



SPECIFIC BIOCHEMICAL MARKERS OF AMNIOTIC FLUID IN PREGNANT WOMEN AFTER COVID-19

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ABSTRACT

A wide specific biochemical examination of amniotic fluid was performed in 2022 at the Republican Perinatal Center on 70 pregnant women who were delivered during this time. All pregnant women were divided into 3 groups. Group I (control) – pregnant women with physiological pregnancy and without a burdened obstetric and somatic history (n=10), group II - pregnant women who underwent COVID–19 during their 2nd-trimester gestation (n=30) and group III - pregnant women who underwent COVID–19 during their 3rd-trimester gestation (n=30). Amniotic fluid sampling was performed using transabdominal amniocentesis in the period from 22 to 38 weeks of gestation. From non-specific indicators in the group of pregnant women who underwent COVID-19, there is a decrease in the level of phosphatidylcholine, sphingomyelin, phosphatidylinositol and lysolecitin, revealing unformed and immaturity of fetal lungs; decrease in the level of the lamellar body, revealing a decrease in the production and release of pulmonary surfactant and immaturity of the fetal lungs.

KEYWORDS: amniotic fluid, non-specific biochemical markers, amniocentesis, COVID-19.

INTRODUCTION

In three large cohort studies of pregnant women with COVID-19 in the United States, the overall rate of premature birth was 7.2% (compared with 5.8% in patients without COVID-19 [1]), 12,9% (compared with 10.1% in patients without COVID-19 [2]) and 14.8% (compared to with 10.2% in patients without COVID-19) [3].

As of August 2021, the CDC's COVID data tracking system showed that among pregnant women in the United States with COVID-19, the preterm birth rate was 11.6 percent (2,697 out of 23,265) among births with known gestational age, and the cesarean birth rate was 33.1 percent (8,077 out of 24,373) [4]. As noted above, these figures for the entire US population were 10.1 and 31.8 percent, respectively [5].

A number of authors note that in case of fetal disorders, the study of amniotic fluid earlier and to a greater extent will reflect changes in the biochemical and acid-base state of fetal blood

than the study of the mother's blood. In addition, the more indicators are analyzed, the more accurate information will be obtained [6].

If there is a potential threat of pneumonia in COVID-positive pregnant women, they should certainly be classified as a high-risk group and be closely monitored until delivery. It has been established that the most common obstetric complications of COVID positive pregnant women are premature birth and premature discharge of amniotic fluid, as well as fetal growth retardation syndrome and perinatal mortality [7].

Intra-amniotic infection and inflammation are associated with fetal lung damage, aberrant lung development and, as a consequence, chronic lung diseases in newborns and adults [8,9]. The degree of damage to the fetal lungs probably depends on the type, time and duration of the infectious-inflammatory reaction. Intrauterine infections are associated with an increase in the frequency of bronchopulmonary dysplasia (BPD) and a decrease in respiratory distress syndrome (RDS). BPD is a multifactorial chronic lung disease in premature infants, which affects 35% of children with a body weight <1500 g [10]. BPD is characterized by impaired development of the alveoli, inflammation of the large respiratory tract, interstitial fibrosis and impaired pulmonary angiogenesis [11,12].

The purpose of these studies was to study the effect of the transmitted viral disease SARS-CoV-2 on the content of phospholipids and lamellar bodies in the amniotic fluid of pregnant women.

MATERIALS AND METHODS

The study included 70 pregnant women aged 21 to 40 years of 2-3 trimesters of pregnancy who were hospitalized at the Republican Perinatal Center in the Department of Pathology of pregnant Women. All pregnant women during gestation were evaluated for specific biochemical markers of amniotic fluid composition.

The patients were divided into the following main groups:

Group I (control) – pregnant women with physiological pregnancy and without a burdened obstetric and somatic history (n=10),

Group II (main) – pregnant women who have undergone COVID-19, at a period of 22 to 28 weeks of gestation (n=30).

Group III (main) – pregnant women who have undergone COVID-19, in the period from 29 to 38 weeks of gestation (n=30).

Amniotic fluid was taken using transabdominal amniocentesis with a Braun G-18 needle in an amount of 10.0ml. All pregnant women before amniocentesis gave written voluntary consent to manipulation.

In the second and third groups of all pregnant women, 73.3% were repeat births, and the remaining 26.6% were primiparous. According to the anamnesis of pregnant women, as well as hospital discharge data, where there was hospitalization for COVID-19, 80.0% of patients had the disease in moderate severity, and 20.0% of patients had pneumonia, that is, in severe form.

High-performance liquid chromatography (HPLC) was used to determine specific parameters such as phosphatidylcholine (PC), phosphatidylinositol (PHI), lysolecitin (LPH) and sphingomyelin (CM). which was carried out on an Agilent 1100 series liquid chromatograph (Agilent Technologies Inc., USA) equipped with a 4-gradient pump G1311A, a degasser

G1322A, a gradient mixer, a variable wavelength detector (VWD) G1314A and a Rheodyne 7725i loop injector (Rheodyne, USA) with a 100 µl loop. Supelco Discovery HS C18 column (4.6x75 mm/3 microns). The lamellar body of the amniotic fluid was counted using a hematological analyzer. The amniotic fluid was centrifuged at 2700 rpm for 5 minutes before the start of the study to separate from cervical mucus, cheese-like grease, meconium, epidermis scales and fetal downy hair.

Statistical analysis of the results obtained was performed by conventional methods with the determination of the average value (M) and the average error of the arithmetic mean (m) using the Microsoft Excel computer program, with the calculation of the Student's t-test for comparing the averages. Statistically significant differences were accepted at the significance level of $p < 0.05$.

RESULTS AND DISCUSSION

According to the results of the conducted studies, in the main groups of patients who underwent COVID-19, in comparison with the control group, there is a change in the specific parameters of the amniotic fluid (Table-1).

Table №1

Characteristics of amniotic fluid surfactant between pregnant women after COVID-19 and the control group.

Phospholipids of amniotic fluid	Control group patients (n =10)	Pregnant women at 22-29 weeks gestation (n=30)	Pregnant women at 29-38 weeks gestation (n=30)
Phosphatidylcholine, mcg/ml	71,30±2,98	34,68±0,83	26,74±1,07
Phosphatidylinositol, mcg/ml	5,28±0,17	4,30±0,15	4,22±0,11
Lysolecitin, mcg/ml	5,13±0,147	4,11±0,153	4,01±0,107
Sphingomyelin, mcg/ml	3,28±0,08	1,17±0,09	0,98±0,05
Plate body (×10 ⁹ /l)	103,0 ±1,15	72,18±1,06	67,0±1,21

The concentrations of phosphatidylcholine and sphingomyelin in the amniotic fluid of the patients of the main group were 34.68±0.83 mcg/ml and 1.17±0.09 mcg/ml in the group of pregnant women at gestation of 22-29 weeks and 26.74±1.07 mcg/ml and 0.98±0.05 mcg/ml in the group of pregnant women at gestation of 29-38 weeks, respectively. These indicators were clearly lower than in the normal control group, amounting to 71.30±2.98 micrograms/ml and 3.28±0.08 micrograms/ml.

Phosphatidylcholine and sphingomyelin are surfactants produced by the fetal lungs. During normal pregnancy, the amount of phosphatidylcholine in the amniotic fluid should increase. In our studies, this indicator decreases, which may indicate the unformed and immaturity of the fetal lungs. The concentration of other studied phospholipids, represented by phosphatidylinositol and lysolecitin, is also lower than that of the control group.

The lamellar body is secreted by type II alveolar cells and attaches to the alveolar surface, then comes into contact with the amniotic fluid. It also gradually increases as pregnancy progresses and the fetus matures. Thus, the concentration of lamellar bodies in the amniotic fluid

reflects the production and release of pulmonary surfactant and can predict the maturity of fetal lungs [13].

The results obtained when comparing pregnant women with physiological pregnancy and without burdened obstetric and somatic anamnesis and pregnant women who underwent COVID-19 at 22 to 38 weeks of gestation show the presence of changes in the content of phospholipids and lamellar bodies in the amniotic fluid of pregnant women who underwent COVID-19. A significant decrease in these indicators in comparison with the control group suggests that the transmitted coronavirus infection affects the pregnant woman's body and the formation of fetal lungs and their maturity.

For the possibility of a more in-depth study of the content of phospholipids and lamellar bodies in the amniotic fluid responsible for the formation and maturity of fetal lungs, both groups of pregnant women who underwent COVID-19 at 22 to 29 weeks of gestation and at 29 to 38 weeks of gestation were additionally divided into groups according to the severity of coronavirus infection.

The mild course of COVID-19 disease was defined as a mild disease (with or without respiratory symptoms) with oxygen saturation >96%. The number of patients with a mild course of the disease in the group of pregnant women in the gestation period from 22 to 28 weeks was 10 people, and in the gestation period from 29 to 38 weeks was 16 people.

The moderate course of the disease was defined as a disease (with a lesion of the lung parenchyma up to 30%) with oxygen saturation of 94%. The moderate form was additionally accompanied by difficulty breathing, an increased level of air shortage. This group of pregnant women in the gestation period from 22 to 28 weeks included 15 patients, and in the gestation period from 29 to 38 weeks - 14 patients.

The severe course of the disease was defined as a disease with severe pneumonia with a lesion of the parenchyma of 40% or more. This group included 5 patients at gestation from 22 to 28 weeks. There were no patients in severe form in the gestation period from 29 to 38 weeks, since they are placed in specialized clinics for constant monitoring of the condition of the pregnant woman and fetus.

In addition, patients with chronic diseases or complicated pregnancy were allocated to a separate group. Of the 30 patients who underwent COVID-19 during pregnancy from 22 to 28 weeks of gestation, there were 5 patients with concomitant diseases. In the group of patients in the gestation period from 29 to 38 weeks of gestation, the number of patients was 12.

According to the results of the conducted studies, in the main groups of patients who underwent COVID-19, in terms from 22 to 28 weeks and from 29 to 38 weeks of gestation, depending on the severity of the disease and concomitant diseases, there is a statistically significant change in specific indicators of amniotic fluid, summarized for convenience in summary Tables № 2 and №. 3.

Table №2

Specific indicators of amniotic fluid in pregnant women who underwent COVID-19 at 22-28 weeks gestation

Indicators	Control group	Mild form	Moderate severity	Severe form	Pregnant women with EGD
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Phosphatidylcholine, mcg/ml	71,30±2,98	36,07±0,57	34,47±0,86	33,53±0,59	30,18±4,19
Phosphatidylinositol, mcg/ml	5,28±0,17	4,26±0,09	4,33±0,17	4,35±0,20	3,95±0,56
Lysolecitin, mcg/ml	5,13±0,147	4,06±0,202	4,14±0,128	4,08±0,131	3,79±0,525
Sphingomyelin, mcg/ml	3,28±0,08	1,13±0,09	1,15±0,09	1,33±0,08	1,19±0,18
Lamellar body (× 10⁹ /l)	103,0 ±1,15	71,24±0,81	72,85±1,20	72,39±0,79	67,42±9,43
Phosphatidylcholine, mcg/ml	71,30±2,98	36,07±0,57	34,47±0,86	33,53±0,59	30,18±4,19

The results obtained in the groups of patients who underwent COVID-19, depending on the severity of the disease and concomitant diseases, confirm the previously obtained data and clarify the picture of COVID-19 disease.

Table №3

Specific indicators of amniotic fluid in pregnant women who underwent COVID-19 at the gestation period of 29-38 weeks

Indicators	Control group	Mild form	Moderate severity	Pregnant women with EGD
Phosphatidylcholine, mcg/ml	71,30±2,98	27,06±0,97	26,39±1,20	24,87±0,35
Phosphatidylinositol, mcg/ml	5,28±0,17	4,23±0,13	4,21±0,10	4,34±0,10
Lysolecitin, mcg/ml	5,13±0,147	3,99±0,199	4,05±0,094	4,0±0,144
Sphingomyelin, mcg/ml	3,28±0,08	0,96±0,04	1,01±0,05	0,95±0,03
Plate body (× 10⁹/l)	103,0 ±1,15	67,50±1,32	66,43±1,07	66,57±1,18

Thus, the content of phosphatidylcholine and sphingomyelin, the main surfactants produced by the lungs, decreases in pregnant groups depending on the severity of the disease.

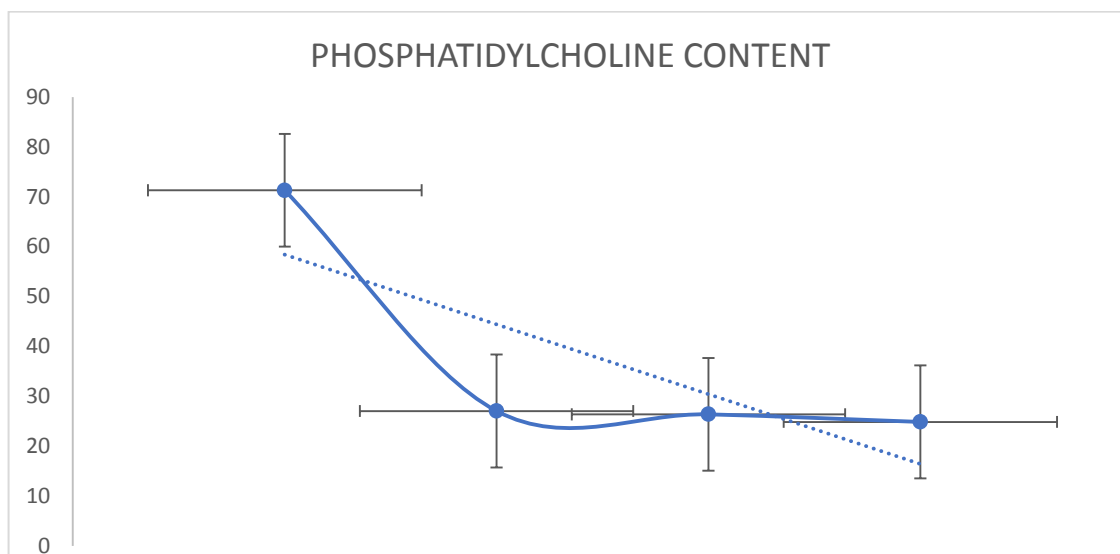


Fig.1. Phosphatidylcholine content in pregnant women who underwent COVID-19 at the gestation period of 29-38 weeks in comparison with pregnant women of the control group.

The concentration of phosphatidylcholine decreases by 1.98 – 2.36 times in the group of pregnant women who underwent COVID-19 at 22-28 weeks gestation. In the group of pregnant women who underwent COVID-19 at the gestation period of 29-38 weeks, this indicator was reduced by 2.6 – 2.87 times (Fig.1).

The concentration of sphingomyelin also decreases in pregnant women who underwent COVID-19 in the range of 2.46 – 3.45 times, depending on the gestation period and the degree of the disease (Fig.2).

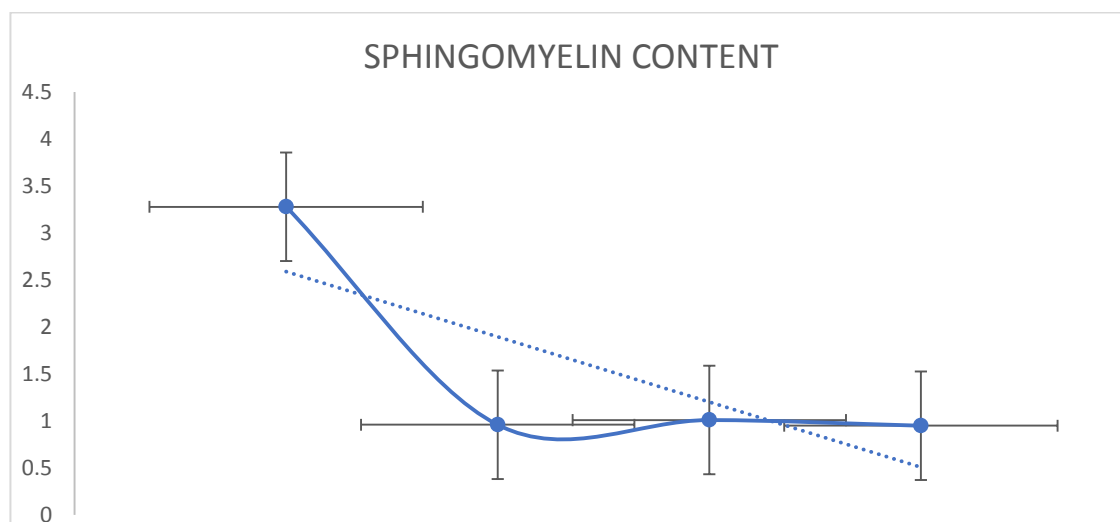


Fig.2. The content of sphingomyelin in pregnant women who underwent COVID-19 at the gestation period of 29-38 weeks in comparison with pregnant women of the control group.

The concentration of these indicators confirms the previously made assumption about the unformed and immaturity of the fetal lungs, and the greatest decrease in the concentration of the studied phospholipids occurs in groups of pregnant women who have concomitant diseases or complicated pregnancy.

The concentration of other studied phospholipids, represented by phosphatidylinositol and lysolecitin, also decreases and amounts to a lower level than in pregnant women of the control group.

The concentration of the lamellar body during the normal development of pregnancy and fetal maturation increases at a period of 34 to 36 weeks.

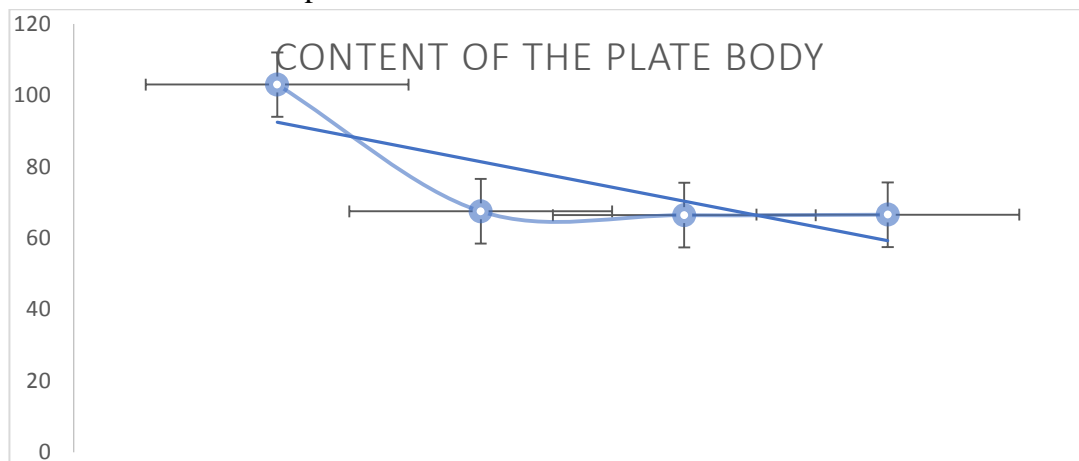


Fig.3. The content of the lamellar body in pregnant women who underwent COVID-19 at the gestation period of 29-38 weeks in comparison with pregnant women of the control group.

However, in the studied groups of pregnant women, at the gestation period of 29-38 weeks, this indicator decreases by an average of 1.5 times, which indicates the immaturity of the fetal lungs (Fig.3).

CONCLUSION

1. The specific composition of the amniotic fluid of pregnant women with coronavirus infection varies and directly depends on the degree of viral load of the body.
2. In comparison to the control group, pregnant women with coronavirus infection had lower levels of phosphatidylcholine, sphingomyelin, phosphatidylinositol, and lysolecitin, indicating that the fetal lungs are unformed and immature.
3. There is a reduction in the level of the lamellar body, indicating a decrease in the generation and release of pulmonary surfactant as well as fetal lung immaturity.

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