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Usefulness of marking alkaline phosphatase and C-reactive protein in monitoring the risk of preterm delivery

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Summary

Background:

The purpose of this paper is to compare the effectiveness of use of alkaline phosphatase (ALP) and C-reactive protein (CRP) levels in marking and monitoring the risk of preterm delivery due to infection.

Material/Methods:

The study involved 83 patients assigned to groups: Group I (n=43) consisted of patients hospitalized for symptoms of preterm delivery, and Group II (control group n=40) consisted of patients controlled or hospitalized delivering on time without complications, whose pregnancy had a physiological course. All patients had a single marking of ALP and CRP levels in serum performed.

Results:

CRP levels were within the range 7 mg/l to 94 mg/l in the study group, and 4.83 mg/l to 90 mg/l in the control group. The level of ALP in the study group ranged from 139 u/l to 368 u/l and from 218 u/l to 321 u/l in the control group. In more than half of women (72.1%) from study group, CRP level exceeded 7 mg/l; in the control group, the CRP level exceeded 7 mg/l in 35% of cases. Significantly higher levels of CRP (above 20 mg/l) and ALP (above 300 u/l) were found in the 18 patients from the study group compared to the control group.

Conclusions:

Although an increase in the level of ALP in serum cannot be an absolute and sole marker of the risk of preterm delivery, it can be used in conjunction with a significantly elevated CRP level.

key words:

C-reactive porotein (CRP) • alkaline phosphatase • preterm delivery

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BACKGROUND

Although obstetric care has undergone significant developments in recent years, the frequency of preterm deliveries has not only not decreased, but in most developed industrialized countries it has slightly increased. Preterm births constitute an important problem of gestational pathology due to the related phenomenon of prematurity in newborns [1]. In developed countries, prematurity remains the main cause of complications and mortality in newborns and is responsible for 60–80% of deaths in children who have not been diagnosed with any developmental defects [2]. Therefore, prevention of preterm deliveries should be a priority for obstetrics. Presently, accumulating scientific data confirm the important role of ascending infections in the pathogenesis of preterm birth and miscarriage. In the course of intrauterine infection, mediators of infectious reaction synthesize with free radicals, stimulating preterm contractions of the uterine muscle [3,4].

Currently, scientists are trying to develop newer methods for the biochemical monitoring of the risk of preterm birth. In the literature, there are few reports on marking ALP levels in serum of pregnant women with symptoms of preterm delivery threat. Alkaline phosphatases belongs to the orthophosphate hydrolases group of enzymes, and occurs mainly in plasma membranes as protein located on the external side of the membrane or as a component of the complex with proteins and phospholipids of the membrane. Thus far 4 structural genes encoding ALP have been defined, the expression of which corresponds to the presence of 4 isoenzymes in plasma [5–7] – hepatic, osseous, intestinal, placental.

For several years, clinical practice has also stressed the importance of C-reactive protein (CRP) in monitoring the threat of preterm birth. CRP belongs to the group of acute phase proteins principally synthesized in the liver during the course of acute and chronic inflammations, which act as a part of the non-specific immune response of the body. The role of acute phase proteins is not sufficiently known. Most of them have the properties of protease inhibitors or act as transporter proteins (eg, haptoglobin) [8]. Concentration of CRP can be 1000-fold in response to infection, anoxia, trauma, burns, and inflammation [9]. CRP is generated in response to infection stimulus as early as 2–5 hours after infection. Increased CRP concentration precedes the occurrence of clinical symptoms of infection by several hours. Among the main stimulants of CRP synthesis is IL-6, which is in turn stimulated by TNF- α and IL-1. All the aforementioned mediators of the inflammatory process play an important role in inducing preterm delivery. The most frequently used methods for CRP marking include immunoturbidimetric or immunonephelometric methods. CRP is a highly stable protein, durable for between 4.5 years and 7 years [6] if stored at the temperature range -70°C to -80°C .

Purpose

The purpose of this paper is to compare the effectiveness of ALP and CRP levels in marking and monitoring the risk of preterm delivery due to infection.

Material and Methods

This prospective cohort study was performed at the Clinic of Obstetrics and Perinatology at Collegium Medicum of

the Jagiellonian University, Cracow, Poland from 1 June 2007 to 30 December 2007. The study sample was random chosen from hospitalized women in different states of advanced pregnancy. Some of them had symptoms of risk of preterm delivery, while others delivered on time without complications; classification into 2 groups was made just after delivering. The study involved 83 patients assigned to 2 groups:

- Group I (study group, $n=43$) — patients hospitalized for displaying symptoms that placed them at risk of preterm delivery,
- Group II (control group, $n=40$) — patients controlled or hospitalized, delivering on time without complications, whose pregnancy had a physiological course.

The patients participating in the study gave their informed consent and could terminate their participation at any stage of the study.

All patients had a single marking of ALP and CRP levels in serum performed. Measurements were made during hospitalization after stabilizing the patient's condition. Therefore, marking ALP and CRP could be preformed at different times during pregnancy.

CRP level was marked on the basis of the immunoturbidimetric or immunonephelometric method, while ALP was marked on the basis of the kinetic method according to DGKC, with pNpp, buf. DEA, and a temperature of 37°C .

Moreover, the following parameters were analyzed:

- age,
- period of gestation,
- obstetric past,
- presence of possible obstetric pathologies,
- hospitalization period,
- general condition of the newborn based on Apgar scale,
- weight and body length of the newborn,
- interdependence of CRP and ALP levels in mother's serum with period of gestation.

A statistical analysis was performed on the basis of the parametric Z test for comparison of 2 proportions for independent samples, non-parametric rank Spearman correlation coefficient as a measure of dependence of 2 attributes when a model of dependence is unknown, χ^2 test of independence for comparison 2 probability distributions, and parametric T test for testing of significance of regression models parameters. Calculation was performed using Statistica version 7.0 software.

RESULTS

The age of hospitalized women ranged from 17 to 42 years, with an average age of 27.9 years. Patients hospitalized due to the threat of preterm delivery were in the age range 17 to 42 years (average: 28.3 years), while those delivering on time were from 18 to 37 years (average: 27.1 years) (Table 1 and Figure 1).

The largest group of pregnant women (67.4%) with symptoms indicating the threat of preterm delivery was formed by women hospitalized between weeks 33 and 36 of the gestation period (Table 2 and Figure 2).

Table 1. Distribution of age of patients classified into cases and control groups.

Age of hospitalized patients	Patients at risk of preterm delivery	Patients delivering without complications
17–20	6	7
21–25	5	12
26–30	14	12
31–35	9	7
36–42	6	5

Figure 1. Distribution of age of patients classified into cases and control groups. **Figure 2.** Distribution of patients depending on period of gestation.**Table 2.** Distribution of patients depending on period of gestation.

Number of week of gestation	Patients at risk of preterm delivery	Patients without complications
22–24	4	0
25–26	0	1
27–28	4	0
29–30	2	0
31–32	3	0
33–34	10	4
35–36	10	5
37–38	4	21
39–40	2	11
41–42	1	1

The percentage of miscarriages in this study was higher in women at risk of preterm delivery (27.9%) as compared to the control group (12.5%) (Table 3 and Figure 3). The difference is significant at the level of $p=0.05$. Comparison between distributions was made by non-parametric χ^2 test of independence.

When analyzing possible complications, 16 women (37.2%) were diagnosed with anemia. Furthermore, in patients hospitalized due to preterm delivery, the following complications were observed:

- Gestational hypertension – 12 patients (27.9%),
- Gestational diabetes – 10 patients (23.3%),
- Urinary tract infections – 7 patients (16.3%),
- Thyroid dysfunction – 6 patients (13.95%),
- Concomitant uterine myomas – 4 patients (9.3%).

The values do not add up to 100%, as some patients experienced more than 1 complication.

The hospitalization time of patients at risk of preterm delivery ranged from 2 to 42 days (on average, 9.5 days) (Figure 4).

In turn, no statistically significant (at the level of $p=0.05$) dependence was found between the hospitalization period and the week of gestation in which the risk of preterm delivery occurred. Correlation between them was performed by non-parametric Spearman rank correlation coefficient.

The data concerning weight, body length and number of points in Apgar scale in both groups of newborns are presented in Table 4.

When marking the CRP and ALP level in serum, it was found that the CRP level fell within the range 7 mg/l to 94

Table 3. Distribution of ratio of miscarriage in previous pregnancy in the tested and control group.

Previous pregnancy	Patients at risk of preterm delivery	Patients delivering without complications
Miscarriage in interview	11	5
Primiparas	21	26
Multiparas	8	12

Figure 3. Distribution of ratio of miscarriage in previous pregnancy in the tested and control group.

mg/l in the study group of patients with a risk of preterm delivery, and 4.83 mg/l to 90 mg/l in the control group of women delivering on time. The level of ALP in the study group ranged from 139 u/l to 368 u/l, and from 218 u/l to 321 u/l in the control group.

Figure 5 presents the dependence between the time of gestation and the levels of CRP and ALP. Due to high dispersion in results, medians were used to observe the general tendency. The figure shows that together with the period

Figure 4. Distribution of hospitalization period.

of gestation, the values of medians grow for both CRP and ALP. The growth in time is statistically significant at the level of $p=0.05$. A standard T test was used for testing of significance of slopes in linear regression models (Figure 5).

In women with a risk of preterm delivery, elevated levels of CRP and ALP were found. However, it must be considered that in the case of ALP all the values exceeded the assumed normal range of 104u/l. Figure 5 shows 2 yellow outliers – points for patients who delivered without complications and were hospitalized after 38 week of gestation (12 patients). The points are placed beneath of regression line calculated for patients at risk of preterm delivery. For the same patients values of CRP (dark green points) are not such obvious indicators. Some of them had CRP values above the regression (mean) line, but another CRP value lies beneath one.

In more than half of women (72.1%) whose pregnancy at risk of preterm delivery or who delivered prematurely, CRP level exceeded the value of 7 mg/l; in the case of the control group, the CRP level exceeded the value of 7 mg/l in 35% of cases. The result is significant at the significance level of $p=0.05$. A standard Z test was used to compare 2 independent samples. Significantly higher levels of CRP (above 20 mg/l) and ALP (above 300 u/l) were found in the 18 patients from Group I who delivered prematurely, as compared to the control group.

Table 4. Data on the newborns delivered.

	Preterm birth			Delivery on time		
	Min.	Max.	Average	Min.	Max.	Average
Weight(g)	1710	3300	2241.3	3000	3600	3280.0
Length(cm)	43	53	47.6	53	65	55.6
Apgar Scale (points)	10	4 newborns		32 newborns		
	9	6 newborns		6 newborns		
	8	5 newborns		–		
	7/8/9	–		2 newborns		
	3/7/8	2 newborns		–		
	2/5	1 newborn		–

Figure 5. Dependence of ALP and CRP levels on week of gestation.

DISCUSSION

In the current literature there is insufficient evidence of the effectiveness of marking ALP in the serum of pregnant women to monitor the risk of preterm delivery, which is related to the fact that the ALP level grows during gestation (placental fraction) [10–12]. The study by Aliyu et al shows that the presence of placenta during gestation causes reference values of ALP to fall within the range 24 u/l to 161 u/l. The authors postulate the necessity of performing research on the effectiveness of the cyclical marking of ALP in monitoring placenta efficiency [13]. In the literature, 1 case of isolated placental excess production of ALP was found, the value of which exceeded reference values by 10-fold [14] during gestation.

Goldenberg et al, in a multicentre study of 3000 women which aimed at assessing biochemical markers of the risk of preterm delivery before week 32 of gestation, proved that alkaline phosphatase and alfa-fetoprotein are among the most effective [15].

In the authors' study, it is shown that the elevated ALP level positively correlates with the risk of preterm contraction activity of uterine muscle, particularly when its value exceeds 300 u/l. In work by Meyer RE et al, where the risk of preterm delivery was defined depending on the serum level of ALP, it was similarly found that a 2-fold or greater excess of reference value of ALP positively correlates with the risk of preterm delivery (a 2.9-fold increase in relative risk) [16]. The literature also stresses the relation between high ALP levels and newborns born on time but with low weight at birth – below 2500 g [17].

The role of CRP in monitoring the risk of preterm delivery becomes increasingly greater, as shown by results of the scientific research. The correct CRP level in pregnant women is hard to define. Bek et al believe the correct concentration of CRP in a pregnant woman's serum falls within the range of 8–20 mg/ml [18]. Watt et al. adopt a top limit value of CRP as 15 mg/ml after week 22 of gestation [19].

In the study performed, it is shown that the risk of preterm delivery grows with CRP levels above 20 mg/l. Also, Hvilsum's study showed statistically significant differences between concentrations of CRP measured in women delivering

prematurely as compared to women delivering on time. It is also concluded that high CRP concentration in the initial period of gestation is related to an almost 2-fold higher risk of preterm delivery [20]. A number of scientists assessed the usefulness of CRP application for the diagnosis of intrauterine infections [20–22]. For the most frequently used CRP value at the level of 20 mg/dl, sensitivity of 50–80% was achieved, and specificity of 68–81% in forecasting histologically confirmed chorion amnionitis [20]. Furthermore, in relation to the opportunity of monitoring the risk of preterm delivery with the level of CRP, the study by Mazor et al seems interesting, as they claim the serum level of CRP in women with preterm amniorrhexis above 8mg/ml positively correlates with the risk of preterm delivery [21].

The above results present many controversies related to ALP and CRP. What cannot be disputed, however, is their role in preterm delivery, and in particular in its early diagnosis.

CONCLUSIONS

On the basis of the study performed and the analysis of the results, it must be concluded that although an increase in the level of ALP in serum cannot be an absolute and sole marker of the risk of preterm delivery, it can be used in conjunction with a significantly elevated CRP level.

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