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NEWBORNS' HEART RHYTHM DISORDERS IN EARLY NEONATAL PERIOD

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Abstract

Heart rhythm and conductivity disorders may occur at different ages. In doctor's practice it is important to determine causative factors, which may lead to newborns' heart rhythm disorder. The research involved examination of 384 newborns at the age of 0-8 days at gestation period of 39.2±1.7 weeks who showed clinical signs of the cardiovascular system (tachy-, bradyarrythmia, systolic murmur, and perioral cyanosis). Thirty seven infants showed heart rhythm disorder.

Evaluation of risk factors, which can occur in perinatal period and potentially affect the development of heart rhythm disorders and conductivity, was performed. The analysis of pregnancy course showed that 83.8% ($p \leq 0.05$) of mothers had fetomaternal disease including hazard of preterm birth in 62.2% ($p \leq 0.05$) of examined mothers, preeclampsia and gestational toxicosis were determined in 37.8%, subcompensated placenta dysfunction in 45.9% and placental abruption in 29.7% of women. Anemia of pregnant women was recorded in 21.6% of expectant mothers. 72.9% ($p \leq 0.05$) of infants were born by Caesarian section. Exacerbation of chronic pyelonephritis at the time of pregnancy was found in 10.8% of women, 10.8% of expectant mothers suffered from phlebeurysm of lower limbs. 17.5% of women had burdened heredity of cardiovascular system represented by heart rhythm and conductivity disorders. There was a domination of sinus bradycardia in 56.8% ($p<0.05$) babies and extrasystole in 32.4% in the heart rhythm disorders. Atrial flutter was observed in one newborn, clinical case is presented; one newborn had QT prolongation syndrome. At the age of 7.2±2.9 days 97.3% ($p \leq 0.05$) of patients had heart rhythm normalization. The development of heart rhythm disorders and conductivity in newborns of mothers suffering from extragenital pathology is affected by different unfavorable ante- and perinatal factors, such as pathological pregnancy and delivery. A baby with arrhythmia firstly found in neonatal period requires mandatory consultation with a cardiologist; it needs further examination in a specialized cardiological center/department, follow-up monitoring in polyclinic.

Keywords: *newborns, arrhythmias, early neonatal period.*

Introduction

Heart rhythm and conductivity disorders may occur at different ages. In doctor's practice it is important to determine causative factors which may lead to newborns' heart rhythm disorder. The first cause is represented by organic congenital abnormalities of the heart anatomy [*Grosse-Wortmann L et al., 2010*], inflammatory and degenerative myocardial diseases, and heart tumors; the second one includes metabolic disorders of homeostasis (hypo- and hyperkalemia, -magnesemia, -calcemia; hypo- or hyperthermia; hyperthyroid conditions and the effect of medications) [*Maulidi H et al., 2012*]. The presence of an arrhythmogenic substrate, hypoxia and morphofunctional immaturity of myocardial tissue is considered to be the principal ethiopathogenetic factors of heart rhythm disorders in babies of early age [*Hoogaars WMH, Tessari A, 2004; Kovalev IA, Usenkov SY, 2013*]. Particularly heart rhythm disorders of posthypoxic

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genesis which developed in newborns after past asphyxia or birth injury are frequently transient and can disappear spontaneously [*Wood CE, 2009; Roos R et al., 2011*]. Autoimmune diseases of connective tissues, diabetes mellitus and thyroid gland diseases in a pregnant woman play an important role in the genesis of arrhythmias [*Kruchina TK, Egorov DF, 2011; Mitchell JL et al., 2012; Yildirim A et al., 2013; Ng O, Shahani SJ 2014*].

Nowadays there are no exact data concerning the prevalence of heart rhythm and conductivity disorders in childhood [*Roos R et al., 2011*]. However, approximately 1% of newborns were found to have heart rhythm disorders during routine screening before discharge from hospital [*Poddar B et al., 2006*]. This problem requires enhanced study of newborns, because some types of arrhythmias may be transient and benign, while others lead to development of newborns' cardiac insufficiency and even to cardiogenic shock and death [*Wood CE, 2009; Safina AI et al., 2010*].

The goal of the research is to improve the diagnostics of and to study the pattern of newborns' heart rhythm and conductivity disorders of women suffering from extragenital pathology in early neonatal period.

Materials and Methods

The research involved examination of 384 newborns at the age of 0-8 days in gestation period of 39.2±1.7 weeks who showed clinical signs of the cardiovascular system (tachy-, bradyarrhythmias, systolic murmur and perioral cyanosis). 37 infants showed heart rhythm disorder, among them 72.9% ($p \leq 0.05$) of boys and 27.1% of girls. The age of the babies at the time of observing rhythm disorders was 5.7±4.5 days. The average weight of patients under examination was 3444.2±427.3 *g* which did not differ from control group values ($p \leq 0.05$). The control group was represented by 20 healthy newborns of the same age.

The evaluation of risk factors which may occur in perinatal period and potentially affect the development of heart rhythm and conductivity disorders was performed. The research involved the analysis of obstetric history, gestation and labor course, clinical examination of a newborn, ECG-examination on 12 deflections and Doppler echocardiography. The research was approved by Institutional Bioethics Committee and conforms the principles provided in the Declaration of Helsinki (*Br. Med. J. 1964; p.177*) with further supplements.

The data obtained were processed using the variational statistical method and the Mann-Whitney nonparametric test for equating two independent abnormally distributed samples.

Results and discussion

The research findings demonstrate that infants suffering from heart rhythm disorders were born to mothers whose average age was 28.4±3.4 years old. 32.4% of the children were from the second and subsequent pregnancies, 10.8% of the infants were from multiple pregnancies. The analysis of pregnancy course showed that 83.8% ($p \leq 0.05$) of mothers had fetomaternal disease including hazard of preterm birth in 62.2% ($p \leq 0.05$) of examined mothers, preeclampsia and gestational toxicosis were determined in 37.8%, subcompensated placenta dysfunction in 45.9% and placental abruption in 29.7% of the women. Anemia of pregnant women was diagnosed in 21.6% of expectant mothers. 72.9% ($p \leq 0.05$) of infants were born by Caesarian section due to increasing severity of preeclampsia and occurrence of the signs of fetal disorders and development of dystocia and presence of severe extragenital pathology in mothers. According to anamnestic and clinical laboratory data fetal distress was found in 39.7% of examined mothers that was a consequence of unfavorable conditions of intrauterine growth associated with fetomaternal disease secondary to concomitant somatic pathology in 56.8% ($p \leq 0.05$) of mothers. Somatic pathologies of expectant mothers are as follows: severe endocrine disorders (obesity, primary hypothyroidism (medically compensated); gastrointestinal tract disorders (cholelithiasis, stomach and duodenum ulcer, and abnormal development of bile ducts); urinary system disorders, chronic pyelonephritis, phlebeurysm of lower limbs, and epilepsy. 17.5% of the women were found to have burdened heredity of cardiovascular system repre-

sented by heart rhythm and conductivity disorders (II grade AV-block), congenital cardiac failure (secondary defect of interatrial septum), vegetative-vascular dysfunction, and hypertonic disease. Exacerbation of chronic pyelonephritis at the time of pregnancy was found in 10.8% of the women, 10.8% of the expectant mothers suffered from phlebeurysm of lower limbs.

Heart rhythm disorders such as tachycardia and episodes of extrasystole were prenatally diagnosed in 10.8% of fetuses. General condition of newborns was considered to be satisfactory. The diagnoses of examined children suffering from heart rhythm disorders included the following: signs of fetal chronic hypoxia in 5.4%, asphyxia in the process of delivery in 10.8%, intrauterine pneumonia in 10.8%, respiratory distress syndrome in 16.2%, hypoxically induced ischemic damage of central nervous system in 29.7% ($p \leq 0.05$) of children, and syndrome of intrauterine growth retardation. Multiple congenital abnormalities were found in one newborn.

Among heart rhythm disorders there was the prevalence of sinus bradycardia in 56.8% ($p \leq 0.05$) of the children, 32.4% suffered from extrasystole, mostly single, nomotopic and atrial. 5.4% of patients were diagnosed with sinus tachycardia; one newborn was found to suffer from atrial flutter and another had QT prolongated syndrome. During the period of monitoring the state of children remained stable, patients did not need antiarrhythmia drug therapy. At the age of 7.2±2.9 days the overwhelming majority of patients, which is 97.3% ($p \leq 0.05$), was found to have heart rhythm normalization. The signs of heart organic damage were found in two children (secondary defect of interatrial septum, aneurism of interatrial septum, and wide open arterial canal).

Thus, the development of heart rhythm and conductivity disorders in newborns of mothers suffering from extragenital pathology is affected by different unfavorable ante- and perinatal factors such as pathological pregnancy and labor. Perinatal damage of CNS hypoxic and ischemic genesis activates the development of 'benign' arrhythmias which do not lead to the damage of central hemodynamics and general state of a child and are transient.

The extract from hospital neonatal record of P., who was born at regional perinatal center of VI pregnancy, II preterm labor at gestation period of 36-37 weeks by Caesarian section (due to development of fetal distress, heart rate (HR) 220 *bpm*), illustrates the above-mentioned data. The patient's medical history reveals that the mother suffered from acute respiratory viral infection during the 35th week of pregnancy. The signs of maternal and fetal infection and congenital abnormality of urinary tract – multicystosis of fetal left kidney were prenatally detected. Mother, who is 28 years old, suffers from congenital abnormality of urinary tract: hydronephrosis of right kidney and chronic pyelonephritis, renal failure 0. A baby was born with the weight of 3200 *g*, height 49 *cm*, head circumference 35 *cm* and chest circumference 34 *cm*. According to Apgar scale, his score was 7/7 grades. The condition was grave at birth; there were signs of respiratory failure, low-pitched crying, and hypomyotonia. Respiration rate was 65-72/*min*. HR was 190-204 *bpm*. Saturation (SaO2) was 88-89%. Heart sounds were rhythmic, soft systolic noise in the second intercostal space to leftward of sternum. Abdomen was soft; liver was enlarged by1.5 *cm* below the costal margin. The values of acid-base balance at birth were as follows: pH 7.33, pCO2 50.0 *mm Hg*, pO2 15 *mm Hg*, HCO2 26.7 *mmol/l*, BE (-0.4). Calcium was 1.4 *mmol/l*. Due to the critical condition the baby was transferred to neonatal intensive care unit, where she was staying for one day.

During the first day of life the general condition remained critical due to respiratory failure, tachycardia with up to 200 *bpm* was still present. Hyporeflexia. Muscle tone and spontaneous motor activity were reduced. Skin was clean, pink and wet. Skin elasticity was normal, turgor was reduced. Subcutaneous fat layer had been sufficiently developed by the gestation period. The edemas were absent. Respiration was spontaneous, rhythmic with the involvement of intercostal spaces. During auscultation, the lung respiration reached all regions from both sides. Respiration rate was 65/*min*. SaO2 97-98%. The borders of deep cardiac dullness were as follows: right – along the right edge of the sternum, upper – II intercostal space,

1.0 *cm* to the left of the midclavicular line. Auscultation detected rhythmic and dull cardiac sounds. HR was 190 *bpm*. Arterial pressure was 69/36 *mm Hg*, average arterial pressure – 36 *mm Hg*. Abdomen was soft. Liver was enlarged by 1.5 *cm* lower the edge of costal arch. Urination was sufficient (1.5 *ml/kg/h*). Clinical analyses of urine and blood and biochemical blood assay (total bilirubin, electrolytes, albumin, C-reactive protein and blood sugar) are without pathological findings. Creatine phosphokinase-MB: 60.1 *u/l*. Doppler imaging for the first day of life: left ventricular end-diastolic diameter 14.9 *mm*, left ventricular systolic length 12.4 *mm*, left ventricular posterior wall thickness 3.9-5.0 *mm*, interventricular septum thickness 3.9-4.9 *mm*, ejection fraction 62%, ΔD 31%, left atrial length 10.3 *mm*, aorta diameter 8.6 *mm*, pulmonary artery diameter 9.2 *mm*, right ventricular end-diastolic diameter 16.0 *mm*. The average pressure in pulmonary artery trunk was 27 *mm Hg*.

Conclusion

Moderate dilatation of right chambers. Aneurism of interatrial septum, wide oval opening, diameter 4.7 *mm*, intensive left-right bypass. Open arterial duct, diameter 3.1 *mm*, pressure gradient of aorta/pulmonary artery 17 *mm Hg*. Cardiac arrhythmia, HR 113-190 *bpm*. Blood flow in abdominal aorta was pulsating.

Under the influence of post syndrome therapy the signs of respiratory failure had been reduced by the 2nd and 3rd days of life, the baby was transferred to the general unit. However tachycardia was still present (HR 200-220 *bpm*) in the absence of hemodynamic disorders, thereat Doppler imaging was performed, cardiologist's consultation was recommended. The results of Doppler imaging by the third day of life (Fig. 1): left ventricular end-diastolic diameter 13.9 *mm*, right ventricular end-diastolic diameter 16.0 *mm*, right atrial end-diastolic diameter 15.0 *mm*, ejection fraction 56%, left atrial length 10.3 *mm*, aorta diameter 8.6 *mm*, pulmonary artery diameter 9.2 *mm*. The average pressure in pulmonary artery trunk was 27 *mm Hg*.

Conclusion: Moderate dilatation of right chambers, wide oval opening with intensive left-right by-

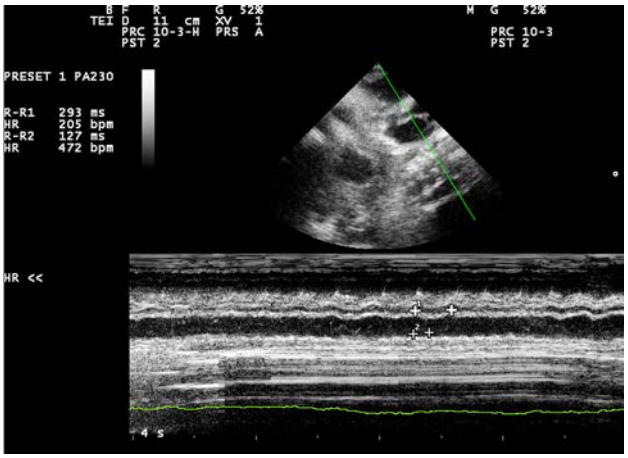

Figure 1. *Doppler imaging (M-mode) of a newborn P. (girl), third day of life.*
Note: *Atrial contraction with the rate of 472 bpm, see mark "2" in echogram of the left atrium; left ventricles deflate rate 205 bpm, see mark "1" at aorta root.*

pass through interatrial septum. Open arterial duct 2.4 *mm* (Doppler color flow mapping mode downstream in pulmonary artery trunk with diameter). Tachycardia. Blood flow in abdominal aorta was pulsating. Investigation in M-mode showed non-compliance of aortic root HR and left atrium in the ratio 1:2 with the aorta deflate rate 190-200 *bpm*, atria 350-400 *bpm* (irregularly), which allowed diagnosing heart rhythm disorders – atrial flutter.

ECG examination showed characteristic atrial waves F which have a specific saw-tooth form that determined the presence of heart rhythm disorder - atrial flutter (Fig. 2). Clinical signs of cardiac failure were not detected.

To normalize baby's HR she was prescribed antiarrhythmic therapy (cordaron with the dosage of 5 *mg/kg/d*). The normalization of heart rhythm was recorded in 20 hours (Fig. 3).

Holter monitoring of ECG during the day detected sinus rhythm (Fig. 4).

After examination the aim of which was to exclude intrauterine infection, myocarditis, consultation with heart surgeon the baby was discharged in

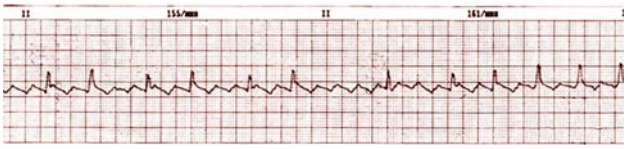

Figure 2. *ECG, of a newborn P (girl), third day of life. Atrial waves F which have a specific "saw-tooth" form.*

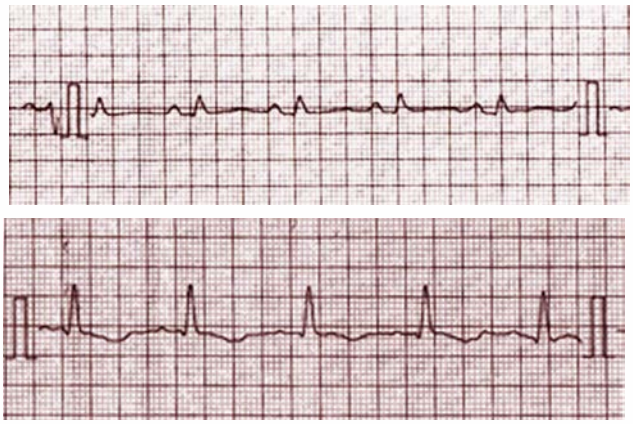

Figure 3. *ECG of a newborn P (girl), fourth day of life (after administration of amiodarone (cordaron). Sinus rhythm. HR is 148 bpm.*

satisfactory condition to be further supervised by pediatrician at the place of residence with diagnosis: rhythm disorders of the heart: atrial flutter. Heart failure (HF0). Patent foramen ovale, functioning patent ductus arteriosus. Abnormal trabecula of left ventricle. Congenital disorder of urinary system: multicystosis of the left kidney. Renal failure 0. During the first month of life the condition was satisfactory, episodes of tachycardia were not recorded, parents did not ask for doctor's help. Routine examination and follow-up monitoring at the regional children's cardiological center during the year is recommended.

ST episode Ch.1. Total number of episodes 10.
Total duration 00:28:18. Heart rate: 171 bpm.

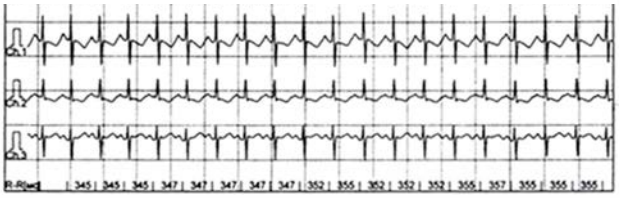

Figure 4. *Holter monitoring of ECG, 12 days of life. Sinus rhythm. HR is 150-171 bpm. Isolated supraventricular premature beats (time of observation 23 hours 53 minutes).*

Given clinical case demonstrates that it is possible to conclude that atrial flutter in newborn may develop in early neonatal period as the result of right atrial overload with the volume associated with actively functioning fetal communications (wide oval opening with left-right bypass, open arterial duct). To the extent of the stabilization of extrauterine hemodynamics, normalization of pulmonary artery pressure, associated with undertaken therapy, atrial flutter was reduced, and the baby was discharged in a satisfactory condition.

Neonatologist and children's cardiorheumatologist should exclude a significant number of diseases of baby and mother to determine the genesis of arrhythmia. The specification of frequency of development and range of fetal and perinatal heart rhythm and conductivity disorders, the investigation of cause-and-effect interactions of their formation, possible consequences, indications and algorithms of medical or surgical treatment will promote reduction of the perinatal and neonatal morbidity rates and have significant clinical, social and economic effects.

A baby with the arrhythmia detected for the first time in the neonatal period requires mandatory consultation of a cardiologist. Later on, it is in need of further examination in a specialized cardiological center/department, follow-up monitoring in an out-patients' clinic.

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MODULATORY ROLE OF BLOOD CYTOKINES IN PREVENTION OF ADRENALINE-INDUCED ACUTE LUNG AND MYOCARDIAL INJURY BY INDOMETHACIN AND MECHANICAL VENTILATION

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Abstract

Objective: the objective of this study is to investigate the role of some cytokines in adrenaline-induced pulmonary and myocardial injury, as well as prevention by treatment with indomethacin and mechanical lung ventilation in rats.

Materials and Methods: experiments were performed in albino male rats divided into: group 1 – control group, group 2 – animals treated with IV adrenaline, group 3 – animals treated with IM indomethacin 30 min prior to adrenaline injection, and group 4 – animals exposed to mechanical lung ventilation (positive end-expiration pressure) 10 min prior to adrenaline injection.

Lung and myocardial tissue sections were stained by Hematoxylin-Eosin. Blood cytokines (IL-1β, IL-6, IL-8, IL-10 and TNF-a) were detected by ELISA method. Statistical analysis was run by Student's t-test in Excel 2007.

Results: showed hemorrhages, inflammation and edema in lungs and neutrophil retention in myocardial sections of adrenaline-treated animals. Pretreatment with indomethacin and mechanical lung ventilation led to almost no pathological alterations in either of the tissues. Blood cytokine analysis in adrenaline-injected group resulted in increase of IL-1β, IL-6, IL-10 and TNF-a and slight suppression of IL-8. Pretreatment with indomethacin or mechanical lung ventilation led to even higher values of IL-1β and IL-6, and reduction of IL-10 and TNF-a compared to adrenaline group; IL-8 was significantly elevated in indomethacin-pretreated rats and normalized in animals exposed to mechanical lung ventilation.

Conclusion: indomethacin and mechanical lung ventilation can prevent adrenaline-induced injury of lungs and myocardium presumably via modulation of inflammatory and anti-inflammatory blood cytokines. This may have impact on the course of stress-related diseases.

Keywords: *adrenaline, acute lung and myocardial injury, cytokines, indomethacin, lung ventilation.*

Introduction

As previously known, a major cause of cardiac mortality is the myocardial infarction. Stressful conditions are associated with hypersecretion of catecholamines (CA) which may induce tachycardia, coronary vasoconstriction, splitting of oxygen supply and consumption resulting in hypoxia, diffuse necrosis of cardiomyocytes, and ventricular fibrillation [*Adameova A et al., 2009*].

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The role of pulmonary ventilation and progression of generalized hypoxia (hypoxemia) in pathogenesis of myocardial infarction is suggested by us as a new hypothesis opposing the well-established concept of myocardial affection under primary influence of CA. High CA levels lead to systemic and pulmonary vasoconstriction resultant in elevated pulmonary capillary resistance followed by increase of capillary permeability, pulmonary inflammation and lung edema, for example, in pheochromocytoma [*Sukoh N et al., 2004*] and experimental CA-stimulation [*Rassler B et al., 2012*]. Activation of phospholipases by CA exerts stimulatory effects on

pro-inflammatory cytokines and also affect number and function of inflammatory cells, the neutrophils in particular [*Rassler B. 2007*].

Acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) is a complex clinical syndrome involving acute inflammation, microvascular damage and increased pulmonary vascular and epithelial permeability, frequently resulting in acute respiratory failure [*Allen TC, Kurdowska A, 2014*]. According to Wheeler and Bernard, the ALI (also called non-cardiogenic pulmonary edema) is characterized by the abrupt onset of significant hypoxemia and diffuse pulmonary infiltrates in the absence of cardiac failure [*Wheeler AP, Bernard GR, 2007*].

The contribution of epithelial injury to progression of ALI/ARDS has become increasingly obvious. Decreases in epithelial cell barrier function facilitate influx of protein rich fluid into alveolar space; furthermore, epithelial injury leads to impaired cell fluid transport and reduced production of surfactant [*Manicone AM, 2009*]. Neutrophils are an important component of the inflammatory response in ALI and demonstrate increased production of pro-inflammatory cytokines [*Gommes J, Soehnlein O, 2011; Williams AE, Chambers RC, 2014*].

Cytokines are small proteins secreted by immune system cells, thereby transmitting signals between them. After an acute insult there is systemic release of cytokines such as TNF, IL-1 and IL-6 that have diverse effects on endothelium, epithelium and on circulating as well as resident immune cells [*Manicone AM, 2009*], and in turn, stimulate release of other pro-inflammatory cytokines as IL-8, which is a potent neutrophil attractant and is produced in lung macrophages performing a crucial role in ALI [*Allen TC, Kurdowska A, 2014; Williams AE, Chambers RC, 2014*].

Among powerful anti-inflammatory mediators IL-10 provides degradation of transcription factors for inflammatory IL genes or activates transcription repression factors [*Murray PJ, 2005*], suppresses inflammatory mediators TNF-a, IL-1, IL-6 and IL-8 [*Yilma AN et al., 2012*].

Arachidonic acid metabolites (prostanoids) that have diverse physiological effects in lungs contribute also to ALI [*Park GY, Christman JW, 2006*] and cardiovascular pathology [*Smyth EM et al., 2009; Qiu H et al., 2012*], trigger acute ischemic tissue injury via activation of endotheliocytes and oxidative stress processes [*Feitoza CQ et al., 2008*].

Treatment of ALI is based on both ventilatory and non-ventilatory strategies. To date, the most significant advances in the supportive care of lung injury patients have been associated with improved ventilator management – positive end-expiration pressure (PEEP) [*Johnson ER, Matthay MAJ, 2010*]. Among non-ventilatory strategies the non-steroidal anti-inflammatory drugs (NSAID) are used to block inflammatory actions of prostanoids and interleukins, and also improve the functional state of the ischemic tissue [*Feitoza CQ et al., 2008*].

Indomethacin is a non-selective inhibitor of arachidonic acid metabolism enzymes cyclooxygenase (COX) 1 and COX 2, which has earlier been proved to prevent the adrenaline-induced life-threatening cardiac arrhythmias in rats [*Sisakian SH et al., 1987*]. Hence, anti-inflammatory agents are suggested as drugs of choice to prevent adrenaline-induced acute lung and heart injury. In addition, protective mode of mechanical lung ventilation (MLV) as a means improving lung ventilation is applied to prevent gas exchange imbalance in the same model of lung injury.

The objective of this study is to investigate the role of indomethacin (IND) and MLV in modulating blood cytokines and preventing adrenaline-induced injury of lung and heart.

MATERIALS AND METHODS

Experiments were performed in male albino rats (150-180 g.) divided into 4 groups: group 1 – control (intact) animals (n=11); group 2 – animals (n=12) treated with IV adrenaline (ADR) 0.09 *mg/kg* (adrenaline hydrotartrate solution 0.18%) under Nembutal anesthesia (40 *mg/kg*, IP); group 3 – animals (n=12) were administered indomethacin (50 *mg/kg*, IM) 30 min prior to adrenaline treatment (IND+ADR); group 4 – animals (n=12) were exposed to MLV through endotracheal intubation to TOPO™ Volume/Pressure Small Animal Ventilator "Kent Scientific, USA" (ventilation rate – 30*/min*, ventilation volume – 2.76 cm^3, inhaled oxygen concentration – 21%, PEEP ventilation mode) 10 *min* prior to ADR injection (MLV+ADR). Animals were sacrificed using Nembutal, 20 *min* following adrenaline injection.

Paraffin-embedded lung and myocardial tissues were stained by hematoxylin-eosin.

Concentration of blood cytokines (IL-1β, IL-6, IL-8, IL-10 and TNF-a) was detected by immune-enzyme analysis method (ELISA) on immune analyzers StatFax-3200 (Awareness Technologies Inc., USA) and Elecsys cobas 2010 (Roche, Germany) in the laboratory of N1 Clinical Hospital of Yerevan State Medical University.

Statistical analysis was performed by Student's *t*-test (2 sided, $\alpha < 0.05$) in Excel 2007. All data are presented as mean ± SD.

Results

Histopathology of the heart following adrenaline injection revealed cardiomyocytes contractures, most of the cardiac muscle cells performed wavy outlines (Fig. 1b) compared to the control group (Fig. 1a). Interrupted intercalated disks leading to cardiomyocytes dyscomplexity were often found, intercellular capillaries were anemic, the myocardial stroma showed neutrophil accumulations (Fig. 1b). The lung specimens of the same animals exhibited pulmonary edema with interstitial and intra-alveolar components (Fig. 2b) in contrast to a control specimen (Fig. 2a). Eosinophilic liquid within bronchi and alveoli as well as thickening of interalveolar septa (due to hyperemia, focal erythrodiapedesis and diffuse inflammatory infiltration presenting recruitment of neutrophils) were observed (Fig. 2b).

Pretreatment with indomethacin led to almost complete restoration of normal cardiac morphology, with minor stromal edema left (Fig. 1c); lung sections performed no hemorrhages, though minor inflammatory reaction was preserved (Fig. 2c). Lung ventilation preventive strategy restored almost normal histomorphological pattern of myocardial sections (Fig. 1d), the lungs showed emphysematous dilations due to certain mechanical stretch (Fig. 2d).

The results of the blood cytokines study showed 2.4 fold increase of IL-1β and IL-6, minor depres-

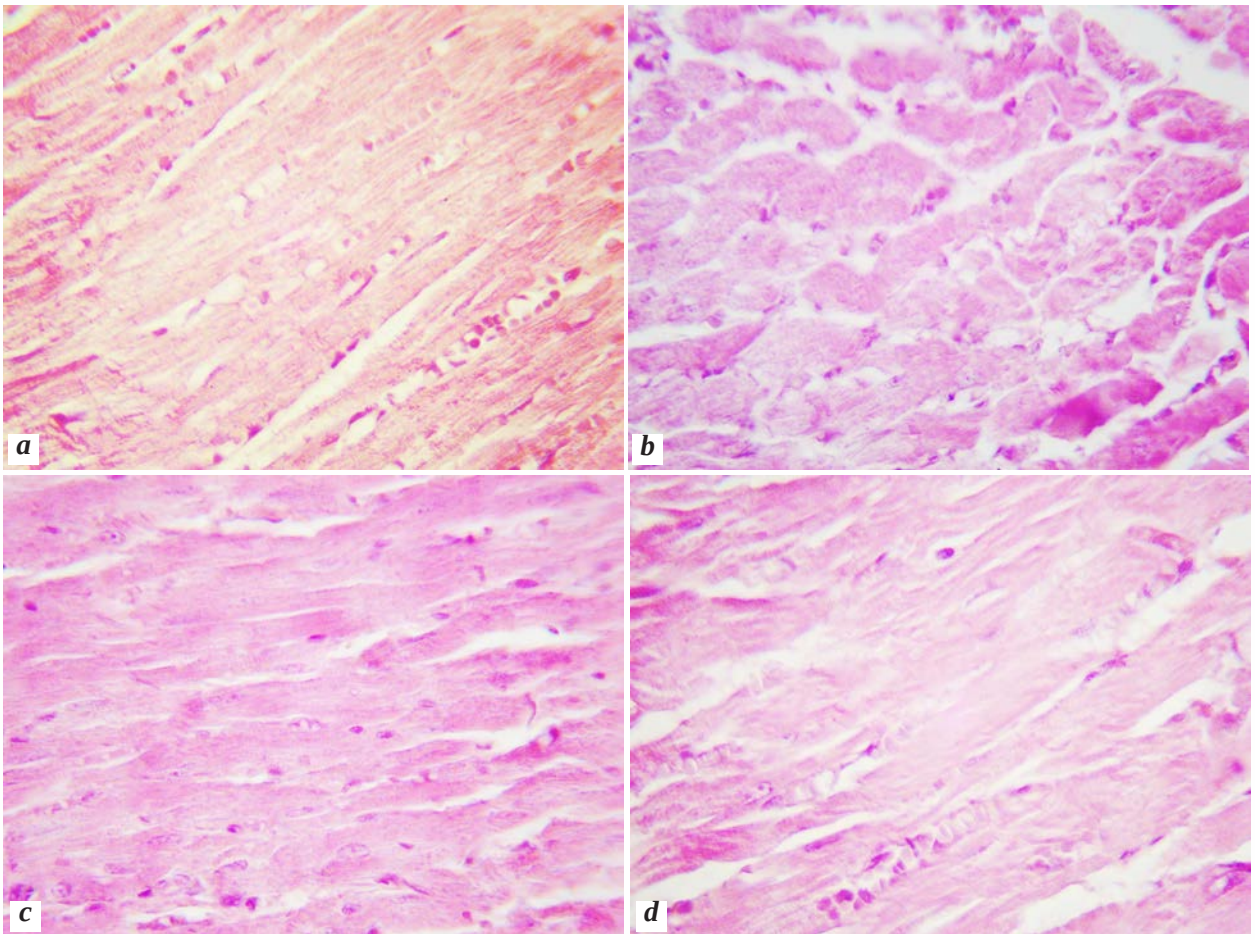

Figure 1. *Myocardium sections stained by hematoxylin-eosin, ob. 40, oc. 10.* ***a)*** *control group,* ***b)*** *adrenaline injection group,* ***c)*** *indomethacin-pretreated group,* ***d)*** *mechanically ventilated animals group.*

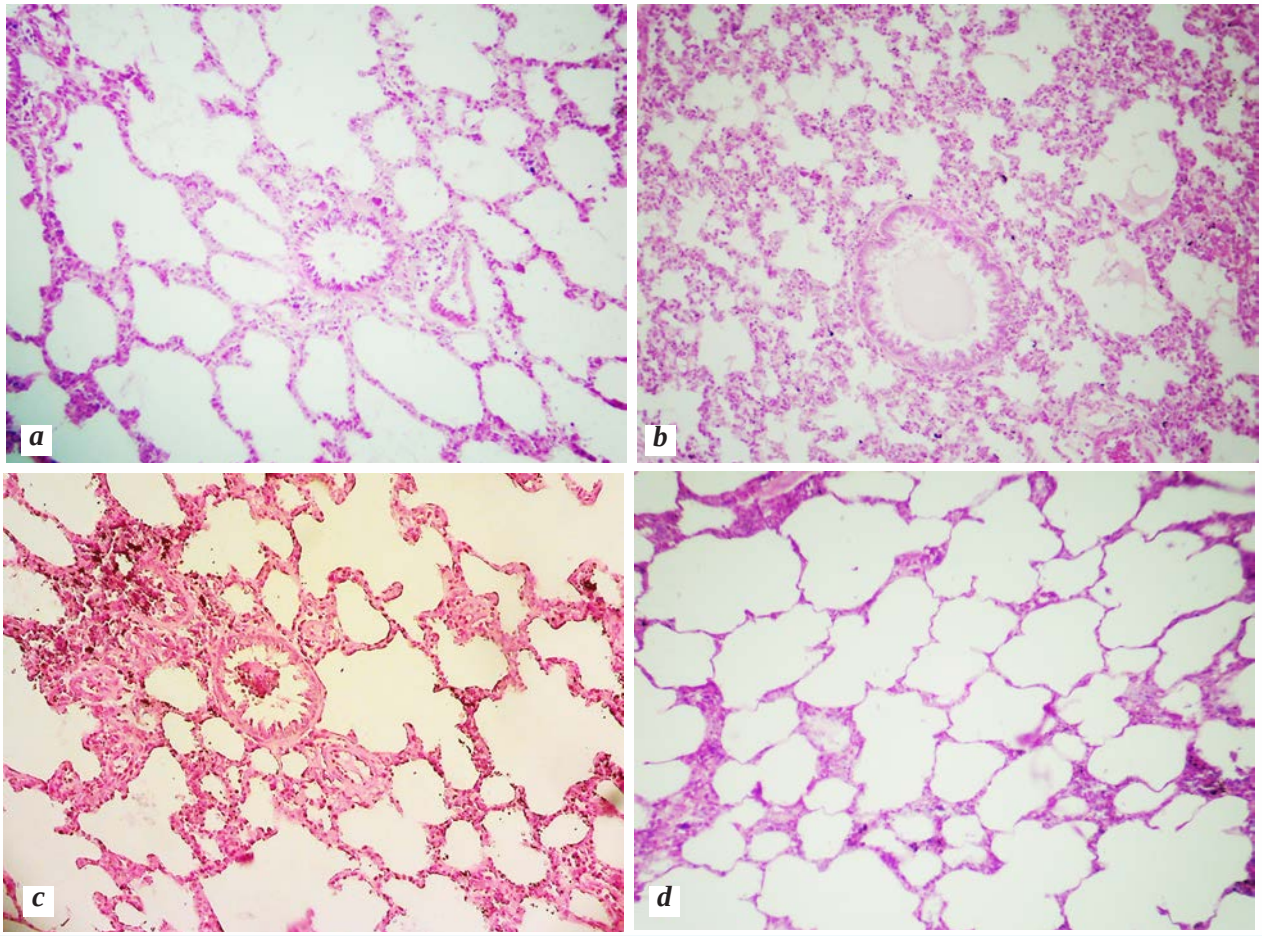

Figure 2. *Lung sections stained by hematoxylin-eosin, ob. 10, oc. 10: a) control group, b) adrenaline injection group, c) indomethacin pretreated group, d) mechanically ventilated animals group.*

sion of IL-8 (by 10%, $p<0.05$), 3.8-fold elevation of TNF-a and 43.7% increase of IL-10 in contrast to ADR group animals (Tab.). Indomethacin and protective mode MLV pretreatment animals developed more expressed elevation of IL-1β and IL-6 vs. ADR group, IL-8 was substantially increased (3.1-fold, $p<0.001$) in IND+ADR animals and was normalized in ventilatory correction. Inverse shift was recorded for IL-10 and TNF-a following NSAID injection before adrenaline exposure and MLV+ADR groups (in IND+ADR group decrease by 64.2% and 60% ($p<0.001$), respectively, and in ventilated rats – by 37.8% and 48%, $p<0.001$, respectively).

Table

Blood cytokine data (M±SD)

Cytokine measurements	Control	ADR	IND+ADR	MLV+ADR
IL-1β, (*pg/ml*)	8.92±0.71	20.98±0.96*	43.56±3.64†	51.5±2.85$^{\#}$
IL-6, (*ng/ml*)	21.12±2.82	51.26±2.39*	81.54±5.42†	84.65±3.07$^{\#}$
IL-8, (*pg/ml*)	43.84±11.81	38.56±3.68**	123.56±4.76†	42.6±3.11
IL-10, (*pg/ml*)	102.86±2.88	147.9±6.84*	53.02±3.21†	92±5.69$^{\#}$
TNF-α, (*pg/ml*)	7.69±0.61	29.12±5.99*	11.66±2.22†	15.15±1.67$^{\#}$

Note: ** - $p<0.001$, ** - $p<0.05$, significance of differences between ADR and control groups; † - $p<0.001$, significance of differences between IND+ADR and ADR groups; # - $p<0.001$, significance of differences between MLV+ADR and ADR groups.*

Discussions

No studies to date, to the best of our knowledge, have investigated the inflammatory changes in lungs, alterations of blood cytokines influenced by high-dose adrenaline during acute lung injury followed by cardiac affection.

Our study investigated the mechanisms involved in the preventive effects of indomethacin and MLV in experimental ADR-induced model of ALI leading to myocardial affection. We observed the effects of high-dose adrenaline, mechanical ventilation and indomethacin on histomorphological status of lungs and myocardium, the modulation of blood cytokine activity.

As stated in the report of "Acute Lung Injury in Animals Study Group", an animal model of ALI should ideally capture one or more features of human ALI, including rapid onset (hours) after an inciting stimulus, evidence of pulmonary physiological dysfunction (e.g., abnormalities of gas exchange), histological evidence of injury to the lung parenchyma (inflammatory response) and evidence of increased permeability of the alveolocapillary membrane [*Matute-Bello G et al., 2011*]. It is noteworthy that, based on more precise definitions, in regard to the recent classification (Berlin 2012) the term "Acute lung injury" is replaced by "Acute respiratory distress syndrome", despite using the same basic pathogenetic principles [*Ranieri VM et al., 2012*]. This term ALI considered in our studies still to apply the term of ALI. With that in mind we used the term ALI in our studies.

Our results showed several evidences for ALI, including histological alterations, inflammatory response, and physiological dysfunction. In particular, high doses of adrenaline resulted in extravasation of neutrophils, erythrocytes in lungs, interstitial and intra-alveolar edema, and diffuse alveolar damage.

The research of Rassler and coauthors showed that infusion of CA (norepinephrine, phenylephrine, isoproterenol) over 72*h* may induce pulmonary remodeling (fibrosis and vascular hypertrophy) in rats. They have concluded that mainly α-adrenergic but also β-adrenergic mechanisms contribute to these processes. In addition, cardiac hypertrophy also developed and was predominantly mediated by β-adrenergic stimulation. Noteworthy, cardiac hypertrophy was considered to be a direct adrenergic effect rather than a consequence of pulmonary fibrosis [*Rassler B et al., 2012*]. Meanwhile, our suggestion to elucidate CA-induced ALI followed by acute myocardial damage is that cardiac affection is secondary to pulmonary gas exchange imbalance. The CA used in our studies was adrenaline in single injection and high doses.

According to Castro et al, the acute exposure of mice to cigarette smoke led to cellular activation of transcription factor NF-kB and p38 mitogen activated protein kinase (regulatory factor in translocation of NF-kB to the nucleus implicated in intracellular signaling during inflammatory stimuli) resulting in neutrophil recruitment into the lungs and oxidative damage. They showed that these effects can be blocked by treatment with indomethacin (10 *mg/kg,* IP), administered during 4 days, 1h before cigarette smoke exposure [*Castro P et al., 2009*]. Treatment with both NSAID inhibited the augmentation of PGE2 in bronchoalveolar lavage fluid. This suggests that COX independent mechanisms are at least as important as COX-dependent ones to the anti-inflammatory effect of indomethacin.

In the last years, growing evidence demonstrated a broad molecular modulation of NSAID by interacting with different intracellular pathways other than COX inhibition, suggesting a new therapeutic potential of these drugs. Particularly, indomethacin has been further described to have an effect on cellular apoptosis and inflammatory cell migration via a COX-independent mechanism [*Standiford TJ et al., 2005*]. Several reports have shown that NSAID can inhibit acute experimental lung inflammation induced by different agents. For example, pretreatment of rabbits with indomethacin under partial lung microvascular recruitment, protects against phorbol myristate acetate-induced pulmonary endothelial enzyme dysfunction, perhaps by diverting flow to the lung tissue previously unperfused, unexposed to phorbol myristate acetate, and hence metabolically healthy vessels [*Chen X et al., 1992*].

An acknowledged way of treatment of ALI in patients is the mechanical lung ventilation, which improves the alveolar gas exchange in turn improving survival and reducing the duration of mechanical ventilation with a lung-protective ventilation strategy [*Johnson ER, Matthay MAJ, 2010*]. In our research we suggest it as a means of prevention rather than treatment of experimental ALI which

further leads to cardiac affection and death in rats. As shown previously in our recent experiments, the mechanical ventilation prior to adrenaline injection prevents the life-threatening cardiac arrhythmias [*Sisakyan SH et al., 2008*].

Blood cytokine analysis in ADR group resulted in increase of IL-1β, IL-6, IL-10, TNF-a, and suppression of IL-8. Bergmann and Sautner demonstrated that activation of particularly TNF-a and IL-1b can be stimulated by alfa-adrenergic effect [*Bergmann M, Sautner T, 2002*]. In the study of DeRijk and coauthors it was revealed that adrenaline, given subcutaneously and IV infusion, increased plasma levels of IL-6, which could be blocked by the beta-adrenergic receptor antagonist propranolol [*DeRijk RH et al., 1994*]. As shown in a number of literature sources, IL-6 was considered as a pro-inflammatory cytokine. Meanwhile, number of researchers referred it as an anti-inflammatory IL in regard to its ability to down-regulate the synthesis of IL-1 and TNF-a [*Xing Z et al., 1998*].

A dynamic balance exists between pro-inflammatory cytokines and anti-inflammatory components of the immune system, and almost all the anti-inflammatory cytokines have at least some pro-inflammatory properties as well [*Opal SM, DePalo VA, 2000*]. Specifically, the lung injury is caused by an imbalance of pro- and anti-inflammatory mediators; and the transcription factor NF-κB has emerged as a likely candidate shifting the balance in favor of a pro-inflammatory state [*Ware LB, 2006*].

In a model of HCl-induced ALI IL-8 overproduction parallel with pulmonary hemorrhages was shown to occur in pigs [*Lampland AL et al., 2014*], and, along with TNF-a and IL-6 it increased following the ischemia-reperfusion lung injury caused by deep hypothermic circulatory arrest cardiopulmonary bypass in humans [*Dong LY et al., 2013*].

In accordance with several studies, stressor exposure significantly augments lipopolysaccharide (LPS)-induced IL-10 expression in mice, and our trials conclude that during sympathetic activation in stress-induced immune regulation, noradrenaline increases LPS-induced IL-10 via activation of beta-adrenoceptors [*Curtin NM et al., 2009*]. We can assume that IL-10 elevation in ALI is mediated by beta- rather than alfa-adrenoreceptor stimulation, and it is a feedback response to neutralize the detrimental effect of adrenaline.

Pretreatment with indomethacin or MLV led to even higher values of IL-1β and IL-6, and reduction of IL-10 and TNF-a compared to ADR group; IL-8 was significantly elevated in IND+ADR and normalized in MLV-treated animals. IND-mediated and MLV-induced suppression of IL-10 in our study can be considered as a feedback to reduced TNF-a activity. Sirota and coauthors showed that spontaneous secretion of IL-1b and TNF-a and LPS-induced production of TNF-a were substantially increased following incubation of adult PBMC with indomethacin, and only the spontaneous synthesis of TNF-a by cord blood monocytes of preterm newborns was suppressed by this drug [*Sirota L et al., 2000*]. Analogously, our findings reveal that indomethacin increased production of IL-1b, whereas TNF-a was reduced in peripheral blood along with elevation of IL-10 in IND+ADR group.

According to Zampronio et al., IL-8 induces fever in rats by a mechanism independent of the release of COX products, since indomethacin could not perform antipyretic effect following injection of IL-8 [*Zampronio AR et al., 1995*]. Interestingly, in our trials, IL-8 was not subject to substantial alterations, except IND+ADR group, where the mediator increased significantly along with IL-6 amount.

A lung-protective strategy of mechanical ventilation may reduce inflammatory response in patients with ARDS through decreasing concentrations of IL-6, soluble TNF-a receptors and IL-1 receptor antagonist [*Ranieri VM et al., 1999*]. The results of studies by Reis et al. showed that aerobic exercise plays an important role in protecting the lung from LPS-induced ALI [*Reis GCT et al., 2012*]. The beneficial effects of exercise are mainly mediated by the increased expression of anti-inflammatory cytokines and antioxidants and modulation of the inflammatory/anti-inflammatory and the oxidative/anti-oxidative balance in the early phase of ALI. These data can additionally support the idea of applying MLV as a method of prevention in our trials.

Petersen and Pedersen have concluded that muscle-derived IL-6 is mediating the health beneficial effects of regular exercise and can have important role in protection against diseases associated with cardiovascular disorders [*Petersen AMW, Pedersen BK, 2006; Pedersen BK, 2013*]. Here, it should be considered the classic signaling of IL-6, which stimulates physiological, regenerative, and anti-inflammatory

responses, whereas IL-6 trans-signaling is used in pathophysiological states and is mainly pro-inflammatory [*Yamamoto K, Rose-John S, 2012*].

CONCLUSIONS

High doses of adrenaline induce ALI followed by myocardial injury manifested in recruitment of neutrophils, increase of capillary permeability, edema and damage of the lung and heart which are accompanied with increase of both inflammatory and anti-inflammatory blood cytokines.

MLV and indomethacin prevent adrenaline-induced experimental ALI and myocardial injury modulating the blood cytokine activity. It can be suggested that this may have an impact on stress-related disease processes. Presumably, the cardio- and lung-protective effects of non-selective NSAI drug indomethacin can be mediated by both COX-dependent (PGE_2 synthesis suppression) and COX-independent mechanisms, since it elevates the inflammatory cytokines, or else, certain pro-inflammatory interleukins (e.g., IL-6) can perform anti-inflammatory response in context of modulation of the rest of cytokines. Both indomethacin and mechanical lung ventilation share preventive mechanisms in suppressing TNF-a and IL-10 caused by adrenaline-induced injury of lungs and myocardium.

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