First Newborn's Aspirate Microbial Analysis and Lysozyme Activity Determination

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Abstract: *The aim of the present study was to investigate the presence of microorganisms in pregnant women's cervicovaginal regions just before delivery and in the first aspirate of the upper respiratory tract and the oral cavity in the newborns immediately after delivery. Microbial analyses of smears taken two hours postpartally from the throat, oral cavity, stomach and ear of neonates showed totally altered findings. Estimating lysozyme activity in the first newborn's aspirate, by the use of turbidimetry, according to the method of Prockop and Davidson (Lysozyme-Testomer-Boehringwerke), we found it's very high value. Our study indicates that there is a strict relationship between microbiological substratum and lysozyme activity in the first newborn's aspirate. (Thai J Obstet Gynaecol 1993; 5: 87-90.)*

Key words : lysozyme activity, first newborn's aspirate, microbial finding

The removal of the content from the oral cavity and the upper respiratory tract (amniotic fluid, blood, mucus) immediately after the birth is called the primary (first) aspiration. In the first aspirate we may expect microorganisms that are found in the cervicovaginal region[1]. Bacterial colonization of the amniotic cavity has been reported in patients with intact membranes during pregnancy, but microbial invasion of the amniotic cavity during labour, prior and especially after the rupture of membranes was found to occur more frequently. In cases of premature membranes rupture, the ascending infection may lead to maternal infection and fetal/neonatal sepsis[2]. But, one must have in mind that lysozyme, transferrin, peroxidase, beta-lysin, beta 1a/beta 1c globulin, IgA, and zinc-protein complex in neonatal oral and rhinal cavity, throat, pharynx and respiratory tract, are assumed to exibit bacterial growth-inhibitory activities[3,4].

The aim of this study was to investigate the microbiologic and nonspecific immunologic aspect of the first aspirate in newborns immediately

after delivery.

Materials and Methods

The prospective study was carried out during a period of 3 years. Samples of the first aspirate of 107 newborn infants immediately after delivery as well as cervicovaginal region smears taken before the expulsion were analyzed in the Clinic of Gynaecology and Obstetrics, University Clinical Center in Belgrade. Smear samples were taken from throat, pharynx, stomach, skin, umbilicus and ear of the newborns 2 hours after the birth at the Department of Neonatology. Lysozyme activity from the first aspirate content and umbilical cord of the neonates was determined in the Laboratory for immunochemistry.

Each sample was centrifuged at 10° C for 10 minutes at 1000 X g to remove particulate materials and was frozen at -70° C until lysozyme concentration testing. Determination of lysozyme level was based on the use of turbidimetry at 546 nm, calculated by a standard curve (phosphate buffer 67 mmol/l, pH 6.3, Micrococcus lysodeicticus 0.2 g/l, sodium azide 8 mmol/l), according to the method of Prockop and Davidson (Lysozyme-Testomer-Boehringwerke)[5].

All samples were cultured in aerobic and anaerobic conditions immediately after they were obtained.

Oral cavity and pharynx smears as well as peripheral blood samples of 30 healthy nongravid adults served as controls for the lysozyme concentrations estimation.

Obtained data were tested by *Student t test* and one way variance analysis.

Results

The results of our investigation showed microbial presence in the throat aspirates of the newborn infants with predominant of E.coli (24.30%) followed by S. albus (14.95%), Enterococcus (7.48%), Diphtheroids (5.61%), etc.. The incidence of haemolytic streptococcus of "B" group colonization was 2.80%, S. pyogenes 1.87%, Klebsiella-Enterobacter 0.93% and Candida 1.87% in the examined samples. Comparing the results of the microbial analyses of the cervicovaginal smears with the samples of the first newborn's aspirate, we observed the decreased incidence of E.coli (19.63%), but elevated percentage of Enterococcus (27.10%), Candida (6.54%) and Streptococcus beta-haemolytic "B" group (7.48%) in the delivery paths (Table 1).

Microbiological examination of the smears taken 2 hours postpartally from the newborns at the Department of Neonatology showed completely different results: the incidence of E.coli was markedly decreased, that is in general, the presence of microbes was significantly lower in all the examined localizations. Furthermore, 60-120 minutes after the birth sterile media were 4 times more frequent than colonised (Table 2).

Table 1 Comparative analyses of the bacterial flora in the first aspirate and the vaginal smear

Bacterial flora	First aspirate		Vaginal smear	
	No.	%	No.	%
E. coli	26	24.30	21	19.63
Enterococcus	8	7.48	29	27.10
Staph. pyogenes	2	1.87	2	1.87
Strept. haemol. (B)	3	2.80	8	7.48
Strept. haemol.	2	1.87	1	0.93
Staph. albus	16	14.95	57	53.27
Diphtheroides	6	5.61	29	27.10
Klebsiella-Enterobacter	1	0.93	1	0.93
Pseudo. aeruginosa	1	0.93	0	0
Proteus mirabilis	0	0	4	3.74
Acinetobacter	2	1.87	0	0
Candida	2	1.87	7	6.54

Table 2 Bacterial flora present in the smear samples of the newborn infants (Percentage and numeric expression in the examined samples)

Bacterial flora	Throat		Stomach aspirate		Skin		Ear		Umbilicus		Anus	
	No	%	No	%	No	%	No	%	No	%	No	%
E.coli	0	0	2	1.87	0	0	0	0	2	1.87	2	1.87
Strept. haemol. (B)	2	1.87	0	0	2	1.87	1	.93	2	1.87	2	1.87
Enterococcus	1	.93	0	0	0	0	0	0	1	.93		1.93
Klebs.-Enterobact.	0	0	0	0	0	0	0	0	0	0	4	3.74
Staph. pyogenes	7	6.54	1	.93	5	4.67	1	.93	5	4.67	1	.93
Staph. albus	0	0	0	0	6	5.61	1	.93	2	1.87	2	1.87
Acinetobacter	1	.93	0	0	0	0	0	0	0	0	0	0
Diphtheroides	1	.93	0	0	0	0	0	0	0	0	0	0.
Pseudo. aeruginosa	0	0	0	0	0	0	0	0	0	0	0	0
Proteus mirabilis	0	0	0	0	0	0	0	0	0	0	0	0
Strepto. haemol.	0	0	0	0	0	0	0	0	0	0	0	0
Candida	0	0	0	0	0	0	0	0	0	0	0	0

Smear samples of the newborns at the Department of Neonatology
(taken 60-120 minutes after birth)

Number of colonised media	n= 22	(20.56%)
Number of sterile media	n= 85	(79.44%)

Lysozyme activity determination in the first newborn's aspirate was statistically higher than in control group of adult persons ($p<0.01$). Although lysozyme levels were higher in umbilical cord samples than in peripheral blood specimens of healthy nongravid persons, the difference was not found to be statistically significant (Fig.1).

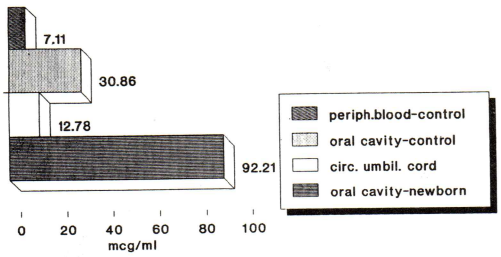


Fig. 1 Lysozyme activity in newborns and adult healthy nongravid persons.

Discussion

The incidence of microbial presence in the first aspirate of the newborn infants is related to the microflora of the maternal cervicovaginal region. However, vaginal flora has variations in different parts of the world. In our study, E.coli was found in 19.63% of cases in the vaginal microflora, and beta-haemolytic streptococcus in 7.48% of cases which is in accordance with the results of other authors[6,7]. The fact is that in the course of delivery a colonization occurs, but a small number of newborns is affected due to the presence of the expressed antimicrobial defence in which beside other factors, lysozyme with its bacteriolytic activity on the peptidoglycans of the microbial walls, has an important role. The analysis of lysozyme values indicated that high levels of this antibacterial enzyme in the investigated substratum can present an important component of the spectrum of nonspecific defence system of the newborn infants, which would probably be confirmed in further clinical and laboratory investigations.

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