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Research Article

CORRELATION OF DIRECT AND INDIRECT BILIRUBIN IN FETUS SERUM OF NORMAL AND PREECLAMPTIC PREGNANCY

Ramadan Dacaj[1]., Donika Shala[2]., Curr Gjocaj[3]., Dervish Gashi[4]., Leopaldina Ramadani[5] and Mentor Malaj[6]

[1]Department of Gynecology and Obstetrics. General Hospital in Peja, Republic of Kosovo
[2]Gynecology with Obstetrics, Department of Obstetrics and Gynecology Peja, Kosovo
[3]Clinic of Gynecology and Obstetrics. University Clinical Hospital Service of Kosovo
[4]Gynecologist Obstetrician in the Department of Gynecology and Obstetrics Peja, Kosovo
[5]Family medicine service, Kosovo
[6]Nephrology Clinic, at the University Clinical Hospital Center of Kosovo

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A B S T R A C T

Introduction. It is known that in severe preeclampsia biochemical and pathohistological changes of hypoxic nature can occur due to changes that occur in the blood vessels as a whole, but also in the liver of the pregnant woman and the fetus. Determination of direct and indirect bilirubin in fetal blood in normal and preeclamptic pregnancies is of great importance to determine the health status of the fetus (newborn), as well as their prognosis, morbidity and mortality.
Purpose of the paper. It is to analyze the level and correlation of direct and indirect bilirubin in the blood of pregnant women and fetuses/newborns in normal and preeclamptic pregnancy.
Material and methods. The study included 80 pregnant women. In 40 pregnant women both pregnancy and fetal development flow in physiological form. While in 40 pregnant women we have selected pregnant women with preeclampsia and intrauterine growth retardation (IUGR - intrauterine growth retardation). The gestational age of pregnant women with normal flow was determined by the method of recent menstruation as well as by determining the biometrics of the fetus by ultrasonography. The processing of the results was done by statistical methods. Scattered, non-parametric numerical variables were analyzed using the Mann Whitney U test for independent samples. The Spearman correlation coefficient was used to calculate the data with normal distribution.
Results. They showed that there is no significant difference in the median of gestational weeks between pregnant women with physiological pregnancies and preeclamptic pregnancies [U = 1455.500, z=-1.827, p=0.068]. There is a very statistically significant difference between the median of newborn body weight between pregnant women with physiological pregnancy and preeclamptic pregnancy [U=489.500, z=-6.883, p <0.001]. Changes in patient age [1,817; 95% CI (-0.216; 3.849)] do not show statistical significance [t (118) =1.770, p=0.079].
The results have determined that the achieved values show that there is no statistically significant difference between the values of serum indirect bilirubin in pregnant women with physiological pregnancy and preeclamptic pregnancy [U=1430.500, z=-1.943, p=0.052]. There is also no statistically significant difference in direct bilirubin (μmol/L) values between pregnant women with physiological pregnancies and pregnant women with preeclamptic pregnancies [U=1537,000, z=-1.578, p=0.115]. The presented changes did not reach the threshold of statistical significance at the level α=0.05, so it can be concluded that there is no statistically significant difference in the values of indirect serum bilirubin between the fetus with increasing stagnation (IUGR) and the fetus with growth and normal development [U=1432.500, z=-1.929, p=0.054], while there is a statistically significant difference in the median values of direct bilirubin in the serum of stunted fetuses (IUGR) and fetuses with normal growth and development [U=1412.500, z=-2.037, p=0.042].
There is no statistically significant linear correlation between weeks of gestation and indirect serum bilirubin values in fetuses / newborns with normal development and growth (rs=-0.063; p> 0.05). There is a statistically significant linear correlation between the week of gestation and indirect serum bilirubin values in fetuses/newborns with increasing stagnation (rs = 0.666; p <0.001). There is a very significant positive linear statistical correlation between weeks of gestation and direct serum bilirubin in fetuses/newborns with increasing stagnation (rs=0.673; p<0.001).
Discussion. The increase in the level of bilirubin directly in the fetus with increasing stagnation (IUGR), is due to preeclampsia, as a consequence of hypoxia of the fetal liver. Due to the growing stagnation and hypoxia of the fetus, from the adrenal gland increases the synthesis of fetal cortizol which stimulates the synthesis of these enzymes uridin-diphosphate-glucuronyl-transferase as well as bilirubin-diglucuronyl-glucuronosyl-transferase which are found in hepatic fetuses. These enzymes conjugate indirect bilirubin to direct bilirubin as the case is followed by an increase in the level of direct bilirubin in the fetal blood with increasing stagnation. The increase in the level of direct bilirubin in fetuses with increasing stagnation is also explained by the fact that fetuses with increasing stagnation are in a state of stress which is caused by hypoxia. Hypoxia stimulates increased levels of prolactin, thyroid-stimulating hormone (TSH), which stimulate microsomes in liver cells to stimulate the synthesis of the enzyme glycuronyl transferase which makes the conjugation of indirect bilirubin to direct bilirubin.
The increase in the level of indirect bilirubin during the development of pregnancy in the fetus with increasing stagnation, is explained by the fact that due to hypoxia of the fetus with increasing stagnation, increases the synthesis of erythropoietin in the fetal kidneys which then stimulates erythropoiesis in the medulla (marrow) bone as the case follows the increase in the number of erythrocytes in relation to plasma as well as the increase in blood viscosity in the fetus with increasing stagnation (IUGR). In the fetus with increasing stagnation, the increase of indirect bilirubin in linear form follows also due to hypoxia and acidosis which exists in the growing stagnation of the fetus, in addition the increase of indirect bilirubin in linear form can also be caused due to hemolysis of erythrocytes as a consequence of oxytocin of fetal origin, which is synthesized in the fetal thymus with increasing stagnation. It has been proven that as the fetus grows, the level of fetal oxytocin also increases.
Conclusions. Based on the results we conclude that the level of direct serum bilirubune is significantly higher (p=0.042) in the fetus/newborn with increasing stagnation (IUGR) compared to the fetus/newborn with normal development. The results showed that there is a significant positive linear correlation (p<0.001) between the age of the fetus/newborn with stagnation (IUGR) and indirect bilirubin. In fetuses/neonates with increasing stagnation during intrauterine life, the level of indirect bilirubin increases with increasing fetal age during intrauterine life. The results also found that there is a positive linear correlation (p <0.001) between direct fetal/neonatal serum bilirubin with increasing stagnation during intrauterine life. Direct bilirubin levels increase significantly with the age of the fetus/neonates with increasing stagnation during intrauterine life.

INTRODUCTION

Preeclampsia in pregnancy is a complicated and not well defined condition in etiologicalterms. It is also associated with many complications on the part of the mother and fetus.

It is known that in severe preeclampsia pathological changes of hypoxic nature can occur due to changes that occur in the blood vessels as a whole, but also in the liver of the pregnant woman. Due to cell and tissue hypoxia and atherosclerotic changes in blood vessels, there are changes in the concentration of biochemical parameters in the body of the pregnant woman and the fetus.

Corresponding author:* **Ramadan Dacaj
Department of Gynecology and Obstetrics. General Hospital in Peja, Republic of Kosovo

Liver hypoxia plays an important role in the synthesis and excretion of biochemical parameters during pregnancy with preeclampsia. It is known that in severe preeclampsia pathological changes of hypoxic nature can occur as a result of changes that occur in the blood vessels of the pregnant woman's liver.

Biochemical parameters (direct and indirect bilirubin) change their concentrations which are related to path morphological changes that occur due to liver hypoxia. Determination of direct and indirect bilirubin in fetal blood in normal and preeclamptic pregnancies is of great importance to determine the health status of the fetus/newborn, as well as their prognosis, morbidity and mortality [1, 2, 3, 4].

Purpose of the Work

The aim of this study is to analyze the level and correlation of direct and indirect bilirubin in the blood of pregnant women and fetuses/newborns in normal and preeclamptic pregnancies.

MATERIAL AND METHODS

The study included 80 pregnant women. In 40 pregnant women both pregnancy and fetal development flow in physiological form. While in 40 pregnant women we have selected pregnant women with preeclampsia and intrauterine growth retardation (IUGR). The gestational age of pregnant women with normal flow was determined bythe method of recent menstruation as well as by determining the biometry of the fetus by ultrasonography. The gestational age of pregnant women with preeclampsia is determined by the Hadlock formula, the presence of proteinuria in the urine in quantities (> 0.5gr/l) as well as the measurement of blood pressure of pregnant womenin values above (TA = 140/90 mm/Hg).

In pregnant women with preeclampsia and pregnant women with normal pregnancies as well as in their fetuses / neonatuses, blood is taken from the cubital vein for the determination of direct and indirect bilirubin.

All pregnant women with the following pathologies were excluded from the study: RH- immunization, pregnancy anemia, pregnant women with fetal infection (especially with viral rubella, cytomegalovirus and herpes simplex virus), pregnant women who had hepatitis (A, B, C, D and E), cholelithiasis, liver cirrhosis, biliary cirrhosis, autoimmune hepatitis, inherited hyperbilirubinemia and pregnant women with anticoagulant syndrome.

The study also eliminated pregnant women who used drug therapy (corticosteroids, penicillamine, methyldopa and azithioprine).

The processing of the results was done by statistical methods. Scattered, non- parametric numerical variables were analyzed using the Mann Whitney U test for independent samples.

The Spearman correlation coefficient was used to calculate the data with normal distribution.

RESULTS

Weeks of Gestation

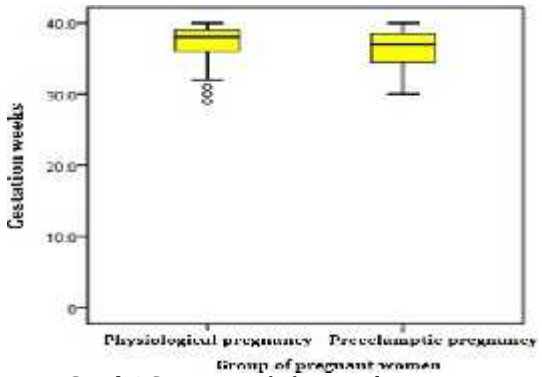
For the processing of the results we have analyzed the gestational age of pregnant women with physiological pregnancy and preeclamptic pregnancy, the weight of the fetus/newborn with normal development and growth and increasing stagnation as well as the age of pregnant women with physiological and preeclamptic pregnancy. We analyzed the level of direct and indirect bilirubin in the serum of pregnant women with physiological, preeclamptic pregnancies as well as the level of direct and indirect serum bilirubin in fetuses/newborns with normal growth and development and fetuses/newborns with increasing stagnation.

Pregnant women with preeclamptic pregnancies had lower median values of gestationalweeks [Me = 37.00 weeks (IQR = 32.45 to 38.75)] compared to pregnant women with physiological pregnancies [Me = 38.00 weeks (IQR = 36.00 to 39.00)]

Table 1 Gestation weeks by type of pregnancy

			Group of pregnant women	
			Physiological pregnancy	**Preeclamptic pregnancy**
Gestation weeks	N		60	60
	Mean		37.03	36.32
	Std. Deviation		2.66	2.55
	Minimum		29.00	30.00
	Maximum		40.00	40.00
	Percentiles	25th	36.00	34.25
		50th (Median)	38.00	37.00
		75th	39.00	38.75
	p - value		>0.05	

There is no significant difference in the median of gestational weeks between pregnant women with physiological pregnancies and preeclamptic pregnancies [U = 1455.500, z = -1.827, p = 0.068].



Graph 1 Gestation weeks by type of pregnancy

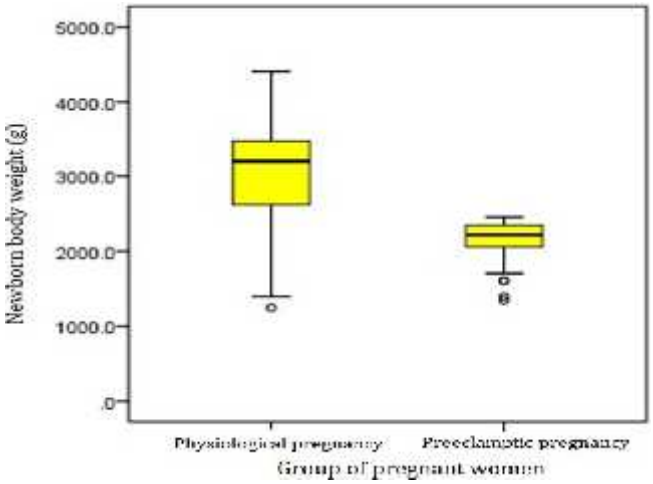
Body Weight of the Newborn

Pregnant women with preeclamptic pregnancies gave birth to newborns with lower body weight [Me = 2220.00g (IQR = 2055.00 to 2350.00)], compared to pregnant women who had physiological pregnancies [Me = 3200.00g (IQR = 2615.00 to 3487.50)].

Table 2 Body weight (g) of the newborn according to the type of pregnancy

			Group of pregnant women	
			Physiological pregnancy	**Preeclamptic pregnancy**
Newborn body weight (g)	N		60	60
	Mean		3010.00	2168.00
	Std. Deviation		670.81	261.49
	Minimum		1250.00	1350.00
	Maximum		4400.00	2460.00
	Percentiles	25th	2615.00	2055.00
		50th (Median)	3200.00	2220.00
		75th	3487.50	2350.00
	p - value		<0.001	

There is a very statistically significant difference between the median of newborn body weight between pregnant women with physiological pregnancies and preeclamptic pregnancies [U = 489,500, z = -6.883, p <0.001].



Graph 2 - Body weight (g) of the newborn according to the type of pregnancy

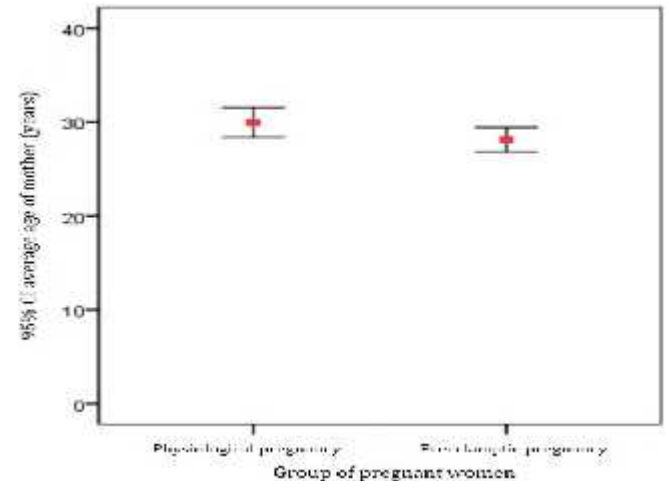
Age of Patients

Pregnant women with physiological pregnancies were older [= 29.95, SD = 6.10; 95% CI (28.37; 31.53)] compared to pregnant women with preeclamptic pregnancies [= 28.13, SD = 5.10; 95% CI (26.82; 29.45)].

Table 3 Age of patients in years by type of pregnancy

Group of pregnant women	N	Minimum	Maximum	Mean	Std. Deviation	p - value
Physiological pregnancy	60	17.00	42.00	29.95	6.10	>0.05
Preeclamptic pregnancy	60	18.00	38.00	28.13	5.10	

Changes in patient age [1,817; 95% CI (-0.216; 3.849)] show no statistical significance
[t (118) = 1.770, p = 0.079].



Graph 3 - Age of patients in years by type of pregnancy

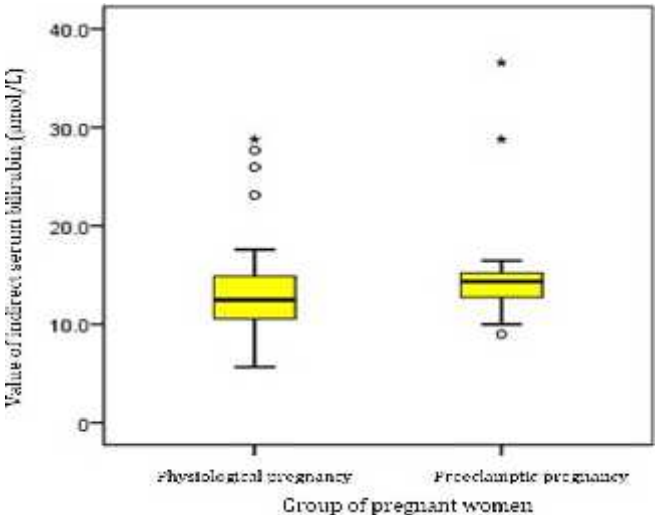
Indirect Bilirubin in Serum

Pregnant women with preeclamptic pregnancies had higher serum indirect bilirubin values [Me = 14.40 µmol/L (IQR = 12.80 to 15.38)] compared to pregnant women with normal pregnancy [Me = 12.55 µmol/L (IQR = 10.53 to 14.98)].

Table 4 Indirect serum bilirubin values (µmol/L l/L) by type of pregnancy.

			Group of pregnant women	
			Physiological pregnancy	Preeclamptic pregnancy
Indirect bilirubin(µmol/L)	N		60	60
	Mean		13.20	14.32
	Std. Deviation		4.52	4.04
	Minimum		5.70	9.00
	Maximum		28.80	36.60
	Percentiles	25th	10.53	12.80
		50th (Median)	12.55	14.40
		75th	14.98	15.38
p - value			>0.05	

The achieved values show that there is no statistically significant difference between the values of indirect serum bilirubin in pregnant women with physiological pregnancy and preeclamptic pregnancy [U = 1430.500, z = -1.943, p = 0.052].



Graph 4 Value of indirect serum bilirubin (µmol/L) by type of pregnancy.

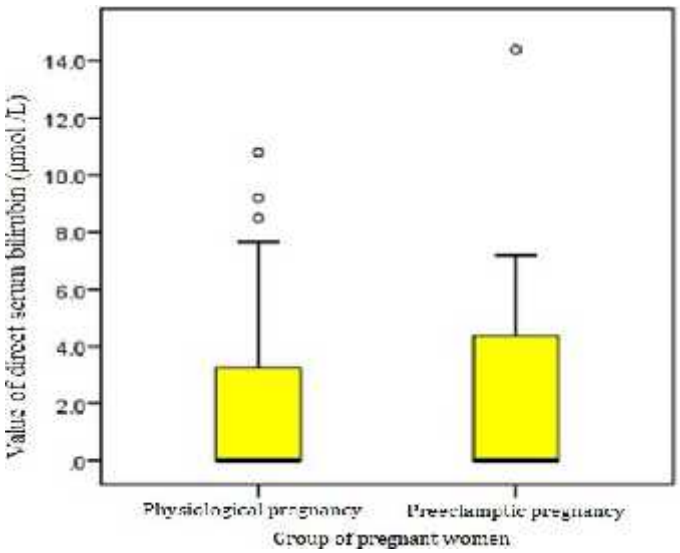
Direct Bilirubin of the Serum

Serum direct bilirubin median values in pregnant women with physiological pregnancyare 0.00 µmol/L (IQR = 0.00 to 3.33), also in pregnant women with preeclamptic. pregnancy the median value of direct bilirubin is 0.00 µmol/L (IQR = 0.00 to 4:43).

Table 5 Value of direct serum bilirubin (µmol/L) by type of pregnancy

			Group of pregnant women	
			Physiological pregnancy	Preeclamptic pregnancy
Direct bilirubin (µmol/L)	N		60	60
	Mean		1.69	2.36
	Std. Deviation		2.88	3.02
	Minimum		0.00	0.00
	Maximum		10.80	14.40
	Percentiles	25th	0.00	0.00
		50th (Median)	0.00	0.00
		75th	3.33	4.43
p - value			>0.05	

There is no statistically significant difference in direct bilirubin (µmol/L) values betweenpregnant women with physiological pregnancies and pregnant women with preeclamptic pregnancies [U = 1537,000, z = -1.578, p = 0.115].



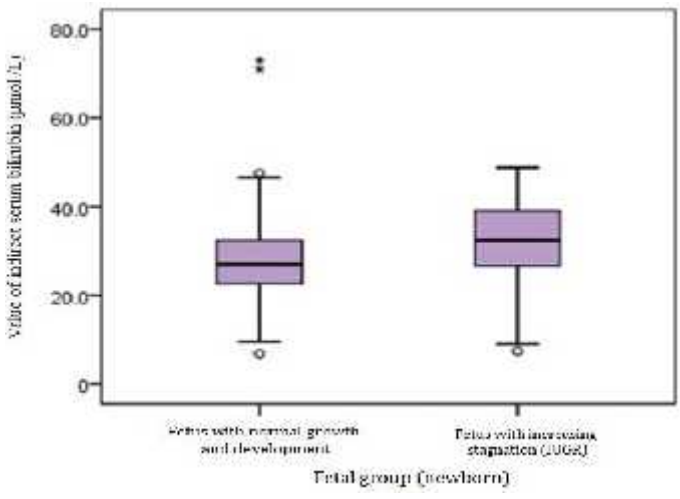
Graph 5 Value of direct serum bilirubin (µmol /L) by type of pregnancy.

Indirect Bilirubin in Serum

Fetuses/neonates with increased stagnation (IUGR) had higher values of indirect serum bilirubin [Me = 32.35 µmol (IQR = 26.43 to 39.00)] compared to fetuses/neonates with normal growth and development [Me = 27.00 µmol (IQR = 22.60 to 32.58)].

Table 6 Indirect serum bilirubin values of fetuses/neonates with increasing stagnation (IUGR)and fetuses with normal growth and development.

			Fetal group (newborn)	
			Fetus (newborn) withnormal growth and development	**Fetus (newborn) with increasing stagnation (IUGR)**
Indirect bilirubin (µmol/L)	N		60	60
	Mean		28.67	30.79
	Std. Deviation		12.53	11.36
	Minimum		6.80	7.40
	Maximum		73.00	48.80
	Percentiles	25th	22.60	26.43
		50th (Median)	27.00	32.35
		75th	32.58	39.00
p - value			>0.05	



Graph 6 Indirect serum bilirubin values of fetuses/neonates with increasing stagnation(IUGR) and fetuses with normal growth and development.

The presented changes did not reach the threshold of statistical significance at the level α = 0.05, so it can be concluded that there is no statistically significant difference in the values of indirect serum bilirubin between the growing stagnant fetus (IUGR) and the growing fetus. and normal development [U = 1432.500, z = -1.929, p = 0.054].

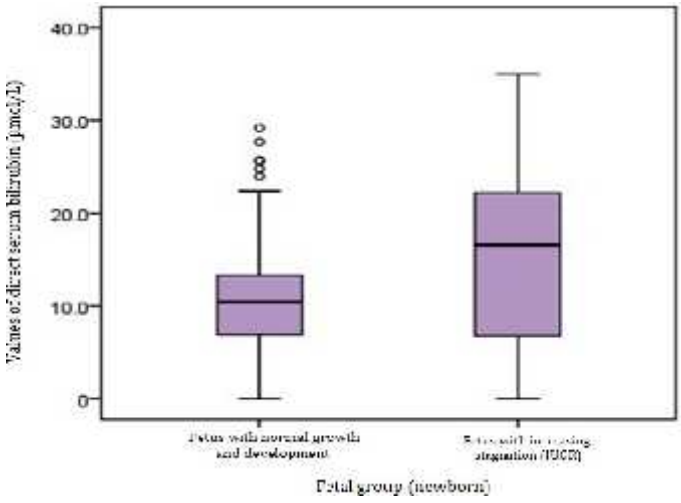
Direct Bilirubin of the Serum

Fetuses/newborns with stagnation had higher serum direct bilirubin values [Me = 16.60 µmol (IQR = 6.75 to 23.13)] compared to fetuses/newborns with normal growth and development [Me = 10.40 µmol (IQR = 6.85 to 13.30)].

Table 7 Direct serum bilirubin values in fetuses/neonates with increasing stagnation (IUGR) and fetuses with normal growth and development.

			Fetal group (newborn)	
			Fetus (newborn) with normal growthand development	**Fetus (newborn) with increasing stagnation(IUGR)**
Direct bilirubin (µmol/L)	N		60	60
	Mean		10.87	14.56
	Std. Deviation		7.27	9.69
	Minimum		0.00	0.00
	Maximum		2 9.20	35.00
	Percentiles	25th	6. 85	6.75
		50th (Median)	10.40	16.60
		75th	13.30	23.13
p - value			<0.05	

There is a statistically significant difference in the median values of direct bilirubin in the serum of fetuses with increasing stagnation (IUGR) and fetuses with normal growth and development [U = 1412.500, z = -2.037, p = 0.042].



Graph 7 Serum direct bilirubin values in fetuses/neonates with increasing stagnation (IUGR) and fetuses with normal growth and development.

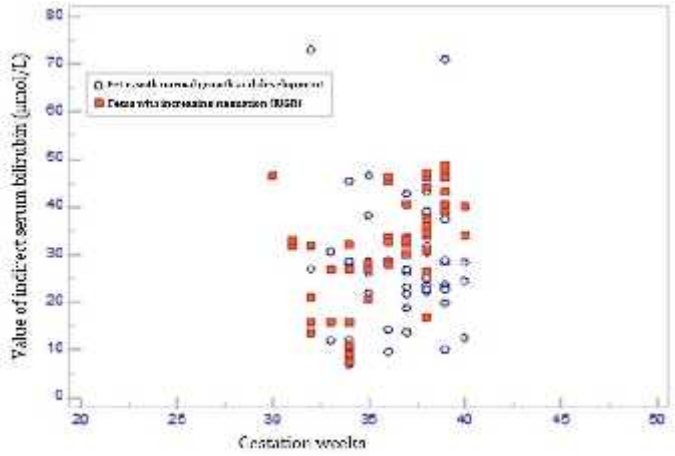
Correlation between Weeks of Gestation And Indirect Bilirubin In Fetus Serum

Table 8 Gestation week of gestation and indirect bilirubin values in fetal/neonatal serum.

Group of pregnant women			**Gestation weeks**	**Indirect bilirubin (µmol/L)**
Fetus with normal	Gestation weeks	Correlation Coefficient	1.000	-0.063
		Sig. (2-tailed)	.	0.633
		N	60	60

Spearman's rho	Fetus with normal growth and development	Indirect bilirubin (µmol/L)	Correlation Coefficient	-0.063	1.000
			Sig. (2-tailed)	0.633	.
			N	60	60
	Fetus with increasing stagnation (IUGR)	Gestation weeks	Correlation Coefficient	1.000	0.666**
			Sig. (2-tailed)	.	0.000
			N	60	60
		Indirect bilirubin (µmol/L)	Correlation Coefficient	0.666**	1.000
			Sig. (2-tailed)	0.000	.
			N	60	60

There is no statistically significant linear correlation between weeks of gestation and indirect serum bilirubin values in fetuses/neonates with normal development and growth (rs = -0.063; p> 0.05). There is a statistically significant linear correlation between gestational week and serum indirect bilirubin values in fetuses/neonates with increasing stagnation (rs = 0.666; p<0.001).



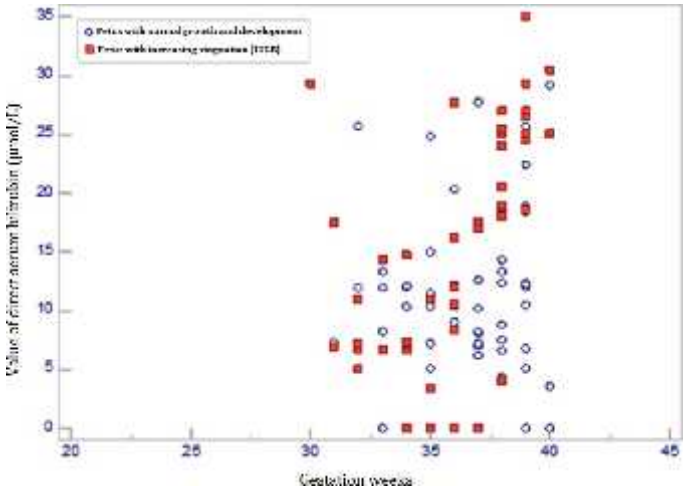
Graph 8 Weekly gestation and indirect bilirubin value serum collection in fetal/neonatalserum.

Correlation between Weeks of Gestation and Direct Bilirubin in Fetus Serum

There is no significant linear correlation between weeks of gestation and direct fetal/neonatal serum bilirubin values with normal development and growth [rs = -0.060; p> 0.05).

Table 9 Correlation of week gestation and direct bilirubin values in fetal/neonatal serum There is a very significant positive linear statistical correlation between weeks of gestation and direct serum bilirubin in fetuses/neonates with increasing stagnation (IUGR) (rs=0.673; p<0.001).

	Group of pregnant women			Gestation weeks	Direct bilirubin (µmol/L)
Spearman's rho	Fetus with normal growth and development	Gestation weeks	Correlation Coefficient	1.000	0.060
			Sig. (2-tailed)	.	0.647
			N	60	60
		Direct bilirubin (µmol/L)	Correlation Coefficient	0.060	1.000
			Sig. (2-tailed)	0.647	.
			N	60	60
	Fetus with increasing stagnation (IUGR)	Gestation weeks	Correlation Coefficient	1.000	0.673**
			Sig. (2-tailed)	.	0.000
			N	60	60
		Direct bilirubin (µmol/L)	Correlation Coefficient	0.673**	1.000
			Sig. (2-tailed)	0.000	.
			N	60	60



Graph 9 Gestation week and direct bilirubin values in fetal/neonatal serum.

DISCUSSION

Preeclampsia in pregnancy is a serious condition in terms of health which presents with a high percentage of morbidity and mortality. Preeclampsia in many cases endangers the health of the mother due to increased blood pressure which can be followed by numerous complications on the part of the mother causing in extreme cases bleeding and cerebral palsy as well as acute pulmonary cor. In the fetus it can also cause complications in the form of increasing stagnation (IUGR) which then manifests itself with neurological problems and psychic retardation.

These changes occur due to the lack of extravasation of the extravilous trophoblast into the spiral artery. Due to non-invasion of the extravilous trophoblast in the spiral arteries will follow the reduction of blood flow in the intervilous spaces. Decreased blood flow in the intervilous spaces causes hypoxia of the placenta which is manifested by ischemia of trophoblastic tissue and therefore in the placenta will appear pathohistological changes in the form of obliteration of blood vessels in the placenta. Hyalinization of chorionic villi, intervilous thrombosis and placental infarction, which reduce the transfer of oxygen and carbon dioxide across the placenta, will occur in the placenta. In these cases, hypoxia and asphyxia of the fetus develop, which is followed by increased fetal stagnation. These hypoxic changes occur in the fetal organs and are most pronounced in the liver, brain, adrenal gland, and kidney. Due to the hypoxia of these organs, biochemical mediators are released first and then histopathological changes follow. As a result of hypoxia in the liver, the concentrations of some biochemical mediators change, which are: indirect bilirubin, direct bilirubin, cholesterol and the level of some enzymes which are: aspartat transaminase (AST) and alanine transaminase (ALT) [5]. Studies have shown that the ratio of fetal liver length (FLL) and aminotransferases (FLL/AST;

FLL/ALT) is lower in fetuses with increasing stagnation compared to fetuses with normal growth [6].

Changes due to hypoxia in the fetal organs can also occur in the increase of erythropoietin as well as cortisol by the adrenal gland.

Bile pigments are formed by the breakdown of the heme molecule which are the product of the degradation of the prosthetic group of hemoproteins. Bile pigments include: bilirubin and biliverdin. The role of the liver in bilirubin excretion is one of the most important liver detoxification functions. Indirect (non-conjugated) bilirubin is toxic to the body's cells. Most indirect bilirubin (80-90%) is formed by the breakdown of heme by hemoglobin. The metabolism of bile pigments goes through three stages: the spleen phase, the hepatic phase (of the liver) and the intestinal phase (of the intestines). In the first lienal phase from heme is formed bilirubin and biliverdin.

In the hepatic phase, liposoluble bilirubin binds to the active glucuronic acid and hydrosoluble bilirubin-diglycuronide (direct bilirubin) is formed, which is released in the bile and then in the intestine.

In the intestinal phase, direct bilirubin is deconjugated and subjected to reduction processes, as the following metabolites are formed: mesobilirubin, urobilongene, stercobilinogen and stercobilin. The urobilinogen in the urine through the middle of the oxidation is then converted to urobilin.

Pregnancy can be associated with an increase in the level of direct and indirect bilirubin due to physiological changes that occur in pregnancy such as: increased levels of hormones (estriol, progesterone, prolactin, thyroxine, thyrostimulating hormone and corticosteroids). During pregnancy due to these changes and the effects of these hormones will follow the slowing of intestinal peristaltics, and bile ducts which in some cases can lead to bile stasis, as the case is followed by an increase in the level of direct bilirubin in the blood. Indirect increase in bilirubin (due to disturbance of bilirubin transport) can also occur in acidosis, hypoxia, and hypoalbuminemia which exist in preeclamptic pregnancy.

Normal and preeclamptic pregnancies are associated with high levels of cortisol and prolactin which stimulate the synthesis of hepatic enzymes uridin-diphosphate-glucuronyl-transferase which stimulates the conjugation process of indirect bilirubin to direct bilirubin.

In fetuses with increasing stagnation there is an increase in the number of erythrocytes (polycythemia) due to hypoxia which is present in the fetus with preeclampsia. Polycythemia is also caused by an increase in erythropoietin which increases in the blood of the fetus with preeclampsia. Erythropoietin is known to stimulate erythropoietin in the bone marrow. The process of erythrocyte hemolysis is increased in the fetus with increasing stagnation and therefore there is an increase in the level of indirect bilirubin in the fetus.

In fetuses with stagnation in the network due to stress increases the level of hormones from theadrenal glands as well as prolactin which stimulate the process of conjugation and transfer fromindirect bilirubin to direct bilirubin.

Other factors that are: nutrition in pregnancy as well as the use of caffeine and alcohol can lead to an increase in direct bilirubin during pregnancy. In addition to these factors, some physiological conditions that may be present during pregnancy can lead to an increase in direct and indirect bilirubin and these are: liver and bile duct diseases, cholelithiasis, preeclampsia, help syndromes, viral infectious diseases (rubella, cytomegalo virus, herpes simplex virus 1 and 2, parvo virus-19 as well as other bacterial diseases). Indirect bilirubin increase is caused by: increase in erythrocyte count, shortening of erythrocyte life in hemolytic disease of the newborn, fetal erythroblastosis, viral infections, hemolytic anemia (spherocytosis, elliptocytosis and hemoglobinopathy).

Elevated bilirubin (due to impaired bilirubin transport) may also occur in acidosis, hypoxia,hypoalbuminemia, and the use of aspirin, diazepam, and sulfunamides.

The increase in bilirubin can be caused due to the lack of conjugation of bilirubin in the liver in the following cases: hypothyroidism, hypoglycemia, glucuronyl-transferase enzyme defect (Sy Crigler-Najar I, II, Sy Gilbert).

Increased bilirubin can also be caused in the following cases: intestinal obstruction, meconiumileus, paralytic ileus.

In our study demographic data of pregnant women have resulted in these characteristics in terms of gestational age, we found that there was no statistically significant difference in the median of gestational weeks (gestational age) between pregnant women with physiological pregnancy and pregnant women with preeclamptic pregnancy [U = 1455.500, z = -1.827, p =0.068].

Our results have concluded that there is a statistically significant difference between the median body weight of the newborn in physiologically pregnant women and pregnant women with preeclamptic pregnancies [U = 489.500, z = -6.883, p <0.001].

Our results have concluded that there is no statistically significant difference between the age of pregnant women with physiological pregnancy and pregnant women with preeclamptic pregnancy [t (118) = 1.770, p = 0.079].

Analysis of the Mann Whitney test found that there is no statistically significant difference between the median values of indirect serum bilirubin, pregnant women with preeclamptic pregnancy and pregnant women with physiological pregnancy [U=1430.500, z=-1.943, p= 0.05].

The Mann Whitney test found that there is no statistically significant difference between the median values of direct serum bilirubin, pregnant women with preeclamptic pregnancy and pregnant women with physiological pregnancy [U = 1537,000, z = -1.578, p = 0.115].

By calculating the Mann Whitney test we have found that there is no statistically significant difference between the values of indirect serum bilirubin in growing fetuses (IUGR) and fetuses with normal growth and development [U=1432,500, z=-1.929, p=0.054].

Through the Mann Whitney test, we found that there is a statistically significant difference between the values of direct serum bilirubin in growing fetuses (IUGR) and fetuses with normal growth and development [U=1412.500, z=- 2.037, p=0.042] [7].

The increase in the level of bilirubin directly in the fetus with stagnation (IUGR), is explained by the fact that due to

preeclampsia and stagnation of the fetus, as a result of fetal hypoxia, hypoxic changes occur in the liver of the fetus. Due to the growing stagnation and hypoxia of the fetus, from the suprarenal gland increases the synthesis of fetal chorizol which stimulates the synthesis of these enzymes uridin-diphosphate-glucuronyl-transferase as well as bilirubin- diglucuronyl-glucuronosyl-transferase which is found in fetus. These enzymes conjugate indirect bilirubin to direct bilirubin as the case is followed by an increase in the level of direct bilirubin in the fetal blood with increasing stagnation. The increase in the level of direct bilirubin in fetuses with increasing stagnation is also explained by the fact that fetuses with increasing stagnation are in a state of stress which is caused by hypoxia. Hypoxia stimulates increased levels of prolactin, thyroid-stimulating hormone (TSH), which stimulate microsomes in liver cells to stimulate the synthesis of the enzyme glycuronyl transferase which makes the conjugation of indirect bilirubin to direct bilirubin. It has been proven that in fetuses with increasing stagnation there are increased values of cortisol and estriol which as precursors have steroids with genesis from the suprarenal gland of the fetus, which is very active during the intrauterine life of the fetus. Our results are consistent with the data of these authors [8, 9, 10].

Using the correlation coefficient according to Spearman we investigated the correlation between gestation weeks and indirect serum bilirubin in fetuses/newborns with normal growth and development and we found that there is no statistically significant linear correlation between gestation weeks (gestational age) and indirect fetal/neonatal serum bilirubin values with normal growth and development ($rs = -0.063$; $p > 0.05$).

Using the correlation coefficient according to Spearman we analyzed the correlation between gestation weeks (fetal age) and indirect serum bilirubin values of fetuses/neonates with increasing stagnation (IUGR), and we found that there is a linear correlation statistically significant between weeks of gestation (gestational age) and indirect serum bilirubin values of fetuses/neonates with increasing stagnation (IUGR) ($rs = 0.666$; $p < 0.001$).

The increase in the level of indirect bilirubin during the development of pregnancy in the fetus with increasing stagnation, is explained by the fact that due to hypoxia of the fetus with increasing stagnation, increases the synthesis of erythropoietin in the fetal kidneys which then stimulates erythropoietin in the medulla (marrow) bone as the case follows the increase in the number of erythrocytes in relation to plasma as well as the increase in blood viscosity in the fetus with increasing stagnation (IUGR).

In the fetus with increasing stagnation, the increase of indirect bilirubin in linear form follows also due to hypoxia and acidosis which exists in the growing stagnation of the fetus, in addition the increase of indirect bilirubin in linear form can also be caused due to hemolysis of erythrocytes as a consequence of oxytocin of fetal origin, which is synthesized in the fetal thymus with increasing stagnation. It has been proven that as the fetus grows, the level of fetal oxytocin also increases.

Using the correlation coefficient according to Spearman we found that there is no statistically significant linear correlation between weeks of gestation and direct serum bilirubin values in fetuses/newborns with normal growth and development [$rs = -0.060$; $p > 0.05$).

Using the correlation coefficient according to Spearman we found that there is a statistically significant linear correlation between weeks of gestation (gestational age) and serum direct bilirubin values in fetuses/neonates with increasing stagnation (IUGR) ($rs = 0.673$; $p < 0.001$).

The increase in the level of direct bilirubin in a linear form with the age of the fetus is explained by the fact that preeclampsia due to hypoxia is followed by increasing stagnation of the fetus. Hypoxia caused due to atherosclerotic changes in uteroplacental and fetoplacental circulation is followed by a decrease in partial oxygen pressure (pO_2) and an increase in partial pressure of carbon dioxide (pCO_2) which is manifested by metabolic acidosis.

Due to metabolic acidosis, there is an increase in the level of thyroid-stimulating hormone (TSH), cortisol and prolactin, which stimulate the growth of uridine-diphosphate-glucuronyl-transferase enzymes and bilirubin-diglucuronyl-glucuronosyl-transferase. It is a known fact that these enzymes are at increased levels in the fetus with stagnation (IUGR).

The increase in the level of these hormones attitude in the fact that the fetus with increasing stagnation lives in stressful conditions due to permanent hypoxia in its organism.

There is evidence that corticosteroids (dexamethasone) increase glucuronyl transferase levelsin fetal livers [10].

There is evidence that increased levels of growth hormone (thyroid hormone) and thyroxine (T4) in fetuses with stagnation stimulate uridine-diphosphate-glucuronyl-transferase synthesis which then transfers indirect bilirubin to direct bilirubin. It has been proven that with the increase of the age of the fetus follows the increase of these enzymes during pregnancy [11, 12].

New studies suggest that stimulation of estrogen receptors ER facilitates (stimulates) bilirubinand liver regeneration [13], which means that estradiol can stimulate the transfer of indirect bilirubin to direct bilirubin. This transfer mechanism of indirect bilirubin to direct bilirubin maybe more successful.

CONCLUSIONS

Based on the results we conclude that the level of direct serum bilirubine is significantly higher ($p=0.042$) in the fetus/newborn with intrauterine growth retardation/restriction (IUGR) compared to the fetus/newborn with normal development. The results showed that there was a significant positive linear correlation ($p<0.001$) between the age of the fetus/newborn with (IUGR) and indirect bilirubin. In fetuses/newborns with increasing IUGR during intrauterine life, the level of indirect bilirubin increases with increasing fetal age during intrauterine life. The results also found, there was a positive linear correlation ($p<0.001$) between direct fetal/neonatal serum bilirubin with IUGR during intrauterine life. Direct bilirubin levels increase significantly with fetal/neonatal age with increasing IUGR during intrauterine life.

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