Assessment of the Functional State of the Endothelium in Patients with Viral Hepatitis before Tooth Extraction

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ABSTRACT

Introduction: Tooth extraction is the most common operation, after which hemorrhagic complications often occur, especially in patients with chronic viral liver disease. This condition is caused by damage to the endothelial lining of blood vessels.

Objective: Based on this, this study aimed to study the features of endothelial dysfunction before tooth extraction in patients with viral hepatitis.

Methods: 58 patients with hepatitis B and C with different duration of the disease were examined. In patients with viral hepatitis before tooth extraction, an increase in platelet aggregation activity on the effect of an ADP inducer (Tma) was noted by 45%.

Results: The observed lengthening of the activated recalcification time (AVR) 37-37% in patients with viral hepatitis reflects a deficiency of plasma factors (XII, XI, XIII) of the blood coagulation system and indicates a state of hypercoagulation. Against this background, high values of alpha-2 macroglobulin in the blood (4 times) and Willebrand factor (15%) and a significant decrease (35%) in the content of protein C in the blood of the examined patients were noted.

Conclusion: The results of the study indicate that these patients have a narrow band of maintaining hemostatic balance, and the existing balance can easily be transformed into hypo- or hypercoagulation, which requires preventive measures to prevent complications after tooth extraction.

Key Words: Viral hepatitis B and C, Endothelium, Fibrinolysis, Thrombotic complications

INTRODUCTION

One of the most common operations in surgical dentistry is the extraction of teeth, which leads to the appearance of defects in the dentition that require orthopaedic treatment. It has been established that the main reasons leading to complications and unsatisfactory quality of dental treatment after a tooth extraction include: imperfection and insufficient information content of both clinical and instrumental-laboratory methods of examining patients, a decrease in complications in the perioperative period caused by disorders of the hemostasis system.¹ As is known, damage to the endothelium of a blood vessel during tooth extraction is a reason for the formation of a blood clot. At the local level, the forming clot is necessary to restore the continuity of the vascular wall, limit blood loss and heal the wound. Clot formation "in vivo" after tooth extraction begins after blood contact with some source of tissue factor (TF), usually subendothelial

cells, exposed when a blood vessel is damaged. TF-initiated blood coagulation occurs in two phases: the first phase is the initiation phase, the second is the amplification phase.^{2,3} The initiation phase begins from the moment when naked TF binds to factor VIIa, which is found in the circulating blood in picomolar concentrations. The formed TF-VIIa complex catalyzes the conversion of a small amount of factor X to Xa, which in turn produces an equally small amount of thrombin. Thrombin starts the amplification phase, during which a large amount of thrombin is generated and its concentration grows like an avalanche.⁴ Thrombin stimulates its production itself by activating platelets and several coagulation factors (V, VIII) and creates conditions for the formation of complex VIIa-IXa, after which the production of factor Xa switches from TF-VIIa-catalyzed mechanism to the internal coagulation pathway, thereby increasing the rate of Xa factor formation in 50 times.^{4,5}

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During the first few hours after surgery, the amount of tissue plasminogen activator (TAP) in the blood approximately doubles, then within 24 hours its level returns to normal. Starting from about the second hour of surgery, the blood level of the type I plasminogen activator inhibitor (IAP-I) begins to rise rapidly, its concentration increases by 4-5 times, and then gradually decreases over a day or so, but on day 7 it is noted new peak. During this time, a high level of IAP-I inhibits the fibrinolytic system - this process is called as turning off fibrinolysis. Currently, there is an optimal approach to the preoperative analysis of the hemostatic system during tooth extraction. It consists of laboratory screening of patients for whom surgery is planned. It is recommended to determine the partially activated thromboplastin time (APTT), prothrombin time (PTV) (expressed in prothrombin according to Quick or in INR), platelet count, blood clotting time (RTC). Preoperative studies of the hemostasis system cannot predict intraoperative hemostasiological disorders, and conventional biochemical tests (APTT, PTT) are ineffective in predicting them.⁶ Their direction and severity depend on many factors: on the nature of the pathology, the volume and trauma of surgery, on organ-specific parameters (different organs and tissues have different thromboplastin and fibrinolytic activity), on the patient's comorbidity. Moreover, the most labile link in the hemostasis system is fibrinolytic. Since it is possible both the inhibition of fibrinolysis by increasing the activity of the inhibitor of tissue plasminogen activator and the activation of fibrinolysis by increasing the activity of tissue plasminogen activator, which is synthesized by vascular endothelial cells.^{6,7}

The physiology of the hemostatic system is closely related to liver function. Liver parenchymal cells produce most of the factors of the coagulation and fibrinolytic systems, as well as thrombopoietin, which is responsible for the production of platelets from megakaryocytes.⁷ Chronic or acute liver diseases are often accompanied by serious disorders of the hemostatic system. In the scientific literature of the last decade, various aspects of disorders of the hemostasis system associated with chronic liver diseases have been actively discussed. The liver plays a key role in primary and secondary hemostasis. It is the site of synthesis of all coagulation factors and their inhibitors except for von Willebrand factor and is responsible for the elimination of activated factor-inhibitor complexes. Liver diseases lead to complex disorders in the hemostasis system, but at the same time a balance is maintained between the coagulation and anticoagulation systems with a reduced reserve, and this balance is easily disturbed in one direction or another. An increase in plasma glycosaminoglycans,⁸ which is observed in patients with liver disease, may reflect a defect in the endothelial glycocalyx. Besides, a decrease in the level of procoagulant proteins is accompanied in this category of patients by a decrease in the level of natural anticoagulants (protein C, protein S and antithrombin).

Thus, the cumulative effect of all changes in hemostasis is often balanced and the system remains functional.⁵ However, the compensatory abilities of the blood coagulation system are markedly weakened in liver diseases.

This leads, in the end, to the coagulation system to bleeding or thrombosis. In viral hepatitis, there is also a decrease in the synthesis of coagulation factors, vitamin K deficiency, thrombocytopenia, etc.³ It should be noted that in chronic liver diseases of viral aetiology, the concentration of procoagulant factors, as a rule, is reduced, however, the level of endogenous anticoagulants (antithrombin, proteins C and S) is also reduced, all this taken together maintain the hemostatic balance, which can be disrupted by structural damage to endothelial cells, which is often combined with infection with dysbacteriosis.^{2,3}

Based on the foregoing, the purpose of this research was to assess dysfunctional endothelial disorders in patients with viral hepatitis before tooth extraction.

MATERIALS AND METHODS

Total 58 patients with hepatitis B and C with different duration of the disease were examined. The studies were carried out in strict accordance with the requirements of biomedical ethics following the Geneva Convention on Human Rights (1997) and the Declaration of Helsinki of the World Medical Association (2000) based on the permission of the local ethics committee. In the group with chronic hepatitis by sex, the patients were distributed as follows: men - 58%, women 42%, the average age of patients was 35.6 ± 10.7 years. Written voluntary informed consent was obtained from all patients to participate in the study. Inclusion criteria: verified diagnosis of chronic viral hepatitis B and C detected by PCR using a test system, patients who have not used drugs for the last six months and have not received antiviral therapy. The study excluded patients under 18 years old with concomitant viral hepatitis D or other diseases that cause liver damage, HIV infection, a history of pulmonary tuberculosis, autoimmune, oncological diseases, as well as pregnant women. In order to determine the control values of the studied parameters of the hemostasis system, 16 practically healthy individuals aged 25 to 45 years were examined after giving an informed consent for examination, who did not differ from patients by sex and age, who, according to the anamnesis, the results of biochemical and serological studies, do not have viral hepatitis, as well as other liver diseases. The work used: instrumental studies: ultrasound examination of the abdominal organs, clinical and laboratory methods (determination of total and direct bilirubin alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, gamma-glutamyl transpeptidase (GGTP), homocysteine, total protein and protein fractions, cholesterol, amylase, urea,

creatinine. Indication. HCV-RNA, determination of the genotype of the virus, the level of viral load by polymerase chain reaction (PCR).

To assess the functional state of the vascular endothelium in patients, blood was taken in the morning, on an empty stomach, by gravity into a plastic tube. 5 ml of blood was stabilized with 3.8% sodium citrate solution in a ratio of 9:1. Centrifugation was performed immediately after blood collection. Plasma samples were analyzed no later than 3 hours after blood collection. The number of platelets in the haematological analyzer was calculated in all the examined patients. To study the adhesive and aggregation properties of platelets using the inducer adenosine diphosphate (ADP). The functional properties of platelets were determined visually using a phase-contrast microscope according to Shitikova T. A. (1997). Determination of activated recalcification time (ABP), fibrinogen (FG) in plasma according to the Clauss method was carried out according to a unified method on a DiaMed CD 4 apparatus (Switzerland). The anticoagulant activity associated with protein C (PS) and antithrombin III (AT-III) was investigated on an automatic digital photometer at a wavelength of 405 nm. [Barkagan Z.S. 2001], quantitative determination of the functional activity of von Willebrand factor using a kit for NPO "Renam" (Moscow), the content of alpha-2-macroglobulin by ELISA using kits from "HUMAN".

Statistical processing of the research results was performed using the STATISTICA 6.0 Microsoft and Office Excel 2003 software packages. When assessing the significance of differences in indicators obtained during dynamic observation, the paired Student's test was used. The null hypothesis was rejected, and the differences between the samples were considered statistically significant at $p < 0.05$.

RESULTS

Liver diseases lead to complex disorders in the hemostasis system, but at the same time, a balance between the coagulation and anticoagulation systems with a reduced reserve is maintained, and this balance is easily disturbed in one direction or the other, therefore, patients with severe liver damage may develop not only bleeding but and thrombosis. Based on the above, to prevent the risk of bleeding after surgery in the dentoalveolar system, we decided to study the nature of changes in the blood: the content and activity of platelets, the level of activated recalcification time (AVR), hemolysate aggregation test, which assesses the functional activity of platelets relative to the adenosine diphosphate ADP inducer and the level of fibrinogen. To determine the risk of thrombosis, we studied the von Willebrand factor activity, the level of protein C, antithrombin-III and the content of alpha-2 macroglobulin. It should be noted that all of the above hemo-

stasis disorders do not always lead to spontaneous bleeding. Infection (sepsis) is one of those factors that shift the balance towards hypercoagulation, significantly impairing clotting, and thus trigger the development of bleeding. The reason for this is the high level of cytokines, which leads to an increase in the level of tissue factor, activation of factors II, V, VII, X and a decrease in the concentration of the latter.

Recently, there has been an increased interest from researchers in assessing the functional state of the vascular endothelium and studying its role in the local regulation of hemostasis, since any damage to the endothelium of a blood vessel during tooth extraction is an incentive for the formation of a blood clot. At the local level, the forming clot is necessary to restore the continuity of the vascular wall, limit blood loss and heal the wound. To prevent excessive activation of the coagulation system and the development of pathological thrombosis, there are several inhibitory mechanisms: intact endothelial cells have anticoagulant properties, inhibitors of activated coagulation factors circulate in the blood, and local fibrinolytic enzymes are present. Considering that many of the studied factors of the coagulation system are synthesized in the endothelium and liver, we decided to assess the state of endothelial dysfunction in patients with viral hepatitis before tooth extraction. It should be noted that the therapy of coagulation disorders in patients with chronic viral hepatitis during surgical interventions in the dentoalveolar system is often carried out with bleeding or thrombosis after invasive procedures. At the same time, intensive therapy is often aimed at compensating for the deficiency of coagulation factors, platelets, fibrinogen, and correcting activated fibrinolysis.

It is known that platelets are strongly adsorbed to the area devoid of the endothelial lining during tooth extraction in patients with viral hepatitis, This leads to an increase in the active forms of platelets. The observed dynamics of the sum of active forms of platelets in patients with chronic viral hepatitis before tooth extraction tended to increase by an average of 1.7 times relative to the indicators of healthy individuals. The destruction and dysfunction of the endothelium activate the process of adhesion, platelets and the release of platelets from the cytoplasm of platelets of endothelial growth factor. As can be seen from the presented research results, the platelet adhesiveness index increases by 23%. A significant number of active platelets, while located not only in the lumen of the vessels of the oral mucosa but also in the gum mucosa itself (Table 1). This may be the reason for the activation of an atherogenic process at the site of endothelial destruction after tooth extraction in patients with viral hepatitis. At the site of surgical intervention, the effusion of red blood cells and its destruction can also occur, which leads to the release of ADP-platelet inducer from the erythrocyte, which enhances platelet aggregation. In patients with viral hepatitis before tooth extraction, we noted an increase in platelet aggrega-

tion activity on the effect of an ADP inducer (Tma) by 45%. The observed lengthening of the activated recalcification time (AVR) by 37% in patients with viral hepatitis reflects the deficiency of plasma factors (XII, XI, XIII) of the blood coagulation system and indicates a state of hypocoagulation. Against this background, coagulopathy of fibrinogen consumption was noted.

Table 1: Indicators of the functional state of platelets in patients with viral hepatitis before tooth extraction

Nº	Indicators	Healthy individuals n=16	Patients with viral hepatitis n=58
1	Sum of active forms platelets (%)	12.42±0.79	21.48±0.91*
2	Adhesiveness index Platelets (%)	42.17±2.12	51.87±1.09*
3	Platelet aggregation to stimulate ADP 1×10^{-3} M Tma%	34.18±2.47	49.51±3.48*
4	Activated time recalcification (sec)	57.81± 5.21	78.93± 6.87*
5	Plasma fibrinogen blood g / l	3.03±0.18	5.78±0.43*

*P <0.05 relative to the indicators of the comparison group

As you know, antithrombin-III is a specific protein of the blood coagulation system, which is synthesized in the vascular endothelium and liver. Its main function is to inactivate several major coagulation factors, including thrombin, and to prevent the increased formation of blood clots, thrombi. In patients with viral hepatitis, as shown in Table 2, there is a statistically significant decrease in the activity of antithrombin III compared with the control group, which reflects a decrease in its release by endothelial cells, as well as the index of the anticoagulant activity of the vascular endothelium. Usually, when a vessel is damaged and the endothelium dysfunctional, the body activates a series of coagulative factors (coagulation cascade) to form a blood clot and prevent further blood loss. Antithrombin III helps regulate this process - it slows down the action of several coagulation factors, including thrombin, as well as factors X, IX, and XI, which are designed to prevent excess blood clots from forming. At the same time, a decrease in the level of antithrombin III indicates the consumption of this anticoagulant, due to the activation of the hemostasis system, which indicates the risk of thrombosis. Lack of natural anticoagulants, primarily AT III, is unable to fully inhibit the increased level of thrombopenia, which can result from thrombotic complications in this pathology, on tooth extraction.

The balance between the state of the pro-and anticoagulant systems determines the risk of bleeding or thrombosis in patients with chronic viral hepatitis. Although bleeding is much more common, hemostatic imbalance in viral liver disease can lead to fatal thrombosis. Increased thrombotic activity is associated primarily with the lack of natural anticoagulants, which correlates with the severity of liver damage. Natural anticoagulants include protein C and alpha-2-macroglobulin. Analysis of the obtained research results shows a significant decrease (by 35%) in the content of protein C in the blood of the examined patients, which indicates a decrease in thrombin inhibition. In addition to the violation of protein C synthesis in the liver in viral liver pathology, the synthesis of defective proteins C with the absence of C-carboxylation of their molecules, which is associated with insufficient intake of vitamin K into the body, is not excluded. Vitamin K is an indispensable cofactor for the synthesis of biologically active forms of factors II, VII, IX and X. When the process of carboxylation in the liver is disturbed due to vitamin K deficiency or the action of its antagonists, inactive forms of these factors are formed.

Alpha-2-macroglobulin is the main inhibitor of a broad spectrum of proteinases, and it acts as a reserve inhibitor for several enzymes, including plasmin. Macroglobulin begins to exert a noticeable effect on the fibrinolysis system when the reserves of a-2-antiplasmin are depleted and the role of the main plasmin inhibitor is transferred to macroglobulin, although its efficiency concerning plasmin is 10 times lower than that of a2-antiplasmin. Macroglobulin plays the role of a trap, capturing the target, as a result of which access to the substrate is blocked from the outside. In our studies, high values of macroglobulin in the blood (4 times higher than those of the comparison group) in patients with viral hepatitis indicate an increase in the activity of an inhibitor of the plasmin system. Also, the development of hypercoagulable status can be facilitated by an increase in the content of procoagulant von Willebrand factor in the blood. As can be seen from the obtained research results, the percentage of von Willebrand factor in patients with viral hepatitis is increased by 15% relative to that in healthy individuals.

The processes of formation and destruction of a clot after tooth extraction in patients with viral hepatitis are closely intertwined with each other. The reason for this lies in the blood coagulation system. Thus, thrombin stimulates the release of tissue plasminogen activator (TPA) from the endothelium, which improves lysis; thrombin activates factor XIII, an enzyme that covalently cross-links the clot, making it more resistant to lysis; thrombin activates thrombin-activated fibrinolysis inhibitor (TAFI), impairing lysis. Plasmin, a nonspecific serine protease, can break down many coagulation factors, leading to its deterioration.

Table 2: Risk indicators of thrombosis in patients with viral hepatitis before tooth extraction

Indicators	Healthy individuals n=16	Patients with viral hepatitis n=58
Antithrombin-III (%)	88.35±6.12	74.23±5.71
Alpha-2-macroglobulin (mg / DL)	121.83±7.51	484.91±10.15*
Protein C	105.23± 7.54	68.15±4.75*
Von Wille brand factor (%)	81.37±4.56	96.82±5.74*

*P <0.05 relative to the indicators of the comparison group

Consequently, patients with chronic viral liver pathology have a variety of hemostatic defects that affect all links of the hemostatic system. Such patients have a narrow band for maintaining hemostatic balance, and the existing balance can easily be transformed into hypo- or hypercoagulation.^{9,10} This group of patients with surgical accommodations in the dentoalveolar system can be one of the causes of the disorder, which is accompanied by increased bleeding, but in some patients, the development of intravascular coagulation syndrome can lead to thrombotic episodes. Only comprehensive studies before surgical placement in the dentition, including the simultaneous diagnosis of the main components of fibrinolysis and thrombosis of the blood coagulation system, can give an overall picture of the state of hemostasis and take preventive measures to prevent complications in this contingent of patients.^{11,12} In this situation, coagulation support during tooth extraction in patients with viral hepatitis should be differentiated. Therefore, oral surgery should be started after correcting hemostasis. It should be noted that timely laboratory detection of existing defects in the hemostasis system helps to prevent both hemorrhagic and thrombotic complications, to improve the quality of life of patients and the results of treatment of pathology of the dentition.

CONCLUSIONS

Patients with chronic viral liver pathology have a variety of hemostatic defects that affect all links of the hemostatic system. Such patients have a narrow band for maintaining

hemostatic balance, and the existing balance can easily be transformed into hypo- or hypercoagulation. Comprehensive studies before surgical placement in the dentition, including the simultaneous diagnosis of the main components of fibrinolysis and thrombosis of the blood coagulation system, can give an overall picture of the state of hemostasis and take preventive measures to prevent complications in this contingent of patients.

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