

DEVELOPMENT OF THE AIRWAYS MATURITY OF NEWBORN WITH MECONIUM ASPIRATION SYNDROME (MAS)

Adnan Bozalija¹, Lirim Mustafa², Ragip Shabani³, Naim Haliti⁴, Hilmi Islami⁵

¹Department of Pharmacy, Faculty of Medicine, University of Prishtina,
Clinical Centre, Prishtina, Kosova

²Institution KFLM, Prishtine, Kosova

³Department of Pathology, Faculty of Medicine, University of Prishtina, Clinical
Centre, Prishtina, Kosova

⁴Department of Forensic Medicine, Faculty of Medicine, University of Prishtina,
Clinical Centre, Prishtina, Kosovo

⁵Department of Pharmacology, Faculty of Medicine, University of Prishtina,
Clinical Centre, Prishtina, Kosova

Abstract: Work shows the role of the epithelial volumetric component, septal and alveolar respiratory spaces in newborn in different weeks of gestation. Overall 36 cases are examined. Material observed were lungs of the newborn, taken after the autopsies, which have died due to different causes. Material was fixed in the 10% buffered formalin. Moulding was done in paraffin, whilst serial cuttings of 10 μ were conducted with microtome. Routine and special histochemical staining was done. Stereometric analyses on the volumetric density were done with the universal visual system, Weibel M 42. Morphologic and histochemical results obtained indicate the presence of argentaffin granules in the amniotic fluid in the newborn's MAS. Morphometric analyses provide data on the significant volumetric density of the alveolar epithelial component in all gestational ages in newborns. It is evident that the alveolar epithelial component (pneumocytes type I + II) have dominant volume in comparison to the alveolar septum and alveolar spaces ($P < 0.001$). There can be seen no relevant statistical difference in between volumetric density of alveolar septum and alveolar spaces in different gestational ages starting from the abortions, immature, premature and mature ($P > 0.05$). Results suggest a continual domination of the alveolar epithelial component with a slight decrease of the linearity at matured ages, whilst the component of the alveolar spaces has tendency of continual growth at matured ages.

Key words: Meconium aspiration syndrome, lungs, stereometry.



INTRODUCTION

Syndrome of the aspiration of amniotic fluid (MAS) is an important cause of the respiratory mortality and morbidity in newborns. Mechanical obstruction of the airways, dysfunction of the pulmonary surfactant, pulmonary inflammation, and vasoconstriction are as patho-mechanisms associated to MAS. Airways obstruction can affect the reflexive alterations of the bronchomotor tonus related to the bronchoactive substances.

Adnan Bozalija-Department of Pharmacy, Faculty of Medicine,
Lirim Mustafa-University of Prishtina, Clinical Centre, Prishtina,
Kosova, Institution KFLM, Prishtine, Kosova
Naim Haliti-Department of Pathology, Faculty of Medicine, University of

Prishtina, Clinical Centre, Prishtina, Kosova

Ragip Shabani-Department of Forensic Medicine, Faculty of Medicine,
University of Prishtina, Clinical Centre, Prishtina, Kosova

Hilmi Islami- Department of Pharmacology, Faculty of Medicine, University
of Prishtina, Clinical Centre, Prishtina, Kosova

Meconium is found to be present from 12th week of gestation. It is a product of amniotic fluid of foetus containing plaque cells of vernix caseosa secretion and gastrointestinal cells (1). Meconium contains 4 different fatty acids (e.g. Choline, henodeoxicholic acid and lithocholic) and minerals of which copper, zinc, manganese, calcium, iron and phosphorus are more frequent component (2, 3). Also contains plasma proteins (alpha 1 antitrypsin) (4,5) and other

active substances such as interleukins IL-1 β , IL-6 and IL8, tumour necrosis factor (TNF-alpha) (6) and phospholipase A₂ (PLA₂) (7) which may induce directly or indirectly pulmonary inflammation, by increasing production of cytokines and by activating white blood cells or epithelial/endothelial cells of lungs. In vitro exposure of meconium increase release of IL-8, TNF-alpha (8), endothelin-1, platelets activating factor (PAF), leukotrienes, thromboxan A₂, induced of NO synthetase (9), NO (10), PLA₂ and other substances which affect the reactivity of respiratory airways and inflammation.

Meconium contains biologically active substances with powerful contractile effect in the vascular and airways smooth musculature, assisted by leukotrienes, PAF, ET-1 etc. In meconium there is high percentage of the fatty acids (11) and biliary acids (12), which induce the contraction of the airways smooth musculature.

Several studies on MAS are related to intrauterine stress and hypoxia. There are many different opinion, even controversy ones, about the pregnancy complication from the impurity of the amniotic fluid. Because of this, following open thesis derive: reasons of the variation of the amniotic fluid flow, pathophysiologic mechanisms of the meconium fluid aspiration, and MASs' development. Mortality and morbidity from the aspiration of the meconium fluid and its role as marker of the delayed pregnancy is as a result of the chronic hypoxia that has a role in the clinic importance and prevalence of this syndrome (13).

Purpose of the work is to show the evaluation of the respiratory volume density (Vv) in the meconium aspiration syndrome (MAS) as a relative variable and the airways components (epithelium, interstitium and alveoli) in different gestational ages in newborns starting with aborts, immature, premature, and mature.

WORK METHODOLOGY

Used was material from the autopsy of newborn, born alive who have died due to different reasons and born dead, in different gestation weeks. Material for research was taken

from all lung lobbies (3+2). Material was fixed for 24 hours in 10% buffered formalin. Case grouping was presented as follows

Table 1. Presentation of cases analyzed in newborn.

Group	Newborn	Weigh (gr)	Gestation weeks
I	Abortus	<500	<22
II	Immaturus	500 – 1100	23-29
III	Praematurus	1100 – 2500	30-37
IV	Maturus	>2500	>38

Hematoxylin and eosin staining were made. Staining for connective tissue was done with VanGizon. Moulding took place in paraffin; serial cuttings of preparation were done in 7 and 10 micron. PAS stains (periodic, acid, Schiff) for neutral mucopoly- ccharides. For research were taken

cases with verified histopathology of the amniotic and meconium aspiration syndrome (MAS), presence of the cell detritus in airways, blood elements, lanugo and vernix.

Stereometric method

For stereometric researches was used the testing universal system Weibel M 42. After calibration of the system with adequate micrometer of the objective, we

have had following values of the measuring diameter (d) (Table 2).

Table 2. Measuring diameter.

The increase of objective	Diameter (d)
---------------------------	--------------

10 x	100 μm
20x	50 μm
40x	25 μm
60x	17 μm
100x	10 μm

Researches were done in **five testing points (Pt)** and in serial cuttings of tissues in 10 micron. Used objective was 40 x, whilst measuring diameter was 25 μm .

Defining of the volumetric density

Volumetric density is a relative variable, which shows how many of the overall space occupy observed phase in the volumetric unit. Universal testing test Weibel M42 is used, numbering was done in five testing points (Pt).

Calculation of the results is done with this formula:

$$V_{vf} = P_f : P_t$$

V_{vf} = Volumetric density of the structure in the observed stage (mm^0);

RESULTS

It is evident that the alveolar epithelial component (pneumocytes type I + II) have dominant volume in comparison to the alveolar septum and alveolar spaces ($P < 0.001$). Alveolar septum in comparison to

P_f = Number of the points of the system in the observed stage.

P_t = Overall number of the testing system points. Obtained results are in mm^0 and expressed in (%).

Obtained results were statistically processed with the assistance of the statistical scientific software INSTAT 3 with t - test (GraphPad InStat 2 tm Copyrin 1990-93, graph Pad software V2,02; Dr Ernberger, Case Western reserve U.930952S).

alveolar spaces occupies no statistically relevant volume ($P > 0.05$).

Table 3 represents respiratory volumetric density (V_v) in different gestational ages of newborn.

IJSER

Table 3. Respiratory volumetric density $V_v = \text{mm}^0$ in newborn (abortus, immature, premature and mature) in 5 testing points (Pt). N = 36.

Weigh (gr)	Pt- alveolar epithelial	Pt- alveolar septum	Pt- alveolar space
275	30	7	5
285	23	4	13
325	18	11	13
350	25	9	8
380	19	9	15
385	22	6	14
400	26	5	11
420	26	7	9
480	29	8	5
840	26	9	7
870	25	13	4
875	15	8	19
900	12	7	13
985	22	12	8
985	20	9	13
990	29	10	3
1000	19	10	13
1050	16	11	15
1070	24	10	8
1350	18	13	11
1450	27	10	5
1600	14	7	21
1850	24	13	5
1950	19	9	14
2030	24	10	8
2050	20	6	16
2350	17	7	18
2500	16	13	13
2600	18	8	16

ABORTUS

IMMATURUS

PREMATURUS

2650	18	12	12
2950	22	9	11
2980	19	10	13
3000	22	11	9
3050	20	12	10
3100	18	9	15
3100	22	11	9

MATURUS

In figure 1, statistical significance of domination of the epithelial alveolar component is evident in comparison to alveolar septum and spaces ($p < 0.001$), whilst volume of the alveolar septum in comparison to alveolar spaces has no relevant statistical difference ($p > 0.05$).

Pt alveolar epithelium: Pt alveolar septum $P < 0.001$
 Pt alveolar epithelium: Pt alveolar spaces $P < 0.001$
 Pt alveolar septum: Pt alveolar spaces $P > 0.05$.

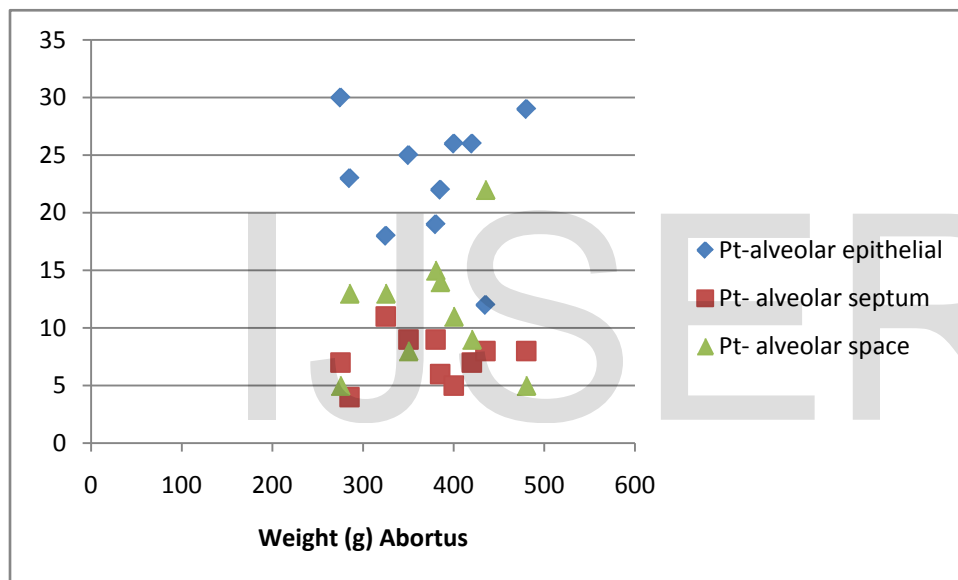


Fig.1. Individual reaction of the respiratory volumetric density (V_v) in early gestational weeks (abortus with the weight below 500 g). $N = 9$.

Figure 2, presents the frequency of the respiratory volumetric density (V_v) in immature newborn. Dominating frequency of the epithelial component is evident in despite of the body weight.

Pt -alveolar epithelium: Pt -alveolar septum $P < 0.001$
 Pt-alveolar epithelium: Pt -alveolar spaces $P < 0.001$
 Pt--alveolar septum: Pt-alveolar spaces $P > 0.05$.

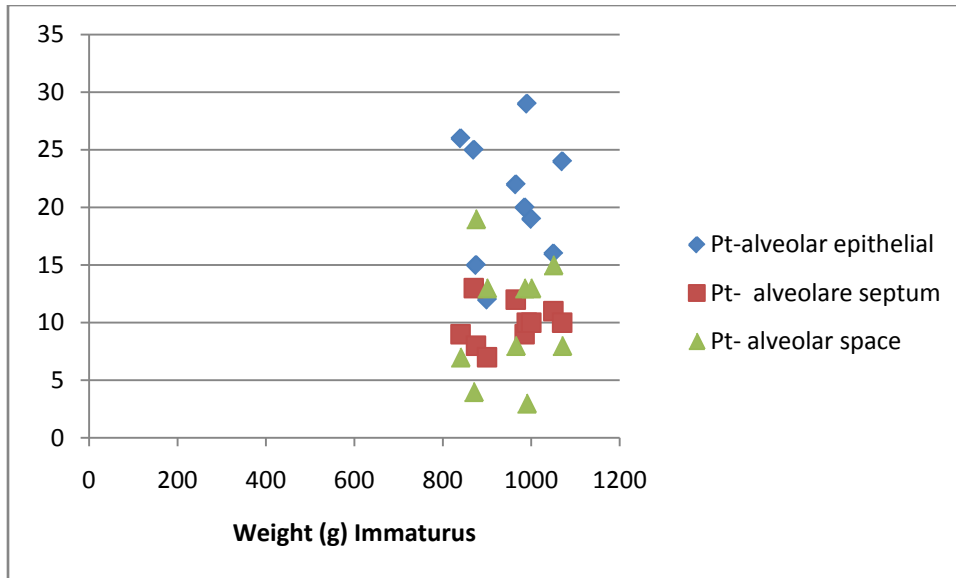


Fig.2. Individual reaction of the respiratory volumetric density (Vv) in immature newborn (with the weight 500-1100 g). N = 9.

Figure 3, represents the frequency of the respiratory volumetric density (Vv) with dominant presentation of the epithelial component.

Pt- alveolar epithelium:	Pt -alveolar septum	P < 0.001
Pt-alveolar epithelium:	Pt-alveolar spaces	P < 0.001
Pt-alveolar septum:	Pt-alveolar spaces	P > 0.05.

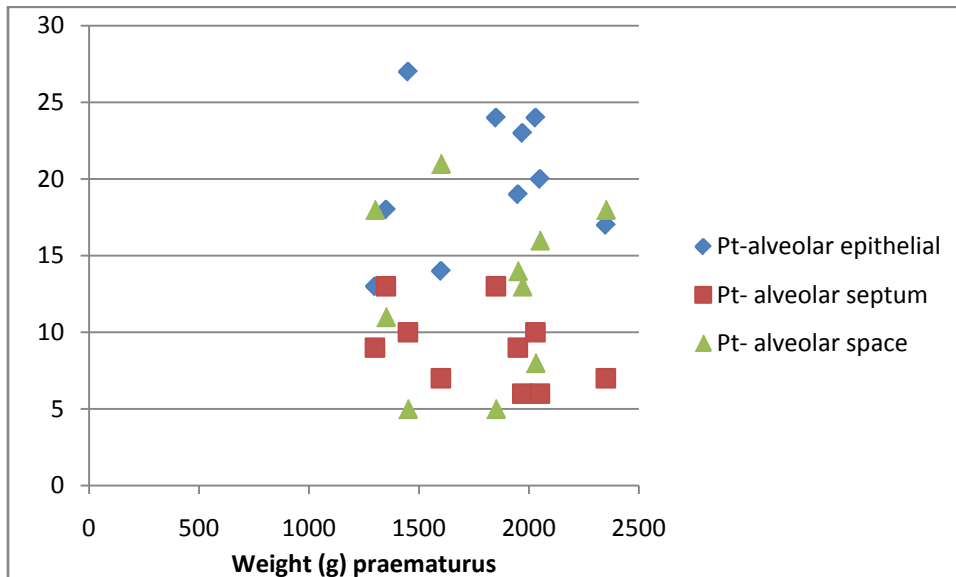


Fig.3. Individual reaction of the respiratory volumetric density (Vv) in premature newborn (with the weight 1100-2500 g). N = 9.

Figure 4, represents graphically the respiratory volumetric density (Vv) in **mature** age newborn and it is evident the significant

dominating presentation of the epithelial component with same type of the alveolar epithelium in despite of the body mass.

Pt alveolar epithelium: Pt alveolar septum P < 0.001

Pt alveolar epithelium: Pt alveolar spaces P < 0.001

Pt alveolar septum: Pt alveolar spaces P > 0.05.

Results indicate continual domination of the alveolar epithelial component with a slight decrease of the linearity at matured ages, whilst the component of the alveolar spaces has tendency of continual increase in matured ages.

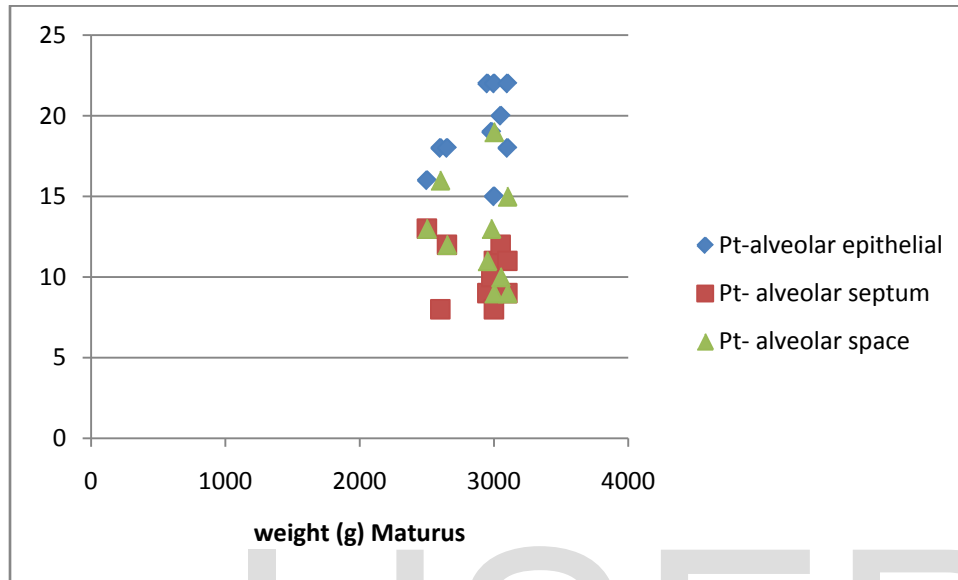


Fig.4. Individual reaction of the respiratory volumetric density (Vv) in mature newborn (with the weight over 2500 g). N = 9.

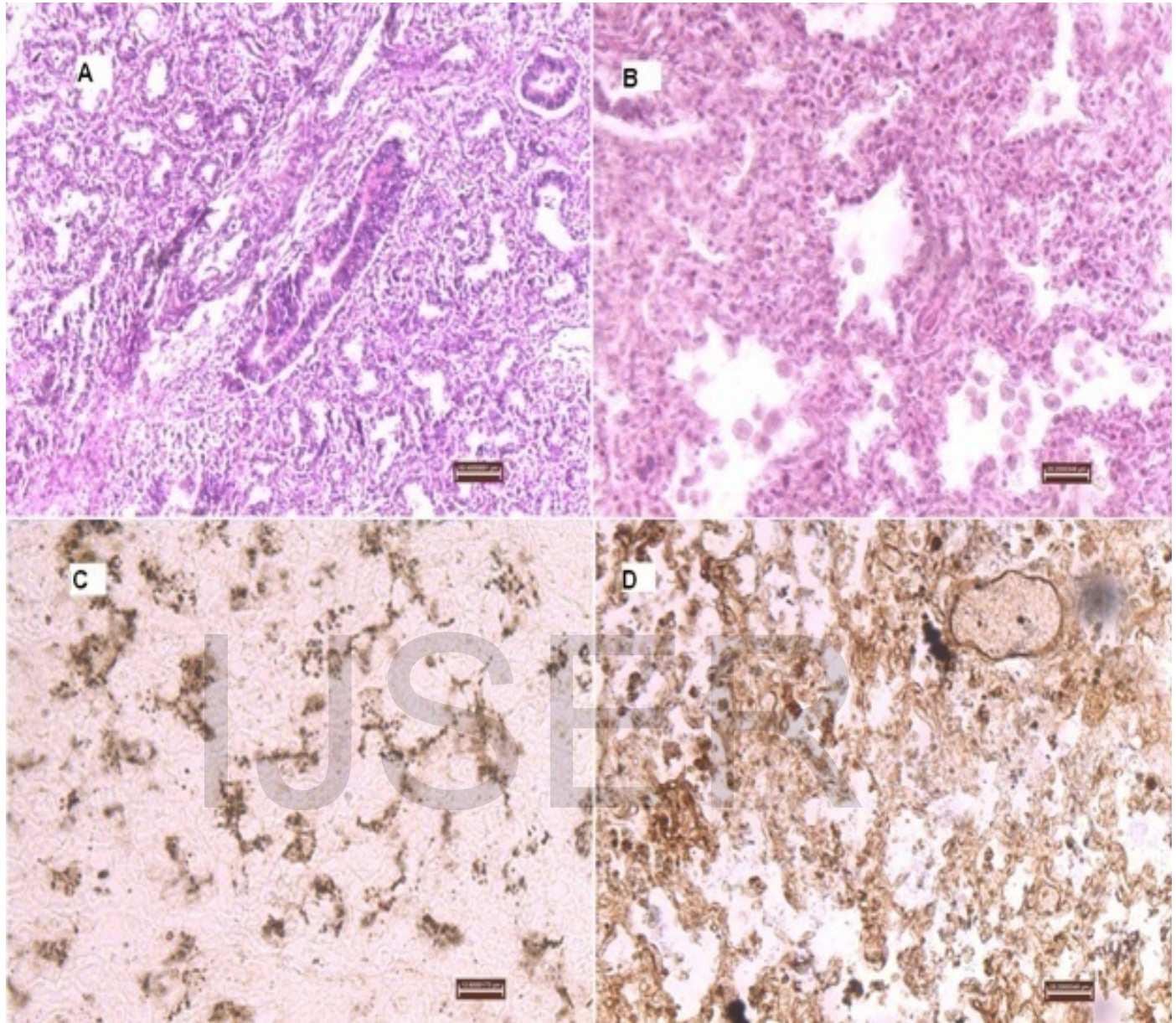


Fig. 5. a. Presentation of the pseudo glandular pulmonary developmental stage in premature newborn (prematurus). Staining: hematoxylin - eosin. 100x. **b.** Histopathology of the aspiration of amniotic liquid and pulmonary meconium in premature newborn (prematurus). Staining: hematoxylin - eosin. 200x. **c.** Emphasized argentaffin reaction in the alveolar epithelium and amniotic fluid in the meconium aspiration syndrome in premature newborn (prematurus). Staining: Grimeliu for argentaffin cells. 200x. **d.** Emphasized reaction of the ATP in the alveolar epithelium and amniotic fluid in the meconium aspiration syndrome in premature newborn (prematurus). Staining: ATP in fresh tissues. 200x.

DISCUSSION

Meconium in meconial fluid is scarce during the normal intestinal foetal function. Meconial passage is rare prior to

34th week of the gestation. After the 37th week of the gestation, it is correlated with gestation weeks. During our

morphologic and functional researches in newborn with the meconium aspiration syndrome, we have ascertained that the development of the alveolar structures is in the process of the permanent growth and it is seen the domination of the epithelial alveolar component. Epithelial alveolar component is emphasized in immature ages. Passage of the meconium in mature newborn (within the time term) is reduced and relates to nerve fibre myelination and influence of the parasympathetic tonus and the concentration of the mitilin (peptide that stimulate smooth musculature). Mitilin as a peptide belongs to the components of the argentaffin intestinal system, which relates to the APUD system. In our morphologic observation, in stained preparation of the pulmonary tissue with special histochemical staining (Grumelius), we see huge deposits of argyrophilic granules especially in mature ages of newborn.

Meconium stasis presents risk for newborn babies; MAS proceed in 10-30% and 19-34% cause mortality. Risk factors in MAS syndrome include: newborn babies 1-3 weeks after birth date period, maternal diabetes, hypertension in gestation, difficult births, respiratory distress syndrome (RDS) in newborn babies, intrauterine hypoxia (14).

Symptoms and signs of MAS syndrome include: tachypnea, nasal secretion, retraction, cyanosis or increased desaturation, also heavy stasis in umbilical cordon with skin spots. Meconium stasis can be noticed in oropharynx, larynx and trachea. Prophylactic suction of nose and mouth before birth of body of the babes decreases risk of MAS (15).

Recently, new therapeutic strategy for the treatment of MAS is suggested including anti-inflammatory drugs, such as antagonists of prostaglandins, high frequency ventilation, exogenous surfactant, nitric oxide and water inhalation (16).

The rings of tracheal tissue and lungs of guinea pigs are incubated for one hour in water bath for isolated organs, in three different concentrations with human meconium to investigate whether there is connection between the cumulative dosages of acetylcholine, histamine and meconium concentrations and contraction response (17). Current studies shows that the contractile response of the rings of tracheal and lungs tissue gradually increases with cumulative doses of acetylcholine and histamine in different concentrations of meconium and in control condition (17). However, there is no complete response in decreased concentration of meconium, which has lower tracheal

reactivity to histamine and acetylcholine (17). High concentration of meconium have tendency to increase tracheal reactivity. Incubation in lower concentration of meconium 1 mg/ml in rabbit trachea act by lowering reactivity in vitro of acetylcholine and histamine (17). In vitro relaxation of tracheal smooth musculature has been demonstrated in rats (18), but relaxation response increases with increasing of meconium concentration. Presence of reduced concentration of meconium in amniotic fluid may present a sign of physiologic maturity in newborn babies, and do not present inflammatory response of tissues. On the other side high concentration of meconium may cause harmful changes resulting in inflammations and related with constriction of vascular and respiratory airways of the smooth musculature. While, lower concentration of meconium increases secretion of surfactant in isolated alveolar tip II cell and inhibit oxidative blast of neutrophils and phagocytosis (18). This anti oxidative capability of meconium could be partially responsible for lower incidence of MAS syndrome in case of amniotic fluid aspiration of newborn babies.

Data on different causes and the mechanism of aspiration are controversy. Here correlate following mechanisms: non adequate maturity of the respiration centre in medulla oblongata and pulmonary tissue, disorder of the foetal intrauterine circulation, mechanic obstruction of the airways, pneumonitis, inactivation of the surfactant, compression of umbilical vessels. This syndrome in newborn causes persistent pulmonary hypertension. In our stereometric researches of different stages of gestational development, starting from weeks under 22 and over 38 of the gestation, we see that alveolar epithelial density occupies an obvious place in comparison to the lung alveolar septum and spaces. Researches of the volumetric density of the epithelial component, septum and alveolar spaces in different ages of the gestation starting from the abort, immature, and newborn born prior and on the time term, does not show relevant statistical difference. From this data it can be concluded that in the meconium aspiration syndrome, epithelial, septal, and alveolar volumetric density have no significant impact.

CONCLUSION

Based on morphologic and stereometric observations of the pulmonary component, with special emphasize on the epithelium, septum and alveolar spaces in newborn of different gestational ages, it can be concluded that:

- Alveolar epithelial component has dominant volume in all weeks of the gestation in newborn in comparison to the volume of septum and alveolar spaces. ($p < 0.001$).
- Volumetric epithelial component has a tendency of continual decrease depending on the maturity of the newborn.

- Volumetric density component of the alveolar spaces has a tendency of continual growth in mature ages of the newborn.
- Volumetric density of the alveolar epithelial component, alveolar septum and alveolar spaces does not indicate relevant statistical difference in different gestational ages in newborn with the meconium aspiration syndrome. ($p > 0.05$).
- With special histochemical staining concluded that amniotic fluid contains argentaffin material (neuroendocrine).

Contributions of authors

NH and PI have provided significant contributions to the conception and design of the study. BK, RSH and HI, have made a significant contribution in data collection, analysis and interpretation of data, and also in drafting the manuscript. All authors have read and approved the final version of the manuscript. The project is funded by the authors themselves. The study was not funded by any Institution or Pharmaceutical Company. The authors did not have

any financial support from any Institution or Pharmaceutical Company regarding the manuscript.

All authors have read and approved the final version of the manuscript.

The authors declare that they have no competing interests.

Ethics:

This study was supported by the Ethics Committee of the University Clinical Center of Kosovo (QKUK) and

the Faculty of Medicine in Pristina.

LITERATURE

1. Soukka H. and Kapa P. Experimental approach to the treatment of the meconium aspiration syndrome. *Archives of Perinatal* 2001; 7(2): 13-18.
2. Alexander Z. et all. Cell death and lung cell histology in meconium aspirated newborn rabbit lung. *European Journal of Pediatrics*. Abstract Volume 159 Issue 11 (2000); 891-96.
3. Eden R. D., Seifert L.S., Winegar A., Spellacy WN. Perinatal characteristics of uncomplicated postdate pregnancies. *Obstet. Gynecol.* 1987; 69: 296-9.
4. Steer P.J., Eigbe F., Lissauer T.J., Beard R.W. Interrelationships among abnormal cardiotocograms in labor, meconium staining of the amniotic fluid, arterial cord blood pH, and Apgar scores. *Obstet. Gynecol.* 1989; 5: 715-721.
5. Usher R.H, Boyd M.E., McLean F.H., Kramer M.S. Assessment of fetal risk in postdate pregnancies. *Am. J. Obstet. Gynecol.* 1988; 2: 259-64.
6. Wiswell T.E., Bent R.C. Meconium staining and

- the meconium aspiration syndrome. Unresolved issues. *Pediatr. Clin. North. Am.* 1993; 5: 955-81.
7. Fleischer A., Anyaegbunam A., Guidetti D., Randolph G., Merkatz I.R., A persistent clinical problem: profile of the term infant with significant respiratory complications. *Obstet. Gynecol.* 1992; 79: 185-90.
8. Katz V.L., Bowes W.A. Jr Meconium aspiration syndrome: reflections on a murky subject. *Am. J. Obstet. Gynecol.* 1992; 166: 171-183.
9. Mitchell J., Schulman H., Fleischer A., Farmakides, G. and Nadeau D. Meconium aspiration and fetal acidosis. *Obstet. Gynecol.* 1985; 65: 352-5.
10. Co E., Vidyasagar D. Meconium aspiration syndrome. *Compr. Ther.* 1990; 16: 34-9.
- (11). Avery G.B., Fletcher M.A., MacDonald M.G. (eds.), *Neonatology: Pathophysiology and Management of the Newborn* (4th Edition), J.B. Lippincott, Philadelphia, 1994.
12. Nathan L., Levino K.J., Carmody T.J. 3rd, Kelly M.A., Sherman M.L. Meconium: a 1990s perspective on an old obstetric hazard. *Obstet. Gynecol.* 1994; 83: 329-32.

13. Mitchell J., Schulman H., Fleischer A., Farmakides, G. and Nadeau D. Meconium aspiration and fetal acidosis. *Obstet Gynecol* 1985; 65: 352-5.
14. Katz V.L., Bowes W.A. Meconium aspiration syndrome. *Am. J. Obstet. Gynecol.* **2002**; 171-183.
15. Dorohoi D.O., Urzica D., Oancea S. Biophysical and clinical evaluation of the meconium aspiration syndrome. *Fizica mediului* **2007**; 27-30.
16. Mokry J., Mokra D., Nosalova G. Effects of meconium on airway reactivity to histamine and acetylcholine in vitro. *J. of Physiol. and Pharmacol.* **2007**; 58: 409-417.
17. Collins L.C., Roberts A.M., Robinson T., Joshua I.G. Direct effects of meconium on rat tracheal smooth muscle tension in vitro. *Pediatr. Res.* 1996; 40: 587-591.
18. Saukka H.R., Ahotupa M., Ruutu M., Kaapa P.O. Meconium stimulates neutrophil oxidative burst. *Am. J. Perinatal.* **2002**; 19: 279-284.

IJSER