

1 **THE ROLE OF HEMIC HYPOXIA IN THE DEVELOPMENT OF**
2 **SENSORINEURAL HEARING LOSS IN CHILDREN ASSOCIATED WITH**
3 **HEPATITIS B**

4
5 **Abstract**

6 **Background.** There are not enough studies and evidenced researches conducted
7 related to this topic. Therefore, we studied fetal hemoglobin in various somatic
8 diseases in children with sensorineural hearing loss associated with hepatitis B.

9 **Material and methods.** 26 children with sensorineural hearing loss associated
10 with hepatitis B, aged from 5 to 18 years, were examined. The comparison group
11 consisted of 8 children with sensorineural hearing loss without concomitant
12 somatic pathology. The control group consisted of 12 healthy children. The
13 compulsory examination plan for patients included generally accepted laboratory
14 and instrumental diagnostic methods: complete blood count, urine, feces,
15 Wasserman reaction, ECG.

16 **Results.** Hb concentration in blood in patient children with sensorineural hearing
17 loss of the associated CHB was reduced significantly by 58% compared with the
18 healthy children. In children with CHT without CHB, the studied parameter
19 decreased when compared with healthy children by 25%.

20 **Key words:** hypoxia; sensorineural hearing loss; hepatitis B; VEGF; PGDF; fetal
21 hemoglobin

22 **INTRODUCTION**

23 Sensorineural hearing loss in children refers to diseases, the problem of
24 diagnosis and treatment of which does not lose its relevance. The significant
25 prevalence of sensorineural hearing loss (CHT) is due to the diversity of both
26 endogenous and exogenous etiological factors that trigger the development of this
27 disease [2, 4]. In the occurrence of CHT, numerous clinical observations and
28 scientific studies have proved the role of perinatal pathology, the effects of toxic
29 and allergic factors, and the role of viral and vascular pathology in the etiology of
30 this disease [6, 9]. The social significance of the problem is due to the effect of
31 hearing impairment in children on their speech development, the formation of the
32 intellect and the personality of the child.

33 In modern scientific literature, it is considered established that there is
34 hypoxia of varying severity in chronic hepatitis, which is characterized by
35 persistent violations of the mechanisms of oxidation in the liver tissue with
36 subsequent progression of dystrophic, fibrotic processes and the development of

37 decompensation of the functional state of the organ [1, 7] also with their high
38 energy consumption.

39 Moreover, as has been shown in a number of studies, **intra-lobular** stellate
40 cells of the liver are more sensitive to oxygen insufficiency compared to cells in
41 the periportal zones and play a key role in angiogenesis with the involvement of
42 vascular endothelial growth factor (VEGF) and platelet growth factor (PGDF) in
43 the pathological process [12, 13, 18]. Under the influence of hypoxia, the release of
44 HIF-1 from stellate cells is stimulated, which affects the progression of
45 angiogenesis and fibrosis. Consequently, the delicate balance between the need of
46 liver tissue for oxygen and its delivery can be disrupted by the pathology of this
47 organ. Endothelial dysfunction resulting from chronic hepatitis B (CHB), causing
48 vasodilation in the lungs, contributes to the development of hypoxemia [3].
49 According to the researchers, hypoxia of the hepatic parenchyma in CHB is a
50 consequence of several mechanisms, including vascular resistance, intrahepatic
51 shunts, intravascular thrombosis, and a venom of the venous process, a venom, and
52 a subjugation of the venom of the procedure. and sinusoidal capillaries [14].

53 Due to the fact that the gas transport function of blood occupies a special
54 place in providing adaptive and compensatory processes, in recent years, scientists
55 have focused on studying the problem of heterogeneity of hemoglobin in various
56 human pathological conditions [1].

57 Numerous studies have shown an increase in the concentration of fetal
58 hemoglobin (HbF) in erythrocytes under various pathological conditions, which is
59 a consequence of the adaptive reactions of the erythron to the hypoxia state [4]. It
60 is known that HbF has an extremely high ability to bind oxygen and the increased
61 affinity of HbF to oxygen helps the body to adapt conditions of relative hypoxia
62 and provide the tissue with sufficient oxygen. It is believed that the increase in
63 HbF level is a biochemical mechanism long-term adaptation to hypoxia, which is
64 based on the phenomenon of “adaptive stabilization structures” is realized upon
65 activation of the genetic apparatus of the cell in response to a change homeostasis
66 [3]. Therefore, HbF is a marker of tissue hypoxia not only in newborns, but also in
67 various adult pathologies.

68 Despite the considerable interest in the study of fetal hemoglobin in various
69 somatic diseases, in the available modern literature we have not found data on its
70 study in children with sensorineural hearing loss associated with hepatitis B.

71 **MATERIAL AND METHODS**

72 To solve the tasks for the period from 2016 to 2018. On the basis of the
73 Republican Scientific and Practical Medical Center of Pediatrics, 26 children with
74 sensorineural hearing loss associated with hepatitis B, aged from 5 to 18 years,
75 were examined. The comparison group consisted of 8 children with sensorineural

76 hearing loss without concomitant somatic pathology. The control group consisted
77 of 12 healthy children.

78 All patients were admitted to the hospital in the acute phase of sensorineural
79 hearing loss associated with hepatitis. A detailed clinical diagnosis was made on
80 the basis of the nature of complaints, anamnesis, clinical examination results,
81 laboratory and instrumental methods of diagnosis, taking into account information
82 from the patient's outpatient card (extracts of case histories of previous
83 hospitalizations, data of dynamic observation of the patient in the clinic). In
84 making the diagnosis, modern classifications of sensorineural hearing loss in
85 children were used.

86 Criteria for the inclusion of patients in the study were proven viral etiology
87 of sensorineural pathology; proven signs of HBV.

88 Exclusion criteria for the study were: primary pathology of the biliary
89 system (primary sclerosing cholangitis, primary biliary cirrhosis), signs of
90 secondary liver damage in patients with chronic diseases of the biliary tract and
91 intestines (cholelithiasis, chronic cholecystitis, stenotic papillitis, Crohn's disease,
92 ulcerative colitis); extrahepatic obstruction of the portal vein, associated with the
93 consequences of surgical interventions, portal vein thrombosis, tum cholic
94 pathology, congenital developmental abnormalities, injuries; Budd-Chiari
95 syndrome; patients with fever associated with concomitant diseases (acute
96 respiratory infections, pneumonia, acute intestinal infections, pyelonephritis, etc.);
97 acute and chronic diseases of the broncho-pulmonary system;

98 All clinical, anamnestic and laboratory and instrumental data were entered
99 into a detailed map developed by us. The map noted the patient's complaints, of
100 which more often there was increased fatigue, weakness, memory and sleep
101 disorders, dyspeptic disorders (nausea, belching, vomiting), pain and heaviness in
102 the right hypochondrium, epigastric pain, nausea, loss of appetite. Complaints
103 about shortness of breath and her temper, cough, pain in the heart area, the
104 presence of heartbeat and rhythm disturbances, and changes in blood pressure were
105 investigated in detail.

106 The presence of jaundice, pruritus, fever, manifestations of hemorrhagic
107 syndrome (gingival, nasal, gastroesophageal, hemorrhoidal bleeding), arthralgia,
108 stool disorders, flatulence, and dysphagia were taken into account. An objective
109 examination focused on manifestations of portal hypertension and signs of disease
110 activity for the presence of liver signs (spider veins, palmar erythema), xanthomas,
111 Dupuytren's contracture, lymph nodes, abdominal veins, ascites, peripheral edema,
112 gynecomastia, and the size of the liver and spleen.

113 The compulsory examination plan for patients included generally accepted
114 laboratory and instrumental diagnostic methods: complete blood count, urine,

115 feces, Wasserman reaction, ECG. A study was conducted in the blood of total
116 protein and protein fractions, immunoglobulins, Circulating Immune Complexes
117 (CIC), lipoproteins, cholesterol, bilirubin, urea, creatinine, amylase of blood and
118 urine, determined the activity of Alanine Aminotransferase (ALT) and Aspartate
119 Aminotransferase (AST), GTP, alkaline phosphatase. The coagulogram was
120 determined: fibrinogen content, XIII coagulation factor, fibrinolytic activity, fibrin
121 monomers using ethanol test, fibrinogen-fibrin degradation products by the
122 method.

123 For the isolation and purification of HbF, alkaline denaturation with 1.2 M
124 NaOH, salting out with ammonium sulfate, gel filtration on a column with
125 Sephadex G-25 (working buffer — 0.05 M phosphate buffer pH 7.4), and ion-
126 exchange chromatography on DEAE Sephadex were used G-50 on 0.01 M Tris-
127 chloride buffer pH 8.1. The quantitative determination of HbF was carried out by
128 electrophoresis on an agar gel with sodium dodecyl sulfate. The HbF level in the
129 control group was 2.26 ± 0.02 g/l, which is corresponds to literary data. Gender
130 differences in the control group were absent.

131 *Statistical analyses*

132 Data analysis was performed using the STATISTICA v.6.0 Windows XP
133 application package. Descriptive statistics of the trait included arithmetic average
134 (M), minimum and maximum values, median (Me) and interquartile range [Q25-
135 Q75]. When comparing the obtained results, the Mann-Whitney test was used due
136 to the inconsistency of the analyzed data with the law of normal distribution. The
137 relationship between signs was studied by the Spearman (R) correlation analysis
138 method. Differences were considered statistically significant at $p < 0.05$.

139 **RESULTS AND DISCUSSION**

140 As can be seen from the presented research results (table 1), the hemoglobin
141 values of blood in the examined children with sensorineural hearing loss of the
142 associated CHB was significantly reduced by 58% compared with the healthy
143 children. In children with CHT without CHB, the studied parameter decreased
144 when compared with healthy children by 25%.

145 Other dynamics was noted with respect to fetal hemoglobin in the blood of
146 the examined children with combined pathology. Analysis of the results showed a
147 significant increase in the level of fetal hemoglobin in the blood of children with
148 CHT associated with hepatitis B on average by 1.5 times, indicating hypoxia.

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153 **Blood biochemical parameters in children with hepatitis B associated hearing**
 154 **loss**

№	Indicators	Healthy children n = 12	Children sensory hearing loss combined CHB n = 26	Children sensory hearing loss without CHB n = 8
1	Hemoglobin content g/l	158,62±8,02	92,05±6,54*	119,32±8,13
2	Fetal hemoglobin (HbF) content (g/l)	2,26±0,12	3,41±0,18*	2,38±0,19
3	pO ₂ of arterial blood (mm Hg)	76,05±6,11	64,82±5,43	72,43±6,32
4	pO ₂ arterio-venous blood difference (mm Hg)	42,35±3,12	24,89±1,57*	38,75±2,87
5	The content of desquamated endothelial blood cells (x10 ⁴ /l)	2,34±0,22	4,78±0,34*	3,01±0,26*
6	Endothelin 1-retention (fmol/l)	0,93±0,12	1,740±,13*	0,96±0,12
7	Von Willebrand factor (%)	76,51±4,27	112,36± 7,11*	91,76± 6,34*
8	Activity lactate dehydrogenase (U/l)	576,12 ±11,3	1028,74± 13,85*	788,63 ±12,34
9	Aspartate aminotransferase activity (U/l)	20,61 ±1,44	69,71 ± 5.32*	32,28 ±2,52*

155 Note: * - significance of differences P <0.05

156 Hypoxia in chronic liver disease can be both local and systemic. Deficiency
 157 of oxygen entering the liver parenchyma occurs during chronic hepatitis as a result
 158 of several mechanisms that include vascular resistance, intrahepatic shunts,
 159 intravascular thrombus formation, reduction in the area of sinusoids and sinusoidal
 160 capillaries. Endothelial dysfunction (ED) plays a significant role in the
 161 development of these processes. ED is involved in the formation of portal
 162 hypertension that develops in chronic hepatitis, which leads to the formation of an
 163 extensive network of portal anastomoses, including and in the lung tissue with
 164 increased hypoxia and hypoxemia.

165 One of the objectives of this study was to establish the association of HbF
166 changes with the concentration of such important markers of endothelial
167 dysfunction as the vasoconstrictor ET-1 and the adhesive protein von Willebrand
168 factor. As can be seen from the data of table 1, the studied markers of endothelial
169 dysfunction were significantly increased when comparing the obtained results with
170 the values of healthy children. Consequently, a comprehensive assessment of HbF
171 levels, markers of endothelial dysfunction, in combination with clinical data,
172 provides much more information about the development of tissue hypoxia and
173 hypoxemia in sick children with combined pathology, and also allows an
174 additional assessment of the severity of the pathological process in the liver.

175 It was found that in children with sensorineural hearing loss associated with
176 hepatitis B, in 30.8% of cases there was a simultaneous increase in the
177 concentration of HbF and a decrease in blood oxygen saturation of 64.82 ± 5.43
178 mm Hg. against 76.05 ± 6.11 mm Hg, indicating moderate hypoxemia. The increase
179 in tissue hypoxia was also indicated by the results of arterial-venous blood
180 difference in the examined children. For a more reliable confirmation of this
181 version, we studied the activity of lactate dehydrogenase and aspartate
182 aminotransferase in the blood of the examined children. As can be seen from the
183 obtained results of the research, the activity of the studied enzymes exceeded the
184 initial indicators, respectively, by 59% and 3.3 times.

185 An indicator of the functional state of endotheliocytes is von Willebrand
186 factor and endothelin-1. As can be seen from the presented research results, the
187 level of endothelin-1 in children with the combined form of the disease was
188 significantly higher in comparison with healthy children. The level of activity of
189 von Willebrand factor in the blood plasma was also significantly higher than in
190 healthy children.

191 Under physiological conditions, the endothelium produces a number of
192 vasodilating and vasoconstrictive substances that support the necessary level of
193 vascular tone. Numerous studies have shown that endothelin-1 is the most potent
194 vasoconstrictor factor currently known. Proved that vascular endothelial is the
195 main source of endothelin-1 in vivo.

196 The von Willebrand factor is a complex multidimensional adhesive
197 glycoprotein synthesized by endothelial cells. Functionally, it is a carrier-stabilizer
198 for procoagulant protein that circulates in the blood serum as a non-covalently
199 bound complex and is an adhesion protein in hemostasis processes. The von
200 Willebrand factor can bind collagen and possibly other endothelial structures and
201 mediate platelet adhesion to the subendothelium through the binding of the
202 glycoprotein Ib surface platelet receptor. Therefore, an increase in the level of von
203 Willebrand factor activity is an indicator of endothelial damage.

204 In this study, in children with sensorineural hearing loss associated with
205 hepatitis B, the reaction of endothelial dysfunction indicators was detected - a
206 significant increase in endothelin-1 level and von Willebrand factor activity in the
207 blood plasma and their interrelation with the level of oxygen partial pressure in the
208 blood, which indicates a violation of vasoconstrictor therapy and adhesive
209 endothelial function in this pathology. This fact is explained by the fact that with
210 this pathology in children there are favorable conditions for the development of
211 endothelial dysfunction, on the background of hypoxia, as well as disruption of the
212 metabolic function of the endothelium, which can lead to an increase in the content
213 of various biologically active substances.

214 In addition, an important sign of endothelial dysfunction is a change in the
215 phenotypic activity of endotheliocytes, which results in the cells losing
216 anticoagulant properties and enhancing the production of coagulation factors.
217 When exposed to a damaging factor (hypoxia), leukocytes, monocytes,
218 mononuclear phagocytes are activated, and damage and proliferation factors are
219 produced: free radicals, interleukin-1, tumor necrosis factor a, tissue factor,
220 tromboocyte growth factor, and other biologists, active substances acting on
221 endotheliocytes. In this situation, endotheliocytes begin to intensively secrete
222 vasoactive and prosclerogenic substances (endothelin, etc.), the accumulation of
223 which stimulates fibrotic changes and vascular remodeling.

224 Thus, an important pathogenetic role of endothelial dysfunction in children
225 with sensorineural hearing loss associated with hepatitis B has been shown.

226 **CONCLUSION**

227 Firstly, dependence of the indices of partial oxygen in the blood and, to a greater
228 extent, HbF, on the blood content of the vasoconstrictor endothelin-1, von
229 Willebrand factor, indicates the pathogenetic significance of the leading markers of
230 endothelial dysfunction in the development of tissue hypoxia in children with
231 sensorineural hearing loss combined liver disease.

232 Secondly, combination of sensorineural hearing loss with hepatitis B in children.
233 Pulmonary hypertension is associated with endothelial dysfunction (increased
234 endothelin-1 concentration and von Willebrand factor activity).

235 Thirdly, determining level of HbF in children with CHT of combined HBV can be
236 used to diagnose chronic tissue hypoxia and helps to clarify the severity of the
237 pathological process, which allows to predict the progression of the disease and
238 their complications.

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