МЕДИЦИНА И СТОМАТОЛОГИЯ

SEROLOGICAL METHODS IN THE DIAGNOSIS OF LIVER FIBROSIS IN PREGNANT WOMEN WITH HEPATITIS B, C AND EVALUATION OF THE SENSITIVITY AND SPECIFICITY OF SWE ELASTOGRAPHY

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Urgency. B, C virus hepatitis being the leading cause of acute and chronic liver disease, causes 1,4 million deaths every year. Currently, 248 million people worldwide live with chronic HBV infection, and 110 million people are positive for HCV-antibodies (in 80 million of them an active viremic infections are found) (1) According to the American College Obstetricians and Gynaecologist (FAQ-Pregnancy 093, November, 2013), chronic HBV infection can lead to complications such as liver cirrhosis, liver cancer and premature death. Infected infants are at high risk (up to 90%) for viral transmission.(2) They also transmit the virus to others and in 25% of cases they die because of liver cirrhosis or liver cancer at reaching adulthood. Hepatitis C also has similar complications, such as Hepatitis B. Chronicity of these diseases contributes to the development of fibrosis in the liver. In scientific sources of recent years (2018), there are reports on chronicity of the disease during pregnancy and deepening of fibrosis in the liver. (3)

There are various methods in the diagnosis of liver fibrosis, and biopsy is considered the "gold standard" among them. However, because the method is invasive, its use in pregnant women is limited. The rapid development of biological medicine in recent years has led to the use of non-invasive serological tests in blood in the diagnosis of liver fibrosis (serum haptoglobin, serological analysis of AST / ALT), and SWE elastography that detects focal lesion in the liver. (4)

Haptoglobin is a major plasma protein that is mainly synthesized in livers and rarely in the lungs, fat tissues, skin, and kidneys. The main function of haptoglobin is to unite and remove the free haemoglobin, which is released as a result of intravascular or extravascular haemolysis, from body, thereby having an antioxidant role in the body. (5**,** 6,7) Haptoglobin-haemoglobin complex is a stable complex and has high affinity. (8)Scientific sources have reported the importance of the determination of haptoglobin in the blood as a major non-invasive serum indicator of hepatic fibrosis in patients with hepatitis B and C. Haptoglobin is a key component of the FIBRO test used in the diagnosis of liver fibrosis. (9,10)

Increasing of levels of alanine aminotransferase and aspartate aminotransferase, the major enzymes of liver in hepatitis B, C, in particular determination of Retis Index-AAN (AST/ALT) ratio as an indicator of liver fibrosis include diagnostic non-invasive methods. (11)

During our study of scientific sources, no scientific research was found in pregnant women with chronic B, C hepatitis that study blood haptoglobulin (Hp), alaninaminotransferase (ALT), aspartate aminotransferase (AST), serum albumin, C-reactive protein (CRP), and SWE elastography of liver in comprehensive and comparative way, on specificity and sensitivity of these methods in the diagnosis of hepatic fibrosis during pregnancy. No scientific research has been carried out in this area in the Republic of Azerbaijan either.

Objective of the study: to clarify the role of serum Hp, ALT, AST, serum albumin as serum biomarkers of liver fibrosis in pregnant women with chronic B, C virus hepatitis, and as well as to evaluate the serological methods shown in the diagnosis of liver fibrosis and the specificity and sensitivity of the SWE elastography of liver. For this purpose, a comparative analysis of non-invasive serological methods and SWE elastography of the liver was performed with specific mathematical-statistical methods in infected pregnant women.

Object and methods of research: Scientific research work was conducted at the II Department of Obstetrics and Gynaecology at the Azerbaijan Medical University, Baku, Azerbaijan. The research was based on the collected material for 2016-2018. This scientific work has been approved by the Ethics Committee of Azerbaijan Medical University. The focus of the study was 100 pregnant women with chronic B, C virus hepatitis in the age group of 18-45 years, and test group consisted of 50 practically healthy pregnant women. Pregnant patients with genital and extragenital infections, severe preeclampsia, and haemolytic pathologies were not included in the group. Pregnant women in the study group were identical to their pregnancy and parity. Diagnostics of viral hepatitis (B, C) were performed by Express Card, immune-enzyme analysis (Automated Biochemical ECLIA Analyzer - Cobas 4000 e411), and Quantitative and Qualitative analysis of HBV, HCV was performed by PZR Reaction (Real Time PZR Detection Systems). PZR-1IU = 4.5 copies for hepatitis B virus; PZR-1IU = 2,5

copies for Hepatitis C virus (National Institute of Biological Standards and Hepatitis B Control for NIBSC WHO International Standard: 97/746) 2,5 ml blood samples were taken from elbow veins for PZR analyzes. The amount of haptoglobins in the blood serum was performed by immunoturbidimetric analysis. (The human haptoglobin causes a precipitate in reaction with a specific antiserum). Immunoturbidimetric analysis (Roshe Company) is based on the principle of immunological agglutination. Blood samples for biochemical analyzes were obtained from elbow veins in the amount of 3 ml after morning starvation. Two reagents were used during the laboratory analysis: R1-phosphate buffer: 12,7 mmol / l, pH 7,2; NaCL: 130 mmol / l; PEQ: 40g/l, preservative; R2 reagent: antibody taken from rabbit blood against human haptoglobin. Blood was centrifuged and plasma removed, placed in a test bottle containing Li-heparin and K_2-EDTA. Determination of haptoglobins in blood serum, liver enzymes, serum albumin, and CRZ in blood was performed on a biochemical automated analyzer of Roche-Hitachi Cobas 4000 c311 (c 501/502) - (USA) by immunoturbidimetric method. (12,13,14)

Scientific literature provides very little information on the level of serum haptoglobins during pregnancy. (15)

The liver density in pregnant women was studied by Shear Wave Elastography, SWE elastography of the liver in pregnant women was performed at the Supersonic Aixplorer multi-Wave (France) device at the AMU Educational Surgery Clinic. The examination was conducted in a left-leaning position lying on the back. The ultrasonic transmitter is located in the VIII-IX hypochondrium area, along the right and front axillary lines. The stiffness of the tissue was measured 2 cm below the liver capsule in the non-vascular area of the right liver lobe. The results were evaluated according to the METAVIR scale. (16)

Statistical methods: Variance, dispersion (F-Fisher), discriminant (χ2-Pearson Chi Square), and correlation analysis (ρ-Spearman) were used in the course of the study. Statistical analyzes were performed in the MS EXCEL-2013 spreadsheet and in the SPSS-20 statistical package software. “O” hypothesis was rejected when $p \leq 0{,}050$ was in statistical analyzes.

An ROC curve is set on the sensitivity and specificity indicators. The ROC curve (receiver operating characteristic) indicates the dependence of the number of cases correctly diagnosed (positive) in the binary classification on the number of inaccurate (negative) cases. The numerical evaluation of the ROC is measured by the area below the curve. The ideal ROC curve has a Γ-shaped form. When the characteristic curve is close to the ideal graphics, the studied method is considered effective.

Results of the study: 150 pregnant women were included in the study object. The studied pregnant women were divided into 3 groups: I group - control group (n = 50). The control group was made up of practically healthy pregnant women. II group - HBV-positive pregnant women (n = 55), III group - HCV-positive pregnant women (n = 45). Only monocyesis pregnant women were included in the study. There were no smokers or alcohol-addicted women in the study group. Demographic data and clinical characteristics of patients indicated that all pregnant women were Azerbaijani. The average age of the control group was 26,7 ± 0,6; in the main group it was 28,8 ± 0,5 years. BKI in practical healthy pregnant women was 24,5 ± 0,5 kg/m²; in the main group, 25,7 ± 0,3 kg/m² (it should be noted that BKI in pregnant women was calculated based on pre-pregnancy weight gain in the second trimester of pregnancy). The clinical characteristics of the groups under study for other indicators are given in Tables 1,2,3,4.

Table 1

Crosstab					
			Groups		Total
			Control	HBV and HCV	
Rh	Rh -	Count	8	9	17
		% within group	16,0%	9,0%	11,3%
	Rh +	Count	42	91	133
		% within group	84,0%	91,0%	88,7%
Total		Count	50	100	150
		% within group	100,0%	100,0%	100,0%

Chi-Square Tests					
	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,625[a]	1	,202		
Continuity Correction[b]	1,003	1	,316		
Likelihood Ratio	1,554	1	,213		
Fisher's Exact Test				,274	,158
Linear-by-Linear Association	1,615	1	,204		
N of Valid Cases	150				
a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 5,67.					
b. Computed only for a 2x2 table					

Table 2

Crosstab					
			Groups		Total
			Control	HBV and HCV	
Blood groups	O(I)	Count	13	37	50
		% within group	26,0%	37,0%	33,3%
	A(II)	Count	18	33	51
		% within group	36,0%	33,0%	34,0%
	B(III)	Count	10	20	30
		% within group	20,0%	20,0%	20,0%
	AB(IV)	Count	9	10	19
		% within group	18,0%	10,0%	12,7%
Total		Count	50	100	150
		% within group	100,0%	100,0%	100,0%

Chi-Square Tests			
	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2,982a	3	,394
Likelihood Ratio	2,947	3	,400
Linear-by-Linear Association	2,354	1	,125
N of Valid Cases	150		
a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 6,33.			

Table 3

Crosstab					
			Group		Total
			Control	HBV and HCV	
Count of pregnancies	First pregnancy	Count	32	42	74
		% within group	64,0%	42,0%	49,3%
	Multiple pregnancy	Count	18	58	76
		% within group	36,0%	58,0%	50,7%
Total		Count	50	100	150
		% within group	100,0%	100,0%	100,0%

Chi-Square Tests					
	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6,454[a]	1	,011		
Continuity Correction[b]	5,604	1	,018		
Likelihood Ratio	6,517	1	,011		
Fisher's Exact Test				,015	,009
Linear-by-Linear Association	6,411	1	,011		
N of Valid Cases	150				
a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 24,67.					
b. Computed only for a 2x2 table					

Table 4

Crosstab					
			Group		Total
			Control	HBV and HCV	
Paritet	Nulliparous women	Count	39	58	97
		% within group	78,0%	58,0%	64,7%
	Multiparous women	Count	11	42	53
		% within group	22,0%	42,0%	35,3%
Total		Count	50	100	150
		% within group	100,0%	100,0%	100,0%

Chi-Square Tests					
	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5,835[a]	1	,016		
Continuity Correction[b]	4,993	1	,025		
Likelihood Ratio	6,097	1	,014		
Fisher's Exact Test				,019	,012
Linear-by-Linear Association	5,797	1	,016		
N of Valid Cases	150				
a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 17,67.					
b. Computed only for a 2x2 table					

In infected pregnant women the average of ALT, AST enzymes was respectfully 27,5 ± 2,5 U / l (range 6-187) (pF = 0,026); 32,0 ± 2,6 U / l (range 3-194) (pF = 0,016) (in control group – 19,5 ± 0,7 U / l (range 11-28); 22,9 ± 0,5U / l (range 16-28). However, it should be noted that in 89% of pregnant women with chronic parenteral hepatitis, ALT remained unchanged and in 83% AST level remained unchanged, and only ALT increase was observed in 11,0 ± 3,1% and an increase in AST at 17,0 ± 3.8%.

However, different types of viremia have been detected in the blood. According to the results of study, virological examination of the blood of pregnant women with HBV in 14 patients (25,5 ± 5,9%) was PZR negative, in 20 patients (36,4 ± 6,5%) the burden of the virus was <2000 IU / ml, in 21 pregnant (38, 2 ± 6,6%) the viral load was> 2000 IU / ml. In 9 (20,0 ± 6,0%) pregnant women with HCV infection, PZR was negative; in 14 persons (31,1±6,9%) the burden of the virus was <4 x10^5 IU/ ml, in 6 pregnant women (13,3±5,1%) it was 4 x10^5 IU/ml - 8x10^5 IU / ml; in 16 pregnant women (35,6 ± 7.1%), the quantity index of the virus in blood was> 8x10^5 IU / ml.

On our part, the level of haptoglobin in the blood was comparatively studied in practically healthy pregnant women and women with chronic HBV and HCV. The results of the study showed that the level of haptoglobin in the blood of pregnant women with HBV and HCV was 1,2 times higher than in practically healthy pregnant women (Fp = 0,049) (Fig.1).

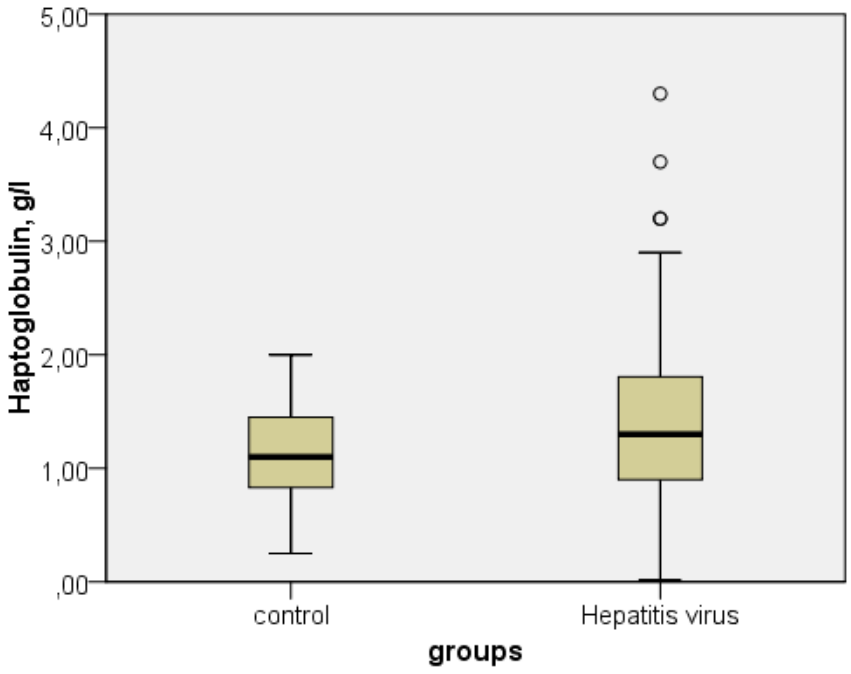

Figure 1. The level of haptoglobulin

It is known from scientific sources that haptoglobin may increase blood levels in inflammatory processes such as acute phase protein. It is even believed that haptoglobulin plays an immunomodulatory role in the pathogenesis of inflammatory diseases. (17)

On our part, we evaluated the correlation with HP and CRP, the primary inflammatory mediator in the blood, in order to study the role of haptoglobin as an inflammatory mediator or a fibrosis biomarker. Mathematical and statistical calculations revealed that positive correlation between HP and CRP was found (ρ = 0,273; ρ = 0,006) (Fig.2).

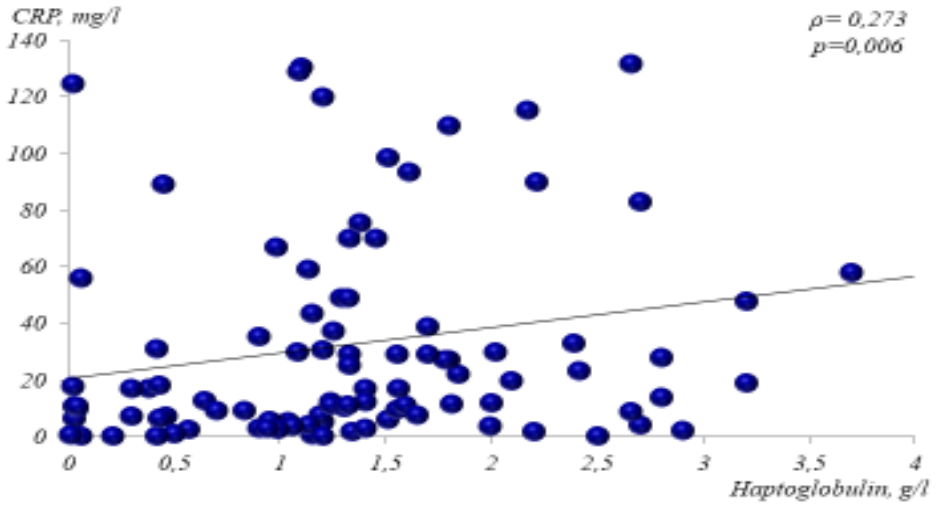

Figure 2. Haptoglobulin~ CRP

A study of liver density in parenteral hepatitis pregnant women with SWE elastography revealed that liver density in the main group was 1,6 times higher than in the control group (Fp = 0,009). It should be noted that comparisons between the main group and the control group on this indicator revealed that the liver density in the HCV group increased 1,8 times (Fp = 0,017) compared to the test group (1,5p) in the HBV group (Fp < 0,001).

On our part, the sensitivity and specificity of serological biomarkers (ALT, AST, haptoglobin) and liver SWE elastography in the diagnosis of liver fibrosis in pregnant women with chronic B, C hepatitis were studied by ROC analysis. Statistic calculation showed that a correctness of integrity indicators of specificity and sensitivity for ALT was p = 0,073; for AST was p = 0,058 in examined groups in diagnostics, which was not statistically correct (Fig.3). However, integral indicators of sensitivity and specificity for albumin were statistically correct (p<0,001) (Fig.4).

ROC ALT AST by group1

ROC Curve

Case Processing Summary	
Group	Valid N (listwise)
Positive	98
Negative	50
Missing	2

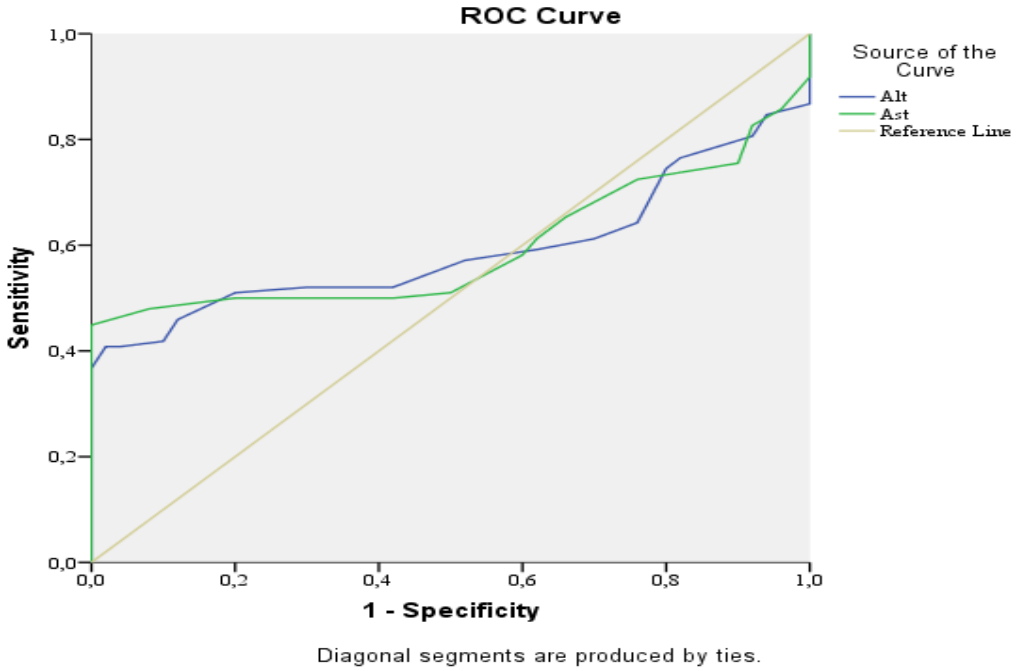

Figure 3. The sensitivity and specificity of ALT, AST

Area Under the Curve					
Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Alt	,590	,046	,073	,501	,680
Ast	,596	,046	,058	,506	,685

ROC Albumin BY group1
ROC Curve

Case Processing Summary	
Group	Valid N (listwise)
Positive	100
Negative	50

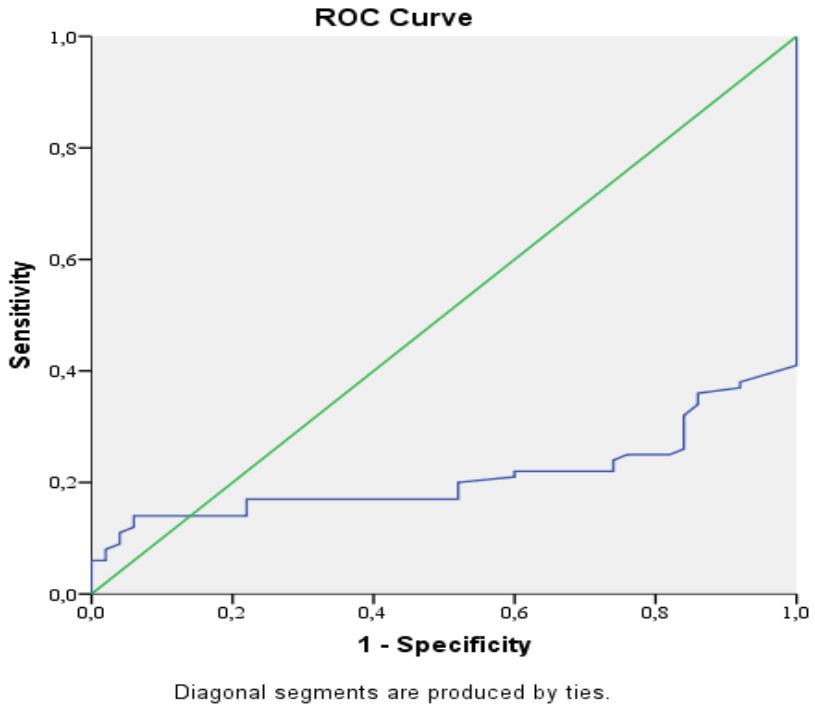

Figure 4. The sensitivity and specificity of albumin

Area Under the Curve				
Test Result Variable(s): Albumin				
Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
,211	,037	,000	,138	,284

Mathematical and statistical analyzes showed that integral indicators of sensitivity and specificity of serum haptoglobin in the setting of hepatic fibrosis in pregnant women were not statistically reliable (p = 0,085). However, integral indicators of sensitivity and specificity of the figures obtained from SWE elastography were statistically correct (p <0,001). (Fig.5)

ROC Haptoglobin Fibrosis by group1

ROC Curve

Case Processing Summary	
Group	Valid N (listwise)
Positive	100
Negative	50

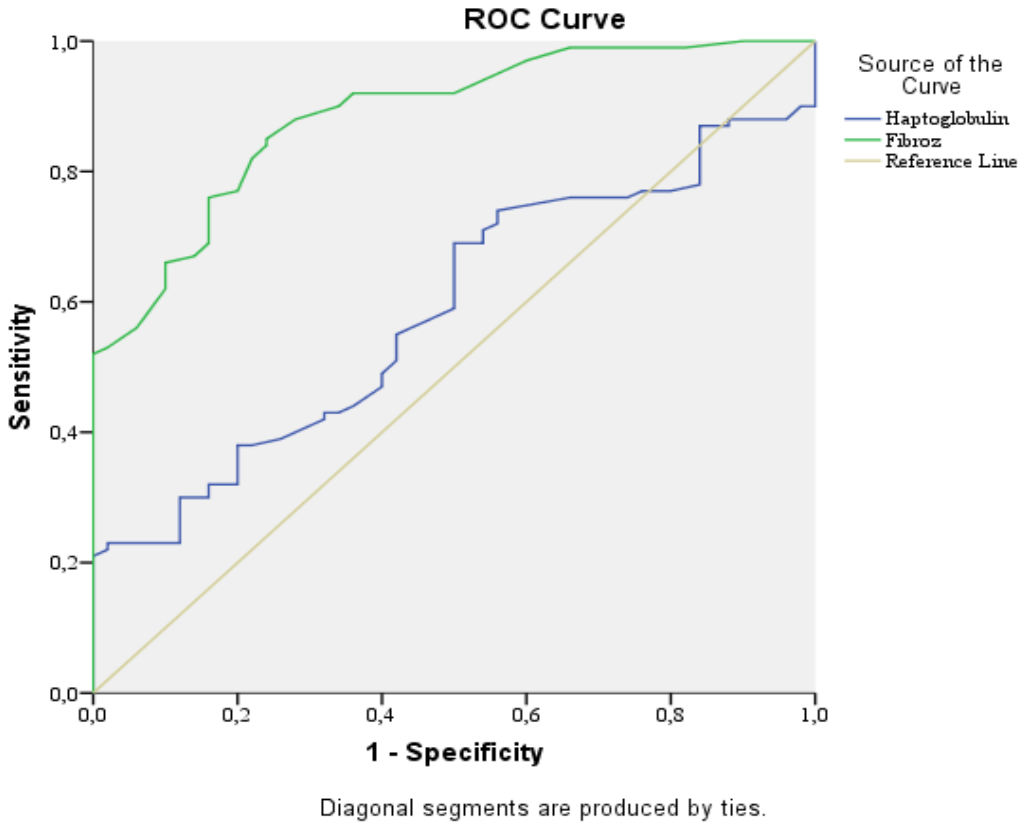

Figure 5. ROC curves for serum haptoglobin and SWE elastography

Area Under the Curve					
Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Haptoglobulin	,586	,047	,085	,495	,678
SWE (fibrosis)	,885	,027	,000	,833	,938

Discussion:

The study found that ALT, AST, and serum haptoglobin in pregnant women with hepatitis B, C were not the most effective method for detecting liver fibrosis. However, serum substances in the detection of hepatic fibrosis in non-pregnant patients with hepatitis B and C are an integral part of diagnostic tests. Serum haptoglobin has become more informative as an indicator of inflammatory process in pregnant women with parenteral hepatitis, which is the object of our study. Our results coincide with those of other authors. Some researchers believe that plasma haptoglobin levels are increased threefold if there are inflammatory processes in the absence of hemolysis in the blood. (18) Recent scientific studies have revealed a positive correlation of haptoglobin with IL-6 and IL-8 in inflammatory processes and severe chronic lung disease. (19)

The determination of serum albumin in infected pregnant women and SWE elastography of the liver has been shown to be highly effective as diagnostic methods for the detection of liver fibrosis. Studies by Fontane H. et al have shown that chronic hepatitis C infection has a negative effect on liver tissue during pregnancy: 25% of non-pregnant women and 83,3% of pregnant women have signs of necro-inflammatory process in the liver, respectively, signs of fibrosis were found in the liver in 8,3% of non-pregnant women and 41,6% of infected pregnant women.(20)

Conclusion: Thus, serum albumin and SWE elastography have high sensitivity and specificity in the diagnosis of hepatic fibrosis in pregnant women with hepatitis B, C. It is advisable to use these examination methods as non-invasive methods in the diagnosis of hepatic fibrosis in pregnant women infected with parenteral (B, C) hepatitis.

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