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Platelet Pathology in Patients with Chronic Diffuse Liver Diseases with Viral Etiology

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Abstract--- Objective of the research: *Study of platelet hemostasis disorders in patients with chronic hepatitis and liver cirrhosis of viral aetiology.*

Materials and methods: *The study included 142 patients with chronic diffuse liver diseases, including 80 patients with liver cirrhosis of viral etiology and 62 patients with chronic viral hepatitis of moderate activity.*

Research results: *Hemorrhagic syndrome was expressed in a group of patients with liver cirrhosis of HBV and HBV+HDV etiology in 63, 3% and 70,0%, respectively. Hematological and cytomorphological studies of plateletes in patients with liver cirrhosis and chronic hepatitis of viral etiology showed significant abnormalities in the amount of blood cells and platelets morphology in liver cirrhosis and minor changes in chronic hepatitis. Liver cirrhosis of viral etiology was accompanied by significant changes in thrombocytopoiesis in the red bone marrow with severe depression of the megacaryocytic lineage and significant abnormal platelet production in the bone marrow.*

Conclusions: *In LC and chronic hepatitis of viral etiology morphological characteristics of platelets and production of platelets in bone marrow are disturbed, which is the cause of hemorrhagic syndrome in 34,3-11,6% of LC patients in the stage of decompensation and in 9,6-3,1% of patients with chronic viral hepatitis.*

Keywords--- *Thrombocytopenias, Hemorrhagic Syndrome, Liver Cirrhosis, Chronic Viral Hepatitis, Mean Platelet Volume, Platelet Anisocytosis, Plateletcrit, Bone Marrow.*

I. INTRODUCTION

Liver cirrhosis is the world's leading cause of death from non-tumor diseases of the digestive system. One of the most common causes of chronic hepatitis and liver cirrhosis is infection with hepatitis B and C. According to the WHO, about 325 million people are infected with parenteral hepatitis viruses in the world, and more than 500 million people are latent carriers of viruses (WHO). 60-80% of infected patients develop chronic viral hepatitis B and C. At the same time, there are 10-15 non-icteric forms per one patient with icteric form, the number of people who have been ill is much higher and it is not uncommon for the process to be chronised after viral hepatitis B and C [4].

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The liver has an important role in hemostasis, so diffuse lesions of its parenchyma lead to complex disorders of blood clotting [6, 8]. Liver produces blood clotting factors and hematopoietic growth factor - thrombopoietin (THPO). Many liver diseases, including viral hepatitis, alcoholic hepatitis, chronic hepatitis and liver cirrhosis lead to thrombocytopenia. According to a number of authors, one of the reasons for the development of thrombocytopenia is the increase of antiplatelet (anti-PLT) immunoglobulin (Ig) in liver diseases [7, 9]. Thrombocytopenia in liver diseases leads to an increased risk of bleeding, and it can be contraindication for antiviral therapy, liver biopsy and surgical [2, 3, 5, 11].

Despite the large number of studies devoted to the study of early diagnosis, treatment, prognosis of complications of chronic viral hepatitis and liver cirrhosis, in recent years the issue of the prognostic significance of a number of hemostatic indicators continues to be discussed in the literature, which indicates the need for further improvement, especially in the early detection of the disease.

Recent data show that changes of hemostasis in liver cirrhosis affect both the procoagulant and anticoagulant systems and, due to the reduced reserve of each of these systems, it easily shifts towards hypo- or hypercoagulation [1]. At the same time, in patients with liver cirrhosis, the hemostatic system is balanced, since the decrease of procoagulant proteins is also accompanied by a decrease in the levels of anticoagulant proteins [10]. According to studies dedicated to blood clotting, patients with decompensated liver cirrhosis show marked deviations in the direction of hypocoagulation, which indicate that decompensated liver cirrhosis is the cause of acquired coagulopathy and is associated with the risk of bleeding [12, 13].

Platelet pathology is one of the pathogenetically significant disorders of hemostasis, which is associated with angiotrophic, adhesive-aggregatory, concentration-transport functions of thrombocytes, their ability to cause vascular spasm and inhibit fibrinolysis, microcirculatory disorders, which contribute to a more severe course of the disease, the occurrence of severe complications, the formation of prolonged and chronic forms of liver disease. With increased histological activity and fibrosis severity, endothelial damage increases, platelet count and their function decreases.

Objective of the Research

To study platelet hemostasis disorders in patients with chronic hepatitis and cirrhosis of the liver of viral etiology.

II. MATERIALS AND METHODS

Clinical studies were carried out at the Department of Hematology and Hepatobiliary Pathology of the Tashkent Medical Academy, 1st Clinic in 2016-2018. The study included 142 patients with chronic diffuse liver diseases, including 80 patients with liver cirrhosis of viral etiology and 62 patients with chronic viral hepatitis of moderate activity. The diagnosis of liver cirrhosis and chronic hepatitis of viral etiology took into account the data of the anamnesis (indication of transfusions of blood components, treatment by a dentist, etc.), characteristic clinical syndromes (hemorrhagic, anemic, astheno-neurotic, jaundice, etc.) and laboratory and instrumental studies. The detection of hepatitis virus markers by ELISA was mandatory, as well as the detection of DNA of hepatitis B virus

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(HBV) and RNA of hepatitis C virus (HCV) and D virus (HDV) by PCR blood tests with the determination of genotypes. Patients with chronic hepatitis and liver cirrhosis of viral etiology had a viral load of more than 1,000,000 IU/ml.

Analysis of the results of the blood test with the calculation of platelets was carried out on the hematological analyzer Mindray 5000 (China). For the cytological study of the bone marrow punctate, a sternal puncture was performed in the area of the manubrium or the upper third of its body (at the level of 3 - 4 ribs) along the middle line.

The diagnosis of cirrhosis and the degree of liver cell insufficiency was established taking into account the recommendations of the WHO (2008), according to the classification of Child-Pugh on the basis of diagnostic criteria.

To determine the degree of fibrosis in patients with liver cirrhosis, ultrasound examination (US), MSCT and liver fibroscan were performed.

Patients with chronic hepatitis and liver cirrhosis, who did not receive antiviral therapy were included in the study. The distribution of patients is shown in figure 1.

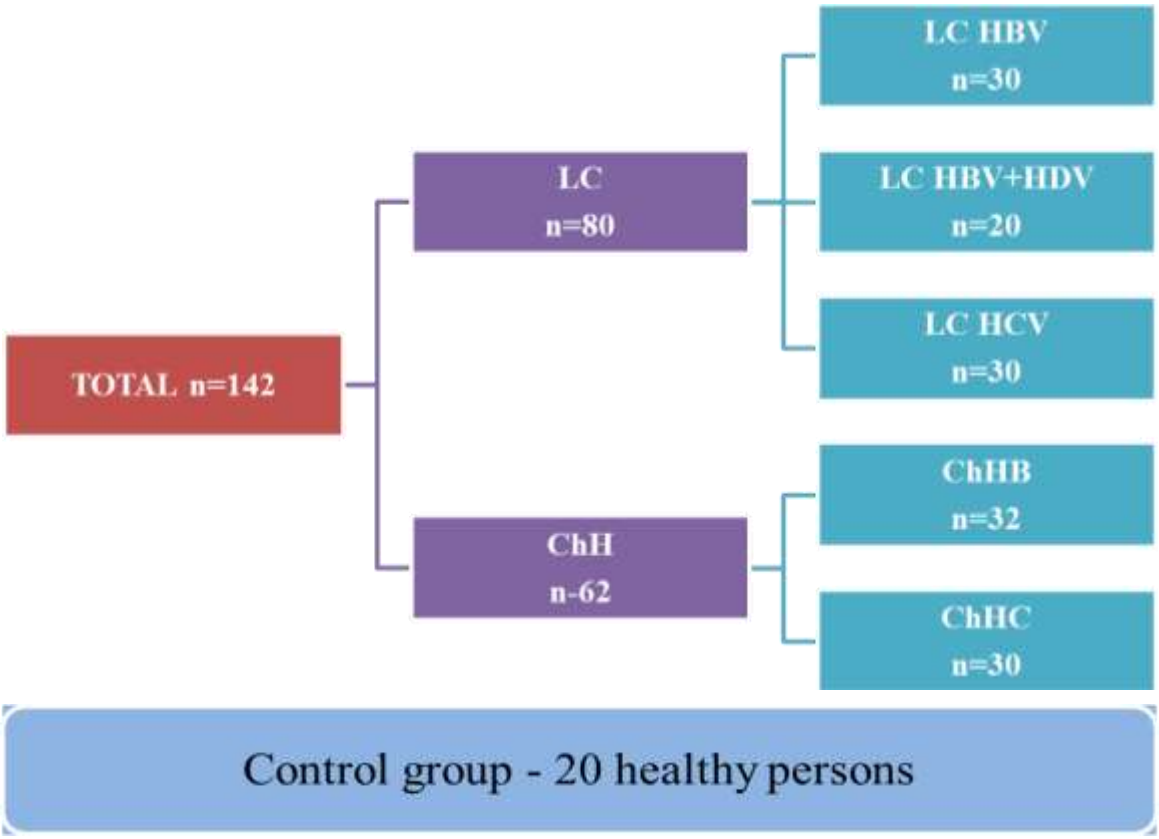

Fig. 1: Research Design

All examined patients were divided into five groups: The 1st group consisted of 30 patients with LC of HBV etiology in the decompensation stage, class B according to Child-Pugh, the 2nd group consisted of 20 patients with

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LC of HBV+HDV etiology in the decompensation stage, class B according to Child-Pugh, the 3rd group consisted of 30 patients with LC of HCV etiology, in the decompensation stage, class B according to Child-Pugh classification. The 4th group included 32 patients with chronic viral hepatitis B of moderateactivity; the 5th group included 30 patients with chronic viral hepatitis C of moderate activity.

Of the 142 patients included in the study, 81 (57,04%) were men and 61 (42,96%) were women. The age of the patients ranged from 21 to 69 years, with an average age of 48,2 ± 12,1 years. Among the patients– 43,97% were of working age.

The control group included 20 practically healthy persons who had no history of liver diseases and fatty hepatosis, with negative results for markers of hepatitis B andC (Fig.2).

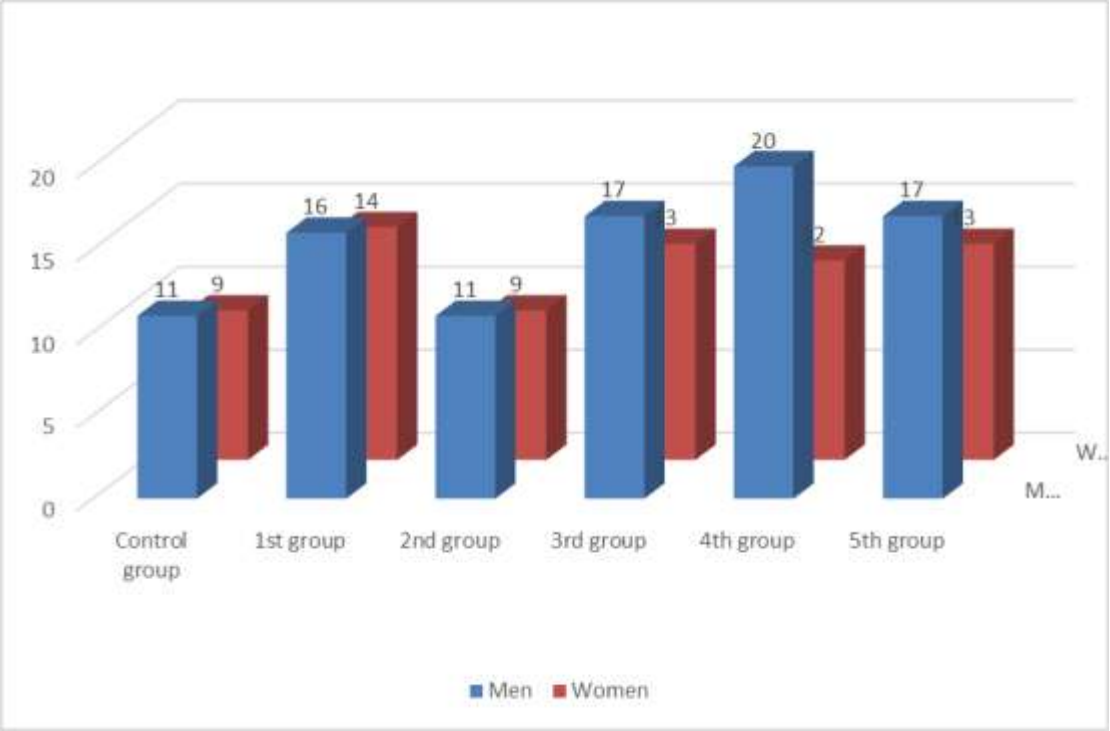

Fig. 2: Distribution of Patients with Chronic Viral Hepatitis and Liver Cirrhosis Depending on Sex

All patients with liver cirrhosis included in the study were with a long duration of chronic liver disease, the duration of the disease averaged 3, 85±1,74 years.

Patients with chronic viral hepatitis, who were in 4th and 5th groups of the study, were newly detected and did not receive antiviral therapy against hepatitis B and C virus.

III. Results of the Research

In order to determine the type of hemorrhagic syndrome, complaints, life and disease history and objective data of patients were studied. The patients examined in our study had characteristic signs of bleeding. Hemorrhagic syndrome was more pronounced in 1st and 2nd groups (Table 1).

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Table 1: Structure of Hemorrhagic Syndrome in Patients with ChVH and LC

Types of bleeding	Groups of people examined									
	1st group (n=30)		2nd group (n=20)		3rd group (n=30)		4th group (n=32)		5th group (n=30)	
Nasal bleeding.	17	56,7	12	60,0	16	53,3	2	6,3	2	6,7
Gingival bleeding	17	56,7	13	65,0	13	43,3	1	3,1	1	3,3
Skin petechiae	13	43,3	11	55,0	12	40,0	2	6,3	1	3,3
Hemorrhoids bleeding	12	40,0	8	40,0	10	33,3	0	0	0	0
Bleeding from esophageal varices	12	40,0	9	45,0	9	30,0	0	0	0	0
Bleeding from GIT	10	33,3	7	35,0	7	23,3	0	0	0	0
Menorrhagia	8	26,7	5	25,0	4	13,3	0	0	0	0

As can be seen from the above data, hemorrhagic syndrome was marked in the group of patients with liver cirrhosis of HBV and HBV+HDV etiology for 63,3% and 70,0%, respectively. In the group of patients with liver cirrhosis of HCV etiology, bleeding symptoms occurred in 53,3% of cases. And, in chronic viral hepatitis B and C of moderate activity, hemorrhagic syndrome occurred in 15,6% and 13,3%, respectively.

Hemogram studies have shown that there is a clear tendency to moderate thrombocytopenia in the main groups with liver cirrhosis. For instance, the average platelet values in patients of the 1st group were 148±25,8×10^9/l, in the 2nd group this value was equal to146±32,9×10^9/l, and in the 3rd group platelet count was significantly reduced – the value was135±33,5×10^9/l. These data differ significantly from the control group, which is equal to 222±21, 21×10^9/l.

Studies have shown that the cause of thrombocytopenia in groups 1, 2 and 3 was largely pancytopenia caused by hypersplenism in liver cirrhosis of viral etiology. This is confirmed by a decrease in the number of erythrocytes and leukocytes (table 2). It is well known that in hypersplenism, the form elements of blood, such as erythrocytes, leukocytes and platelets are trapped and destroyed in the hypertrophied spleen.

Table 2: Peripheral Blood Count in Patients with Chronic diffuse Liver Disease

Groups	Platelets, ×10^9/л	Erythrocytes, ×10^{12}/л	*Leukocytes, ×10^9/л*
Control group (n=20)	222±21,21	4,22±0,37	*5,95±1,01*
1st group(n=30)	148±25,8*	2,98±0,16**	*3,54±0,32**
2nd group(n=20)	146±32,9	2,83±0,21**	*3,49±0,19**
3rd group (n=30)	135±33,5*	3,07±0,34*	*3,66±0,36**
4th group (n=32)	216±22,8	3,47±0,53	*6,06±1,99*
5th group (n=30)	187±32,9	3,46±0,35	*5,48±1,17*

Note: * – differences in the control group data are relevant (* – P<0,05, ** – P<0,01)

A study of hemogram platelet indexes produced by a hematology analyzer has shown that patients with CP have significant abnormalities in mean platelet volume (MPV), platelet distribution width by volume (anisocytosis) (PDW) and plateletcrit (PCT).

The mean platelet volume (MPV) indices showed that the mean platelet volume increases significantly in cirrhosis of the liver viral aetiology, which, in turn, indicates the prevalence of young platelet forms in the blood in response to the shortening of their life expectancy. The mean platelet volume (MPV) in patients with chronic hepatitis was within normal values. The PDV indicator shows that in liver cirrhosis and chronic viral hepatitis, there

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is a significant increase in the platelet distribution width by volume, which indicates a marked platelet anisocytosis (table 3).

Table 3: Platelet Indexes of the Hematology Analyzer in Patients with Chronic diffuse Liver Disease

Hemostasis indices	MPV, fl	PDV, %	PCT, %
Control group (n=20)	8,25±0,64	13,45±0,51	0,28±0,01
1st group (n=30)	13,27±1,17***	29,30±1,21***	0,10±0,008***
2nd group (n=20)	13,57±0,70***	30,57±0,82***	0,10±0,008***
3rd group(n=30)	10,52±0,76*	22,91±0,92***	0,08±0,008***
4th group(n=32)	8,52±0,62	29,30±1,21***	0,18±0,01***
5th group(n=30)	7,95±0,66	30,57±0,82***	0,20±0,01***

Note: * – differences in the control group data are relevant (* – P<0, 05, *** – P<0,001)

In the study of plateletcrit (PCT) it was found that it also decreased significantly in liver cirrhosis and chronic hepatitis of viral aetiology, especially in LC of HCV etiology. These results of the PCT study indicate a decrease of platelets in groups with liver cirrhosis of viral etiology.

When studying the morphological features of platelets, it was found that the amount of macroplates of platelets was increased in liver cirrhosis. In the control group, macroplates accounted for 8%. In the 1st group the macroplates were 36±4,8%, in the 2nd group - 42±5,8%, in the 3rd group - 18±2, 2%. (fig. 3, 4).

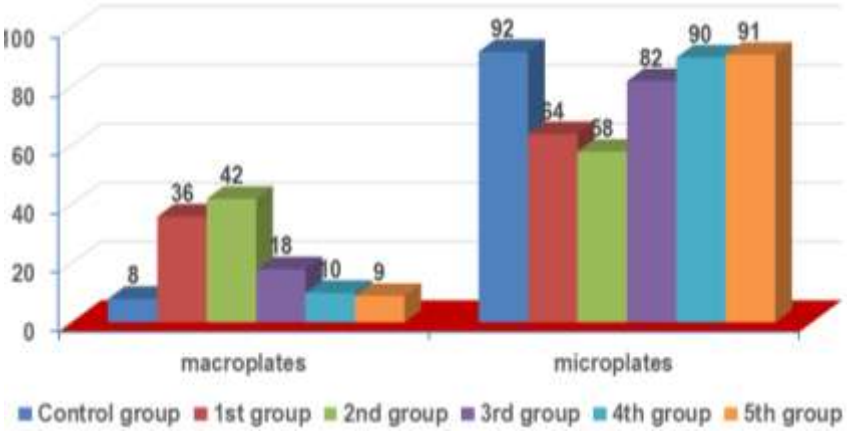
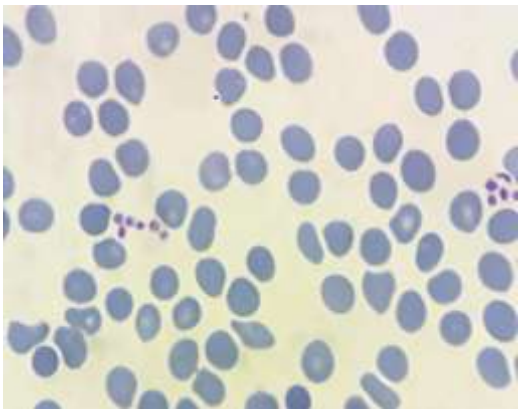
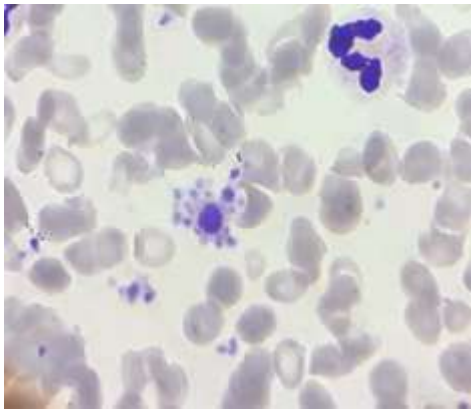


Fig. 3: Platelet Anisocytosis



Normal platelets



Macroplate of thrombocyte

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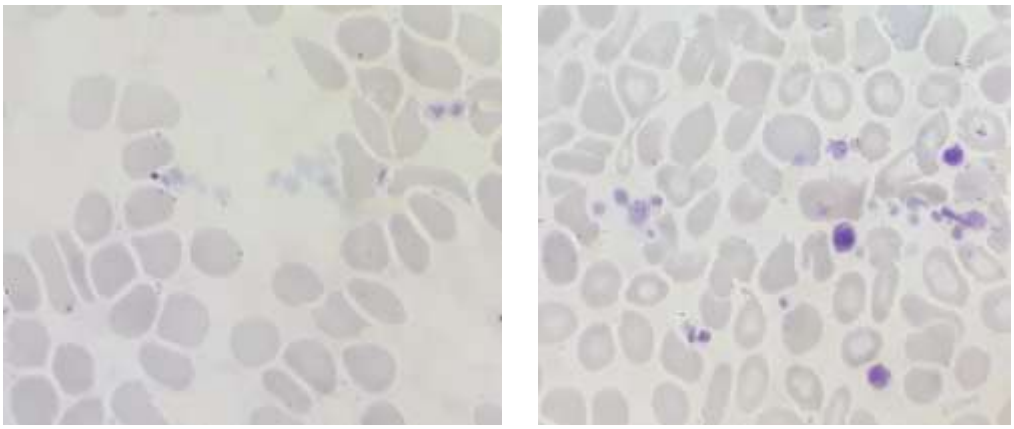

Platelets without granulomers Plateletan isocytosis

Fig. 4: Morphological Features of Platelets

When studying the morphological features of platelets it was found that in liver cirrhosis of viral etiology platelets with no or reduced granulomere prevail (Table 4)

Table 4: Platelet Morphology in Patients with Chronic diffuse Liver Disease

Hemostasis indices	Platelets with granulomers, %	Platelets with reduced granulomers, %	Platelets without granulomers, %
Control group (n=20)	95,15±2,32	3,21±0,34	1,64±0,08
1st group(n=30)	58,74±5,21***	21,83±1,54***	19,43±1,96***
2nd group(n=20)	53,32±4,32***	25,12±1,68***	21,56±1,02***
3rd group(n=30)	66,17±3,70***	17,29±1,67***	16,54±1,88***
4th group, CVHB (HBV) (n=32)	85,72±2,78*	8,44±0,90***	5,84±0,69***
5th group, CVHC (HCV) (n=30)	87,59±2,66*	7,19±0,68***	5,22±0,82***

Note: * – differences in the control group data are relevant (* – P<0, 05, *** – P<0,001)

As can be seen in Table 4, all of the above indicators differ significantly from those of the control group. In chronic hepatitis, platelet morphology disorders were not detected.

Thus, hematological and cytomorphological studies of thrombocytes in patients with liver cirrhosis and chronic hepatitis of viral etiology have shown significant disturbances in the number of blood cells and thrombocyte morphology in liver cirrhosis and inexpressive changes in chronic hepatitis.

In order to determine the cause-effect relationships of thrombocytopenia and bone marrow lesions in liver cirrhosis of viral etiology, we carried out a detailed cytological analysis of the red bone marrow with the calculation of the number of cells of all hematopoietic sprouts in 15 patients with LC of HBV etiology in 14 patients with LC of HCV etiology.

Analysis of the megacaryocyte series of the bone marrow showed that in groups with liver cirrhosis of viral etiology in the bone marrow, holonuclear cellsand inactive megacaryocytes prevailed and the number of platelets containing megacaryocytes and megacaryocytes with lacing of platelets was almost decreased in two times. These data indicate a marked depression of this lineage and a significant abnormality of platelet production in bone marrow patients with LC of viral etiology (Table 5, Figure 5).

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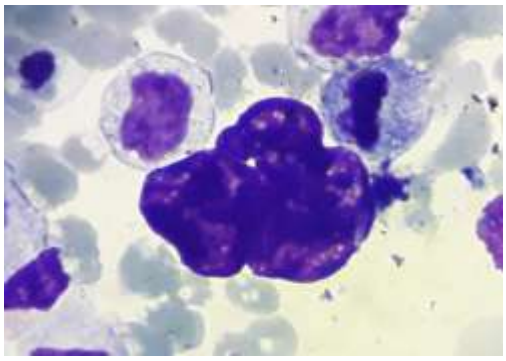
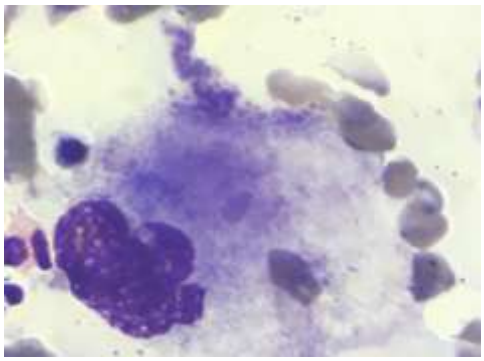
Table 5: Megacariocyte Analysis of the Bone Marrow

Megacariocytes	Control Group	1st group, LCHBV (n=15)	3rd group, LCHCV (n=14)
Megacariocytes with the lacing of the platelets, %	30,3±1,7	17,9±2,1***	16,6±0,5***
Platelet containing megacariocytes, %	33,3±1,8	19,5±1,9***	17,8±0,6***
Inactive megacariocytes,%	24,1±1,6	32,2±1,5**	34,4±0,9***
Holo-nucleic (almost non-existent cytoplasm) megacariocytes,%	12,3±1,1	30,4±1,3***	31,2±0,8***

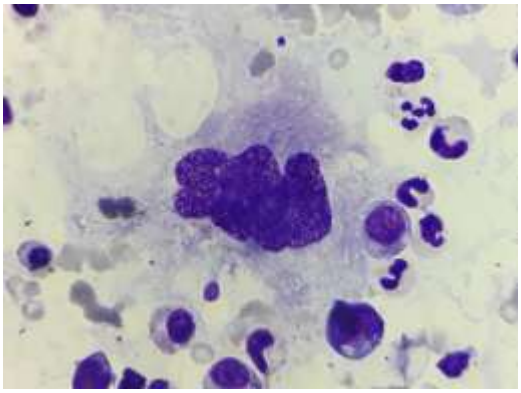
Note: * – differences in the control group data are significant (*** – P<0,001)



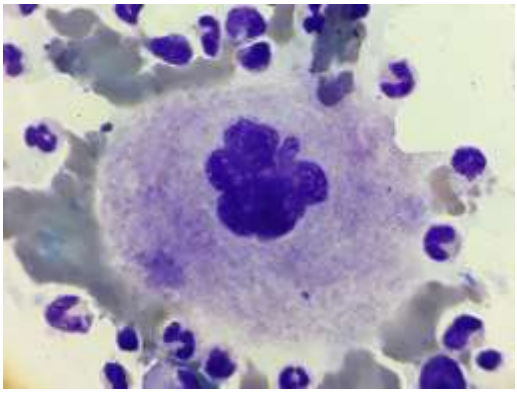
Holo-nucleic Megacariocytes



Megacariocytes with Lacing



Inactive Megacariocytes



Platelet-containing Megacariocytes

Fig. 5: Types of Megacariocytes

Summarizing the above, it can be concluded that liver cirrhosis of viral etiology was accompanied by significant changes in the process of thrombocytopoiesis in the red bone marrow. First of all, it should be noted that there is a marked depression of the megacariocytic lineage and a significant disturbance of platelet production in the bone marrow.

IV. Conclusion

1. Hemorrhagic syndrome in patients with LC in the stage of decompensation (class B according to Child Pugh) was manifested by nasal bleedings in 34,3%, gingival bleedings in 30,2%, skin petechiae in 26,7%, hemorrhoids in 20,9%, esophageal varices bleedings in 20,4%, gastrointestinal bleedings in 16,9% and menorrhages in 11,6% of patients. Nasal bleeding and skin petechiae were found in 9,6% of patients with HBV, gingival bleeding in 3,1% of patients.

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2. In LC of viral etiology, the morphological characteristics of platelets are disturbed due to the predominance of young platelet forms in the blood.
3. In liver cirrhosis of viral etiology, the function of the megacaryocyte lineage is suppressed and the formation of platelets is reduced.

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