polyacrylamide gel (PAGE) and stained with silver nitrate for visualization. Lipid profile was analyzed through Advia 1800, plasma homocystein levels were measured on Advia centaur XP and anticardiolipin and anti-phospholipid antibodies were detected by enzyme linked immunosorbant assay (ELISA). Prothrombin time (PT) and activate partial thromboplastin time (APTT) were performed manually at 37 oC. While protein C, protein S and AT deficiencies were analyzed by Sysmex CA 1500.

## **Data Analysis**

The data was entered and analyzed by using statistical package for social sciences (SPSS) version 22. Frequency and percentages were calculated for qualitative data while for quantitative variables, mean and standard deviation was calculated. Various groups were compared and Independent t-test was applied.

## **RESULTS**

A total of thirty patients aged 18 to 45 years with history of CVA were included in this study. Age range was 23 to 44 years (mean 36.6 and standard deviation of  $\pm 6.83$ ). Out of these 30 patients there were 26 (86.7%) males and 04

(13.3%) were females (male to female. ratio 6.5:1). Twenty one out of 30 (70%) were smokers. Mean hemoglobin was 13.83 g/dl with minimum 8.0 g/dl and maximum 16.2 g/dl and standard deviation of  $\pm 2.05$ . Minimum TLC was  $4.0 \times 10^9 /l$  and maximum was  $16.5 \times 10^9 /l$ , with mean of  $8.33 \times 10^9 /l$  and standard deviation of  $\pm 2.61$ . Mean Platelets count was  $169.60 \times 10^9 /l$  with minimum of  $30 \times 10^9 /l$  and maximum of  $399 \times 10^9 /l$ , while the standard deviation of  $\pm 79.81$  (table-I). PT and APTT were normal in 21 (70%) and abnormal in 09 (30%). While 27 (90%) patients had normal lipid counts and only 03 (10%) had raised levels. All the patients showed

homocysteine with a mean of  $22.21 \mu\text{mol}/l$  (SD  $\pm 10.63$ ). Independent t-test was performed to compare the genders and positive homocysteine patients. There was a significant association between the genders, males (M=24, SD=10.29, N=26) and females (M=10.57, SD=1.20, N=04) and the homocysteine (M=22.21, SD=10.63, N=30) with 'p' value of  $<0.001$ , two tailed with 95% of confidence level around the difference.

## DISCUSSION

We planned this study to see the contribution of major causes of thrombophilia in young adult patients of CVA admitted to our

**Table-I : Mean of Hb, TLC and Platelets in all the patients.**

	Minimum	Maximum	Mean	SD
Haemoglobin	8.0	16.2	13.83	2.05
TLC	4.0	16.5	8.33	2.61
Platelets	30	399	169.60	79.81

**Table-II: Thrombophilia profile of the patients.**

Test	Result	Frequency	Percentage (%)
Protein C	Normal	30	100
Protein S	Normal	30	100
Prothrombin gene mutation	No mutation	100	
Antiphospholipid and Anticardiolipin Antibodies	No Antibodies detected	30	100
Antithrombin III	Normal activity	30	100
Factor V Leiden mutation	No mutation detected	30	100
Plasma Homocysteine	Normal	10	33.3
	Moderate	13	43.3
	High	07	23.3

normal results for protein C, protein S and AT activity. No gene mutation was detected for prothrombin or factor V leiden. anticardiolipin and antiphospholipid antibodies were not detected (table-II).

The levels of homocysteine were categorized into three groups: normal levels ( $5-15 \mu\text{mol}/l$ ), moderately high levels ( $15-30 \mu\text{mol}/l$ ) and very high levels ( $>30 \mu\text{mol}/l$ ). Therefore in our study we found 10 (33.3%) patients with normal levels of homocysteine, while 13 (43.3%) patients had moderately high levels and 07 (23.3%) patients had very high levels of plasma homocysteine. Result shows a minimum value of  $9.10 \mu\text{mol}/l$  and maximum value of  $47.90 \mu\text{mol}/l$ , for

teaching hospital. Protein C, protein S, homocysteine, antithrombin, factor V leiden mutation, prothrombin gene mutation, antiphospholipid antibodies and anticardiolipin antibodies were calculated in all the young adults of CVA. Male patients were more than the female patients as CVA in young adult is commonly seen in females in our population<sup>11</sup>. Frequency of Factor V Leiden mutation ranges from 5% to 8% in European populations. Its frequency in Pakistan has been determined to be 1.2% in local study<sup>2,10,15</sup>. Another study conducted on young patients with thrombophilia by *Junaid et al* found FVL mutation in 4.3% of patients<sup>16</sup>. *Ali et al* found FVL mutation in 5% of

their patients with CVA of all age groups. We did not find any association of CVA with FVL in our young patients. Prevalence of F2G20210A mutation in white population is 0.7% to 6.5%. It is somewhat more common in northern as compared to southern Europeans and is rare in nonwhite population<sup>10,13</sup>. None of our young patients with CVA had F2G20210A mutation. Multiple studies done earlier have also failed to associate FVL and F2G20210A with CVA in the young<sup>12,16-18</sup>. Low frequency of these mutations in our population may also contribute to the lack of their association with CVA<sup>17,18</sup>.

Protein C and protein S deficiencies are rare disorders with each having a prevalence of less than 0.15%. AT is also a rare cause of hereditary thrombophilia with a prevalence of around 0.05% in the normal population<sup>2,10,19</sup>. Data is extremely conflicting regarding the association of protein C, protein S and AT deficiencies with CVA. The prevalence of protein C, protein S and AT deficiencies in patients with CVA has been reported as high as 23% in different studies<sup>9,13,19</sup>. A study done at Armed Forces Institute of Pathology Rawalpindi Pakistan on prevalence of prothrombin gene mutation in general population suggested 1.0% of mutation in the target population<sup>17</sup>. In our study none of our patient with CVA showed any relationship with heritable causes of thrombophilia. *Morris et al* pointed out that testing for inherited thrombophilia is of little value in CVA of arterial origin. They did not find any convincing association between the inherited thrombophilias and ischemic stroke in the analysis of multiple case-control studies<sup>5</sup>. *Giordano et al* found that less than 10% patients with acute ischemic stroke tested positive for one of the inherited thrombophilias, but the relationship was likely to be coincidental rather than causal<sup>19</sup>. Anticardiolipin antibodies (IgG/IgM) can result in thrombotic disorder in neonates and young adults<sup>20</sup>. We could not find any relation of antiphospholipid and anticardiolipin antibodies to young patients of CVA in our study. We found raised levels of homocysteine in 66.6% of our

patients. As the number of females was less therefore levels of homocysteine was found to be elevated in only males. Hyperhomocysteinemia being a cause of thrombus formation can be regarded as a warning in a young person regardless of arterial or venous thrombus<sup>21</sup>. It can be related to malnutrition and malabsorption of vitamin B12 and folate which can magnify the hypercoagulable state along with MTHFR mutation genotype<sup>22</sup>. We found normal levels of homocysteine in 04 females however out of 26 males 16 had hyperhomocysteinemia. This study is of vital significance because it studies all major causes of inherited thrombophilia in young patients of CVA in our population. An important feature of our study was measurement of homocysteine which was estimated primarily in association with thrombophilia in the young. A limitation of our study is that it was conducted on a small number of patients as compared to other studies on the subject.

## CONCLUSION

Raised plasma homocysteine levels were most common finding in young patients of CVA. All other laboratory parameters of thrombophilia were negative in young patients of CVA in our study.

All major causes of inherited thrombophilia have low contribution in young CVA in our population. Consequently it is recommended that Thrombophilia workup should be carried out in patients with contributory family history only. Hyperhomocysteinemia is a curable, cause of CVA, in young patients when rehabilitated with vitamin B12 and Folate management. However raised levels of Homocysteine still show some correlation in males in our population and it is recommended that measurement of Homocysteine levels should be included in the list of investigations of young adult CVA patients in our set up.

## RECOMMENDATIONS

Diagnostic workup for thrombophilia in young patient of CVA can assist in prospects of manageable therapeutics. Since screening for

all parameters of thrombophilia is costly for a developing country like ours, it is recommended that these tests should not be carried out routinely unless there is a clear indication.

### CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

### REFERENCES

- Meyer MR, Witt DM, Delate T, Johnson SG, Fang M, Go A, et al. Thrombophilia testing patterns amongst patients with acute venous thromboembolism. *Thrombosis research* 2015; 136(6): 1160-4.
- Ali N, Ayyub M, Khan SA. High prevalence of protein C, protein S, antithrombin deficiency, and Factor V Leiden mutation as a cause of hereditary thrombophilia in patients of venous thromboembolism and cerebrovascular accident. *Pak J Med Sci* 2014; 30(6): 1323.
- Han L, Wu Q, Wang C, Hao Y, Zhao J, Zhang L, et al. Homocysteine, ischemic stroke, and coronary heart disease in hypertensive patients: A population-based, prospective cohort study. *Stroke* 2015 1; 46(7): 1777-86.
- Kutteh WH. Inherited and acquired Thrombophilias and adverse pregnancy outcomes. In *Recurrent Pregnancy Loss*. Springer 2016 (pp. 67-73).
- Morris JG, Singh S, Fisher M. Testing for inherited thrombophilias in arterial stroke: can it cause more harm than good?. *Stroke* 2010; 41(12): 2985-90.
- Buchanan GS, Rodgers GM, Branch DW. The inherited thrombophilias: genetics, epidemiology, and laboratory evaluation. *Best Pract Res Clin Obstet Gynaecol* 2003; 17(3): 397-411
- Mendis S, Norrving B. Organizational update: World Health Organization. *Stroke* 2014; 45(2): e22-3.
- Ng KW, Loh PK, Sharma VK. Role of investigating thrombophilic disorders in young stroke. *Stroke research and treatment* 2011; 670138; 1-9.
- Mishra MN, Kalra R, Rohatgi S. Clinical profile, common thrombophilia markers and risk factors in 85 young Indian patients with arterial thrombosis. *Sao Paulo Med J* 2013; 131(6): 384-8.
- 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): 353-60.
- Nomani AZ, Iqbal M, Jamil U, Nabi S, Mughal S, Badshah M, et al. Etiology of stroke in young Pakistani adults; results of a single center study. *Pak J Neuro Sci* 2015; 10(4): 18-22.
- Connors JM. Thrombophilia Testing and Venous Thrombosis. *N Engl J Med* 2017; 377(12): 1177-87.
- Taveira I, Vicente C, Sobral S, Nzwalo H, Costa JS. Multiple inherited thrombophilias in a young patient with striatocapsular stroke. *J Neurosci Rural Pract* 2017; 8(Suppl 1): S154.
- Mendis S, Davis S, Norrving B. Organizational update: The world health organization global status report on non-communicable diseases 2014; one more landmark step in the combat against stroke and vascular disease. *Stroke* 2015; 46(5): e121-2.
- Nasiruddin, Zah-ur- Rehman, Anwar M, Ahmed S, Ayyub M, Ali W. Frequency of factor V leiden mutation. *JCPSP* 2005; 15(1): 15-7.
- Junaid A, Ahmed S, Anwar M, Ali W, Nasiruddin AM. Factor V Leiden in young patients with Thrombophilia. *J Coll Physicians Surg* 2004; 14: 698-9.
- Naeem MA, Anwar M, Ali W, Ayyub M, Nasiruddin. Prevalence of prothrombin gene mutation (GA 20210 A) in general population: A pilot study. *Clinical and applied thrombosis/ hemostasis* 2006; 12(2): 223-6.
- Adams A, Roels C. Abstract TP215: Increased Economic Burden and Low Clinical Impact With Thrombophilia Testing in Adult Acute Ischemic Stroke Patients. *STROKE* 2018, ATP215-A.
- Giordano C, Penna O, Amato A, Bruno R, Tripodi VF, Vadalà EG, Pennisi NC, Mondello E, Fodale V. Combined Intestinal Ischemia, Cerebral Stroke and Thrombosis of Thoracoabdominal Aorta and Splenic Artery in a Thrombophilic Woman: 2015. A Case Report.
- Ahmad, A. Genetics of cerebral venous thrombosis. *J Pak Med Assoc* 2006; 56(11): 488-90.
- Raziel A, Friedler S, Schachter M, RonEl R, Kornberg Y, Sela BA. Hypercoagulable thrombophilic defects and hyper-homocysteinemia in patients with recurrent pregnancy loss. *Am J Reprod Immunol* 2001; 45(2): 65-71.
- Daraban AM, Trifa AP, Popp RA, Botezatu D, erban M, Uscatescu V, et al. Thrombophilia genetic testing in Romanian young women with acute thrombotic events: Role of Factor V Leiden, Prothrombin G20210A, MTHFR C677T and A1298C polymorphisms/Evaluarea genetică a trombofililor la femeii tinere din România cu evenimente acute trombotice: rolul Factorului V Leiden, Protrombinei G20210A, polimorfismelor MTHFR C677T i A1298C. *Rev Romana Med Lab* 2016; 24(3): 291-305.