ORIGINAL RESEARCHES

A.Z. Mestnikova, S.A. Artyushkin, V.K. Artyushkina

Pathogenetic Aspects of Nasal Lesions in Patients with Chronic Hepatitis C

ABSTRACT

The article reflects the problem of nasal bleeding in patients with chronic hepatitis C. The aim of the study was to determine the presence of HCV fragments in the nasal mucosa, change the local T-cell immunity, identifying morphological changes in the nasal mucosa in chronic viral hepatitis C. At the result we revealed the presence of HCV nonstructural protein NS3, local activation of T-cell immunity and metaplasia of the epithelium of the nasal mucosa.

Keywords: chronic hepatitis C (HCV), virus of hepatitis C (HCV), nosebleeds, NS3 protein of HCV, epithelial metaplasia, hypocoagulation.

INTRODUCTION

With the hepatitis C virus (HCV) around 500 million in the world are infected. In Russia, the number of patients with a chronic form of hepatitis C and carriers reaches more than 2 million people [8].

An early sign of liver cirrhosis with chronic viral hepatitis may be nosebleeds [6,12]. The incidence of nasal bleeding in liver disease varies from 1% to 13.4% [14].

As known, several causes of nasal bleeding in liver disease: the violation of the coagulation system, as in the direction of anticoagulation due to insufficient synthesis function and reduction of vitamin K-dependent coagulation factors II, VII, IX, X, abnormalities in platelet caused by stagnation in the portal vein, including hypersplenism, which are characterized by a decrease in the level of platelets. [5]. So toward hypercoagulable state, the reduction of synthesis of plasminogen hepatocytes and appear tromboplastin and platelet activating substances into the systemic circulation in the syndrome of portal hypertension [7].

For local reasons of epistaxis include the development of degenerative changes in the nasal mucosa (in particular, sub-and atrophic processes), and malformations of the vascular wall (microangiomatosis and varicose) that are observed in the allocation of toxic substances that lead to violation of angiogenesis [3].

However, in chronic viral hepatitis C (CHC) are appeared extrahepatic manifestations of hepatitis C immunocomplex genesis found in 19-56%, which can be determined before the manifestation of the liver. Vasculitis is associated with hepatitis C from the immune complexes affecting small-caliber arteries in the skin of petechiae to necrotic ulcers. It can also affect the

kidneys, joints, peripheral nervous system, salivary glands, sometimes the lungs, gastrointestinal tract and brain [1].

Patients with hepatitis C virus in the blood often determined Ig G to nonstructural proteins NS3 [2], and due to the fact that in previous studies [10] ribonucleic acid (RNA) in the nasal mucosa have not been detected, there was considered the definition of non-structural HCV proteins in mucosal nose.

It is known that the NS3 / 4A protein inhibits the activation of complement component C4 and reduces the activity of the classical pathway of complement activation [13]. Thus, the authors Amr El-Shazly et al. in studying the nasal mucosa did not found C3b, which eliminates the impairment of the immune complexes based on immunological reactions in the nose [11].

PATIENTS AND METHODS

The study involved 42 people aged 25 to 66 years (20 men and 22 women), with hepatitis C, of whom 21 were in cirrhosis. And it was recruited control group of 30 people (18 men and 12 women) with no liver disease and hepatitis C. Patients were divided into 3 groups, in connection with the signs of liver damage and the presence of hepatitis B virus.

Group 1 - Patients with chronic hepatitis C, 21 people;

Group 2 - Patients with viral liver cirrhosis, 21 people;

3 group - the control group, 30 people.

All patients underwent a complete clinical evaluation: an objective examination of the patient, laboratory methods of investigation - blood count, urinalysis, blood chemistry, coagulation. The study included coagulation: ACHTV, PTT, prothrombin by Quick, INR, fibrinogen, platelet count.

The main screening test for hepatitis C virus is the determination of HCV antibodies by ELISA. In the case of confirmation of the diagnosis carried out qualitative determination of HCV-RNA. To confirm cirrhosis performed abdominal ultrasound, EGD, liver scintigraphy, CT of the abdomen, elastography.

ENT examination was carried out by the usual method using videoendoscopy equipment. Endoscopic examination of the nasal cavity was evaluated the following signs: the state of the mucosa of the nasal cavity, the presence of subatrophy areas, symptoms of nasal bleeding, the location of the blood vessels of the nasal mucosa.

Exclusion criteria were: age over 66 years since involutive changes in the nasal mucosa, the presence of HIV, the mixed hepatitis, the presence of autoimmune diseases.

In patients after obtaining informed consent under local application anesthesia 10% lidocaine was carried out biopsy from the inferior turbinate size 3 * 4 mm, opened nosebleeds

stoped after decongest nasal cavity and cauterization with silver nitrate in the form of "pearls". Complications after this manipulation was not obtained in any case. In the control group the fence material during scheduled surgery for nasal septum deviation.

A portion of the nasal mucosa biopsy was fixed in 10% neutral buffered formalin, after which the material passed standard treatment for producing histological and immunohistochemical preparations with thick serial 3-5 micron paraffin sections. Another part of the biopsy material was placed in Eppendorf, containing 0.5 ml sterile solution version (Biolot) to determine the local amount lymphocytes by flow cytometry.

For microscopic studies were stained with hematoxylin and eosin. To identify collagen fibers and assess the degree of fibrosis was used histochemical staining method Van Gison. Using histological examination determined the degree of inflammatory infiltration of the nasal mucosa by lymphocytes, plasma cells, histiocytes, neutrophilic leukocytes, eosinophils, fibrosis and atrophy rate.

Immunohistochemical study included the determination of the expression of NS3 antigen of hepatitis C virus using monoclonal mouse antibody produced by clone MMM33 NovocastraTM in dilution 1:50. Setting three-stage reaction is carried out by indirect enzyme-linked immunosorbent LSAB (Eng. Labeled streptavidin - biotin, DakoCytomation, LSAB 2 System - HRP) imaging technique, detecting peroxidase activity was carried out using 3,3-diaminobenzidine, drugs dokrashivali Mayer's hematoxylin.

To determine the local amount of lymphocyte cells from biopsy material obtained using a mechanical disintegrator Medimachine (BD). We used antibodies: HLA DR-FITC, CD4-PE, CD3-ECD, CD56-PC5.5, CD25-PC7, CD8-APC, CD19-APC-AF700, CD45-APC-AF750 (Beckman Coulter). The samples were analyzed by flow cytometry Navios (Beckman Coulter) in multi-protocol.

These clinical results were processed using c system STATISTICA for Windows (version of the Faces. BXXR310F964808FA-V).

RESULTS OF THE STUDY

From the anamnesis of patients significant differences in the incidence of nasal bleeding, depending on the stage of chronic hepatitis C has not been received (Table. 1). However, nosebleeds occur more frequently in patients suffering from liver disease in contrast to the control group ($p < 0{,}001$).

Table 1

The frequency of nasal bleeding in patients with CHC and the control group

	I group, *n=21* % (abs. n.)	II group, *n=21* % (abs. n.)	III group, *n=30* % (abs. n.)
Patients received wit hepistaxis	24% (5)	14% (3)	0
Patients with usual epistaxis	38% (8)	24% (5)	0
Patient with single epistaxis	38% (8)	33% (7)	0

Endoscopic examination of the nasal cavity (Table. 2) in patients with chronic viral hepatitis and cirrhosis are dominated subatrophic processes mucosa ($p << 0,001$) compared with the control group; surface vessels located in places bare ($p <0,01$), crusts in the nose occur with equal frequency in the group with viral hepatitis.

Table 2

These endoscopic examination of the nasal cavity of patients with chronic hepatitis C

	I group % (abs. n.)	II group % (abs. n.)	III group % (abs. n.)
Subatrophy of nasal mucosa	**67%** (14)	**67%** (34)	**0%**
Superficial vessels of the nasal mucosa	**43%** (9)	**43%** (9)	**7%** (2)
Crimped vessels of the nasal mucosa	**5%** (1)	**0%**	**3%** (1)
Crusts in the nasal cavity	**62%** (13)	**57%** (12)	**10%** (2)
Nasal septum deviation	**57%** (12)	**43%**(9)	**100%** (30)

As a result of coagulation investigation (Table. 3) significant differences between patients with chronic hepatitis C and the control group ($p >> 0.05$) didn't find, so, the function of the coagulation system is not broken.

Table 3

The coagulation system in patients

index	I group	II group	III group	Normal values
Prothrombin for Quick, %	97,90±3,13	68,08±7,76	101,09±3,16	70-120
Fibrinogen, mg/dl	367,32±24,11	246,45±26,75	415,83±19,39	200-400
ACHTV, seconds	29,35±0,7	32,52±1,1	27,24±0,38	25-33
Prothrombin time, sec	12,47±0,2	21,23±6,62	11,83±0,025	11-16
Platelets, x 10^9 g / l	226,90±29,28	136,10±23,06	253,83±9,43	160-320

When comparing the patients with cirrhosis of the liver with the control group and viral hepatitis marked reduction of prothrombin Quick and prolonged prothrombin time ($p < 0,01$), indicating hypocoagulation due to clotting of the external type.. Reducing the number of platelets ($p < 0,01$) constitutes a violation of the vascular-platelet clotting mechanism. From the data obtained it follows that a violation of coagulation occurs only in the terminal stages of hepatitis C. Data of hypercoagulability has been received.

7 immunohistochemical studies were performed patients with chronic hepatitis and cirrhosis of the liver the presence of NS3 protein, and found in 6 patients the expression of HCV NS3 protein in the cytoplasm in the epithelial metaplasiaand protein-mucous glands of the lamina propria of the nasal mucosa (Fig. 1, а, б). What it confirms the presence of HCV non-structural fragments in the nasal mucosa and does not exclude the manifestation of nasal bleeding as a result of extrahepatic manifestations of hepatitis.

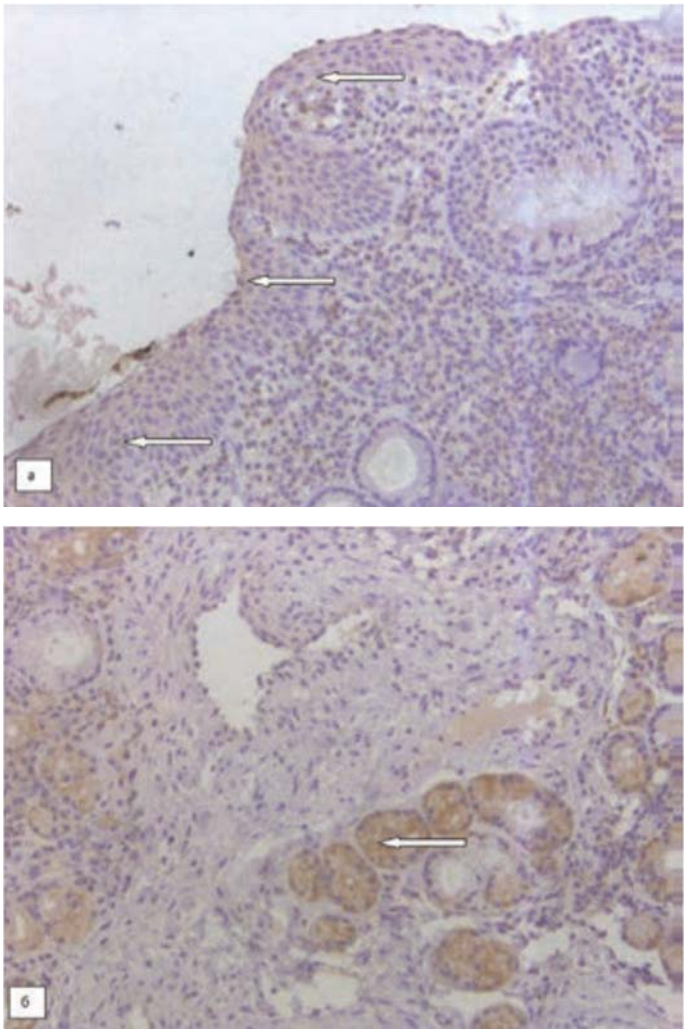

Fig. 1. The mucous membrane of the nose. The expression of NS3 antigen: a) metaplasia of the epithelium; b) mucous glands. Immunohistochemical study. The increase of 200.

In determining the amount of the local subpopulation of lymphocytes in the nasal mucosa was obtained (Table 4).

Table 4

Parameters of cellular immunity in the biopsy in patients with chronic hepatitis C and controls

Index	patients with CHC, M±m, *n=6*	control group, M±m, *n=6*
CD3+ (T-limp)	87,8±2,36	85,2±3,96
CD3+CD4+ (T-hel)	26,3±7,31	33,4±1,69
CD3+CD8+ (T-kills)	59,83±8,72	49,8±3,99
CD4/CD8	0,63±0,27	0,67±0,07
CD3+CD56+ (TNK-sell)	8,05±3,49	6,62±1,44
CD19+ (B-limp)	2,22±0,69	5,2±1,77
CD 25+ (IL-2)	1,37±0,68	3,68±1,13
CD3+25+	1±0,48	3,32±1,1
CD3-CD8+ (activ. NK-sells)	1,57±0,77	1,78±0,78
CD3-CD56+ (NK-sells)	8±1,55	8,1±3,06
HLA DR+	35,53±6,64	18,2±2,25
CD3+HLA DR+ (activ. T-sells)	26,6±9,34	11,68±2,29
CD56+HLA DR+ (activ. NK-sells)	6,43±1,54	2,82±0,37

Predominance of T cell composition on B-lymphocytes, confirming the dominance of the cellular immunity over humoral immunity in the nasal mucosa. Increase in viral hepatitis C T-killer cells, CD3 + HLA DR +, CD56 + HLA DR + ($p < 0,05$), in comparison with the control group, characteristically for later activation of T-lymphocytes with a predominance of T-cell immunity, which is typical for chronic persistent infection, while a decrease in T-helper cells ($p < 0,05$) indicates the weakening of the regulatory processes of cellular immunity. High-grade chronic HCV infection is associated with low CD4 + and CD8 + T-lymphocytes, poorly immunogenic viral proteins and the ability to mutate [4]. Also, in patients with chronic hepatitis C in comparison with the control group decreased content of CD25 + ($p < 0,05$) - a marker of the receptor IL-2, which indicates the early activation of cellular immunity.

Table 5

Parameters of cellular immunity in the nasal mucosa biopsy and serum in patients with chronic hepatitis C

Indices	In the nasal mucosa, *n=6*	In serum, *n=8*
CD3+ (T-limp)	87,8±2,36	75,38±9,88
CD3+CD4+ (T-hel)	26,3±7,31	47,38±3,05
CD3+CD8+ (T-kills)	59,83±8,72	26,36±3,66
CD3+CD56+ (TNK-sell)	8,05±3,49	9,1±2,33
CD19+ (B-limp)	2,22±0,69	10,6±1,6
CD 25+ (IL-2)	1,37±0,68	4,68±0,63
CD3+25+	1±0,48	3,88±0,58
CD3-CD8+ (activ. NK-sells)	1,57±0,77	4,88±0,88
CD3-CD56+ (NK-sells)	8±1,55	12,75±1,99
HLA DR+	35,53±6,64	21,38±3,53
CD3+HLA DR+ (activ. T-sells)	26,6±9,34	9,16±2,39
CD56+HLA DR+ (activ. NK-sells)	6,43±1,54	2,33±0,69

In comparing T-cell immunity nasal mucosa and serum (Table. 3) is determined by the predominance in the first case of T-lymphocytes, T-killer cells, CD56 + HLA DR + ($p < 0.01$), indicating that activation of T-cell immunity, with activation of killer T-cells and NK-cells, and decrease the early activation marker CD25 + CD3 + ($p < 0.01$) in biopsy confirms the presence of the virus and a long persistence in the nasal mucosa. In serum B-cell immunity ($p < 0,01$) more pronounced, however, in view of the fact that there is a constant antigenic variation of the virus develop antibodies to lose its relevance, and thus the virus escapes the control of humoral immune system, so the most important in the study elimination of the virus is the T-cell immunity. Also in the serum increased helper T cell activity ($p < 0.01$), which causes increased sensor detection and regulatory activity, due to a load on the immune system.

Presence NS3 HCV protein and increase of T-killer cells, activated T-lymphocytes and NK-cells in the nasal mucosa of viral hepatitis C can be treated as a result of the damaging effect Thelper-1 mediated pathway of the immune response, where the presentation of antigens on the surface of the damaged cell, in this case mucosal epithelium and lamina propria glands, causes the activation of CD8 + T cells [9].

Table 6

Evaluation of epithelial metaplasia of the nasal mucosa

epithelial metaplasia	**1 group,** *n=7*	**2 group,** *n=7*	**3 group,** *n=6*
absent	43% (3)	0	83% (5)
DPer	0	14% (1)	0
DPl	14% (1)	72% (5)	0
OPl	43% (3)	14% (1)	17% (1)

Abbreviations: ДDPer – Diffuse transitional cell metaplasia of the epithelium; DPl – diffuse squamous; OPl – Local squamous.

Histological examination in comparison with the control group was found (tab. 6): metaplasia of the epithelium of the nasal mucosa in patients with viral hepatitis ($p < 0.05$), and prevails in stratified squamous metaplasia of epithelium (Fig. 2), which subsequently can lead to atrophic processes mucosa. Atrophic processes ($p << 0.01$) in 57% (4) cases were weakly expressed in group 1 and pronounced at 43% (3) Group 2 patients. Moderate to severe fibrosis effects ($p < 0,001$) in both groups appear the same in 86% (6) cases. Determined relationship: the more expressed effects atrophy and fibrosis of the mucosa, the smaller vessels in the mucosa. The number of vessels in the mucosa is significantly reduced ($P < 0.001$), in chronic viral hepatitis, and cirrhosis in step. When viral hepatitis C prevails lymph plasmocytic infiltration of the stroma of the mucous membrane ($p < 0,01$).

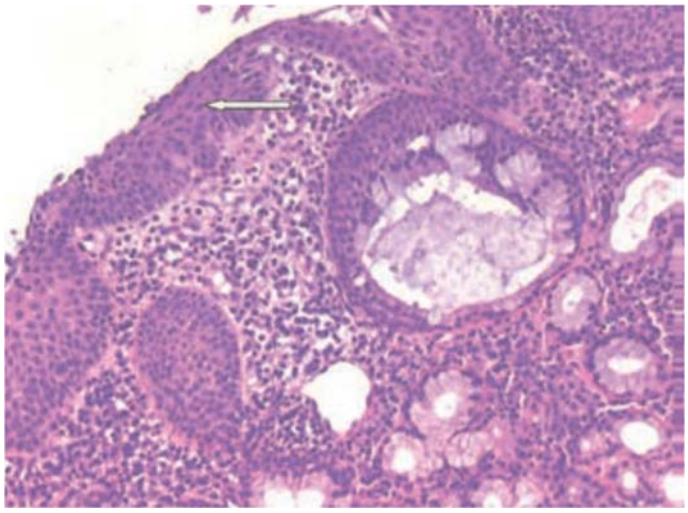

Fig. 2. The mucous membrane of the nose. Diffuse squamous metaplasia of the epithelium. H & E stain. The increase of 200.

CONCLUSIONS

1. Nosebleeds are more common in patients with persistent HCV, regardless of the stage of the disease, in spite of the clotting disorder by type of anticoagulation in the stage of cirrhosis. This fact indicates that the coagulation disorder is not the main cause of nosebleeds.
2. In patients with chronic hepatitis and cirrhosis in mucosal epithelium and protein-mucous glands of the lamina propria in the nasal mucosa HCV NS3 proteins were detected, indicating the presence of hepatitis C virus in the nose and it does not exclude secretion in the nasal cavity.
3. The presence of a fragment of HCV NS3 contributes local activation of T-cell immunity with increase T-killer cells, NK-cells with viral hepatitis C.
4. Metaplasia of the epithelium of the nasal mucosa can be caused by the presence of HCV NS3 protein, resulting in increased T cell activity. A change in the surface layer may lead to superficial vessels of which occur nosebleeds.

REFERENCES

1. Baykova T.A. Lopatkina T.N. Mnogoobrazie vnepechenochnyh proyavlenij hronicheskih virusnyh gepatitov B i C, obshchie principy lecheniya [The variety of extrahepatic manifestations of chronic viral hepatitis B and C, the general principles of treatment] Terapevticheskij arhiv [Therapeutic archive], 2013, №4, Pp. 106-107.
2. Baranov A.V. Shishkina L.V. Soderzhanie antitel klassa Ig G k NS3, NS4 i NS5-antigenam virusa gepatita C i RNK HCV v syvorotkah krovi bol'nyh s latentnym techeniem hronicheskogo gepatita [The contents of class Ig G antibodies to NS3, NS4 and NS5-antigens of the hepatitis C virus and HCV RNA in the serum of patients with latent chronic hepatitis] Materialy konferencij Fundamental'nye issledovaniya [Conference materials Basic research]. Moscow,2008, №1, pp. 98-99.
3. Volkov A.G. Boyko N.V. Kiselev V.V. Nosovye krovotecheniya [Nosebleeds]. Moscow: Jagar, 2002, 270 p.
4. Dudanova O.P. Kiselev O.I. Hronicheskij virusnyj gepatit S: diagnostika i osobennosti klinicheskogo techeniya [Chronic hepatitis C: diagnosis and clinical features]. Petrozavodsk: Izd-vo PetrGU [Petrozavodsk State University Publishing House] 2013, 12 p.
5. Palchun V.T. Nacional'noe rukovodstvo po otorinolaringologii [National manual of otorhinolaryngology]. Moscow: GEOTAR-Med, 2009, 960 p.

6. Palchun T.V. Kryukov A.I. Otorinolaringologiya. Klinicheskie rekomendacii [Otorhinolaryngology. Clinical recommendations]. Moscow: GEOTAR Media, 2013, 368 p.
7. Petrov V.V. Aktual'nye voprosy patogeneza nosovyh krovotechenij [Actual questions of pathogenesis of nasal hemorrhage] Klinicheskaya medicina [Clinical Medicine]. 2006, V.84. (4), pp.13-17.
8. Radchenko V.G. Stelmach V.V. Kozlov V.K. Optimizaciya ehtiopatogeneticheskoj terapii hronicheskogo gepatita C [Optimization of etiopathogenetic therapy of chronic hepatitis C]. St. Petersburg: Tactic studio, 2004, 166 p.
9. Osnovy klinicheskoj immunologii: perevod s anglijskogo [Fundamentals of Clinical Immunology: translated from English] Chepel E. [et al.]. Moscow: GEOTAR Media, 2008. 416 p.
10. Abbas F. Hepatitis C virus induces nasal epithelial erosion and sub-epithelial rhinitis / F. Abbas [et al.] // Rhinology. – 2009. - Vol. 47 - N4. - P. 438-443.
11. Amr El-Shazly. Characterization of hepatitis C virus-induced nasal mucosa remodelling / Amr El-Shazly [etc.] // Histopathology. – 2010. - Vol. 57. - N3. – P. 488-492.
12. Barrio J. Epistaxis as an initial sign of hepatic disease/ Barrio J. [et al.] // *An Pediatr* (*Barc*) - 2009 Vol. 70. - Issue 6. - P. 599-601.
13. Mawatari S. Hepatitis C virus NS3/4A protease inhibits complement activation by cleaving complement component 4 / Mawatari S. [et al.] // PLoS One. – 2013. - 8 (12).
14. Urvashi Razdan. Epistaxis: Study of aetiology, site and side of bleeding/ Urvashi Razdan, Zada R., Chaturvedi V.N. // Indian Journal of Medical Science -1999 – Vol.53. - Issue 12. - p.545-552.

The authors:

Mestnikova Aina Zakharovna – post-graduate student of the department of otorhinolaryngology Mechnikov Northwestern State Medical University, 195067, St. Petersburg, Piskarevskiy pr., D. 47; tel .: (812) 543-94-13, e-mail: Aino4ka_13@mail.ru

Artushkin Sergey Anatolevich – MD, Professor, Head of department of otorhinolaryngology of MechnikovNorthwestern State Medical University, Russia, 191015, St. Petersburg, ul.Kirochnaya, d. 41; tel .: (812) 303-50-00, e-mail: Sergei.Artyushkin@spbmapo.ru

Artushkina Valentina Kuzminichna - PhD, Associate professor of the department of otorhinolaryngology of Mechnikov Northwestern State Medical University, Russia, 191015, St. Petersburg, ul.Kirochnaya, d. 41; tel.: (812) 303-50-00, e-mail: Sergei.Artyushkin@spbmapo.ru.