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Research Article,

Factor V Gene Polymorphism (G1691A) Among Sudanese Patients with Intracerebral Hemorrhage, Khartoum State, 2022

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Abstracts:

Background

Intracranial hemorrhage refers to any bleeding within the intracranial vault, including the brain parenchyma and surrounding meningeal spaces. In Sudan, there is no published data regarding factor V Leiden mutation. Therefore this study was designed to detect the possible present of factor V polymorphism (G1691A) among Sudanese patients with intercereberal hemorrhage.

Material and method

This study was a cross sectional hospital-based study, conducted at the research laboratory of the national center of neurological sciences (NCNS), Khartoum, Sudan during the period June 2022 to August 2022.It included all patients attended with Intracerebral hemorrhage, DNA extraction was done from blood of all patients and controls.PCR for *factor V gene* was done and thus Sanger sequencing.

Results

The PCR results showed; 100% were positive for *factor V gene.* Sequencing results revealed single based exchange in *factor V gene*G to A (G1691A).

Conclusion

Factor V gene polymorphism (G1691A) was detected and might be in association with intercereberal hemorrhage among Sudanese patients

Keywords: Hemorrhage, DNA, PCR, Factor V, Polymorphism

Introduction:

Any bleeding that occurs inside the intracranial vault, which includes the brain parenchyma and surrounding meningeal spaces, is referred to as intracranial hemorrhage. Incidence of spontaneous ICH is 24.6 per 100,000 person-years globally, with 40,000 to 67,000 instances reported annually in the US [1,2].

Only 20% of survivors are anticipated to have fully recovered functionally after six months, with a 30-day death rate ranging from 35% to 52%. In addition to Asia, the total occurrences are 18–24%, respectively, in Japan and Korea. In low- and

middle-income nations, the incidence rates of primary ICH were twice as high as those in high-income countries. In addition, the yearly incidence rate per 100,000 people was greater in males than in women. [3,4]

The medial and smooth muscle degeneration that leads to ruptured vessels that constitutes the typical pathophysiology of ICH. Some patients may experience localized dilations, micro aneurysms, and fibrinous necrosis of the sub endothelium. While cerebral amyloid angiopathy (CAA) is comparatively more common in lobar ICH, lipohyalionoses, which are significantly associated to long-standing hypertension, are most frequently observed in non-lobar ICH. [5,6] Rarely referred to as proaccelerin or labile factor, factor V is a protein of the coagulation system. It serves as a cofactor instead of being an active enzyme, unlike the majority of other coagulation factors. Deficiency increases the risk of hemorrhaging. First chromosome contains the *factor V gene (1q24)*. The gene is 70 kb long, has 25 exons, and produces a protein with a molecular weight of about 330 kDa. [7]

Numerous studies show that the most prevalent coagulation deficiency in people with venous thrombosis is the factor V Leiden mutation, which has a high prevalence. In adults, there is currently no evidence linking this mutation to cerebrovascular illness. The findings did not support a link between the factor V Leiden mutation and intracerebral hemorrhage or ischemic stroke. In subgrouping the study by age, smoking status, myocardial infarction, hypertension, diabetes mellitus, or coronary disease, there was no evidence of a connection. There is no evidence linking the factor V Leiden mutation to an increased risk of cerebrovascular illness. Factor V deficiency is an uncommon bleeding illness that can cause symptoms to appear at any age and an increased risk of internal bleeding in the skull, according to published data. The vast majority of research indicates that there is no link between the factor V leiden mutation and Intracerebral hemorrhage. Regarding the factor V leiden mutation, no published information exists for Sudan. Therefore, the purpose of this study is to identify Sudanese individuals with intercereberal hemorrhage who may have factor V polymorphism (G1691A). [8]

Material and methods:

This study was a cross sectional hospital-based study, conducted at the research laboratory of the national center of neurological sciences (NCNS), Khartoum, Sudan during the period June 2022 to August 2022.All patients attending NCNS and diagnosed with ICH during the aforementioned period were included. In addition to that, apparently healthy individuals with no history of ICH were selected as control group.

From each participant 3 ml of venous blood was collected from the antecubital vein using a dry sterile disposable syringe and needle. Blood samples dispensed into sterile containers with Ethylene Diamine Tetra-acetic Acid (EDTA), labeled with subject's age, sex and identification number stored at -20°C for molecular analysis.

DNA was isolated by G-DEX IIB Genomic DNA extraction Kit. Primers were designed by using Prime3 software. The forward primer for FV (G-A) was designed as "5- CAT ACT ACA GTG ACG TGGAC -3"and reverse as "5- TGTT CTC TTG AAG GAAA TGC 3"with product size of 206 bp fragment.

The PCR was done in commercial thermal cycler machine (Swift^{TM}MaxPro SWT-MXP-BLC-4)at following condition: Denaturation temperature 94^{o}C for 30 secs, annealing temperature at 61^{o}C for 30 sec and extension temperature at 72^{o}C for 30 secs, the final elongation was adjusted for 5 minutes at 72 °C.PCR reaction was set at 35 cycles. The PCR amplification product was separated on agarose gel. Products were sent for sequencing to Macro gene Europe Laboratory.

The data was collected using pre-designed structural questionnaire, the demographic and clinical data concerning each participant was obtained from the registry data base office, which included the following information: (Gender, age and medical history). The laboratory data included hematological results, PCR findings and sequencing results.

The study was approved by the ethical committee of the National Center for Neurological Sciences and ethical review committee of National University, faculty of medical laboratory, and the participants were fully informed about the advantages and disadvantages before participation in the research (verbal informed consent).

Data was entered and organized into Microsoft Office Excel 2010 data sheet, then for the analysis, SPSS version 23 statistical software (SPSS Inc., USA) was used for statistical analysis.

The sequencing results analyzed using different bioinformatics soft-wares and tools. To examine the presence of polymorphisms the obtained sequences, were aligned using BioEdit-ClustalW software with a normal sequence from GenBank (National Center of Biotechnology Information).

Results:

Socio-demographic study

In the present study 100 participants were enrolled, 50 were selected as cases and 50 were selected as control group. In the case group; 64% were male and 36% were female, the most affected age group more than 70 years (38%) , followed by less than 50 years and 50-70 years (34%, 28%) respectively. Most of them from Khartoum state (66%). In addition to that about 42% hadn't history of chronic disease, 26% hypertensive and 24% diabetic. For the types of cerebral hemorrhage; 64% had a subdural hemorrhage and 30 % had intercereberal hemorrhage. (Table 1, 2)

Table (1) Socio-demographic data of the cases

Socio-demographic		Frequency	Percent
Gender	Male	32	64.0
	Female	18	36.0
	Total	50	100.0
Age	< 50 years	17	34.0
	50 – 70 years	14	28.0
	> 70 years	19	38.0
	Total	50	100.0
Residence	Khartoum	33	66.0
	Aljazerah	10	20.0
	White Nile	1	2.0
	River Nile	2	4.0
	Kassala	1	2.0
	Korodofan	3	6.0
	Total	50	100.0

Table (2) Distribution of the Associated Diseases in the Case Group

Chronic Disease	Frequency	Percent
Hypertension	13	26.0
Diabetic	12	24.0
Diabetic & hypertension	4	8.0
Absent	21	42.0
Total	50	100.0

Table (3) Distribution of Cerebral Hemorrhage in the Case Group

Hemorrhage	Frequency	Percent
ICH	15	30.0
SDH	32	64.0
EDH	2	4.0
IVH,ICH,EVD	1	2.0
Total	50	100.0

3.2 Molecular studies

Visualizing PCR results revealed *factor V gene* detection in all patients and controls

In gel electrophoresis 206 bp of *factor V gene* was detected after PCR (Figure 1).

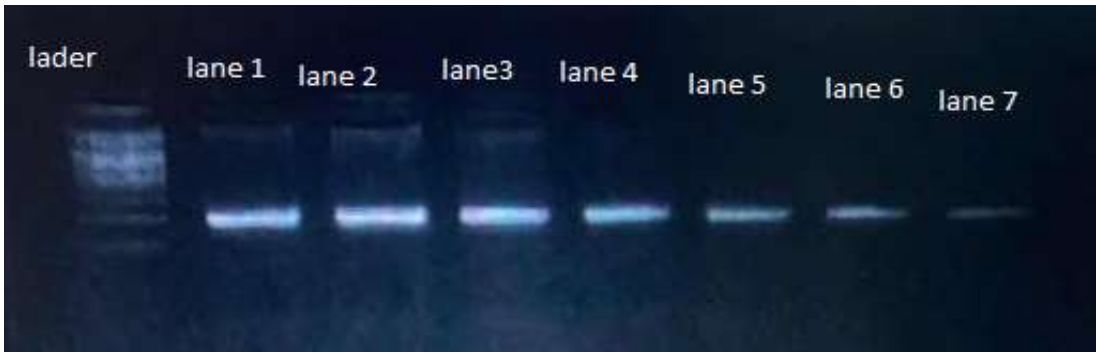

Figure (1) *Factor V gene (*206 bp*)* inGel electrophoresis

Sequencing

The sequencing results were analyzed using different bioinformatics soft-wares and tools. The obtained sequences aligned using BioEdit-ClustalW software with a normal sequence from Gen Bank gene (accession number NC_00001.11 in NCBI).
When the cases were compared with the normal reference one single base exchange was found G to A (G1691A). While when the controls were compared with normal reference, no any single base exchange was found among the control groups (figure 2,3)

Mutation Taster

Mutation taster was used to confirm the mutation which revealed; G>A polymorphism was predicted, amino acid sequence was changed, protein features might be affected and splice site also was changed, alteration location was at chromosome 1, alteration type was single base exchange. (Figure 4)

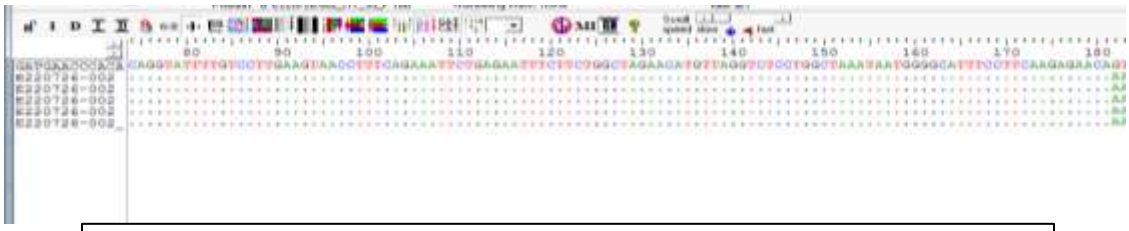

Figure (2) multiple sequence alignment using Bio-Edit clustal W for cases group with reference gene sequence of *FV gene.*

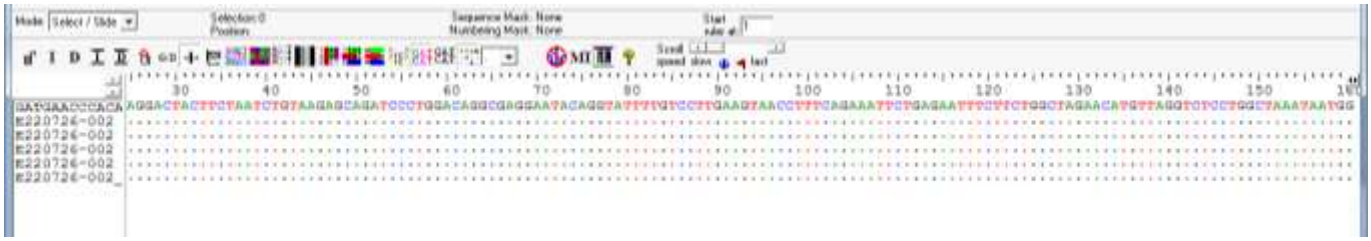

Figure (3): Multiple sequence alignment using Bio-Edit clustal W for control group with reference gene sequence of *FV gene*.

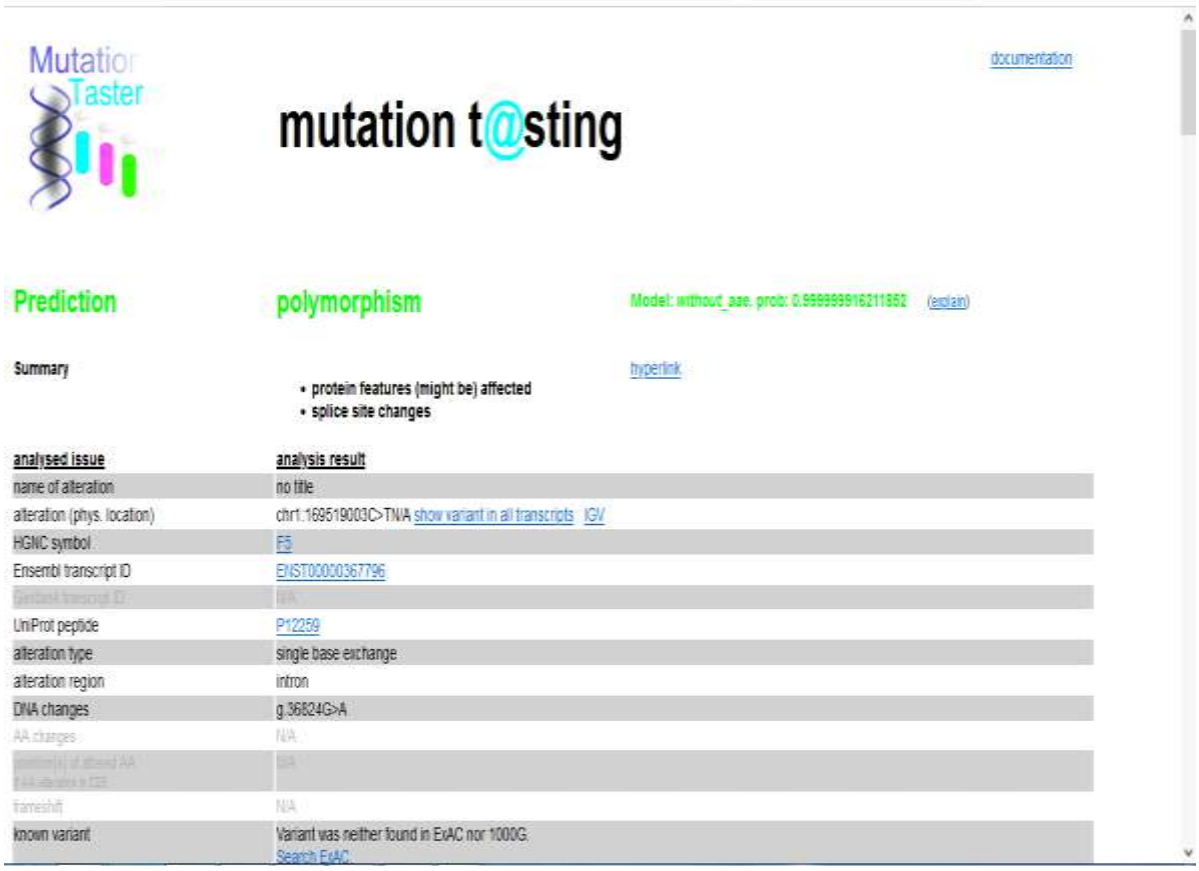

Figure (4): result of G>A singles Base Exchange tested in mutation taster application

Discussion:

Intercereberal hemorrhage is a severe disease resulting in high mortality and a large proportion of unfavorable functional outcomes, sometimes being recurrent and causing even more devastating consequences, such a disorder requires timely risk factor evaluation and prevention [9]

This was a cross sectional hospital-based study conducted at the research laboratory of the national center of neurological sciences (NCNS), Khartoum, Sudan, to detect factor V gene polymorphism (G1691A) among Sudanese patients with intracerebral hemorrhage.

Age related strokes are one of the leading causes of death in the United States, and intracerebral hemorrhage is the deadliest type of stroke. Age affects the body in many ways, including changes to the cardiovascular and neural systems that interact with other risk factors. Age is a significant risk factor for intracerebral hemorrhages. Understanding how age affects risk and results can help inform future clinical studies and treatment. For risk factors for intracerebral hemorrhage, we searched the literature. In addition to characterizing the most often used age cut-off points in the literature, this review sought to define the function of age. According to a recent analysis of the literature, the age cut-off for mortality and morbidity ranges from 60 to 80 years of age, with 65 and 70 years being the most common numbers. [10]

However, the age cutoff threshold in patients with intercerebral hemorrhage is not indicated in the current study. Our current study revealed that male gender was twice as prevalent as female, despite the fact that there is no evidence to suggest that female gonadotropin is unlikely to be neuroprotective in patients with intercerebral hemorrhage and that several Western studies showed reduced morbidity

and mortality in female ICH patients compared to their male counterparts. [11]

Activated factor X and thrombin combine to form activated factor V from factor V. Factor V is subjected to proteolytic activity by activated factor X and thrombin, which removes a domain from factor V and causes it to become activated factor V. Another component in the coagulation cascade before factor V is activated factor X.

A downstream product of activated factor V and factor X activity, thrombin functions as a positive feedback mechanism to further boost its own synthesis. In a prothrombinase complex, which is formed when activated factor V and factor X combine, prothrombin is converted to thrombin. [12]

It has been demonstrated that the production of thrombin is greatly reduced when activated factor V is not present, and this finding confirms the importance of activated factor X in the coagulation cascade. Then, fibrin is formed from fibrinogen by thrombin. A fibrin clot is created when platelets and fibrin protein bond to one another. The clump of fibrin aids in stopping bleeding.

The impact of coagulopathy and platelet dysfunction on the bleeding diathesis has not been thoroughly discovered; the molecular genetic investigations of ICH were initially undertaken as linkage studies. Among Sudanese patients with various types of intracerebral hemorrhages, the factor V gene polymorphism (G1691A) was identified in the current study. In this context, our mutation was confirmed using various bioinformatic tools, and G>A polymorphism was predicted; this could change the protein's features and possibly the splicing site.

One study by Sykes et al. revealed a single point mutation, a guanine to adenine alteration at nucleotide position 1691 in the factor V gene, now known as Factor V Leiden, after identifying people with activated protein C (APC) resistance. 2 Due to the substitution of glutamine for arginine at position 506, caused by this mutation, APC is unable to recognize a major cleavage site on factor V, allowing Prothrombin activation to go unchecked. [13]

A variant mutant version of human factor V (one of numerous substances that aid in blood clotting) called factor V leiden also contributes to an increase in blood clotting (hypercoagulability). This mutation prevents protein C, an anticoagulant protein that typically suppresses factor V's proclotting action, from binding to factor V normally, resulting in a hypercoagulable state. [14]

In addition to this, Shang X et al said *Factor V Leiden (FVL) gene* mutation is a common polymorphism in FV that results in a guanine to adenine substitution at the nucleotide position, interestingly; exchange was found G to A (G1691A). [15]

The prevalence of factor V Leiden (FVL) in people of northern and central European descent suggests that FVL bestowed a survival advantage on those populations. In one study done by Corral et al, the presence of FVL reduced the risk of spontaneous intracranial hemorrhage by 5-fold.1 and was found that , FVL protected against hemorrhagic transformation of ischemic events associated with artherosclerotic cerebrovascular disease in subjects with a mean age of 66.4 years. Despite, our results in this current study revealed half of the patients have this polymorphysim, and the control group do not having it, this mutation can be taken as a risk factor in development of spontaneous intracranial hemorrhage.

In the present study, Factor V gene polymorphism was seen in the half of the patients,and never polymorphism seen in the control group.. our results providing sequencing analysis, one single Base Exchange was found G to A (G1691A). Mutation taster was used to confirm the mutation which revealed; G>A polymorphism was predicted, amino acid sequence was changed protein features might be affected and splice site also was changed.

Sykes et al mentioned; after the identification of individuals with activated protein C (APC) resistance, analysis of DNA revealed a single point mutation, a guanine to adenine transition at nucleotide position 1691 in the factor V gene, now known as Factor V Leiden.2 This mutation causes a substitution of arginine by glutamine at position 506, resulting in a failure of APC to recognize a major cleavage site on factor V, which allows Prothrombin activation to continue out of check [13]

A guanine to adenine substitution at nucleotide position 1691 (G1691A polymorphism), a missense mutation FV Arg506Gln, is caused by the Factor V Leiden (FVL) gene mutation, according to Shang X et al. As a result, intravascular coagulation problems

are brought on by the alteration of a single inactivation site of FV by activated protein C, which results in thrombophilia (an increased propensity to form thrombi). [15]

Furthermore Liu B, Zhang L, Yang Q study revealed; significant effect for Factor V Leiden with ICH, also Corral et al said; for factor V Leiden, the carriers seemed to be at lower risk of developing ICH, FVL protected against hemorrhagic transformation of ischemic events associated with artherosclerotic cerebrovascular disease in subjects with a mean age of 66.4 years. [16] In the other hand Nur Buyru et al reported; Factor V Leiden mutation doesn't seem to be associated with a risk of cerebrovascular disease. Also honghua Yi Xue Za Zhirevelated Factor V Leiden mutation is not a crucial risk factor of cerebral venous thrombosis.[17]

The prevalence of factor V Leiden (FVL) in people of northern and central European descent suggests that FVL bestowed a survival advantage on those populations. In one study done by Corral et al, the presence of FVL reduced the risk of spontaneous intracranial hemorrhage by 5-fold.1 and was found that , FVL protected against hemorrhagic transformation of ischemic events associated with artherosclerotic cerebrovascular disease in subjects with a mean age of 66.4 years. Despite, our results in this current study revealed half of the patients have polymorphysim in factor V, and the control group do not have any, this mutation can be taken as a risk factor in development of spontaneous intracranial hemorrhage.

Conclusion:

From the results of this study the following conclusions were conducted: Factor V gene polymorphism (G1691A) was detected and might be in association with intercereberal hemorrhage among Sudanese patients.

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