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Research Article

Vitamin D Receptors Gene Polymorphisms among Sudanese Patients with Intracerebral Hemorrhage, Khartoum State 2022.

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Abstract

Background:
Cerebral bleed, intraparenchymal bleed, and hemorrhagic stroke are all terms for intracerebral hemorrhage. It accounts for about 10% of all strokes. The prevalence of stroke among Sudanese people with diabetes was 2.5% in that country. A neuroprotective hormone called vitamin D receptor (VDR), also called the calcitriol receptor, controls the differentiation of monocyte-derived macrophage from monocytes, which aids in the removal of hematomas and neurological recovery following intracerebral hemorrhage (ICH).

The objectives of this study are to estimate the prevalence of Taq I gene polymorphism (rs731236) in Sudanese patients with intracerebral hemorrhage, estimate the prevalence of Taq I gene polymorphism (rs731236) in Sudanese people without intracerebral hemorrhage, and compare the two participant groups to determine whether there are any differences.

Material and methods:
This study was cross sectional hospital-based study, conducted at the research laboratory of the national center of neurological sciences (NCNS), Khartoum, Sudan. Patients attended to the center and diagnosed with intracerebral hemorrhage were included. DNA extraction was carried out from blood of all patients and controls, PCR for Vitamin D receptor gene was done and thus Sanger sequencing.

Results:
The PCR results revealed242 bp VDR gene presence in both cases and controls. Sequencingoutcomes showed the detection of VDR gene (T>C) rs731236 and (G>T) rs7975232 polymorphisms.

Conclusion:
VDR gene polymorphism TaqI (T>C rs731236) was found in 86%of cases suggesting this mutation might be a risk factor for Intracerebral hemorrhage.

Keywords: Intracerebral hemorrhage, VDR, gene, polymorphism, PCR, mutation

Supplementary information The online version of this article (https://doi.org/xx.xxx/xxx.xx) contains supplementary material,which is available to autho-rized users.

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Introduction:

The term "intracerebral hemorrhage," often referred to as "cerebral bleed," "intraparenchymal hemorrhage," and "case hemorrhagic stroke," refers to an unexpected bleeding into the brain's structures, ventricles, or both. Small penetrating artery rupture as a result of hypertensive alterations or other vascular anomalies is typically the cause of [1,2]. Symptoms can include a headache, neck stiffness, vomiting, one-sided weakness, seizures, and diminished level of awareness. [3,4]

Hypertension, cigarette smoking, excessive alcohol consumption, low low-density lipoprotein cholesterol, low triglycerides, and medications such as anticoagulants, antithrombotic agents, antiplatelet agents, and sympathomimetic medicines are all modifiable risk factors (Cocaine, heroin, amphetamine and ephedrine).

Old age and male sex are two risk variables that cannot be changed. Asian ancestry, cerebral microbleeds, chronic kidney illness, and cerebral amyloid angiopathy (CAA). Multi-parity, poor working conditions (blue-collar occupation, longer working hours), and prolonged sleep are some additional characteristics that have been linked to risk. [5,6]

A class of fat-soluble secosteroids called vitamin D is in charge of enhancing the intestinal uptake of calcium, magnesium, phosphate, and many other biological consequences. The primary natural source of the vitamin is the chemical reaction that produces cholecalciferol in the lower layers of the epidermis of the skin in response to sun exposure (specifically UVB radiation). Ergocalciferol and cholecalciferol can be consumed as supplements and in food. The biological activity of vitamin D obtained through food or skin production is inactive. Two protein enzyme hydroxylation processes—the first in the liver and the second in the kidneys—activate it. [7]

The calcitriol receptor, often known as the vitamin D receptor (VDR). is a transcription factor that belongs to the nuclear receptor family. The VDR gene on chromosome 12q13 encodes the vitamin D receptor in humans. 11. The VDR gene polymorphism (rs731236 [TaqI]), which is the most frequent cause of intracerebral hemorrhage, was previously thought to be associated with hypertension. When there is a variant in the VDR gene, this results in functionally abnormal Vitamin D, which increases the risk of hypertension-mediated intracerebral hemorrhage and represents poor outcomes for the patients. The VDR gene provides instructions for making a protein called VDR, which allows the body to respond to Vitamin D. [8]

According to published data, intracerebral hemorrhage (ICH) is the second most common cause of stroke, accounting for between 10-15% of all strokes. ICH is also associated with much higher mortality, ranging from 35-52%, and some studies have suggested that Vitamin D Receptor (VDR) variants may act as a novel facilitator of hypertension-mediated intracerebral hemorrhage. This study will be designed to highlight these points and will attempt to determine the relationship between VDR

Material and methods:

This was a cross sectional hospital-based studyconducted at the research laboratory of the national center of neurological sciences (NCNS), Khartoum, Sudan during the period from 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 controls group.

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. This 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 was fully informed about the advantages and disadvantages before participation in the research (verbal informed consent).From each participant 3 ml of venous blood was collected from the antecubital vein using a dry sterile disposable syringe and needle. Blood samples was dispensed into sterile containers with Ethylene Diamine Tetra-acetic Acid (EDTA), label with subject's age, sex and identification number and was stored at -20°C for molecular analysis. Then, G-DEX IIB Genomic DNA extraction Kit was used. Primers were designed by using Prime3 software. The forward primer for rs731236 was(5-CTGCCGTTGAGTGTCTGTGT-3),reveres primer was (3- TCGGCTAGCTTCTGGATCAT-5) with product size of 242bp fragment. Forpolymerase chain reaction PCR preparation14 ul double distilled water wasplaced in PCR tube, then 4 ul of master mix, 1 ul of forward primer, 1 ul of reverse primer and 2 ul of DNA sample was added then vortex. The PCR tube containing this mixture wasplaced in commercial thermal cycler (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 wasadjusted for 5 minutes at 72 °C.PCR reaction was set at 35 cycles.The PCR amplification product was separated on agarose gel (0.7g of agarose gel + 28 ml of DW + 7 ml T.E buffer, this mixture wasplaced in microwave for 1 minute and then 1 ul of ethidium bromide was added and mixed then poured on gel-electrophoresis tray and left to dry). After it dried the PCR amplification products and 100-base-pair DNA ladder were filled in the wells, then the running buffer was poured and ran at 150V for 16 minutes and the results were trans-illuminated with UV light and visualized through gel documented system, products were sent for sequencing to Macro gene Europe Laboratory.

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. Data was expressed as means with standard deviations (SD). The statistical analysis was performed by the analysis of variance. A value of $P < 0.05$ was Considered statistically significant. The sequencing results were analyzed using different bioinformatics soft-wares and tools.The obtained sequences, were aligned using BioEdit-ClustalW software with a reference sequence from GenBank (National Center of Biotechnology Information), to examine the presence of polymorphisms.

Results:

In the case group; 64% were male and 36% were female, the most affected age group were more than 70 years (38%), followed by less than 50 years and 50-70 years (34%, 28%) respectively. Most of them were from Khartoum state (66%). In addition to that about 42% hadn't history of chronic disease, 26% had a hypertension and 24% were diabetic.Regarding the types of cerebral Hemorrhage; 64% had a subdural hemorrhage and 30 % had intercereberal hemorrhage. (Table 1,2,3)

Table (1) Socio-demographic data of the cases

Sociodemographic		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**
Residance	Khartoum	33	66.0
	Aljazirah	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 chronic disease 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**

Molecular studies:VDR (TaqI) allele(242) bp was detected with gel electrophoresis after PCR. It was positive in all the population of the study.

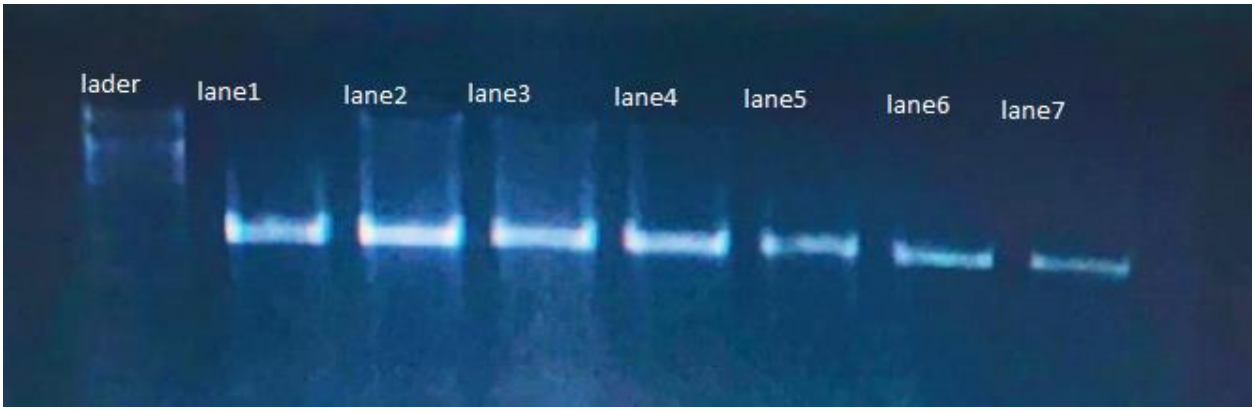

Figure1: *Shows 242 bp of factor VDR gene detected with gel electrophoresis*

Sequencing results of the cases were compared with the normal reference gene. Two single base exchanges were found(G>T) and (T>C). While when the controls were compared with normal reference, no single base exchange was found among all thecontrols group. (Figure 2,3)

Mutation taster was used to confirm the mutations .T>C Base Exchange polymorphism outcomes were predicted. The prediction confirmed the presence of **rs731236**polymorphism at chromosome 12cDNA changes at position 1336. (Figure 4) This SNP was found in 86**%**of cases.

Also G>T base exchange polymorphism was predicted, protein features might be affected and alteration location was at chromosome 12, alteration type was single base with reference ID**rs7975232**. (Figure 5)

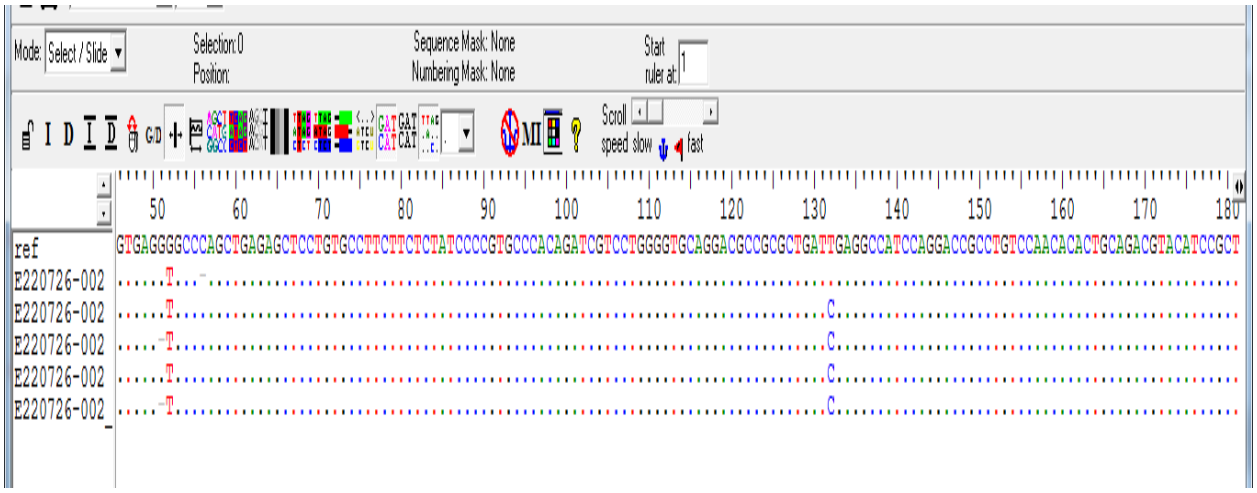

Figure 2: *Shows multiple sequence alignment using Bio-Edit clustal W for cases group with reference gene sequence of VDRTaqIgene.*

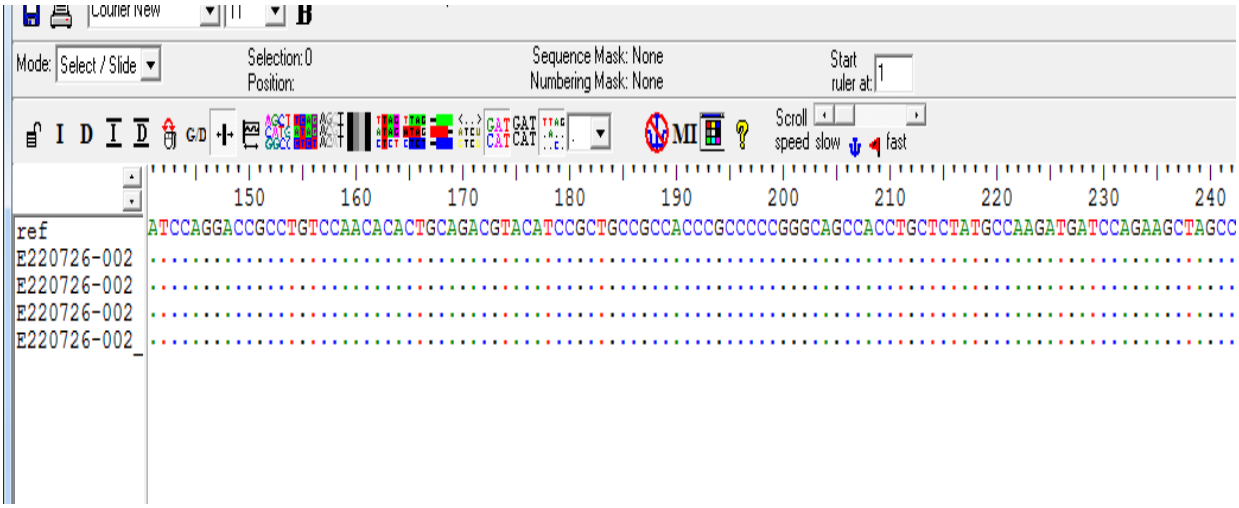

Figure 3: *Shows multiple sequence alignment using Bio-Edit clustal W for control group withreference gene sequence of VDRTaqI gene*

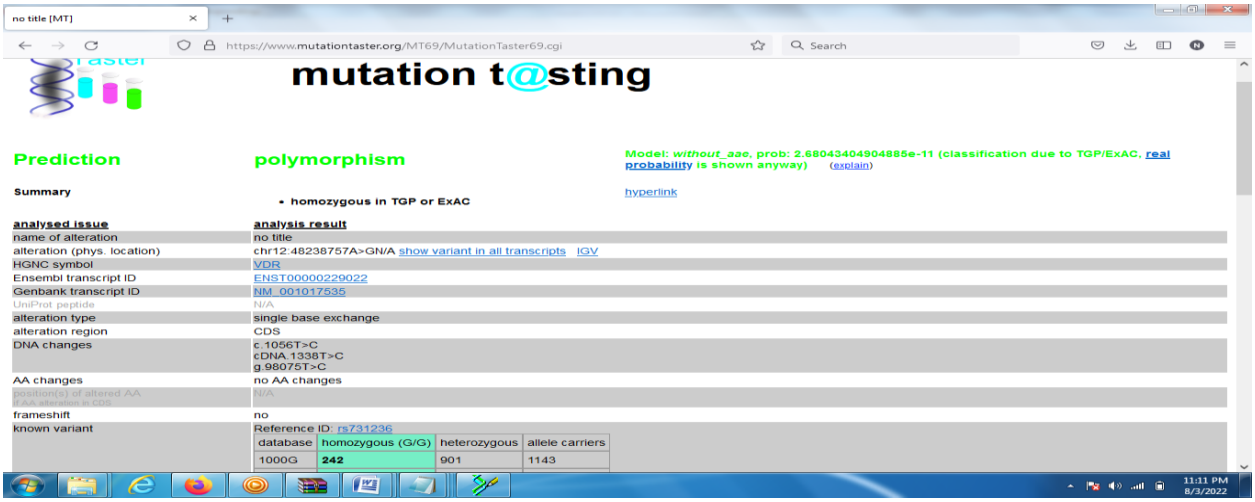

Figure 4: *Shows result of T>C singles base exchange tested in mutation taster application.*

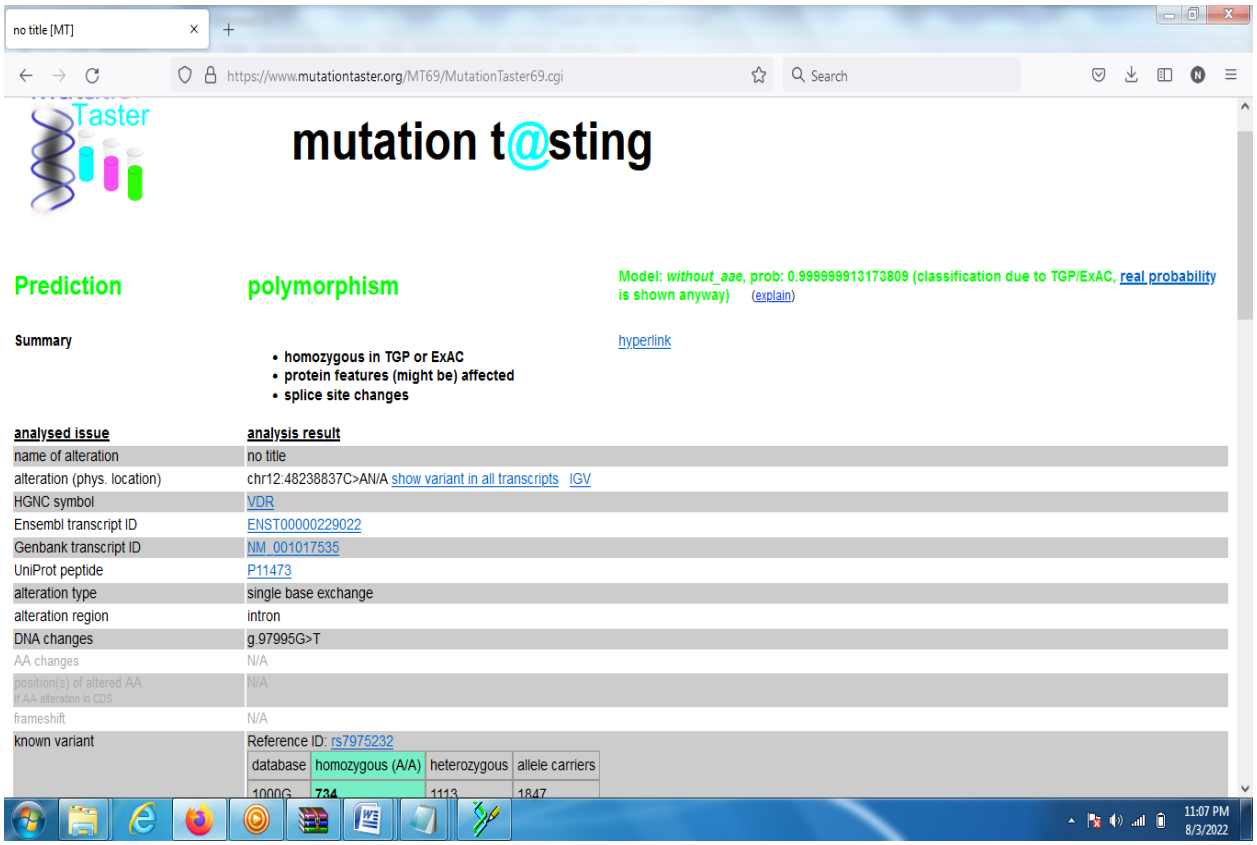

Figure 5: *Shows result of G>T singles base exchange tested in mutation taster application.*

Discussion:

The severe condition known as primary intracerebral hemorrhage (ICH) is widespread. There has been minimal research into sex differences in incidence, pathogenesis, or result after ICH, despite the fact that gender differences can predispose people to developing ICH and alter outcomes. Following a brain injury, loss of female hormones, such as menopause, has an impact on outcomes in both humans and preclinical animals. [9] In addition, the incidence of stroke is higher in men, but the severity and case fatality at one month are worse in women. Intracerebral hemorrhage (ICH) can account for 10 to 15% of all strokes, depending on the population studied. In this study, there were almost twice as many men as women [10,11].

As people age, their risk of acquiring ICH rises. Compared to younger people, the risk of ICH is five times higher in the elderly. [34] It is well recognized that increases in erythrocyte fragility are caused by. [12] There is proof that advancing age is linked to increased complement activation. This contributes to the development of membrane attack complexes (MAC). causing erythrocyte lysis [13,14] and, as a result, hemoglobin- or iron-mediated neurotoxicity. [39] and the development of cerebral edema following ICH. [15]

Additionally, aging increases the chance of systemic diseases including diabetes and hypertension as well as chronic illnesses. [16] that may be involved in the pathophysiology of ICH. An important number of ICH patients are elderly; according to a recent study, 34% of ICH patients were 80 years of age or older. [17] The most prevalent impacted age in the current study is over 70 years old.The most frequent factor leading to primary intracerebral hemorrhage is hypertension (ICH).[18] This condition is combined with diabetes mellitus, which has

been linked to an increased risk of spontaneous intracerebral hemorrhage and, in one study, to mortality within 30 days of hospital discharge.[19]

According to the most recent research on these chronic conditions, hypertension is a major risk factor for intracerebral hemorrhage development. However, a large portion of the individuals in this study do not have either diabetes or hypertension.

Notably, vitamin D deficiency is linked to an increase in renin expression and the production of the angiotensin II converting enzyme, which results in hypertension.On the other hand, type 2 diabetes has been linked to low vitamin D levels.[20,21]Interestingly, our findings indicated that patients with hypertension and diabetes mellitus had an increased risk of intracerebral hemorrhagic stroke.

Furthermore, the vitamin D receptor (VDR), a protein expressed by the VDR gene, mediates both the physiological function and the biological activities of vitamin D. The antioxidant properties of this gene (vitamin D) protect nerve cells. [22] After an intracerebral hemorrhage, vitamin D, a neuroprotective hormone that controls the differentiation of monocyte-derived macrophage from monocytes and aids in hematoma clearance, aids in neurological recovery (ICH). [23]Regardless of the kind of stroke, intracerebral hemorrhage has been linked to vitamin D receptor mutations in our study. Vitamin D is an organic substance made up primarily of fat-soluble secosteroids that regulates calcium and phosphorus levels in addition to other physiological processes. The active form of vitamin D is calcitriol [1,25(OH)2D3], which dimerizes with the retinoid X receptor (RXR) and translocates to the nucleus where it binds to VD response elements (VDRE). [24,25,26,27]

Interestingly, our research has been more focused on the connection between VD and stroke. Numerous G>T and T>C mutations were discovered in this setting. It was predicted that there would be a T>C base exchange polymorphism, which validates the existence of the rs731236 polymorphism at the 1336 position of the chromosomic DNA alterations. Additionally, G>T Base Exchange Polymorphism was predicted, protein characteristics may be impacted, and chromosome 12 was the site of the mutation. The single base modification had the reference ID rs7975232.

Conclusion:

In the conclusion VDR gene (T>C) **rs731236** and (G>T) **rs7975232**polymorphisms were detected and had a clear relation with Intracerebral hemorrhage in Sudanese patients.VDR gene polymorphisms **TaqI (**T>C **rs731236)**were found in 86**%**of cases suggesting this mutation might be a risk factor for Intracerebralhemorrhage.

Reference:

[1]-Hemphill JC, 3rd; Greenberg, SM; Anderson, CS; Becker, K; Bendok, BR; Cushman, M; Fung, GL; Goldstein, JN; Macdonald, RL; Mitchell, PH; Scott, PA; Selim, MH; Woo, D. "Guidelines for the Management of Spontaneous Intracerebral Hemorrhage. Stroke 2015; 46 (7): 2032–60. doi:10.1161/str.0000000000000069.

[2]-Naidich, Thomas P.; Castillo, Mauricio; Cha, Soonmee; Smirniotopoulos, James G. (2012). Imaging of the Brain, Expert Radiology Series,1: Imaging of the Brain. Elsevier Health Sciences. p. 387. ISBN 978-1416050094. Archived from the original on 2016-10-02.

[3]-Qureshi AI, Mendelow AD, Hanley DF. Intracerebral haemorrhage. *Lancet.* 2009;373:1632–1644. [PMC free article] [PubMed] [Google Scholar]

[4]-Garcia JH, Ho KL. Pathology of hypertensive arteriopathy. Neurosurg Clin N Am. 1992;3:497–507.

[5]-Ariesen MJ, Claus SP, Rinkel GJ, Algra A. Risk factors for intracerebral hemorrhage in the general population: a systematic review. *Stroke*. 2003;34:2060–2065.

[6]-Sturgeon JD, Folsom AR, Longstreth WT, Jr, Shahar E, Rosamond WD, Cushman M. Risk factors for intracerebral hemorrhage in a pooled prospective study. *Stroke*. 2007;38:2718–2725.

[7]- Norman AW . "From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health". The American Journal of Clinical Nutrition. 2008; 88 (2): 491S–499S. doi:10.1093/ajcn/88.2.491S. PMID 18689389.

[8]- Sabooh, M.F., Iqbal, N., Khan, M., Khan, M. and Maqbool, H.F. Identifying 5-Methylcytosine Sites in RNA Sequence Using Composite Encoding Feature into Chou's PseKNC. JournalofTheoretical Biology2018; 452, 1-9 https://doi.org/10.1016/j.jtbi.2018.04.037.

[9]-Herson PS, Koerner IP, Hurn PD. Sex, sex steroids, and brain injury.

[10]-Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, de Simone G, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Greenlund KJ, Hailpern SM, Heit JA, Ho PM, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, McDermott MM, Meigs JB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Rosamond WD, Sorlie PD, Stafford RS, Turan TN, Turner MB, Wong ND, Wylie-Rosett J: Heart disease and stroke statistics – 2011 update: a report from the American Heart Association. Circulation 2011;123:e18–e209.

[11]-Appelros P, Stegmayr B, Terent A: Sex differences in stroke epidemiology: a systematic review. Stroke 2009;40:1082–1090.

[12]-van Asch, C. J., Luitse, M. J., Rinkel, G. J., van der Tweel, I., Algra, A., and Klijn, C. J. (2010a). Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. *Lancet Neurol.* 9, 167–176. doi: 10.1016/S1474-4422(09)70340-0.

[13]-Orbach, A., Zelig, O., Yedgar, S., and Barshtein, G. (2017). Biophysical and Biochemical Markers of Red Blood Cell Fragility. *Transfus Med. Hemother.* 44, 183–187. doi: 10.1159/000452106.

[14]-Gong, Y., Xi, G., Wan, S., Gu, Y., Keep, R. F., and Hua, Y. (2008). Effects of aging on complement activation and neutrophil infiltration after intracerebral hemorrhage. *Acta Neurochir. Suppl.* 105, 67–70. doi: 10.1007/978-3-211-09469-3_14.

[15]-Hua, Y., Xi, G., Keep, R. F., and Hoff, J. T. (2000). Complement activation in the brain after experimental intracerebral hemorrhage. *J. Neurosurg.* 92, 1016–1022. doi: 10.3171/jns.2000.92.6.1016.

[16]-Yang, S., Nakamura, T., Hua, Y., Keep, R. F., Younger, J. G., He, Y., et al. (2006a). The role of complement C3 in intracerebral hemorrhage-induced brain injury. *J. Cereb. Blood Flow Metab.* 26, 1490–1495. doi: 10.1038/sj.jcbfm.9600305.

[17]-Yuan, J. J., Zhang, Q., Gong, C. X., Wang, F. X., Huang, J. C., Yang, G. Q., et al. (2019). Young plasma ameliorates aging-related acute brain injury after intracerebral hemorrhage. *Biosci. Rep.*39:BSR20190537. doi: 10.1042/BSR20190537.

[18]-Xi, G., Hua, Y., Keep, R. F., Younger, J. G., and Hoff, J. T. (2002a). Brain edema after intracerebral hemorrhage: the effects of systemic complement depletion. *Acta Neurochir. Suppl.* 81, 253–256. doi: 10.1007/978-3-7091-6738-0_66.

[19]-Ariesen, M. J., Claus, S. P., Rinkel, G. J., and Algra, A. (2003). Risk factors for intracerebral hemorrhage in the general population: a systematic review. *Stroke* 34, 2060–2065. doi: 10.1161/01.STR.0000080678.09344.8D.

[20]-Stein, M., Misselwitz, B., Hamann, G. F., Scharbrodt, W., Schummer, D. I., and Oertel, M. F. (2012). Intracerebral hemorrhage in the very old: future demographic trends of an aging population. *Stroke* 43, 1126–1128. doi: 10.1161/STROKEAHA.111.644716.

[21]-Mohr JP, Caplan LR, Melski JW, Goldstein RJ, Duncan GW, Kistler JP, Pessin MS, Bleich HL. The Harvard Cooperative Stroke Registry: a prospective registry. **Neurology**.1978; *28*:754-762.

[22]-Kornum JB, Thomsen RW, Riis A, Lervang HH, Schønheyder HC, Sørensen HT. Type 2 diabetes and pneumonia outcomes: a population-based cohort study. Diabetes care. 2007 Sep 1;30(9):2251-7.

[23]-AFZAL S., BOJESEN S.E. and NORDESTGAARD B.G.: Low 25-hydroxyvitamin D and risk of type 2 diabetes: A prospective cohort study and meta-analysis. Clin. Chem., 59: 381-391, 2013.

[24]-Song Y., Wang L., Pittas A.G., Del Gobbo L.C., Zhang C., Manson J.E. And Hu F.B.: Blood 25-Hydroxyvitamin D Levels And Incident Type 2 Diabetes: A Meta-Analysis Of Prospective Studies, 2013.

[25]-Kesby JP, Eyles DW, Burne TH, McGrath JJ (2011). The effects of vitamin D on brain development and adult brain function. *Mol Cell Endocrinol,* 347: 121-127.

[26]-Liu J, Li N, Zhu Z, Kiang KM, Ng ACK, Dong CM, Leung GK. Vitamin D Enhances Hematoma Clearance and Neurologic Recovery in Intracerebral Hemorrhage. Stroke. 2022 Jun;53(6):2058-2068. doi: 10.1161/STROKEAHA.121.037769. Epub 2022 May 6. PMID: 35514286.

[27]- Kongsbak M, Levring TB, Geisler C, von Essen MR. Thevitamin D receptor and T cell function. Front Immunol. (2013)4:148. doi: 10.3389/fimmu.2013.00148.