Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

The role of different matrixin gene expressions on cerebral bleeding among patients with deficiency of coagulation factor XIII

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ARTICLE INFO

Original paper

Article history:
Received: July 13, 2022
Accepted: August 14, 2022
Published: August 31, 2022

Keywords:

Cerebral hemorrhage, matrix metalloproteinase, coagulation factor XIII

ABSTRACT

Enzymes of the matrixin family could be seen as a critical determinant in the breakdown of the extracellular matrix, cell membrane, and tissue regeneration and are interned in the process of brain bleeding. On the other hand, coagulation factor XIII deficiency is a sporadic hemorrhagic disease with an estimated prevalence of 1 in 1-2 million people. Cerebral hemorrhage is the leading cause of death in these patients. This study investigated the relationship between the expression of matrix metalloproteinase 9 and 2 genes with cerebral hemorrhage in these patients. For this purpose, in this case-control study, by examining the clinical and general findings of the studied patients, the Q-Real-time RT-PCR method was used to quantitatively examine the mRNA levels of matrix metalloproteinase 9 and 2 in 42 patients with hereditary deficiency of coagulation factor XIII, including two groups with and without a history of cerebral hemorrhage (case and control groups, respectively). A comparative method (2-ΔΔCT) was used to check the expression level of the target genes. The GAPDH gene expression levels were used to standardize the expression of the measured matrix metalloproteinase genes. The results showed that bleeding from the umbilical cord was the most common clinical symptom among all patients. High levels of MMP-9 gene expression were observed in 13 patients of the case group (69.99%) and three patients of the control group (11.9%). which showed a significant difference (CI: 2.77-95.3, P=0.001) Patients with coagulation factor XIII deficiency show a wide range of clinical symptoms crucial in screening and diagnosing this group of patients. Based on the results of this study, it seems that the increased expression of the MMP-9 gene is due to polymorphism or inflammation related to the pathogenesis of cerebral hemorrhage in this category of patients. It may be conceivable to diminish this impact by utilizing MMP-9 inhibitors and offering assistance to diminish these patients' hospitalization and passing rates.

Doi: http://dx.doi.org/10.14715/cmb/2022.68.8.23 Copyright: © 2022 by the C.M.B. Association. All rights reserved.

Introduction

Coagulation factor XIII (FXIII) is a protein from the group of transglutaminases that has two catalytic A subunits (FXIII-A) and two transporter B subunits (FXIII-B) which its presence is required in the final stages of the coagulation cascade (1). Active factor XIII mechanically strengthens the fibrin clot with gamma glutamyl-lysine bridges and, in this way, stabilizes the clot (2). In addition, by increasing the binding of z-plasmin to the newly formed fibrin network, the activated coagulation factor XIII prevents its destruction by the fibrinolytic system (3). Hereditary deficiency of coagulation factor XIII is inherited as an autosomal recessive trait. It is scarce and, at the same time, one of the categories of severe and fatal hemorrhagic diseases. The average prevalence of the severe form of the disease varies among different world populations, but in general, the frequency of 1 in 3-5 million people has been reported (4).

Depending on the factor XIII plasma levels, the clinical symptoms of factor XIII deficiency will vary from gentle to serious (5). The disease is related to serious bleeding, unconstrained central anxious framework (CNS) dying, unconstrained premature birth, deferred wound recuperating, and umbilical line dying that happens some days after birth (6). Cerebral hemorrhage is the first cause of death and the last symptom of a patient suffering from coagulation factor XIII deficiency. This symptom is life-threatening and the most important cause of death in this group of patients due to bleeding causes. In general, despite modern treatments, intracranial hemorrhage, for whatever reason, is recognized as one of the most severe diseases related to the central nervous system (7).

Cerebral hemorrhage occurs in 30% of patients with hereditary factor XIII deficiency, and it is the most common cause of death in these patients compared to other genetic hemorrhagic diseases (3). All patients with severe deficiency of coagulation factor XIII (FXIII <1 U/dl) receive replacement treatments with products such as cryoprecipitate, fresh frozen plasma (FFP), or purified concentrate of factor XIII (8).

Additionally, matrix metalloproteinases (MMPs) are a large family of calcium-dependent, zinc-containing endopeptidases that play an essential role in tissue regeneration and the breakdown of the extracellular matrix, including collagens, gelatin, glycoproteins, and matrix proteogly-

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cans (9). The basement membrane has an abundant type of elastin, collagen, laminin, and fibronectin. The interesting thing to note in this regard is the excellent ability of two enzymes, 2-MMP and 9-MMP, to break down collagen type 4 (the most significant protein in the extracellular matrix), which is more than all other proteinases (10). This ability can be essential in breaking down the blood-brain barrier and causing damage. There is a lot of information about the increased expression of 2-MMP and 9-MMP genes have many effects on the pathogenesis of intracranial hemorrhage (10, 11).

Many studies have shown that matrix metalloproteinases cause the breakdown of essential lamin compounds, which ultimately leads to the destruction of the blood-brain barrier and is also involved in inflammatory responses to some neurological diseases (11-13). While to prevent fatal clinical complications, patients with severe factor XIII deficiency receive alternative preventive therapies so that factor 13 concentration compensates for their deficiency or factor deficiency. Still, intracranial bleeding is the leading cause of death related to bleeding in these patients (13). Cerebral hemorrhage frequently occurs in these patients and affects about 30% of patients (14). This study aimed to investigate the effects of changes in the expression level of MMP-2 and MMP-9 genes on the pathogenesis of cerebral hemorrhage in patients with severe hereditary deficiency of factor XIII.

Materials and Methods

Study population

This case-control study was conducted on 42 patients with severe hereditary deficiency of coagulation factor XIII over ten months. Overall, forty-two patients were selected for the present research, and the history of intracranial bleeding in 18 was confirmed by medical records (Computerized tomography scan, CT scan). The remaining 24 patients were selected as the control group after age and gender matching. To guarantee the conditions of the patients for their support within the consider, MRI L. CT scan was performed 24 or 48 hours after the onset of cerebral hemorrhage symptoms was examined. A signed consent letter was received from all the participants.

Patient information was extracted from medical records and questionnaires completed by the attending physician. All patients with factor XIII deficiency and a history of intracranial bleeding were selected to enter the study. Exclusion criteria included incomplete medical records, factor XIII deficiency with other bleeding disorders, and any evidence of amyloid angiopathy, liver disease, or brain tumor. In addition, all patients were interviewed by trained personnel, and they completed a questionnaire including demographic information, age, gender, symptoms of bleeding during life, and period at the time of cerebral hemorrhage. All patients were regularly subjected to diagnostic tests for meningoencephalitis, bacterial meningitis, HIV, HBV, HAV, and HCV.

Clinical examinations

Patients with severe deficiency of factor XIII often had an abnormal 5 M urea solubility test, indicating their strong tendency to bleed in different tissues. Full medical examinations were performed on all patients participating in the study by a doctor specializing in bleeding diseases and documented. Laboratory and clinically examined patients for all evidence of amyloid, liver disease, brain tumors, and viral infections.

RNA extraction

After collecting blood samples in vacuum tubes containing EDTA by personnel trained in blood collection using sterile methods, the samples were placed in a -75°C freezer for RNA isolation at another time. Total RNA was extracted using the Invitrogen kit (TRIzol reagent) according to the manufacturer's instructions. The quality of the extracted RNA was verified using a spectrophotometer and 2% agarose gel.

DNA production and PCR analysis

Using the RT-PCR one-step script Super kit (Bioneer, South Korea) and suitable primers, the amount of 10A RNA was converted into cDNA (Complementary DNA). The primers used for GAPDH (Glyceraldehyde P-phosphate dehydrogenase) (Clontech) were used as the housekeeping gene (control). The obtained product had a single and specific band in agarose gel electrophoresis.

Real-time quantitative RT-PCR

The primers used to analyze the expression of 9-MMP-2 MMP, and GAPDH genes are listed in Table 1. All the primers were designed using specific primer analysis software (Gene runner), and the desired sequence was blasted in the relevant database (http://www.ncbi.nlm.nih.gov/pubmed). Real-time RT-PCR was performed using the SYBR green kit (Fermentas, UK) in the ABI machine, step one (Applied Biosystems). After PCR, the analysis curve (Melting curve) was checked to confirm the product's characteristics to be tested.

The expression level of genes was calculated in comparison with the changes in the expression level of the GAPDH gene as a control gene using the formula 2-ΔΔCT in Light cycler apparatus software (Roche, Germany). To ensure the specificity of the amplified product, all samples were examined in 2% agarose gel electrophoresis. The results were calculated using a percentage, median, mean, and t-test in SPSS software (version 18, SPSS Inc., Chi-

Table 1. Primers were used to analyze the expression of MMP-9, MMP-2, and GAPDH genes.

Gene	Gene Full Name	Primer Sequences (5'-3')		Product Length
MMP-9	Matrix metalloproteinase-9	Forward	GTGGTTCCAAACCTCAAGAA	216 bp
		Reverse	CAGCGGTCCTCAGTGACACAT	
MMP-2	Matrix metalloproteinase-2	Forward	ACAAAGGGATTGCCAGGACC	402 bp
		Reverse	ACCTCTAGCGTCAATCGTAGC	
GAPDH	Glyceraldehyde 3-phosphate	Forward	CCGGAGTCACATTGGTATGTG	550 bp
		Reverse	TCAGCCTTCTGGTCAAGACGGG	

cago, IL). P<0.050 was considered a significant level.

Results

Forty-two coagulation factor XIII deficiency patients, including 22 men and 20 women, were selected for the study. The clinical information of these people is shown in Figure 2. The average age of the patients at the time of entering the study was 1.15 years (between 14.2-25.0 years). Patients were equally divided in terms of age and gender. The average age in the case group at the time of cerebral hemorrhage was 37 months. The first clinical tissue related to the disease was umbilical cord bleeding, which was recorded in more than 80% of patients. In general, in the case and control groups, the most common clinical finding in the patients was bleeding from the umbilical cord. Routine tests were performed to reveal the individual's infection with HBV, HIV, and HCV during six-month intervals for all patients who received blood and were reported negative. Also, laboratory studies showed that viral meningoencephalitis or meningitis did not cause brain bleeding in patients. Depending on the severity of the disease, all participants in the survey received alternative treatments in the form of Fibrogammin P (XIII) (FFP, Behring, Germany) or cryoprecipitate to prevent bleeding.

Intracranial bleeding in patients

At the time of cerebral hemorrhage, patients showed symptoms including seizures (83.33%) and nausea and headache (11.11%). Bleeding between parenchymal tissues was the most common type of intracranial bleeding in 72.22% of patients. Other forms of cerebral hemorrhage included subdural hemorrhage in 22.22% of people and simultaneous intraparenchymal and subdural tissue hemorrhage in 5.55% of patients. Intracranial bleeding was detected in 2 patients using MRI and in 16 patients using CT scan images. The hematoma volume was more than 30 ml in 8 patients, more than 20 ml in 3 patients, and between 10-15 ml in 6 patients. Bleeding recorded during the patients' lifetime with deficiency of coagulation factor XIII is shown in Figure 1.

Among all patients, alternative treatments were prescribed up to 2 days after the diagnosis of a cerebral hemorrhage. 72.22% of the patients in the case group received fresh frozen plasma and 17.78% cryoprecipitate after cerebral hemorrhage. Other patients received combined treatment with coagulation factor XIII concentrate with or without cryo or plasma. After treating patients, 27.33% of people had seizure complications. One patient also had complications related to mental retardation and microcephaly, while others had almost no side effects.

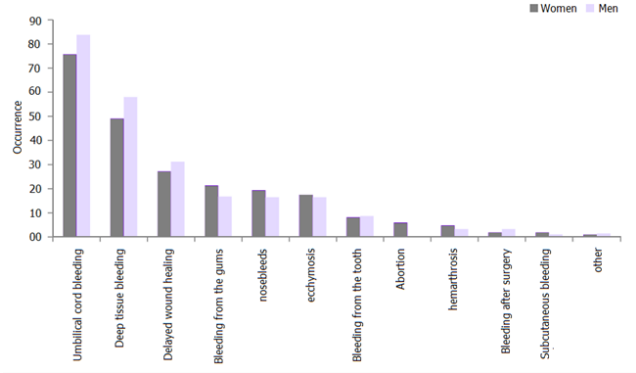

Figure 1. Bleeding was recorded during the patients' lifetime with deficiency of coagulation factor XIII.

Gene expression evaluation

After the numerical values of mRNA obtained in 2-MMP and 9-MMP genes were normalized according to the numerical values of GAPDH, it was observed that the high mRNA values of the 9-MMP gene in the case group compared to the control group showed a significant difference. It shows (CI, P = 0.001: 2.8-95.3), although, there was no significant difference between the expression of the MMP-2 gene and the occurrence of a cerebral hemorrhage. Also, there was no significant difference between the expression level of the target genes with the volume of bleeding, frequency of occurrence of clinical indications, gender, age, racial characteristics, and hemorrhage from specific tissues.

Discussion

Clinical studies show various complications and clinical signs in coagulation factor XIII deficiency patients (1, 6, 15, 16). Preventive treatment compensates for the deficiency of coagulation factor XIII (in the form of replacing it with other products), preventing bleeding. Nevertheless, 30% of patients with coagulation factor XIII deficiency still experience cerebral hemorrhage (17). It is the first cause of death in these patients. Finally, the study's laboratory results showed a significant relationship between the increase in MMP-9 gene expression and the pathogenesis of intracranial bleeding (18). In the central nervous system, it has been shown that matrix metalloproteinases 9 and 2 (gelatinases) cause the breakdown of basal lamina components, secondarily leading to the destruction of the blood-brain barrier (19). The effect of gelatinases on cerebral ischemic stroke and intracranial bleeding has been proven in many studies (15, 17, 20).

Abilleira et al. (21) first observed an increase in the gene expression of MMP-9 mRNA concentration in people's blood after intracranial cerebral hemorrhage. This study's results agree with the investigations of Abilleira et al. (21), Hernandez-Guillamon et al. (22), and other studies showing that increased MMP-9 gene expression is directly related to severe cerebral hemorrhage (21, 22). Gelatinases are involved in the destruction of the extracellular matrix, which plays a crucial role in the occurrence of intracranial hemorrhage. It should be mentioned that matrix metalloproteinases increase in various infections and inflammations. The patients of the study group are exposed to frequent preventive injections of different blood products, which may cause people to become infected with viruses or bacteria that are usually transmitted during blood transfusion due to infection during the injections or contamination of the products themselves and they are not screened (23).

These infections or other inflammations due to blood transfusions can lead to a sharp increase in gelatinase gene expression levels (24). In addition to these, many polymorphisms in MMP genes are involved in increasing the expression level of genes. These polymorphisms provide the basis for further studies in this matter (25). Finally, it was concluded that the increased expression of the matrix metalloproteinase-9 gene is due to polymorphism or in-

flammation related to the pathogenesis of cerebral hemorrhage in patients with coagulation factor XIII deficiency, and perhaps the use of gelatinase inhibitor drugs can reduce the number Death and hospitalization frequency of these patients.

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