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INTER COLLEGAS, VOL. 9, No.1 (2022)
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Table of Contents

INTER COLLEGAS, VOL. 9, No.1 (2022)
ISSN 2409-9988

SURGERY

OBSTETRICS & GYNECOLOGY

EPIDEMIOILY & PUBLIC HEALTH

OPHTHALMOLOGY

THEORETICAL & EXPERIMENTAL MEDCINE

CLINICAL SIGNIFICANCE OF NATRIURETIC PEPTIDES (review)

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Abstract

Natriuretic peptides are widely known for their protective effects against the development of metabolic syndrome and cardiovascular disease. The important role of determining the levels of natriuretic peptides in the diagnosis, assessment of severity, prognosis and effectiveness of treatment of heart failure has been proven in many studies and noted in clinical guidelines worldwide. Visceral obesity reduces the production and action of natriuretic peptides, increasing the risk of heart failure and 2 type diabetes mellitus. Metabolic syndrome is present in 60% of patients with chronic heart failure, most of its components contribute to the development and progression of chronic heart failure and include obesity, hypertension, dyslipidemia, insulin resistance, impaired glucose tolerance. The prevalence of metabolic syndrome in the general population is 34% and is constantly increasing due to unhealthy diet, sedentary lifestyle and chronic stress. The prevalence of chronic heart failure is increasing rapidly in all industrialized countries, affecting 2% of adults and 10% of the elderly. Therefore, the problem of early diagnosis and treatment of metabolic syndrome and chronic heart failure is relevant nowadays. The review focuses on the pathophysiological relationships between natriuretic peptides, heart failure and metabolic syndrome, and the approaches to the correction of natriuretic peptides' metabolism.

Keywords: *2 type diabetes mellitus, heart failure, metabolic syndrome, natriuretic peptides*.

INTRODUCTION

Natriuretic peptides (NUPs) are widely known for their protective effects against the development of metabolic syndrome (MS) and cardiovascular remodeling. Physiological levels of NUPs in serum are effective for prevention of arterial hypertension, sodium and fluid overload, obesity, vascular inflammation, dyslipidemia, insulin resistance (IR), hyperglycemia, myocardial hypertrophy and fibrosis [1]. Excessive visceral adiposity decreases synthesis of NUPs and impairs tissue sensitivity to them by inhibiting NUPs receptors, increasing renal clearance of NUPs, thus contributing to higher risk of heart failure (HF) and 2 type diabetes mellitus (DM-2) [2]. The importance of determining serum natriuretic peptides (NUPs) levels for the diagnosis, assessment of severity, prognosis of HF, and also for the assessment of treatment efficacy, has been extensively investigated in cardiovascular studies worldwide. Over the recent 20 years, the prevalence of chronic HF has substantially increased in all industrialized countries, and in recent years has been 3 to 20 cases per 1,000 adults and 100 cases per 1,000 elderly people [1]. The presence of chronic HF impairs quality of life and significantly increases overall morbidity and mortality. The main risk factors for HF are closely related to MS and include visceral obesity, arterial hypertension, dyslipidemia, IR, hyperglycemia, and DM-2. The prevalence of MS in the general population is 34% and is constantly increasing due to poor nutrition, sedentary lifestyle and chronic stress. MS is observed in 60% of patients with chronic HF, most of its components contribute to the development and progression of chronic HF. Therefore, the concern about early diagnosis and treatment of MS and chronic HF is very serious nowadays, and NUPs play a significant role in managing these healthcare problems [2, 3]. The review focuses on the pathophysiological relationships between natriuretic peptides, heart failure and metabolic syndrome, and approaches to the correction of natriuretic peptide metabolism.

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GENERAL INFORMATION

NUPs are produced in the atria and ventricles of the heart as well as in the endothelium. These hormones act as vasodilators, diuretics and natriuretics, activate lipolysis of adipose tissue and insulin secretion, increase oxidation of free fatty acids (FFA) in skeletal muscles and liver. The main stimulus for their secretion is elevation of myocardial tension with increasing pressure or volume overload. In cardiovascular diseases NUPs reflect contractile capacity of a myocardium [4]. Brain natriuretic peptide (BNP) and N-terminal pro b-type natriuretic peptide (NT-proBNP) are widely used for the diagnosis of HF in clinical practice, since their elevation is observed in the majority of HF types, they can exclude the presence of heart failure with a high sensitivity, in case when the diagnosis is uncertain [5, 6]. The use of these biomarkers has the highest class of recommendations for confirming the diagnosis or exclusion of HF in the latest clinical guidelines [4, 6].

The NUPs family includes a group of hormones that have a similar molecular structure and are natural antagonists of the renin-angiotensin and sympatho-adrenal systems, aldosterone and vasopressin. There are four members of this family: Atrial Natriuretic Peptide (ANP), Brain Natriuretic Peptide (BNP), Natriuretic Peptide-C (CNP) and Dendroaspis Natriuretic Peptide-D (DNP) [4].

NUPs: physiological and pathophysiological mechanisms of action

The main stimulus for increased secretion of ANP and BNP is stretching of the atria, caused by pressure or volume overload. BNP synthesis and secretion can also be triggered by hypoxia and ischemia of cardiomyocytes, angiotensin II, endothelin-1, glucocorticosteroids and tachycardia, even in the absence of dilation of the heart chambers [2]. Significant increase of BNP levels in serum within 1 hour and ANP levels within 3 hours is observed in response to hemodynamic stress [9]. ANP and BNP are known to reduce the sympathetic tone and suppress both renin and aldosterone secretion [3]. Increased levels of proinflammatory cytokines, especially tumor necrosis factor-α (TNF-α), interleukin-1 and 6 (IL-1, IL-6) or thyroid hormones impair release of ANP and BNP in response to mechanical stretching and ischemia of the heart [1]. Physiologically, ANP and BNP secretion is stimulated by estrogen, thus ANP and BNP levels are higher in women. On the other hand, CNP synthesis and release is activated by testosterone and growth hormone [3]. CNP release and production in endothelium and vascular smooth muscle cells is stimulated by transforming growth factor-β1 (TGF-β1), TNF-α, and IL-1, which are released in response to hypoxia, hyperglycemia, pressure and volume overload [8]. CNP plays an important role in vasorelaxation, acting together with another potent vasodilators – nitric oxide (NO) and prostacyclin [11].

ANP and BNP provide cardiovascular and kidney protection and metabolic effects by multiple physiological mechanisms:

1. Reduction of systemic blood pressure and venous return (preload) to the heart, balancing electrolyte homeostasis by:

• increase in glomerular filtration;

• inhibition of sodium reabsorption and enhancement of natriuresis and diuresis; inhibition of vasopressin / antidiuretic hormone reactions;

• relaxation of vascular smooth muscles.

2. Reducing the impact of the sympathetic nervous system on the heart and blood vessels by inhibiting the effects of RAAS by inactivating renin and aldosterone, angiotensin II.

3. Prevention of remodeling of the myocardium and vascular walls as a result of:

• inhibition of inflammatory, hypertrophic and proliferative processes in the endothelium, smooth muscle cells, myocardium;

• reduction of the activity of RAAS and cytokine systems, growth factors, matrix metalloproteinases, catecholamines, etc.;

• decrease of hypercoagulation and atherothrombosis.

4. Prevention of obesity, IR and DM-2 by stimulation of lipolysis in adipose tissue, increasing oxydation of FFAs in skeletal muscles, liver and adipose tissue, increasing sensitivity to insulin, decreasing production of proinflammatory cytokines [10–15, 18, 35–39].

Clinical significance of NUPs in the diagnosis of heart diseases

BNP is important in the diagnosis of heart failure, for risk stratification and control of the effectiveness of heart failure therapy [1, 2]. BNP increases in serum before clinical and instrumental signs of LV dysfunction and congestive HF become apparent, thus BNP is indispensable for early diagnosis of this pathology, being more sensitive than echocardiography. The results of The Breathing Not Properly Multinational Study showed that elevated serum BNP indicates the presence of HF. In addition, the BNP levels have clear correlation with the functional class of HF according to the classification of the New York Heart Association [16]. Similar data were obtained

in a study involving 250 older patients (94% were men), 97 of who had congenital and acquired heart defects. In this study, BNP levels in patients with heart disease were significantly higher than in the group of patients with respiratory pathology [17].

Diagnostic levels of NUPs

The latest ESC and ACC/AHA guidelines for the diagnosis and treatment of heart failure identified diagnostic threshold values of NUPs in different clinical situations. Thus, for exclusion of acute HF, the diagnostic threshold for NT-proBNP less than 300 pg/ml and for BNP less than 100 pg/ml; for exclusion of chronic HF, the cut-off NT-proBNP level was less than 125 pg/ml and BNP less than 35 pg/ml were determined. Normal serum levels of NUPs in the absence of previous treatment exclude significant cardiac damage [2, 3, 18].

Evidence based data about diagnostic significance of NUPs

Multiple clinical conditions, besides HF, such as chronic kidney disease (CKD), atrial fibrillation (AF), pericardial disease, pulmonary embolism, and even age >75 y.o., can be the cause of increased levels of NUPs. On the other hand, MS is associated with decreased levels of NUPs, emphasizing the importance of establishing comorbidities [19].

NT-proBNP is excreted only through the kidneys. Therefore, kidney dysfunction can significantly increase NT-proBNP serum levels. In addition, neprilysin increases production of BNP, thus BNP levels can increase during ARNI (sacubitrile/valsartan) therapy. However, NT-proBNP is not a substrate for neprilysin, a successful decrease in NT-proBNP is observed with effective ARNI treatment [18].

BNP level >17.9 pg/ml is reported to be a significant independent predictor of mortality from all causes in patients with chronic HF with reduced ejection fraction (HFrEF) (p=0.006). The mean BNP is 7.8 (3.4–13) pg/ml in 4-year survivors with HFrEF (p<0.0001) [25]. Reduction of NT-proBNP to <1000 pg/ml is associated with significant reverse myocardial remodeling in response to treatment and more favorable prognosis [12].

The PRIDE study showed that NT-proBNP levels >300 pg/ml in 600 patients with dyspnea were 90% sensitive and 85% specific for the diagnosis of acute HF [1]. Also, BNP>100 pg/ml has 83.4% probability of acute HF, while BNP <50 pg/ml excludes acute HF with 96% sensitivity [3]. Body mass index affects the threshold level of BNP for the diagnosis of acute HF: for 90% sensitivity for the diagnosis of acute HF, BNP>170 pg/ml cut-off point should be used for patients with BMI 18.5 to <25 kg/m^2, 110 pg/ml for BMI 25 to <40 kg/m^2 and 54 pg/ml in morbid obesity with BMI>40 kg/m^2. BNP is also useful for the differential diagnosis of acute HF. BNP ≤200 pg/ml provides 91% specificity for acute respiratory distress syndrome, while BNP≥1200 pg/mL has 92% specificity for cardiogenic pulmonary edema [27].

The 2021 ESC guidelines state that the sensitivity and specificity of BNP and NT-proBNP for the diagnosis of chronic HF are higher during exacerbations and lower in the compensation phase. The threshold BNP level for the diagnosis of chronic HF was set at 35 pg/ml [30]. BNP threshold for the diagnosis of congestive heart failure is 80 pg/ml (95% accuracy). NT-proBNP cut-off point 125 pg/ml rules out chronic HF with a 97% probability [2]. BNP levels increase significantly according to each of the following NYHA functional class of HF: class I – 21.6±2.8 pg/ml, class II – 108.6±16.3 pg/ml, class III – 197,1±27.2 pg/ml and class IV – 363.0±67.8 pg/ml, p<0.0001) [28].

Determining serum levels of NUPs allows to clarify treatment strategy and to avoid unnecessary or inappropriate therapy. The latest ESC clinical guidelines for the management of patients with chronic HF emphasize the importance of assessing BNP and NT-proBNP in patients with acute dyspnea in order to exclude other causes of dyspnea not related to HF [20]. Also, in the same clinical guidelines, serum BNP and/or NT-proBNP levels greater than 35 and 125 pkg/ml, respectively, are considered to be necessary criteria for the diagnosis of HF with preserved ejection fraction (HFpEF), in addition to clinical manifestations and certain echocardiographic parameters [2].

In the latest version of the American (ACC/AHA) guidelines for the management of patients with HF, published in 2017, determining BNP levels in serum in order to exclude or confirm the diagnosis of HF in patients with dyspnea, has class I recommendation with the evidence level A [4].

Low level of NUPs, if adjusted to BMI, can rule out HF with high level of confidence [21]. But sensitivity of elevated NUPs levels to confirm the presence of HF is not so high, because increase of NUPs levels can be caused by comorbid non-cardiovascular conditions [9]. Causes of increased BNP and NT-proBNP levels in serum include cardiomyopathies, myocarditis, acute coronary syndrome, chronic coronary syndromes, valvular heart disease, atrial fibrillation and flutter, cardiotoxicity, kidney failure, anemia, sepsis, severe burns,

adult respiratory distress syndrome and other severe forms of pulmonary diseases [27].

Determining BNP or NT-proBNP levels for the assessment of HF severity and prognosis has class I recommendations with evidence level A in latest ACC/AHA and ESC guidelines [2, 4]. Patients with HFpEF usually show 2 times lower level of NT-proBNP (median level 2723 ng/l) compared to patients with HF with reduced ejection fraction (HfrEF), whose median NT-proBNP is 5644 ng/l ($p<0.001$) [22]. The latest ACC/AHA clinical guidelines emphasize the purpose of determining BNP, NT-proBNP and cardiospecific troponins at the time of hospital admission and several weeks after initiation of treatment for the assessment of prognosis in patients with acute heart failure (class of recommendations I, level of evidence A) [10, 12].

High concentrations of BNP and NT-proBNP at the time of hospitalization are often accompanied by pathological elevation of cardiospecific troponins in patients with acute HF decompensation, even in the absence of obvious signs of myocardial ischemia or concomitant coronary heart disease [10, 12]. Such clinical situations are usually associated with a high risk of adverse clinical outcomes, including death from all causes and death from cardiovascular complications in patients with decompensated HF [2, 4, 12, 23, 24].

For certain level of NT-proBNP, prognosis is similar regardless the ejection fraction: survival rates are similar for patients with HFpEF and HFrEF in terms of death from any cause (88.4% vs. 86.9%; $p=0.471$) or cardiovascular death or HF decompensation at 1 year (73.8% vs. 70.6%; $p=0.225$) [23]. NT-proBNP is a strong and independent prognostic factor for all-cause death and major adverse cardiovascular events (MACEs) in elderly patients and MACEs in younger individuals. A 4.8-year study has shown 0.8% all-cause mortality in low NT-proBNP group (<19.8 pg/ml) and 7.8% high NT-proBNP group (≥81.9 pg/ml; $p<0.001$). The rate of MACEs was reported to be 3.1% in low NT-proBNP group and 18.9% in the high NT-proBNP group ($p<0.001$) [25]. In get-ABI-study with 7-year follow-up the rates of all-cause and cardiovascular mortality were: 35.4% and 6% for NT-proBNP > 300 pg/ml; 16.2% and 40% for NT-proBNP 125–300 pg/ml and 11.4%/4% for NT-proBNP ≤ 125 pg/ml [26]. High serum BNP levels are reported to be a reliable predictor of poor outcome not only in HFrEF but also in HFpEF. BNP level shows good correlation with LV end-diastolic pressure. BNP levels are associated with HF severity across the spectrum of HF stages [27].

The PEACE study, which involved low-risk patients with stable coronary artery disease and preserved ventricular function, elevated BNP and NT-proBNP has shown association with increased risk of HF development; higher NT-proBNP was also associated with increased risk of cardiovascular death, HF and stroke [28].

Recent studies indicated that NT-proBNP levels were significantly associated with cardiovascular outcomes and mortality in patients with DM-2 [2, 4, 12-15]. In a cohort study elevated NT-proBNP levels are reported to be independent predictors of major adverse cardiovascular events (MACEs) in patients with chronic coronary syndromes (CCS), preserved systolic function and prediabetes or DM-2 [29]. NT-proBNP was suggested to be superior to traditional risk factors for predicting cardiovascular events in prediabetic or diabetic patients with CCS. A group of patients with CCS and normoglycemia did not show significant associations between NT-proBNP levels and the risk of MACEs, which may need further investigations with a larger size of cohort [29].

Additionally, the evidence is provided that the NT-proBNP level has prognostic value in patients with ACS [14, 30]. Moreover, NT-proBNP has shown to be a strong predictor of MACEs and mortality in ACS patients, who underwent PCI with stent placement. NT-proBNP >1568 pg/ml is related to the high risk of all-cause death in nonST-elevation ACS patients [31]. Newly published study reported that high NT-proBNP level before primary PCI was independently associated with poor myocardial reperfusion in patients with ST-elevation MI [32].

The purpose of determining BNP and NT-proBNP levels in the assessment of treatment effects in HF patients

PARADIGM-HF study has detected statistically significant direct association between the reduction in NT-proBNP to ≤1,000 pg/ml and the 59% reduction in overall mortality [33]. In this trial 2/3 of investigated patients had baseline NT-proBNP values >1,000 pg/ml, which reflects high risk of complications. All the patients were reassessed at 1 and 8 months after the initiation of the treatment. Patients, who received sacubitril/valsartan treatment, had significantly lower NT-proBNP levels after 1 month of therapy, compared to patients, who received enalapril, also sacubitril/valsartan group showed significantly higher rate of NT-proBNP decrease below ≤1,000 pg/ml

compared to enalapril group: in 31% versus 17% of patients [33].

In addition, the results of a recent meta-analysis have shown that the decrease in BNP and NT-proBNP levels in HFrEF patients is associated with lower risk of HF decompensation, which requires hospitalization [34].

NUPs in regulation of lipid and glucose metabolism

NUPs increase lipolysis in adipose tissue, oxidation of FFA in skeletal muscles, liver and adipose tissue, sensitivity to insulin, insulin production and secretion, adiponectin secretion, decrease the secretion of proinflammatory cytokines and excessive leptin production. Visceral obesity has inverse association with levels of ANP and BNP and sensitivity of their receptors. Decreased levels of ANP and BNP are directly associated with the development of arterial hypertension, proatherogenic dyslipidemia, obesity and DM-2 [35]. But loss of visceral fat as a result of healthy diet and moderate physical activity is capable to restore physiological levels of NUPs and appropriate function of the receptors [34–36].

Glucagon-like peptide-1 (GLP-1) and physical activity increase ANP release from cardiac atrium, thus healthy dieting habits and avoiding sedentary lifestyle are cornerstones in maintainance of proper ANP level. The less known stimulators of ANP secretion are vitamin D, retinoids and glucocorticoids. Physical activity increases BNP expression and secretion [36]. C-type natriuretic peptide (CNP) increases in serum in case of endothelial damage, sepsis, hypoxia and chronic renal failure, due to influence of various cytokines and growth factors such as tumor necrosis factor (TNF-α), lipopolysaccharide (LPS), basic fibroblast growth factor (bFGF), interleukin-1 (IL-1), transforming growth factor beta (TGF-β) and thrombin [37]. ANP, BNP and CNP regulate metabolic processes by interaction with their receptors: natriuretic peptide receptor A (NPR-A), natriuretic peptide receptor B (NPR-B) and natriuretic peptide receptor C (NPR-C) [36]. Angiotensin II, endothelin, and endothelial NOS regulate NPR-A expression [36].

The expression of NPR-C significantly increases in patients with MS, which stimulates FFA oxidation and energy expenditure, in order to prevent the progression of obesity, excessive visceral adiposity, hyperproduction of proinflammatory cytokines. All the mentioned protective effects of NPR-C decrease insulin resistance, improve glucose tolerance and thus decrease the risk of new-onset DM-2 and diabetic complications [37]. Sedentary behavior, high-fat and high-sugar diet increase the expression of NPR-C in adipose tissue, whereas regular physical activity and healthy diet restore their physiological levels [38].

NUPs effects in adipose tissue

ANP is the most potent activator of lipolysis, followed by BNP and CNP [36].

Hyperinsulinemia caused by MS or intensive insulin therapy can attenuate NUPs –mediated lipolysis by reducing the level of circulating NUPs [35]. Increase of ANP levels and NPR-A activation in response to hypocaloric diet and moderate aerobic exercise decreases accumulation and inflammation of visceral adipose tissue, decreases release of proinflammatory cytokines from human adipocytes and adipose tissue macrophages, improves sensitivity to insulin according to HOMA-IR index, improves the results of oral glucose tolerance test (OGTT), thus decreasing the risk of DM-2 and cardiovascular diseases [36, 39].

NUPs effects on lipid oxidation and mitochondrial function

NUPs-induced lipolysis dramatically increases the availability of FFA for metabolically active tissues such as skeletal muscles and liver. Moreover, studies in humans and mice show that NUPs enhance oxidation of lipids in adipose tissue, skeletal muscles and liver, allowing these tissues to oxidate FFA more effectively, and use them as the predominant energy substrate [40].

It was found that short-term intravenous administration of ANP significantly increases lipid oxidation and energy expenditure after meals in healthy volunteers [41]. In addition to enhancing acute lipid oxidation, ANP and BNP induce mitochondrial biogenesis in skeletal muscles, increase lipid oxidation in human and rodent cells in vitro and in vivo. Chronic overexpression of BNP leads to increase in amount of mitochondria in skeletal muscles. Increased oxidative metabolism is known to be protective against obesity and IR caused by a high-fat diet [42].

NUPs interaction with adipokines

ANP significantly increases serum levels of adiponectin [36]. ANP reduces the release of leptin from human adipocytes [37]. Significant inverse correlation between serum BNP and leptin levels was reported in patients with chronic HF [39]. In case of NUPs deficiency, these mechanisms may contribute to the development of metabolic syndrome [36].

NUPs, insulin secretion and glucose homeostasis

A number of studies suggest that NUPs directly

and indirectly affect glucose metabolism. Clinical studies revealed an increase in serum insulin level during the infusion of ANP [43]. The effect can be explained by a direct stimulating effect on the β-cells. ANP directly enhances glucose-stimulated insulin secretion in cultured islets. In addition, ANP induced β-cell growth in isolated islets of the rat pancreas, whereas significantly smaller islets with reduced β-cell mass were found in ANP deficiency [44]. Finally, ANP increases glucose uptake by human adipocytes, preventing postprandial hyperglycemia [43].

It is interesting to note that infusion of NUPs in ten healthy young people on an empty stomach slightly increased blood glucose [45]. This effect can be explained by potent lipolytic effect of ANP on adipose tissue, which can dramatically increase the influx of FFAs to insulin-sensitive metabolic organs, and thus induce insulin resistance. In contrast, short-term infusion of BNP, without increasing FFA levels, slightly reduces circulating glucose concentrations during the initial phase of glucose and glucose tolerance test. This effect may be mediated by increased peripheral vasodilation and improved glucose transport through the capillary wall into the interstitial space [36]. Together, these studies suggest that NUPs can increase insulin secretion and insulin-stimulated glucose uptake, thus preventing DM-2 and diabetic complications.

NUPs can reduce lipid-induced IR by activating lipid oxidation in liver and muscle tissue. NUPs also preserve mitochondrial function and insulin sensitivity while using high fat diet in mice [39].

NUPs in obesity

In recent years, numerous studies have shown an inverse relationship between the levels of circulating NUPs and body weight [46]. This correlation may also be observed in patients with chronic HF, despite elevated levels of NUPs, due to oxidative stress in the myocardium [47].

Genetic polymorphism of CNP receptors is associated with lower prevalence of obesity and visceral adiposity compared to individuals with intact CNP receptors [48]. Another genetic polymorphism in the ANP promoter is associated with higher ANP levels and a favorable cardiometabolic phenotype, including lower prevalence of MS [49].

Visceral adiposity is reported to reduce NUPs release, increase their clearance, negatively affects receptor responses to NUPs in subcutaneous adipose tissue, liver and skeletal muscles. In contrast to patients with normal body weight, in obese patients serum NUPs levels are reduced and rapid NUPs responses to stimuli are blunted [50], but they can be restored by regular physical activity [51]. A survey of 7,770 patients and volunteers from the Framingham Heart Study and the Malmö Diabetes and Cancer Study found that obesity and IR were associated with markedly reduced plasma NUP levels [52]. ARIC study (Atherosclerosis Risk in Communities) has shown that NT-proBNP levels were inversely related to the risk of new-onset DM-2 during 12 years of follow-up [53].

Aerobic exercise and hypocaloric balanced diet can significantly increase NT-proBNP levels and tissue responses to ANP [53]. In patients with moderate obesity and HFpEF regular aerobic exercises, especially swimming, significantly increase ANP and BNP levels [27]. This effect may be explained by increase in venous return and cardiac filling pressure as a result of physical activity. Exercise-induced ANP secretion can be increased by taking β-adrenoblockers [29].

NUPs in insulin resistance and diabetes

In the latest studies, decreased levels of NUPs were associated with chronic hyperglycemia and hyperinsulinemia, regardless of body composition and distribution of adipose tissue [54]. Framingham Heart Study and Malmö Diet and Cancer Study have shown that low levels of BNP and NT-proBNP are strongly associated with the development of IR and new-onset DM-2 in both lean and obese patients [56, 59]. Moreover, in patients with NT-proBNP levels at the upper reference level, the risk of new-onset diabetes is significantly lower [57]. In healthy individuals' acute elevation of serum glucose causes rapid increase in ANP levels in response to hyperglycaemia-induced sodium and fluid retention, but this response is blunted in MS [55]. Heinisch et al. reported that BNP infusion during glucose tolerance test temporarily decreases serum glucose levels in healthy men [58].

These data suggest that NUPs may protect against the development of DM-2 due to antihyperglycemic effect and increasing lipid oxidation and mitochondrial function, as described above. On the contrary, NUPs deficiency probably contributes to IR and DM-2.

NUPs and Lipid Profile

The results of meta-analysis of studies, which investigated the relationship between NUPs and the components of serum lipid profile, demonstrated inverse association between NUPs levels and serum levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides, and direct

association with high-density lipoprotein cholesterol levels. These data underline the association between decreased NUPs levels and a proatherogenic lipid profile [40].

NUPs in arterial hypertension

The prevalence of AH in obese patients is 2–3 times higher than in people with normal body weight [48]. In lean healthy patients, sodium load or vasopressors induce release NUPs in order to enhance natriuresis, but in obese patients this response is blunted [26]. Partially, obesity contributes to the development of AH by reduction of vasodilation and natriuretic responses to NUPs and attenuation of NUP-mediated inhibition of RAAS and sympatho-adrenal system [18]. Brunner-La Rocca et al. demonstrated inhibitory effects of BNP on systemic and cardiac sympathetic nervous system activity. Thus, insufficient response to NUPs may increase the activity of the sympathetic nervous system in obesity [8].

In addition to regulation of blood pressure, NUPs also have a beneficial effect on heart remodeling in AH, reducing left ventricular hypertrophy and fibrosis. Rubattu et al. demonstrated that hypertensive patients with MS have lower levels of ANP and NT-proBNP, higher myocardial mass and a higher prevalence of left ventricular hypertrophy compared to hypertensive patients without MS [60].

In general, these results suggest that decreased levels of NUPs are associated with obesity and an increased risk of MS and cardiovascular diseases, while physiological levels of NUPs are associated with more favorable cardiometabolic phenotype.

CONCLUSIONS

NUPs show multifaceted protective cardiovascular and metabolic effects, which prevent myocardial remodeling, obesity, insulin resistance, hyperglycemia, dyslipidemia and hypertension. MS and cardiovascular diseases have close pathophysiological association and cause serious public healthcare system burden worldwide. Recent studies reported decreased NUPs levels and impaired action of NUPs receptors in patients with MS, which can increase cardiovascular risk. Healthy diet and rational physical activity together with pharmacological interventions, recommended by the latest cardiologic guidelines, show beneficial effects on NUPs production and effects. More wide use of NUPs for early diagnosis, risk stratification, and guided pharmacological intervention in patients with MS and HF has a significant potential for improving the cardiovascular care worldwide.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

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NEW MARKERS FOR DIAGNOSIS AND PROGNOSIS OF COGNITIVE IMPAIRMENT IN PATIENTS WITH MULTIPLE SCLEROSIS (review)

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Abstract

The article presents modern approaches to the diagnosis of brain damage and development of cognitive impairment in patients with multiple sclerosis. Neurodegenerative changes, which take place in the early stages of the disease and play an important role in the formation of irreversible neurological deficits, are considered. Cognitive impairment in patients with multiple sclerosis is quite common, but they are not always noticed, but they significantly reduce patients' quality of life. The article describes the possibilities of neuroimaging methods to identify structural changes in the parts of the brain responsible for cognitive functions. The importance of brain neurotrophic factor (BDNF) as a promising biomarker of multiple sclerosis is presented. Further study of BDNF remains interesting, which will allow to develop algorithms for early diagnosis and prediction of disease progression, that will provide an opportunity to deepen the understanding of the place of BDNF in the pathomorphological chain of nervous system damage in multiple sclerosis.

Keywords: *multiple sclerosis, cognitive impairment, brain neurotrophic factor (BDNF), neurodegeneration, neuroimaging.*

INTRODUCTION

Multiple sclerosis (MS) is the most common of the demyelinating diseases, characterized by formation of multifocal foci of demyelination in the brain and spinal cord, optic nerves associated with inflammatory cell infiltrates, reactive gliosis and axonal damage [1, 2].

The prevalence of multiple sclerosis in the world has recently tended to increase. This is due not only to the increase in morbidity, but also in improving the diagnosis of this pathology in the early stages [3, 4].

Researches of recent years have shown the presence, along with demyelinating processes, neurodegenerative changes in the substance of the brain in MS. It was found that neurodegeneration occurs in the early stages of MS, and plays an important role in the formation of the irreversible neurological deficit. Neurodegenerative changes are the main factor that leads to atrophy of the brain and exacerbates the severity of the neurological deficit [5].

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The peculiarity of development of MS is the simultaneous involvement of several different parts of the nervous system, which leads to the appearance of various neurological symptoms depending on the location of the pathological process. Visual, oculomotor, cerebellar, motor disorders, pelvic dysfunction, sensitivity disorders are most often observed in multiple sclerosis.

In recent years, researchers and clinicians have paid special attention to the study of psycho-emotional and cognitive disorders in patients with MS, given the significant impact of these disorders on the patients' quality of life and the necessity for their therapeutic correction. It has been found that depression and anxiety occur in 62% of cases, which is associated with demyelination in the temporal lobes. The occurrence of "constant fatigue" in patients (60–80%) is associated with rapid exhaustion of mental processes and causes drowsiness, difficulty in performing repeated actions, etc. [6, 7]. The prevalence of cognitive dysfunction ranges from 43% to 70% and it is observed at all stages and clinical types of MS [8].

The most vulnerable cognitive domains in MS are the domains of information processing speed, memory and attention [9, 10]. Thus, the typical picture of cognitive impairments in patients with MS includes decreased information processing

speed, speech impairment, lack of verbal and visual episodic memory, impaired attention, executive functions and visual-spatial skills [11, 12]. Speech disorders are usually associated with impaired speech fluency and poor word memorization (phonological and semantic fluency) [13]. The severity of cognitive impairments, which progresses over time, may be one of the determining factors in the deterioration of patients' quality of life [14, 15].

Cognitive impairments in patients with MS may occur in the early stages of the disease in addition to motor disorders or even precede them. This is due to the fact that at the beginning of the disease there is a loss of brain tissue in the structures responsible for cognitive functions, which reduces the social and psychological patients' quality of life, even after monitoring the severity and duration of the disease [16–18].

However, the peculiarities of formation of cognitive disorders, their early diagnosis need further study, especially given the fact that patients with MS in the presence of cognitive dysfunction are more disabled and socially maladapted than patients with MS without cognitive impairment.

In recent years, there has been a growing interest in identifying the possibilities of neuroimaging techniques to detect structural changes in the parts of the brain that are responsible for cognitive functions. Magnetic resonance imaging (MRI) is the most informative method of studying the structure of the substance of the brain and serves as the main neuroradiological marker for the diagnosis of MS. With the help of MRI it is possible to identify both old and new foci of demyelination, their total number and location in the brain and make a differential diagnosis of MS with other diseases. Gadolinium-based contrast agent is additionally used to determine the activity of the pathological process [19–21]. MRI is important for clinical monitoring of MS patients in facilitating the process of relapse and disease progression. MRI criteria for the damage of the brain is strategically important for cognitive functioning areas, but they still need to be further determined.

Recently, researchers have focused on identifying biological markers for the diagnosis of various diseases. Some biomarkers have been proposed for MS [22]. However, there are currently no confirmed biomarkers for diagnosing of the disease, monitoring it, predicting the rate of disease progression and assessing its consequences.

In foreign and domestic literature there is more and more data of the importance of brain-derived neurotrophic factor (BDNF) as a promising biomarker of MS.

Brain-derived neurotrophic factor (BDNF) is a protein from the class of cytokines, the family of growth factors and the subfamily of neurotrophins, which is found in glial and mainly in neuronal cells. BDNF is synthesized as a precursor protein with a molecular weight of 32–35 kDa (pro-BDNF), which is subsequently converted in the Golgi complex to biologically active mature BDNF (mBDNF) with a molecular weight of 13 kDa. Mature BDNF contacts the tyrosine kinase B receptor (TrkB), which induces phosphorylation cascades and leads to protein synthesis, axon growth, dendritic maturation, and increased synaptic plasticity. TrkB and BDNF induce intracellular cascades that control neuronal development and plasticity, cell cycle, and apoptosis [24].

From the pathogenetic point of view, formation of cognitive impairment in neurodegenerative diseases is closely related to such processes as neurogenesis and neuroplasticity [25].

It is known that BDNF affects the mechanisms of neuroplasticity, regulating the formation of new synapses and their plasticity, stimulating the survival, migration, proliferation and regeneration of neurons. Neuroplasticity is the basis of human development, learning and the formation of memory [26]. In addition, BDNF affects the processes of myelination and remyelination, regulating the rate and severity of apoptosis, controlling development and survival of cholinergic and GABAergic neurons in the brain, which play an important role in learning and memory. It was found that the content of BDNF in serum shows its concentration in brain tissue [27], which can be used in practice.

One of the main processes of neuroplasticity is neuronogenesis – the constant generation of nerve cells in the dentate gyrus of the hippocampus, olfactory bulb and striatum. About 700 nerve cells are formed daily in the human hippocampus (which is 1.75% of the total number of cells in the hippocampus). There are some factors that lead to the activation of neurogenesis, including physical activity, continuing education, diet, the formation of certain substances (GABA, glutamate, neurotrophic factors) [26].

Thus, the search of biomarkers for the diagnosis, prediction and evaluation of the effectiveness of MS therapy is a very promising area of research. In this regard, a promising marker may be a brain-derived neurotrophic factor (BDNF), which shows the condition of neuroplasticity [22]. Disorders of neuroplastic processes can be an im-

portant part of the pathogenesis of cognitive impairments.

There are few literature data of the possibility of using BDNF as a marker of MS. Sarchielli P. and co-authors examined 35 patients with relapsing-remitting and secondary-progressive MS. An increased BDNF's level in the supernatant of stimulated mononuclear cells of peripheral blood (MCPB) during relapse in relapsing-remitting type of MS compared with during the phase of clinical stability was found. Also, the authors obtained data about decreased concentration of BDNF in stimulated MCPB in the secondary-progressive type of MS, compared with the control group [28, 29].

Azoulay D. and co-authors found reduced serum and cerebrospinal fluid BDNFs' levels in patients with relapsing-remitting MS. The authors noted that BDNF may have a neuroprotective effect in MS, and immunomodulatory therapy may increase the effect of this mechanism [30].

Yoshimura S. and co-authors measured serum BDNFs' levels in 74 patients with MS and 86 patients with other neurological diseases. Evidence of a significant increase in BDNF's production in MS patients compared with control group and patients with other neurological diseases was found. Young people with MS had the highest level of BDNF. Based on the data obtained on the high concentration of BDNF in patients with a large number of relapses of MS, the authors suggested the correlation between BDNFs' levels and disease severity [31].

Comini-Frota E.R. and co-authors determined the serum BDNF's level of 28 patients with MS and 28 healthy people. A relationship was found between serum BDNFs' levels in patients with relapsing-remitting MS and the number of foci in the brain that accumulates gadolinium during T2 / FLAIR MRI. Based on these data, the authors suggest that the concentration of BDNF in serum can be considered a promising biomarker in patients with multiple sclerosis, which indicates severity of CNS damage [22, 23].

Thus, the clinical picture of MS has a very wide variety and in addition to motor, sensory and cerebellar disorders is often accompanied by cognitive impairment. Detection of cognitive dysfunction in the early stages should be an important part of assessing the patient's clinical status.

Further research of biomarkers for the diagnosis and prognosis of MS can be interesting. A promising marker may be a brain-derived neurotrophic factor (BDNF). However, the possibility of using BDNF requires further study, which will allow to develop algorithms for early diagnosis and prediction of disease progression, that will provide an opportunity to deepen the understanding of the place of BDNF in the pathomorphological chain of nervous system damage in MS.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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COMPARATIVE ANALYSIS OF CLINICAL AND LABORATORY SIGNS OF LUNGS AFFECTION IN PATIENTS WITH COVID-19 WITH THOSE OF PANDEMIC INFLUENZA A/H1N1

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Abstract
Influenza viruses, in particular A – A(H3N2) and A(H1N1)pdm09, as well as influenza B virus, mainly (98%) of the B/Victoria line, continue to circulate during the current epidemic season. The level of influenza vaccination remains low, about 0.6% of the population of Ukraine, and among occupational and epidemiological risk groups – 22.8%, according to the Public Health Centre of the Ministry of Health of Ukraine. In the COVID-19 pandemic, simultaneous circulation of influenza viruses and SARS-CoV-2 can lead to difficulties in differential diagnosis and treatment. Comparison of clinical and laboratory features of severe influenza complicated by pneumonia caused by pandemic influenza virus A(H1N1)pdm09 in the epidemic season of 2015/2016 Kharkiv RCIDH with COVID-19 on clinical and laboratory data was the aim of the work. **Patients and research methods.** The analysis of clinical symptoms and laboratory examination data of 19 patients with influenza complicated by community-acquired pneumonia of clinical group IV who were treated at the Kharkiv Regional Hospital and their comparison with those of patients with COVID-19 according to the literature. **Results and discussion.** Among the studied patients, men predominated – 12 persons (63.2%) aged 50.68±11.95 years. The predominant number had concomitant diseases. At the beginning of the disease, moderate weakness, headache, fever, minor catarrhal phenomena and, as a result, delayed hospitalization prevailed. From 3–4 days of the disease the condition significantly worsened, shortness of breath and cyanosis joined. Typical initial symptoms of COVID-19 are fever of varying degrees (73%), unproductive cough (59%) and shortness of breath or shortness of breath. **Conclusions.** In patients with COVID-19 and severe influenza, a more acute onset of the disease was reported, with moderate weakness, headache and fever up to 38°C and symptoms of pharyngitis. Influenza patients often show a delay in seeking medical attention and hospitalization for 6.21±1.46 days of illness. The severity of the disease in influenza is due to the accession of community-acquired pneumonia, in contrast to COVID-19, where the typical features are diffuse, mostly subpleural lung affection. Vaccination of people at risk before the start of the epidemic season is necessary to prevent severe complications of influenza caused by the pandemic virus A(H1N1)pdm09 in the context of the COVID-19 pandemic.
Key words: *influenza, pneumonia, COVID-19, diagnosis.*

INTRODUCTION

According to the WHO European office, influenza activity, based on patients in sentinel primary care settings testing positive for influenza virus infection, crossed the epidemic threshold of 10% set for the region in week 49/2021. For the region as a whole influenza activity had been increasing, with different levels of activity across the countries and areas of the region, with a dominance of A(H3) viruses [1] that each year infects approximately ten to thirty per cent of European population, and causes hundreds of thousands of hospitalizations across Europe. Both influenza type A and type B viruses were detected with a dominance of A(H3) viruses across all monitoring systems and in all cases. For week 49/2021, 185 (11%)

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of 1 650 sentinel specimens tested positive for an influenza virus; 182 (98%) were type A and 3 (2%) were type B. Of 67 subtyped A viruses, 3% were A(H1N1) pdm09 and 97% A(H3) [2]. Older people, younger children and those with chronic conditions suffer the most, but everyone is at risk of developing serious complications – which include pneumonia, myocarditis and encephalitis – that may result in death [3].

According to the Centre for Public Health of the Ministry of Health of Ukraine, and data of the results of laboratory research by PCR in 13 of 45 samples of materials in the last epidemic season identified influenza viruses: 6 – type A(H1) pdm09, 2 – type A(H3), 3 – type A not subtyped and 2 – type B [4]. It is noted that in the context of the COVID-19 pandemic, monitoring of the data may be inaccurate, both due to the difficulty of differential diagnosis and the reduction in the number of studies for seasonal respiratory viruses' detection.

During the last epidemic season of 2020 in Kharkiv region, 72,936 people fell ill with acute respiratory viral diseases (ARVD) and influenza in the region – 2.8% of the regional population. The largest proportion of patients was registered in the age group 5–14 years. The incidence rate in the region was 57.4% lower than the epidemic threshold and 8.2% lower compared to the incidence rate for the same period in 2019.

According to the Center for Public Health of the Ministry of Health of Ukraine, the level of influenza vaccination remains low, accounting for about 0.6% of the population of Ukraine, and among occupational and epidemiological risk groups – 22.8% of all those vaccinated [4].

However, circulation of influenza A(H1N1) pdm09 virus continues. This virus is known to differ from the already "classic" strains of influenza A1 in genetic and antigenic properties [5]. According to the world literature, the spectrum of clinical manifestations of this infection ranges from mild upper respiratory tract infection to the development of severe pneumonia [5, 6]. The onset of the disease mostly has no pathognomonic signs. In cases of lethal outcome, the deterioration of patients occurs on the 4th day of the disease due to the development with a subsequent lightning course of subtotal or total bilateral pneumonia with the phenomena of hemorrhagic pulmonary edema. Complicated forms of the disease are registered in patients of a group of risk, as a rule, in pregnant women, patients with chronic respiratory diseases, diabetes mellitus and cardiovascular diseases [7]. According to the world and domestic literature, delayed hospitalization and administration of neuraminidase inhibitors are a risk factor for complications and unfavorable course of influenza [8].

In modern conditions of pandemic, simultaneous circulation of influenza viruses and SARS-CoV-2 can lead to difficulties in differential diagnosis and treatment.

Thus, Ukraine takes the same situation as in other countries of the world – dominant has become more aggressive and pain contagious variant of the Delta, which requires more vigilant conducting anti-epidemic health authorities [9].

According to our own data, the clinical picture of pandemic influenza during the 2009–2010 epidemic was also characterized by the onset of disease with moderate intoxication and fever and minor upper respiratory tract affection, leading to delayed hospitalization and specific therapy, which is a risk factor for adverse events, especially in patients of risk groups [10].

Purpose, subjects and methods

1. The purpose of the study was to compere clinical and laboratory features of severe influenza complicated by viral pneumonia caused by pandemic influenza virus A(H1N1)pdm09 in the epidemic season of 2015/2016 CNE KRC Regional Clinical Infectious Diseases Hospital, Kharkiv with COVID-19 by clinical and laboratory data.

2. Subjects & Methods

The clinical symptoms and data of laboratory examination of 19 patients with influenza complicated by community-acquired pneumonia of the IV clinical group, who were treated in the CNE KRC Regional Clinical Infectious Diseases Hospital, Kharkiv during the epidemic season 10.2015–01.2016 were analyzed.

The average age of patients in the study group was 50.68±11.95. Among the patients of the experimental group, men predominated – 63.2%.

In the period from 2015 to 2016, patients underwent clinical studies according to a unified clinical protocol (blood, urine, chest radiography, clinical and bacteriological analysis of sputum, creatinine, urea and biochemical blood tests in dynamics). Statistical data processing was performed using the program MS Excel 2010. Analysis of clinical and laboratory data of COVID-19 was performed as described in the literature.

Results and discussion

During the epidemic season of 2015–2016, there was a significant increase in the incidence of influenza-like illnesses. In January 2016, 235 people

with influenza-like illnesses were hospitalized. Influenza caused by undifferentiated virus was diagnosed in 108 patients, influenza complicated by pneumonia – in 35 patients. Influenza caused by pandemic strain A(H1N1)pdm09 by PCR and immunofluorescence was confirmed in 22 patients. The symptoms and laboratory parameters of 19 people with a confirmed diagnosis of influenza who died in the CNE KRC Regional Clinical Infectious Diseases Hospital, Kharkiv were analyzed.

The mean age of patients of the group was 50.68±11.95 years. Among the studied patients, men predominated – 12 people (63.2%). Concomitant chronic diseases were detected in the vast majority of patients (94.7%). In particular, 13 people (68.4%) were obese, 9 people (47.4%) had coronary artery disease, 7 people (36.8%) – arterial hypertension. Chronic kidney disease was observed in 5 patients (26.3%) and other chronic diseases in 6 (31.6%). In 6 people (31.6%) three or more chronic diseases were observed simultaneously. Thus, all patients of the cohort belonged to the risk group according to the criteria of the unified clinical protocol of medical care for influenza patients according to the Order of the Ministry of Health of Ukraine on July 16, 2014 No.499 "On approval and implementation of medical – technological documents for standardization of medical care for influenza and acute respiratory infections". Data on prophylactic influenza vaccinations are not available in all patients.

Late admission to the hospital was very typical, and as a result, hospitalization was delayed – 6.21±1.46 days from the onset of the disease. This can be partly explained by the fact that the first symptoms of the disease were mainly: moderate weakness in 94.7% of cases, moderate headache – in 89.5%. The temperature at the beginning of the disease was mostly up to 38°C in 17 patients (89.5%). Airway lesions during the first day were characterized by moderate throat pain and discomfort in 7 patients (42.1%), moderate dry cough was reported in all cases. Hyperemia of the pharyngeal mucosa was observed in 13 patients (68.4%). Mucous secretions from the nasal passages were noted by only 5 patients (26.3%).

Thus, in most cases, the onset of the disease was relatively mild, thus the patients did not seek for medical help in time. All of them received only symptomatic treatment. According to the history, 7 patients (36.8%) at the beginning of the disease refused to be hospitalized. A threatening factor is that patients with widespread lung damage in the first 3–4 days subjectively felt well and often tried to refuse treatment. This dissonance, as well as the lack of characteristic stethoacoustic signs of pneumonia at the beginning of the disease is also noted by other authors [11]. But, from days 3-4, the disease was accompanied by fever above 39.1ºC in 11 cases (57.9%), attacks of painful cough, with blood in the sputum in 9 cases (47.4%) myalgia in 17 – (89.5%), nausea and vomiting – in 3 cases (15.8%). All patients noted the accession of shortness of breath during this period, cyanosis of the skin was observed in 9 (47.4%) patients.

At the initial examination, the condition was mostly moderate or severe, cyanosis of the skin and shortness of breath 33.7±9.01 per minute were observed. Tachycardia (106.2±28.3)/min, attenuation of heart tones was determined. Moist rales and crepitation were heard at auscultation in 18 patients (94.7%), percussion sound dulling in all cases. X-ray in all cases revealed bilateral pneumonia, lobar or sublobar in 18 patients (94.7%). The lower lobes of both lungs were mainly affected with further spread of infiltration despite antiviral and antibacterial therapy. SpO2 was 81.5±21.8% with a further decrease to 62.94± ±16.8%, despite the fact that all patients received oxygen therapy throughout their hospital admission. Liver enlargement was detected in 7 patients (36.8%). In the clinical analysis of blood, despite the severity of the condition and the duration of the disease, no significant inflammatory reaction was identified. The total number of leukocytes was 5.38±1.44·109/l, bent – 17.4±4.7%, segmented – 62.3±16.6%, lymphocytes – 17.5±4.7%, monocytes – 3.1±0.82%, ESR – 23.67±6.3 mm/hr. There was a moderate increase in urea 8.85 ± 2.36 mm/l and creatinine – 124.8±33.35 μM/l.

According to data of various researchers, common symptoms of influenza and COVID-19 are acute onset, dry cough and shortness of breath, which is associated with severe disease. Relatively mild onset of the disease with moderate symptoms of upper respiratory tract lesions is common. However, with COVID-19, development of lung damage and acute respiratory distress syndrome is observed on 7–9 day of the disease, which is significantly longer than same with influenza [12]. Typical initial symptoms of COVID-19 are fever of varying degrees (73%), unproductive cough (59%) and shortness of breath or feeling of lack of air. Taste disturbances and anosmia may appear on day 2–14 of the disease in 50–61.2% of severe cases [13]. However, these symptoms can also occur in 17.3% of people over

40 who have various chronic diseases. Computed tomography of the lungs plays an important role in the early diagnosis of severe forms of COVID-19. The most typical sign is opacity of the type of ground glass (65%) with affection from one to several segments of the lungs depending on the severity. In a significant number of severe forms of the disease the lesions were bilateral [12]. In contrast, in case of influenza caused by pandemic strain A (H1N1) pdm09, typical radiographic features are multifocal foci of consolidation. In the progress of the disease, the appearance of pleural effusion and cavity formation was possible. Involvement of four or more zones up to day 7 of the disease was considered one of the signs of adverse course [12, 14, 15].

Conclusions

1. COVID-19 and severe influenza are characterized by an acute onset of the disease, accompanied by moderate weakness, headache and fever up to 38°C. Upper respiratory tract affection was characterized mainly by symptoms of pharyngitis. Classic symptoms of tracheal lesions in influenza have not been reported. As a result, patients with influenza postpone admission to the hospital by 6.21±1.46 days from the onset of the disease.

2. All patients with adverse disease outcomes belonged to the risk group by age and the presence of comorbidities, mainly obesity, coronary heart disease, hypertension, and others. The further severity of influenza was due to community- acquired pneumonia, lobular or sublobular, mostly bilateral with characteristic stethoacoustic signs and symptoms of respiratory failure, in contrast to COVID-19, where the typical features are diffuse, mostly subpleural lung affection.

3. Vaccination of persons of risk groups before the beginning of the epidemic season is necessary to prevent severe complications of influenza caused by the pandemic virus A/H1N1pdm in the conditions of the COVID-19 pandemic, and to increase medical education of the population about behavior in case of symptoms of influenza appearance. Neuraminidase inhibitors should also be prescribed to persons at risk within 48 hours of the onset of the disease.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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CURRENT ISSUES OF RESISTANT TUBERCULOSIS AND SMOKING (review)

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Abstract

Tuberculosis and smoking are among the most pressing issues in the modern health care system in the world. Tuberculosis patients who smoke are confirmed to demonstrate reduced effectiveness of anti-tuberculosis therapy, unsatisfactory prognosis of a specific disease, higher risk of treatment failure and mortality from this disease. Diseases such as HIV and alcoholism increase ineffective treatment in tobacco smokers. In addition, smoking leads to increased virulence of the causative agent of tuberculosis – Mycobacterium tuberculosis.

Key words: *tuberculosis, smoking, Mycobacterium tuberculosis, multidrug-resistant tuberculosis, extensively drug-resistant tuberculosis.*

Tuberculosis (TB) is a communicable disease that is a major cause of ill health and one of the leading causes of death worldwide. Until the coronavirus (COVID-19) pandemic, TB was the leading cause of death from a single infectious agent, ranking above HIV/AIDS. TB is caused by the bacillus *Mycobacterium tuberculosis* (MTB), which is spread when people who are sick with TB expel bacteria into the air (e.g. by coughing). The disease typically affects the lungs (pulmonary TB) but can affect other sites. Most people (about 90%) who develop the disease are adults, with more cases among men than women. About a quarter of the world's population is infected with MTB [1].

Since 1995, the WHO has recorded a TB epidemic in Ukraine and today this problem is relevant in our country [2].

The resistance of MTB to anti-tuberculosis drugs is one of the most serious problems of modern tuberculosis. The structure of resistance of MTB to drugs is of great importance for making specific decisions about the treatment of patients with tuberculosis. According to international standards, the following categories of resistance of MTB to anti-tuberculosis drugs are distinguished:

- Mono-resistance: resistance to one first-line anti-TB drug only [3].
- Poly-resistance: resistance to more than one first-line anti-TB drug, other than both isoniazid and rifampicin [3].
- Multidrug-resistant TB (MDR-TB) is: resistance of MTB strains to at least isoniazid and rifampicin, the cornerstone medicines for the treatment of TB [4].
- Rifampicin-resistant TB (RR-TB) disease on its own requires similar clinical management as MDR-TB [5].
- Pre-extensively drug-resistant tuberculosis (XDR-TB) is: TB caused by MTB strains that fulfill the definition of multidrug resistant and rifampicin-resistant TB (MDR/RR-TB) and which are also resistant to any fluoroquinolone [5].

XDR-TB is TB caused by MTB strains that fulfill the definition of MDR/RR-TB and which are also resistant to any fluoroquinolone and at least one additional Group A drug (Group A drugs are the most potent group of drugs in the ranking of second-line medicines for the treatment of drug-resistant forms of TB using longer treatment regimens and comprise levofloxacin, moxifloxacin, bedaquiline and linezolid) [5].

Ukraine is among the top 10 countries with the highest burden of TB and M/XDR-TB in the world. In addition, Ukraine belongs to the group of 18 high-priority countries in the European region [6]. Every year, the rates of resistant TB in Ukraine are growing steadily.

The emergence of drug-resistant strains of MTB, the isolation of which is a characteristic feature of resistant tuberculosis, has become an obstacle to the elimination of the disease. M/XDR-TB

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is an extremely important part of the current TB epidemic. The spread of M/XDR-TB is facilitated by the high reproductive capacity of resistant strains of MTB [2].

Today, the problem of tuberculosis has gone beyond the purely medical field and has acquired the status of a nationwide problem, given that TB is a socially dangerous infectious disease, and the consequences pose a threat to the economy and national security of our country [6].

Today in Ukraine, as well as around the world, there is a tendency to the increase in the number of patients with M/XDR-TB, which is one of the most unfavorable forms of the disease due to epidemiological danger [7]. Of particular concern is the increase in the number of patients with new cases of M/XDR-TB against a background of a decrease in the number of patients with preserved susceptibility to MTB in general. In 2014, Ukraine was among the top five countries in the world with the highest number of M\XDR-TB cases. According to the WHO estimates, in 2016 the incidence of TB in Ukraine is at the level of 87 per 100 thousand population, with Ukraine ranked 4th in the world in the number of cases of M/XDR-TB and last in terms of effectiveness of treatment of this category of patients in world [6]. According to the national and international experts, the main causes of the epidemic situation in Ukraine, as well as in Europe are low detection and inadequate treatment of TB and M/XDR-TB [6]. This problem causes global economic losses for Ukraine. Thus, over the past few years, the losses from TB in Ukraine amount to about UAH 3.5 billion. per year [2].

Treatment of patients with M/XDR-TB is characterized by low efficiency (below 50%), long duration and requires long-term use of toxic drugs. The results of treatment of patients depend on a number of social and economic and medical factors, the severity of the tuberculosis process, the variant of resistance of MTB, concomitant pathology, antimycobacterial therapy and tolerability, the patient's commitment to treatment, and the presence of bad habits such as smoking [8, 9].

Thus, M/XDR-TB remains an extremely important issue of tuberculosis, timely diagnosis and effective treatment of which by reducing the overall duration of treatment of patients and improving the regime require further research and implementation of their results in practice.

Another problem in the world is smoking. Smoking is an acquired habit of inhaling the smoke of smoldering dried tobacco leaves. Tobacco use in any form kills and sickens millions of people every year [10]. Over 8 million people died from a tobacco-related disease in 2019 [11]. The number of annual deaths can be expected to keep growing even once rates of tobacco use are in decline, because tobacco kills its users and people exposed to its emissions slowly [12]. There is a lot of convincing scientific evidence in recent years about the harmful effects of smoking on human health. In this regard, the constant increase in the number of proponents of smoking in many countries, and especially in Ukraine, cannot be ignored [13].

According to the WHO, in 2020, about a third of the adult population of the Earth smoked, which is the numerical equivalent of 1.37 billion people aged 15 and older. In 2019, 8 million people died as a result of smoking, which is about 22 thousand deaths daily [14].

Smoking is the most common bad habit in the world. Tobacco addiction is included in the International Classification of Diseases [14].

According to statistics, 40.4% of adult men and 9% of adult women smoke daily in Ukraine; among young people – 45% of boys and 35% of girls. In total, there are about 9 million active smokers in the country, which is a third of the total working population. At least 100,000 Ukrainians join the ranks of smokers every year. Ukraine's economic losses from tobacco are about $ 2 billion annually. According to official statistics, 120,000 people die each year from smoking-related diseases in Ukraine [14–16].

There is also a direct link between smoking and an increased risk of developing tuberculosis in people who smoke [17].

Tobacco smoke plays a special role in the pathogenesis of tuberculosis. For example, there were significantly more alveolar macrophages from smokers compared with nonsmokers or ex-smokers. Alveolar macrophages from smokers could not control intracellular MTB growth. Nonsmokers' alveolar macrophages generated significantly more tumor necrosis factor (TNF)-α, IFN-γ, and IL-1β after MTB infection compared with uninfected alveolar macrophages. However, MTB-infected alveolar macrophages from smokers did not secrete significantly more TNF-α, IFN-γ, and IL-1β compared with uninfected smokers' alveolar macrophages. Alveolar macrophages taken from ex-smokers also failed to secrete significantly increased TNF-α, IFN-γ, and IL-1β after MTB infection. Both smokers' and nonsmokers' alveolar macrophages induced T-regulatory cell phenotype responses in allogeneic admixed

T-cells. Even after MTB infection, alveolar macrophages continued to drive this regulatory phenotype [18].

Thereby, in smokers, the pulmonary compartment has a number of macrophage-specific immune impairments that provide some mechanistic explanations whereby cigarette smoking renders a patient susceptible to TB infection and disease [18].

The role that cigarette smoke plays in the pathogenesis of TB is related to ciliary dysfunction, to a reduced immune response, and to defects in the immune response of macrophages, with or without a decrease in the CD4 count, increasing susceptibility to infection with MTB [19]. The alveolar macrophage binds to the bacillus through complement receptors 1, 3 and 4. Activated lymphocytes release cytokines while recruiting macrophages, fibroblasts, and other lymphocytes. The major cytokine involved in granuloma formation is TNF-α, which is released by macrophages immediately after exposure to M. tuberculosis antigens. The TNF-α activates macrophages and dendritic cells. In smokers, nicotine, acting through the α7 nicotinic receptor, reduces the production of TNF-α by macrophages, thereby preventing its protective action and favoring the development of tuberculosis [20,21]. In addition, the secretion of interleukin-12 (IL-12) by macrophages induces the production of gamma-interferon (IFN-γ) in natural killer cells. This aspect of the immune response aims to destroy MTB by forming a fibrous granuloma. Cigarette smoke selectively promotes low production of IL-12 and TNF-α, preventing the formation of granulomas, which would contain infection at this stage in immunocompetent individuals, creating conditions for the development of active tuberculosis [22].

Several epidemiological studies have identified the cigarette smoke as a risk factor for the infection and development of tuberculosis. Nicotine is considered the main immunomodulatory molecule of the cigarette. A study from Mexico found that nicotine decreases the production of HBD-2, HBD-3, and LL-37 in T2P during the infection with MTB promoting its intracellular growth [23].

Some studies have shown that men with a history of TB are four times more likely to develop airway obstruction than men without it [24].

Passive and active exposure to cigarette smoke is associated with an increased risk of TB. This is shown by some previous studies, which show a strong correlation between smoking and TB. In addition, secondhand smoke is moderately correlated with TB and the need for re-treatment [25]. At the same time, the risk of MTB is higher in children living in a region with a high incidence of TB, parents who smoke [26]. A study of children who have had family contacts with TB patients has shown that secondhand smoke, as evidenced by the measurement of nicotine levels in the urine, is a major risk factor for active tuberculosis [27].

Clinical studies have shown that smoking in TB patients has a significant effect on one of the main indicators of recovery in a specific process, namely the conversion of MTB. Thus, TB patients who smoke tobacco are at high risk for late conversion of MTB culture [28, 29]. In addition, some studies have shown that smoking is a risk factor for drug-resistant forms of TB [30].

Failure to treat is an important element in the treatment process for TB patients. Some studies have found that smoking, HIV-positive status, and positive sputum smear microscopy were important factors in TB treatment failure [31, 32]. In addition, according to some authors, the increased risk of treatment failure in TB and smoking is alcohol abuse [33]. Some studies have also shown an association between smoking and tuberculosis recurrence [34, 35].

Another crucial point in the control of tuberculosis is the abandonment of treatment. Smoking has been associated with the abandonment of tuberculosis treatment, and that association has been found to be independent of alcohol or illicit drug use. Therefore, abandonment of tuberculosis treatment might be related to the psychosocial aspects of smoking, the predominance of males, and the lower socioeconomic status of the affected populations, all of which are factors associated with lower rates of adherence to treatment [36].

A prospective study conducted in China shows that smoking is an independent risk factor for TB infection, especially in the elderly, and has shown a direct correlation between smoking history (pack-years) and the risk of latent TB infection [37]. Some studies have shown that when latent TB is diagnosed with IFN-γ in blood, the proportion of false-negative results is higher among smokers than among non-smokers [38]. In addition, this study shows that smoking has a negative effect on the results of tuberculosis treatment [38, 39].

Another study not only shows that smoking reduces the chances of recovery from tuberculosis, but increases the chances of quitting smoking than inactive smokers [40]. Another study found that patients with TB who stop smoking may have better outcomes than those who do not. Health

professionals should support patients in stopping smoking [41].

Some studies note that the direct effects of cigarette smoke on infected cell culture treated with anti-TB drugs interfere with TB treatment and weaken the host's immunity [42]. This can be very important for patients who smoke and do not smoke, but are in the same ward where second-hand smoke can contribute to slower recovery or even failure of TB treatment.

According to the WHO, the mortality rate associated with tuberculosis is significantly higher in smokers than in those who do not have this bad habit. Among people with tuberculosis, smokers are nine times more likely to die from tuberculosis than non-smokers [43–45]. When smokers quit smoking, the risk of death due to tuberculosis drops significantly (by 65% compared with that observed for those who continue smoking), which indicates that smoking cessation is an important factor in reducing TB-related mortality [46].

TB, COVID-19 and smoking are high-prevalence entities with public health consequences. All of these diseases have a great impact on the immune system. There is also upcoming evidence which suggests that smoking and TB increase the risk of severe COVID-19 symptoms [47].

Smoking is considered to be one of the main causes of COPD. At the same time, TB-pulmonary makes a significant contribution to the causal relationship of COPD. However, the underlying pathogenesis of TB-associated COPD is unclear. The study authors showed that patients with TB-related COPD have enhanced inflammatory responses that may be linked to fatty acid pathways and tryptophan catabolism [48].

Some authors have analyzed the impact of cigarette smoke components on MTB, the causative agent of TB. The authors report the impact of cigarette smoke condensate (CSC) on survival, mutation frequency, and gene expression of MTB in vitro. Authors show that exposure of virulent MTB to cigarette smoke increases the mutation frequency of the pathogen and strongly induces the expression of the regulon controlled by SigH – a global transcriptional regulator of oxidative stress [49]. Also, these authors have previously shown that SigH to be required for to respond to oxidative stress, survival, and granuloma formation in vivo [50]. A high-SigH expression phenotype is known to be associated with greater virulence of MTB. In patients with pulmonary TB who smoke, these changes may therefore play an important, yet unexplored, role in the treatment efficacy by potentially enhancing the virulence of tubercle bacilli [49]. Some studies have shown that CSC does not affect the growth of colonies of the standard MTB H37Rv strains in vitro [51]. But at the same time, some studies show that CSC increases the multiplication of MTB in epithelial cells [52].

Thus, reducing the number of people who smoke tobacco can increase the effectiveness of treatment, better prognosis and reduce mortality from TB. In addition, prevention of smoking will not increase the virulence of MTB.

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Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

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SEX-RELATED DIFFERENCES IN THE LEVELS OF URINE 6-SULFATOXYMELATONIN IN VERY LOW BIRTH WEIGHT INFANTS

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ABSTRACT

Background. The sex-related differences of the urinary 6-sulfatoxymelatonin have not been studied in premature infants yet. The purpose of the work was to measure the daily urinary 6-sulfatoxymelatonin in premature infants with a very low birth weight.

Materials and Methods. Fifty premature infants (28 males and 22 females) with gestational age less than 33 weeks and body weight from 999 g to 1499 g were involved in the study. Urine 6-sulfatoxymelatonin was assessed using urine collection on the 1st day and on the 10th–14th days of life.

Results. The level of urine 6-sulfatoxymelatonin on the 1st day of life showed a significant increase in its excretion in females compared to males. The median values in males were 202.0 (95% CI 77.1–390.9) pg/ml and in females 437.0 (279.6–501.0) pg/ml, p=0.0103. Its level on the 10th–14th days of life significantly decreased both in males 57.0 (95 % CI 45,0–99.7) pg/ml, p=0.0028 and in females 90.0 (51.9–160.7) pg/ml, p=0.0021 without differences in sex-related distribution, p=0.3940.

Conclusions. The melatonin metabolite as urinary 6-sulfatoxymelatonin in premature infants with a very low birth weight demonstrates sex-related differences with significant increase in females compared to males on the 1st day of life and no sex-related difference on the 10th–14th days of life. The trend of reduced pineal function is a key point in understanding the neuroendocrine reactivity in male preterms. Future investigation of sex-related aspects of urinary 6-sulfatoxymelatonin excretion in children, especially premature infants, is required.

Key words: *urine, 6-sulfatoxymelatonin, premature infants, very low birth weight.*

INTRODUCTION

It is known that one of the main functions of melatonin in the organism is regulation of "sleep–wake". Melatonin is synthesized both by the pineal gland and other tissues. Its metabolism leads to formation of 6-sulfatoxymelatonin (aMT6s), which can be excreted in various fluids, mainly urine [1].

Due to the differences in the endocrine system in females and males, the role of gender differences in the functional activity of melatonin has been studied. The published findings are rather contradictory even in the adult population. One published study has found that females exhibited significantly greater levels of plasma melatonin than males and there are differences between males and females in its circadian rhythm [2]. Meanwhile, the effect of different light intensities on blood melatonin concentrations in females and males has been, and there were no gender differences in light sensitivity [3].

The recent studies of melatonin effects have shown not only its biological "sleep-wake" function. In the last decades, its role as a powerful antioxidant protection has been demonstrated in both adults and children [4–6]. Despite the fact that positive results have been obtained in the use of melatonin in newborns, sex-related differences in them, and especially in premature babies, have not been sufficiently studied [7].

Purpose, subjects and methods:

1. The purpose of the study was to measure the daily urinary aMT6s in premature infants with very low birth weight (VLBW) as well as evaluate its sex-related differences.

2. Subjects & Methods. *Design.* This simple, cohort, one-center, descriptive, retrospective study involved 50 premature infants with VLBW.

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Inclusion criteria: premature infants with gestational age less than 33 weeks, body weight at birth from 999 g to 1499 g.

Exclusion criteria: gestational age more than 33 weeks and body weight ≥ 1500 g, degenerative and congenital diseases of the nervous system, chromosomal diseases; diseases with impaired renal function; orphan diseases.

Interventions. The data of a detailed case history and objective examinations, medical records, anthropometric measurements, and daily urine collection were studied. aMT6s in the 24-hour urine collection from premature infants was assessed by enzyme-linked immunosorbent assay using the BÜHLMANN 6-Sulfatoxymelatonin ELISA (BUHLMANN Diagnostics Corp., USA), offered by the manufacturer. The urine was collected on the 1st day and the 10th–14th days of life.

Statistics. Statistical analysis was performed with MedCalc version 14.8 (©1993–2014) MedCalc Software bvba (Acacialaan 22 B-8400 Ostend, Belgium). Descriptive analysis such as median (Me), maximum (max), minimum (min), lower quartile (Lq), upper quartile (Uq), 95% Confidence Interval (CI) were used. Fisher's test to calculate the difference in two proportions, Mann-Whitney test (MW test) to compare of two independent samples, and Wilcoxon Rank-Sum Test (WRST) to compare of two dependent samples were used. The difference in parameters was considered statistically significant at $p<0.05$.

Results and discussion

The maternal pregnancy and delivery of infants with VLBW included the following conditions: premature rupture of membranes –19 (38.0%), abortion risk – 13 (26.0%), urogenital infections – 10 (20.0%), multiple pregnancy – 12 (24.0%), cesarean section – 29 (58.0%), fetal distress – 13 (26.0%). The demographic and clinical data of infants with VLBW are presented in *table 1*.

There was no significant difference between males and females among VLBW infants (p= =0.2330). Among them there was preference of moderate preterm: 32 weeks to <34 (p=0.0002) according to the WHO classification [8]. Table 1 presents the ranking of perinatal pathology in infants depending on the frequency of its prevalence. The presence of one or a combination of several pathological perinatal conditions was characterized by changes in the acid-base state of umbilical blood in infants with VLBW (*table 2*).

Table 1. Clinical and demographic data of the premature infants

Data	n (%)	95% CI
Males	28 (56.0)	41.2–70.0
Females	22 (44.0)	29.9–58.7
Gestation age		
≤29 weeks	8 (16.0)	7.0–29.0
30 weeks	12 (24.0)	13.0–38.1
31 weeks	7 (14.0)	5.8–26.7
32 weeks	8 (16.0)	7.0–29.0
33 weeks	15 (30.0)	17.8–44.6
Respiratory distress syndrome	50 (100)	92.8–100.0
Anemia of premature	29 (58.0)	43.2–71.8
Hypoxic-ischemic encephalopathy	26 (52.0)	37.4–66.3
Congenital pneumonia	19 (38.0)	24.6–52.8
Intraventricular hemorrhage	17 (34.0)	21.2–48.7
Sensorineural hearing loss	13 (26.0)	14.6–40.3
Neonatal sepsis	11 (22.0)	11.5–35.9
Severe asphyxia	9 (18.0)	8.5–31.4
Retinopathy of premature	7 (14.0)	5.8–26.7
Bronchopulmonary dysplasia	3 (6.0)	1.2–16.5
Necrotizing enterocolitis	3 (6.0)	1.2–16.5
Lethal outcome	2 (4.0)	0.4–13.7

Table 2. Acid-Base state in the umbilical blood in premature infants with VLBW

Parameter	pH, units	BEb, mEq/l
Median	7.1	–16
Lq; Uq	6.8; 7.3	–19; –11
min; max	7.0; 7.2	–22; –2

The combination of severe perinatal pathology led to the lethal outcome of 2 infants: hypoxic injury due to severe asphyxia and respiratory distress syndrome, second had moderate asphyxia and early neonatal sepsis.

The level of urine aMT6s on the 1st day of life showed a significant increase in its excretion in females compared to males (*Fig. 1*). The median values in males were 202.0 pg/ml (95% CI 77.1–390.9)

and in females 437.0 (279.6–501.0) pg/ml, MW test p=0.0103.

The level of urine aMT6s excretion on days 10^{th}–14^{th} of life significantly decreased both in males 57.0 (95% CI 45.0–99.7) pg/ml, WSRT p=0.0028 and in females 90.0 (51.9–160.7) pg/ml, WSRT p=0.0021. However, we did not find differences in sex-related distribution of urine aMT6s at the 10^{th}–14^{th} days of life, MW test p=0.3940 (*Fig. 2*).

Why did we study this particular question? In animals sex-related histological differences in the thymus after ectopic pineal gland was established [9].

One study in the Netherlands on a population of 94 healthy children aged 2 to 18 years evaluated actigraphic data on 24-hour sleep-wake rhythm as a function of urinary aMT6s levels, sex differences, and body mass index. The methodology of the study included the children wearing of an actigraph device, collection of morning urine for 7

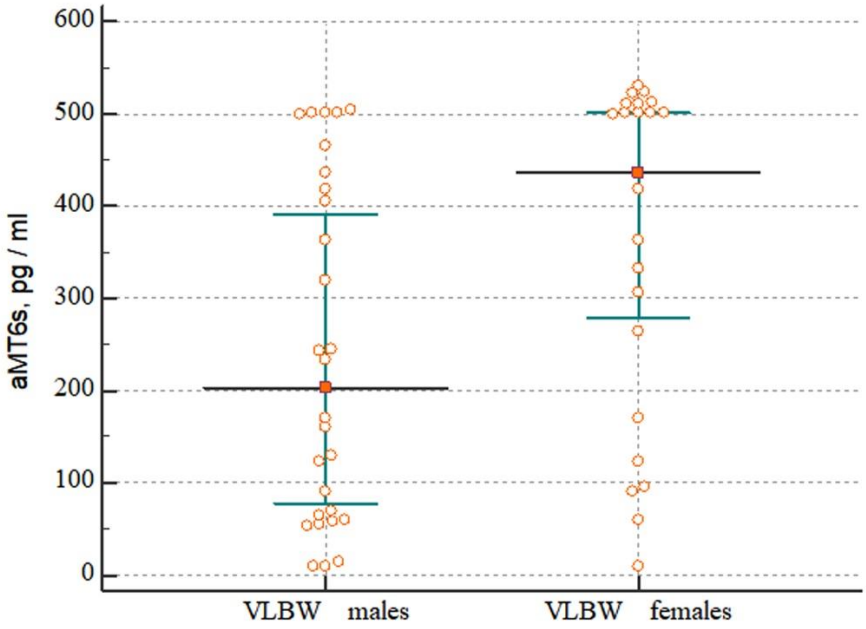

Fig. 1. The sex-related distribution of urine aMT6s in premature infants with VLBW on the 1^{st} day of life.

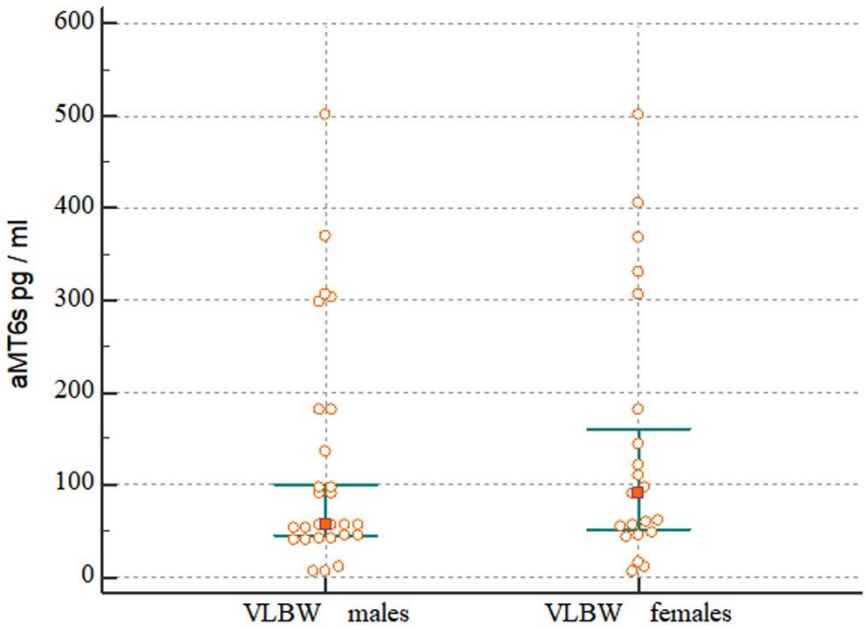

Fig. 2. The sex-related distribution of urine aMT6s in premature infants with VLBW on the 10^{th}–14^{th} days of life.

consecutive days, and a questionnaire. It was found that the levels of urine aMT6s decreased significantly as the child grew older. The authors also concluded that neither gender nor an increase in body mass index was associated with a difference in excretion of urine aMT6s [10].

Another study of the relationship between the excretion of the urine aMT6s, and prenatal, and intranatal, and postnatal changes and psychomotor development was carried out on a fairly large population of term infants (n=355). Poor child neurodevelopment has been associated with low levels of the urine aMT6s at 16 weeks, 3 and 6 months of age. The authors conclude that this association suggests a causal or prognostic relationship between melatonin and neurodevelopment in infants [11]. However, this study did not include premature infants, especially with LBW.

One study showed results about maturation of fetal melatonin synthesis by measuring the urinary excretion of aMT6s in males aged 2–7 days and gestational age 26–42 weeks. A negative correlation was established between an increase in gestational age and the urine aMT6s excretion, which was also shown in our study. We demonstrated the same data but, on the 10^{th}–14^{th} days of life, and reduction of the urine aMT6s excretion. However, in contrast to the study above, we have demonstrated this phenomenon in both males and females [12].

Our study allowed to partially reject the hypothesis, that premature infants with VLBW have no sex-related differences in the profiles of urinary aMT6s in neonatal period. We showed a significant increase in urinary excretion of 6 in females compared to males on the1^{st} day of life, and the absence of sex-related differences of urinary aMT6s between males and females on days 10–14 of life.

Some publications suggest that melatonin plays a major antioxidant role in postnatal adaptation. It is possible that labor stress leads to the release of this hormone for antioxidant protection [4–6]. We consider another important issue for discussion, i.e. why we need to measure the level of the melatonin metabolite.

Low levels of metabolite in the urine in premature children provide for a subsidy of this hormone. But for today the melatonin supplementation for the prevention of hypoxic ischemic injury in newborns has been demonstrated at a high evidence level [13].

The effectiveness of melatonin supplementation as a neuroprotector in the first two weeks of extrauterine life in premature infants is just beginning to be studied. Our study showed that premature babies cannot produce enough melatonin, which is another contribution to research on the effectiveness of melatonin supplementation. Its antioxidant mechanism may be useful in preventing neurodevelopmental disorders. That is why the first study on the role of melatonin in preterm infants with very low birth weight was planned in 2020. It may serve as a basis for further research on melatonin as a neuroprotective strategy in this vulnerable population. [14]. Some publications showed the fact that premature infants have long-term neurodevelopmental disorders, cognitive and motor problems, and a low level of socialization and communication, it is extremely important to evaluate the role of melatonin as a neuroprotector during the first weeks of extrauterine life, to prevent disorders in the development of the nervous system [15–18]. We studied the relationship between the levels of the urine melatonin metabolite in the short-term formation of perinatal pathology in premature infants in the early and late neonatal period. We believe that the results of our study open up more prospects regarding the study of the effect of melatonin on the long-term aspects of the development of a child who was born prematurely. The limitation of our study was due to a small sample and time shortage.

Conclusions

The melatonin metabolite, urinary 6-sulfatoxymelatonin, in premature infants with a very low birth weight showed sex-related differences with significant increase in females compared to males on the 1^{st} day of life and no sex-related difference on the 10^{th}–14^{th} days of life. The trend of reduced pineal function is a key point to understanding the neuroendocrine reactivity in male preterms. Further studies of sex-related aspect of urinary 6-sulfatoxymelatonin excretion in children, especially premature infants are required.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

The authors have no potential conflicts of interest to disclosure.

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Data Transparency

The data can be requested from the authors.

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CHARACTERISTICS OF PSYCHO-EMOTIONAL STATE OF PARENTS OF CHILDREN WITH PARALYTIC SYNDROMES AND ITS CHANGES DURING REHABILITATION MEASURES

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ABSTRACT

The current strategy for the treatment of chronic pain in children with paralytic syndromes and neurological lesions was defined by the WHO recommendations in 2012. These recommendations provide a multimodal approach of analgesia for the chronic pain treatment, including the widespread use of non-pharmacological methods and pharmacological therapy.

Aim. Assessing the subjective vision of parents of children with paralytic syndromes regarding the impact of physical rehabilitation on chronic pain and their psycho-emotional state.

Materials and methods. The study involved data from 64 children and their mothers (64 persons). The age of the children ranged from 1 to 6 years, the median age was 3 years and 3 months.

Results. The changed psycho-emotional state of parents of children with paralytic syndromes is determined. If the child has chronic pain, the parents' "Concern about the treatment of the child" is doubled (RR=2.1; p=0.0024), "Hyperopia of the child" – twice (RR=1.9; p=0.0094), "Intolerance to observe the suffering of the child" - one and a half times (RR=1.6; p=0.0017), and "Sadness and anger when a child cries" (RR=1.5; p=0.0122), "Internal tension" (RR=1.5; p=0.0029), "Insomnia" (RR=1.5; p=0.0215), "Inability of the child to lead a normal image life" (RR=1.5; p=0.0035), "Search for compassion or understanding" (RR=1.5; p=0.0446), "Inner anxiety, feeling of possible trouble" (RR=1.5; p=0.0074) increase one and a half times. After receiving rehabilitation measures, the psycho-emotional state of the parents of children with paralytic syndromes was determined to decrease: "It is intolerable to observe the child's condition" decreased by 31.3% (p=0.0012); "Feelings of inner anxiety" decreased by 24.5% (p=0.0125); "I fall asleep badly due to the child's condition" decreased by 20.6% (p=0.0109); "Internal tension" decreased by 19.6% (p=0.0269); "Sleepless nights due to the condition of the child" decreased by 18.6% (p=0.0401).

Conclusion. The authors consider that physical rehabilitation in children with paralytic syndromes reduces the incidence of moderate chronic pain and improves the emotional state of parents. So, we think that new approaches to rehabilitation should be applied with daily management of the children with paralytic syndromes' needs and their families with increased psychological and social support. Perhaps the searching for new approaches that optimize more intensive and effective rehabilitation strategies using the family reserve will provide the potential for adaptability of nerve plasticity and recovery in such a contingent of children and prospects for the future.

Keywords: *children with paralytic syndromes, chronic pain, psycho-emotional state, physical rehabilitation.*

INTRODUCTION

Chronic pain in children with paralytic syndromes affects various aspects of the child's life and family, such as sleep, emotional state, relationships, development and functional status. It is usually caused by muscle spasticity, contractures, vertebral deformities, bedsores or maceration of the skin, hypersalivation and/or hyperproduction of bronchial secretions, tube feeding, tracheostomy, gastrostomy, colostomy, convulsions, central and peripheral nervous system damage [1].

The current strategy for the treatment of chronic pain in children with paralytic syndromes uses a multimodal model of analgesia, namely, the widespread use of non-pharmacological methods along with pharmacological therapy [2]. This

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model includes: psychotherapy, game therapy, physical and occupational therapy, music therapy, aromatherapy. It is believed that each component of multimodal analgesia relieves pain [2, 3].

The role of physical therapy in relieving chronic pain in children with paralytic syndromes is to overcome spasticity and reduce muscle tone [4]. Individual physical treatments for children with paralytic syndromes are selected depending on age, leading pathological syndrome in the motor area, the degree of motor impairment (Gross Motor Function Classification, GMFCS), the presence of complications of the underlying pathological condition (e.g., seizures or secondary skeletal deformities), the presence of comorbidities [4, 5] Concomitant diseases and their treatment should also be taken into account when choosing rehabilitation tactics [6].

Purpose, subjects and methods:

1. The purpose of the study: assessing the subjective vision of parents of children with paralytic syndromes regarding the impact of physical rehabilitation on chronic pain and their psycho-emotional state.

2. Subjects & Methods

Study design and setting. This study is a non-interventional, descriptive, cross-sectional, single-center. We provided the individual rehab plan to each child, depending on age, motor dysfunction and comorbidities. Pain assessment and questionnaires of children's parents with paralytic syndromes were performed at the beginning of rehabilitation and after 6 months (end-point).

Ethical approval. This study was approved by the Local Ethics Committee (Protocol No.9 on 16 October 2018), which was conducted with the involvement of underage patients and did not include measures that could harm their health and safety. Both parents of the patients were informed about the methods and scope of the study and agreed to their children participation in this research.

Sampling. Data from 64 children and their mothers (64 persons) were included in the study. The age of the children ranged from 1 to 6 years, the median age was 3 years and 3 months. Group Ia included 38 surveyed children with paralytic syndromes and chronic pain: 24 (63.2%) boys and 14 (36.8%) girls, p=0.1212. To group Ib – 26 children with paralytic syndromes without chronic pain, 15 (57.7%) boys and 11 (42.3%) girls, p= =0.4200. Inclusion criteria: children 1–6 years old with paralytic syndromes according to ICD-10 (cerebral palsy G80, hemiplegia G81, paraplegia and tetraplegia G82, other paralytic syndromes G83) on the background of CNS damage caused by hypoxia, bleeding, thrombosis, trauma; congenital brain defects. Exclusion criteria: malignant neoplasms; HIV/AIDS; degenerative diseases of the nervous system, demyelinating diseases; chromosomal diseases; orphan diseases; and those who did not consent to participate in the study.

Data collection. The research included a detailed evaluation of medical records, including an "Individual Rehabilitation Plan". The Gross Motor Function Classification System (GMFCS) was used to provide a standardized assessment of limitations in gross motor function in patients with paralytic syndromes. The parents' interview was conducted using the "Original Questionnaire for Mothers/Legal Representatives (Guardians)" (Copyright No.88107, May 2019). It includes five domains: Domain 1 – "Condition of child" (mobility, walking; pain or discomfort; child's ability to calm down; scream baby while feeling pain or discomfort); Domain 2 – "Score of Rehabilitation" (evaluation of the child condition before and after rehabilitation on a scale from 0 to 10, and number of rehabilitation courses, and follow rehabilitation recommendations at home); Domain 3 – "Parenteral emotional state" (anxiety, agitation, sleep and support – 13 items). Each item provided at least three choices: "Most of the time", "Sometimes" or "Almost never". This publication contains the results of the analysis of Domain 3 depending on Domain 1 (item Pain or discomfort).

Analysis. Statistical analysis was performed with the programs Statistica 7.0 StatSoft Inc. 1984–2004, (Serial No. 12255555555, USA) and MedCalc version 14.8 (©1993–2014) MedCalc Software bvba (Acacialaan 22 B-8400 Ostend, Belgium). Descriptive analysis, comparison of two proportions were used. In-group changes were estimated with the help of frequency tables and crosstabulations function to have an opportunity to connect the frequency of display of observations at different levels. Logistic regression analysis using relative risk index (RR) and its 95% confidence interval (CI). The difference in parameters was considered statistically significant at $p<0.05$. To identify the relationship between adverse signs of psycho-emotional state of parents and the presence of chronic pain in their children before and after rehabilitation, the function of cross-tabulation of values of different frequencies of psycho-emotional state of parents from these questionnaires with only 100% answers. The con-

struction of the conjugation table allowed to combine the frequencies of observation at different levels of factors and to determine the relationship between the cross-tabulated values.

Results

The results of the survey of parents of children with paralytic syndromes of the general cohort (n=64) were developed in the logistic regression model to determine the relative risk of adverse events. And although we understand that this is a subjective opinion of parents, it nevertheless gives us a more detailed assessment of their psycho-emotional state, anxiety, satisfaction with rehabilitation measures. It is parental feedback that can improve approaches to treating their children and supporting families. The relative risk of motor activity and chronic pain was determined by subjective assessment of parents of children with paralytic syndromes (*Table 1*).

Although no chronic pain was detected on the r-FLACC and NCPCC-R scales in the assessment of children in group Ib, parents nevertheless believe that their children are in pain. According to our study, 34 (89.0%) children with group Ia had severe problems with motor activity, and it is important that parents subjectively admit severe disorders 22.5 times (RR=22.5; p=0.0015).

The subjective assessment of parents of the psycho-emotional state of their children with paralytic syndromes was analyzed (*Table 2*).

According to the subjective assessment of the psycho-emotional state of their own child, there were no differences between the parents of both groups, except for one symptom: the ability to calm down, which was characteristic of children of group IB (RR=3.9; 95% CI 1.7–13.0 p=0.0259).

The general condition of own children with paralytic syndromes "eyes of parents" before rehabilitation and after rehabilitation is analyzed (*Table 3*).

The general cohort did not identify significant changes in the general condition of children before rehabilitation and after rehabilitation "through the eyes of parents".

According to the subjective assessment of the condition of parents of their own children before rehabilitation and after rehabilitation showed that 3 (7.8%) children from group Ia improved their condition: from the worst condition to the average condition of 2 (5.2%) children and 1 (2.6%) the child is in the best condition. And among the children of Ib group also improved their condition 3 (11.5%) children: from the average to the best condition 3 (11.5%) children.

Table 1. Relative risk (RR) of movement disorders and chronic pain according to the subjective assessment of parents of children with paralytic syndromes

Indication	Data-out				RR	95% CI OR	p
	a	b	c	d			
The child has significant problems with walking	33	5	1	25	22.5	3.3–154.9	0.0015
The child is experiencing chronic pain	35	3	18	5	1.2	0.9–1.4	0.1737

Notes: a – children of group Ia with the presence of a sign; b – children of group Ia without a sign; c – children of Ib group with the presence of a sign; d – children of Ib group without a sign.

Table 2. Relative risk (RR) of signs of psycho-emotional state of children with paralytic syndromes according to subjective assessment by parents

Indication	Data-out				RR	95% CI OR	p
	a	b	c	d			
Ability to calm down	3	31	9	17	0.3	0.1–0.8	0.0259
Ability to calm down with periodic contact	21	17	14	12	1.1	0.6–1.6	0.9112
The difficulty of calming down	11	27	2	24	3.7	0.9–15.5	0.0677
Moaning or whining	30	8	16	10	1.3	0.9–1.8	0.1575
Constant shouting	5	33	3	23	1.1	0.3–4.4	0.8478

Notes: a – children of group Ia with the presence of a sign; b – children of group Ia without a sign; c – children of Ib group with the presence of a sign; d – children of Ib group without a sign.

Table 3. Relative risk (RR) of subjective parental assessment of children with paralytic syndromes before and after rehabilitation

Indication	Data-out				RR	95% CI OR	p
	a	b	c	d			
Before rehabilitation							
The worst condition	29	9	13	13	1,5	0.9–2.3	0.0502
The best condition	2	36	1	25	1,3	0.2–14.3	0.7935
After rehabilitation							
The worst condition	26	12	13	13	1,3	0.8–2.1	0.1632
The best condition	3	35	4	22	0,5	0.1–2.1	0.3542

Notes: a – children of group Ia with the presence of a sign; b – children of group Ia without a sign; c – children of Ib group with the presence of a sign; d – children of Ib group without a sign.

In general, according to the parents, 6 (9.4%) children with paralytic syndromes improved their general condition after rehabilitation.

Thus, we see that an important component of medical care for children with paralytic syndromes is the involvement of parents in the rehabilitation of their children, taking into account their views, which can improve the psycho-emotional state of parents and improve the psycho-emotional state of children.

When comparing the frequency of care and rehabilitation at home among a cohort of children with paralytic syndromes, we obtained the following data: 21/38 parents of children in group Ia do not worry about the condition of their children (RR=2.3; 95% CI 1.1–5.1; p=0.0239), this can be explained by the fact that parents already have emotional burnout or that parents perceive their own child's condition and feel humble. That is why we see that 20/38 parents of children in group Ia do not perform rehabilitation measures at home compared to 6/26 Ib group (RR=2.2; 95% CI 1.0–4.8; p=0.0344).

When comparing the frequency of conditions that affect parents among a cohort of children with paralytic syndromes showed that parents of children in group Ia are concerned about 5/17 delay in physical development (RR=0.5; 95% CI 0.2–1.1; p=0.0813) and 3/14 convulsions (RR=1.1; 95% CI 0.3–5.0; p=0.8277), and parents of children in group Ib 10/10 delayed physical development (RR=2.2; 95% CI 0.9–5.3; p=0.0813) and 4/16 general condition of the child (RR=0.3; 95% CI 0.1–2.3; p=0.2520). We analyzed the answers to 12 questions of the domain of psycho-emotional state of parents (*Table 4*).

Table 4. Relative risk (RR) of psychoemotional disorders of parents of children with paralytic syndromes according to subjective vision

Indication	Data-out				RR	95% CI OR	p
	a	b	c	d			
Helplessness or fear	36	3	20	6	1.2	0.9–1.5	0.1190
Sadness and anger when a child cries	36	3	16	10	1.5	1.1–2.1	0.0122
Internal tension	38	0	17	9	1.5	1.2–2.1	0.0029
Insomnia	36	3	17	9	1.4	1.1–1.8	0.0215
It is unbearable to watch a child's suffering	38	0	16	10	1.6	1.2–2.2	0.0017
Concerned about the child's treatment	32	6	10	16	2.1	1.3–3.6	0.0024
Hyperopia of the child	29	9	10	16	1.9	1.2–3.3	0.0094
The inability of the child to lead a normal life	37	1	16	10	1.5	1.2–2.2	0.0035
Search for compassion or understanding	28	10	12	14	1.5	1.0–2.5	0.0446
Inner anxiety, a sense of possible trouble	35	3	15	11	1.5	1.1–2.2	0.0074

Notes: a – children of group Ia with the presence of a sign; b – children of group Ia without a sign; c – children of Ib group with the presence of a sign; d – children of Ib group without a sign.

According to the frequency of psycho-emotional disorders of parents of children with paralytic syndromes, according to subjective vision, "Helplessness or fear" is common in both groups. If children with paralytic syndromes have chronic pain, it affects not only the condition of children but also the psycho-emotional state of the parents themselves, namely: "Concern about the treatment of the child" is reduced by half (RR=2.1; p==0.0024), "Hyperopia of the child" – twice (RR==1.9; p=0.0094), "Intolerance to observe the suffering of the child" – one and a half times (RR==1.6; p=0.0017), "Sadness and anger when a child cries" – one and a half times (RR=1.5; p=0.0122), "Internal tension" (RR=1.5; p=0.0029), "Insomnia" (RR=1.5; p=0.0215), "Child's inability to lead a normal life" (RR=1.5; p=0.0035), "Search for compassion or understanding" (RR=1.5; p=0.0446), "Inner anxiety, a sense of possible trouble" (RR=1.5; p=0.0074). Of the 12 questions asked, 9 showed profound violations of the psycho-emotional state of parents of children with paralytic syndromes.

We tabulated the values of different frequencies of psycho-emotional state of parents of children with paralytic syndromes before rehabilitation and after rehabilitation. Only questionnaires with 100% answers were involved in the cross-tabulation function. The aim was to find out the relationship between the unfavorable signs of psycho-emotional state of parents and the presence of chronic pain and their children before and after rehabilitation. The construction of the conjugation table allowed to combine the frequencies of observation at different levels of factors and to determine the relationship between the cross-tabulated values.

We did not compare the objective assessment of chronic pain in children with the help of scales and the subjective vision of the parents of children with paralytic syndromes, but only the subjective vision of parents before rehabilitation and after rehabilitation (*Table 5*).

Table 5. Conjugation of observation frequencies at different levels of factors between crustabilized values

Question	Answer	Cross-tabulated values						p
		Before rehabilitation	Total n=51	%	After rehabilitation	Total n=34	%	
Feelings of inner anxiety	Sometimes	35	44	86.2	15	21	61.7	0.0125
	Most of the time	9			6			
Conversation with a psychologist	Sometimes	25	33	64.7	11	15	44.1	0.0591
	Most of the time	8			4			
Search for compassion or understanding	Sometimes	33	34	66.6	16	17	50.0	0.1205
	Most of the time	1			1			
Concern to lead a normal life	Sometimes	24	45	88.2	14	29	85.2	0.6900
	Most of the time	21			15			
Concerns about over-care	Sometimes	23	35	68.6	12	23	67.6	0.8467
	Most of the time	12			11			
Concerns about pain management	Sometimes	25	36	70.5	12	21	61.7	0.3883
	Most of the time	11			9			
It is unbearable to monitor the child's condition	Sometimes	23	46	90.1	11	20	58.8	0.0012
	Most of the time	23			13			
I do not sleep well because of the child's condition	Sometimes	33	48	94.1	16	25	73.5	0.0109
	Most of the time	15			9			
Sleepless nights due to the condition of child	Sometimes	31	44	86.2	16	23	67.6	0.0401
	Most of the time	13			7			
Internal tension	Sometimes	32	46	90.1	17	24	70.5	0.0269
	Most of the time	14			9			
I feel upset or angry	Sometimes	30	44	86.2	17	25	73.5	0.1691
	Most of the time	14			8			
Helpless or scared	Sometimes	13	31	60.7	11	16	47.0	0.2069
	Most of the time	18			5			

According to the conjugation table, we obtained the following values of the psycho-emotional state of parents of children before rehabilitation by rank: "I do not sleep well because of the child's condition" 44 (94.1%); "It is intolerable to observe the condition of the child" 46 (90.1%); "Internal tension" 46 (90.1%); "Concern to lead a normal life" 45 (88.2%); "Feelings of inner anxiety" 44 (86.2%); "Sleepless nights due to the condition of the child" 44 (86.2%); "I feel upset or angry" 44 (86.2%); "Concerns about the treatment of pain" 36 (70.5%); "Concerns about excessive care" 35 (68.6%); "Search for compassion or understanding" 34 (66.6%); "Conversation with a psychologist" 33 (64.7%); "Helplessness or fear" 31 (60.7%).

It is worth noting that the two psycho-emotional states "Unbearable to watch the child's condition" and "Helplessness or fear" parents experienced most of the time.

After rehabilitation measures, psycho-emotional disorders of parents remained, but we recorded a statistically significant decrease in their frequency among 5 signs: "Intolerable observation of the child's condition" decreased by 31.3% (p=0.0012); "Feelings of inner anxiety" decreased by 24.5% (p=0.0125); "I fall asleep badly due to the child's condition" decreased by 20.6% (p=0.0109); "Internal tension" decreased by 19.6% (p=0.0269); "Sleepless nights due to the condition of the child" decreased by 18.6% (p=0.0401).

Not statistically significant, but the following signs tended to decrease: "Conversation with a psychologist" decreased by 20.6%; "Search for compassion or understanding" decreased by 16.6%; "Helplessness or intimidation" decreased by 13.7%; "Anxiety to lead a normal life" decreased by 3%; "Feeling upset or angry" decreased by 12.7%; "Concerns about pain treatment" decreased by 9.0%; "Concerns about excessive care" decreased by 1.0%.

Discussion

According to one study, the psychological and physical health of caregivers, most of whom were mothers, was highly dependent on the child's behavior and care requirements. Problems with children's behavior were an important factor in the psychological well-being of caregivers, both directly and indirectly, because of their impact on self-perception and family functions. Care requirements directly affect both the psychological and physical health of caregivers. The practical daily needs of the child created problems for parents [7, 8].

These data confirm the influence of the emotional state of parents directly on the emotional state of children [9, 10]. Strategies for optimizing the physical and psychological health of the caregiver include support for behavioral management and daily functional activity, as well as stress management techniques [11, 12].

Most rehabilitation programs for children with paralytic syndromes for several decades have been aimed at normalizing motor functions, ensuring normal posture and independent functional activity of the child, regulating muscle tone, improving visual and auditory responses, supporting motor development and motor control, preventing joint contractures. and more. According to experts, setting individual realistic goals, setting priorities, informing the family and strengthening its participation increase the effects of physical therapy [13, 14].

But the effectiveness of physical therapy in children with paralytic syndromes, according to many researchers, is not based on sufficient scientific evidence. To assess the effectiveness of physical therapy, the use of indicators such as type of exercise, their frequency, intensity and duration has been demonstrated [14].

For example, a recent review of evidence-based approaches to physical therapy for children with cerebral palsy, published in 2019, demonstrates that the effectiveness of most interventions is limited. Despite the recognition of the effectiveness of individual targeted approaches to rehabilitation, future research is needed to determine the best ways to improve functional outcomes in children [15, 16].

Our own study did not include a thorough analysis of a particular approach, type of exercise and their intensity in the provision of rehabilitation services to children with paralytic syndromes. We used a multidisciplinary approach based on the individual needs of a young patient, used a time interval of 6 months, GMFCS, and, the main task was to determine the impact of physical therapy on chronic pain in children with paralytic syndromes, objective and subjective assessment by parents, and their emotional state.

A review of publications from 1990 to 2011, which included research to determine the experience of parents with the treatment of their child, where a quarter of the child population was under five, children received physical and/or occupational therapy in the rehabilitation program, provided a conceptual basis for that the experience of parents is closely related to the quality of interven-

tion for the child [16–18]. This review evaluated 13 studies (eight qualitative and five quantitative). Parents expressed different aspects of their own experiences, had different needs, and needed time to establish a relationship with their child's physical therapist [19].

Our questionnaire created the conditions for closer interaction between the physical therapist and parents, as we took into account the experience of parents (their assessment of rehabilitation), their emotional state, awareness that the child has chronic pain and the ultimate importance of the wider family context.

Equally important is the fact that physical therapists have received feedback on the subjective assessment of children's pain "through the eyes of parents" and their emotional state. A Finnish survey of 201 members of a multidisciplinary team and 311 physiotherapy providers found that they tended to be positive about their family-oriented service. However, research has shown that a family-oriented approach increases with increasing experience of the specialist [17, 20].

Therefore, our research and the proposed questionnaire can be used to identify areas for improvement not only by our team of professionals, but also for use by other rehabilitation practices.

Children with paralytic syndromes have a number of factors that cause pain [21]. To date, there is little empirical evidence that pain is better assessed clinically, and the search for pain assessment in recent decades has led to the development and use of standardized pain assessment tools, and research and broad international cooperation continue and cognitive impairment [22].

Although researchers recognize that pain in children with intellectual disabilities is a common and complex phenomenon, there are no standard educational components for caregivers or guardians of such children [23, 24]. Our study focuses on information for parents and professionals that nonverbal children (1 to 6 years old) with paralytic syndromes may have chronic pain, and they should be monitored for pain that parents can use with a physical therapy professional. pain assessment tool and gain experience in measuring it.

Of the existing tools for assessing pain in nonverbal children, we selected two scales, r-FLACC and NCCPC-R. The selection of the r-FLACC scale was based on the findings of clinicians from three medical centers who reviewed 15 videos of observations of children with neurological disorders using three pain assessment tools and preferred this scale. In addition, clinicians' scores correlated with parental scores ($p < 0.001$) and the reliability of testing and retesting was confirmed by strong correlations ($r=0.8–0.883$; $p<0.001$) [25–27].

In our opinion, the study from 2011 to 2014 on the determination of pain in a cohort of children with developmental disorders (n=544) who visited an outpatient clinic is interesting. In contrast to our study, the mean age of children was 14 years, with one-third of all children having cerebral palsy. Along with emotional disorders (anxiety and depression), children were diagnosed with chronic pain and even one that required treatment at tertiary care. The authors of the study believe that pain assessment should be a routine practice of all multidisciplinary teams [28, 29].

According to one study, the psychological and physical health of caregivers of children with paralytic syndromes was highly dependent on the child's behavior and childcare requirements. Problems with children's behavior have been an important factor in the psychological well-being of caregivers, both directly and indirectly, because of their impact on self-perception and family functions [30, 31]. These data confirm the influence of the emotional state of mothers directly in the first place on the emotional state of children and vice versa [8].

Strategies to optimize the physical and psychological health of the caregiver include support for behavioral management and daily functional activity, as well as stress management techniques. Therefore, it is very important for specialists of multidisciplinary teams to know the emotional state of parents to correct their quality of life, the effectiveness of clinical interventions and care [32].

Conclusions

The authors consider that physical rehabilitation in children with paralytic syndromes reduces the incidence of moderate chronic pain and improves the emotional state of parents. So, we think that new approaches to rehabilitation should be applied with daily management of the children with paralytic syndromes' needs and their families with increased psychological and social support. Perhaps the searching for new approaches that optimize more intensive and effective rehabilitation strategies using the family reserve will provide the potential for adaptability of nerve plasticity and recovery in such a contingent of children and prospects for the future.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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FORENSIC MEDICAL DETERMINATION OF SEVERITY OF CHEST INJURIES WITH THORAX TRAUMA

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ABSTRACT

Background. Closed chest trauma with rib fractures is a common injury to the external respiratory system. Victims with such an injury may be subject to forensic examination.

The aim of the study was to determine the severity of injuries in patients with closed chest trauma with violation of the integrity of the costal skeleton, treated in a specialized surgical hospital, to establish additional criteria for forensic diagnosis of such trauma.

Materials and Methods. 71 medical cards of inpatients, patients with chest injuries who were treated at Kharkiv Institute of General and Emergency Surgery named after V.T. Zaitsev were analyzed. A forensic medical evaluation of closed chest injuries with rib fractures was performed according to the severity of injuries according to clinical observations. Morphological and clinical approach was used to determine severity of injuries.

Results. Severe injuries were found in 14 (19.7%) cases of injuries, mainly with the occurrence of a life-threatening phenomenon, namely acute respiratory failure. Moderate injuries were found in 57 (80.3%) cases of chest injuries with rib fractures in the absence of danger to life.

Conclusions. It is established that in the available scientific and methodological literature there are no diagnostic morpho-clinical signs for qualitative forensic assessment and prediction of the final results of closed chest injuries, there are different views on forensic assessment. It is determined that when applying the morphological and clinical approach of forensic assessment, additional diagnostic criteria for closed chest injuries, should be considered when determining the severity of injuries: dynamics and duration of recovery of post-traumatic morphological and functional changes of injured organs. or the entire respiratory system (lungs), the occurrence of life-threatening phenomena. The ways of further improvement of forensic diagnostics in the assessment of these injuries by the degree of their severity are identified.

Keywords: *forensic examination, chest injury, diagnostic criteria, severity of injuries*.

INTRODUCTION

Chest injuries are a common type of injury, both in peacetime and in wartime [1, 2]. At the closed injuries of the thorax first of all its bone basis is broken [3, 4]. This circumstance is the main cause of trauma to the thoracic cavity [5]. Closed chest trauma can primarily adversely affect the smooth functioning of the external respiratory system [6, 7]. Victims with non-fatal closed blunt chest trauma (CBCT) with fractures of the ribs require a forensic examination to determine severity of injuries [8–10].

In accordance with the current regulations of Ukraine, in particular, according to the "Rules of forensic determination of the severity of injuries" (enacted by the order of the Ministry of Health of Ukraine No.6 from 17 Jan 1995) in classifying such injuries to one or another severity, criteria "danger to life", "permanent loss of general ability to work", "duration of health disorder" may be used [11]. However, as shown by the study of special literature, there are two opposing approaches in the forensic assessment of CBCT and, accordingly, the application of certain qualification criteria [12, 13]. Some experts propose the so-called morphological approach, in which may be used morphological, post-traumatic changes, lung rupture, hemopneumothorax to identify the damage as life-threatening and allow it to be assessed as severe [10, 14]. According to other experts, it is necessary to apply a morpho-clinical approach, in which in addition to the morphology of the injury, it is necessary to assess the presence of clinical signs of life-threatening conditions, including

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acute respiratory failure (ARF) [11, 12]. And only in the case of its presence can the damage be assessed as life-threatening and be classified as grievous bodily harm. Otherwise, the criterion of "duration of health disorder" or "permanent loss of general ability to work" of regulatory documents should be used for forensic assessment. Thus, the presence of these two opposing views shows the lack of a unified scientific and methodological approach in the forensic assessment of CBCT. This can in some cases lead to underestimation or overestimation of the severity of injuries and, accordingly, to expert errors [13]. This in turn can lead to incorrect classification of the crime by law enforcement agencies.

Purpose, subjects and methods:

1. The purpose of the study was to determine the severity of injuries in patients with closed blunt chest trauma with a violation of the integrity of the costal skeleton, treated in a specialized surgical hospital, to establish additional criteria for forensic diagnosis of such trauma.

2. Subjects & Methods

The material for the analysis was medical records of inpatients who were treated during the recent decade at Kharkiv Institute of General and Emergency Surgery named after V.T. Zaitsev. In total, medical records were processed retrospectively in 71 patients. The study material was divided into 3 groups depending on the dynamics of morpho-functional post-traumatic changes in the thoracic organs responsible for respiratory function, the final results and the presence of life-threatening phenomena in patients. The first group included patients with positive dynamics, but incomplete recovery functions of the chest and the disappearance of post- traumatic morphological changes: up to 6 days (subgroup "a"), during from 7 to 21 days (subgroup "b"), in the period over 21 days to 31 days (subgroup "c"), for a period of more than 1 month, up to 2 months (subgroup "d"), for a period of more than 2 months, up to 3 months (subgroup "e"), for a period of more than 3 months, up to 1 year (subgroup "f"). The second group included patients who lost part or all of their respiratory organ (lung) as a result of the injury. The third group included the patients who developed an acute life-threatening condition against a background of CBCT, namely ARF. The following methods were used in the study: registration method – the obtained data were entered into specially developed registration cards; standard method of descriptive statistics; forensic – determined the nature of the injuries, determined the severity of injuries. This study was permitted by the commission on ethics and bioethics of Kharkiv National Medical University. During the examination, oral and written consent was obtained from all victims.

Results and discussion

The analysis of observations showed that among the causes of CBCT domestic injuries occupied the first place. According to the mechanism of damage in all cases there was shock effect of blunt solid objects on the chest. CBCT prevailed in males – 58 (82%) cases, of working age 20–50, had 35 (49%) patients. It should be noted that the distribution of patients obtained by the mechanism of damage, age and sex in general coincides with the literature [1, 4].

The lesions detected in patients in the observation groups were systematized and morpho-clinical variants of CBCT were identified (*Table*). The table shows that 16 (22.5%) patients with rib fractures from the first group did not have any intrapleural injuries and complications. Intrapleural injuries and complications occurred in 55 (77.5%) patients with rib fractures of the first, second and third groups. Hemothorax occurred in 9 (12.7%) cases of CBCT with fractures of several ribs, in 1 (1.4%) case with fractures of one rib. Coagulated hemothorax occurred in 8 (11.3%) cases of CBCT with fractures of several ribs. Pneumothorax occurred in 10 (14.1%) cases of CBCT with fractures of several ribs, in 1 (1.4%) case with fractures of one rib. Hemopneumothorax occurred in 18 (25.4%) cases of CBCT with fractures of several ribs, in 3 (4.2%) cases with fractures of one rib. Post-traumatic pleurisy occurred in 6 (8.5%) cases, mainly in patients of the first group, encapsulated hydrothorax occurred in 1 (1.4%) case in a patient of the first group. Post-traumatic chondroma of the ribs occurred in 1 (1.4%) case in a patient of the first group, bronchopleurothoracic fistula occurred in 1 (1.4%) case in a patient of the third group. Pulmonary contusion was reported in 4 (4.2%) patients, mostly of the third group. Post-traumatic pneumonia occurred in 4 (5.6%) cases in patients of the first group.

Existing post-traumatic morphological changes were detected mainly by X-ray examination of the chest, which was performed in all patients. This study revealed fractures of the ribs, air, blood, fluid in the pleural cavity, changes in the parenchyma of lung tissue. Computed tomography was performed in some observations in order to clarify the results of X-ray examination, or in cases where the X-ray examination was not infor-

Table. Variations of closed chest injuries in the observed groups.

The nature of the injury	Observation groups								Total	%
	I						II	III		
	a	b	c	d	e	f				
CBCT, fractures of several ribs, without complications	3	12							15	21.2
CBCT, fracture of one rib, without complications	1								1	1.4
CBCT, fractures several ribs, the presence of intrapleural complications and injuries:										
- hemothorax		4	1					1	6	8.6
- hemothorax, exudative pleurisy		3							3	4.2
- clotted hemothorax		1		1				1	3	4.2
- clotted hemothorax, pleural empyema		1	1						2	2.8
- clotted hemothorax, lung contusion				1					1	1.4
- pneumothorax		2						2	4	5.6
- pneumothorax, sternal fracture		1	1					2	4	5.6
- hemopneumothorax	2	4		2				1	9	12.8
- hemopneumothorax, lung contusion							1		1	1.4
- hemopneumothorax, hemothorax								1	1	1.4
- hemopneumothorax, pleurisy				1		1			2	2.8
- hemopneumothorax, pleurisy, diaphragm rupture	1	1							2	2.8
- hemopneumothorax, pneumonia						1			1	1.4
- hemopneumothorax, sternal fracture, contusion of lungs								2	2	2.8
- clotted hemothorax, pneumothorax				1					1	1.4
- clotted hemothorax, pneumothorax, wound lungs, pleurisy								1	1	1.4
- encapsulated pleurisy, pneumonia				1					1	1.4
- sacculated hydrothorax					1				1	1.4
- post-traumatic chondroma of the ribs				1					1	1.4
- bronchopleurothoracic fistula								1	1	1.4
- pneumonia, sternal fracture						1			1	1.4
CBCT, fracture of one rib, the presence of complications and combined injuries:										
- hemothorax, pleurisy					1				1	1.4
- pneumothorax		2						1	3	4.2
- hemopneumothorax	2								2	2.8
- hemopneumothorax, pneumonia					1				1	1.4
Total	9	31	3	8	3	3	1	13	71	100

mative enough. In this case, computed tomography is the most informative in the diagnosis of such injuries [15, 16].

Among the medical care provided to patients in the observation groups, conservative therapy was provided in 25.4% of cases. The main method of surgical treatment of CBCT in the observation groups was drainage of the pleural cavity according to Bülau (73.2% of cases), more severe surgery, resection of the lower lung, was performed in 1.4% of cases. It should be noted that our established terms of hospital stay, types of medical care provided to patients, the duration of the disorder in the observation groups, which can be seen from the table above, generally do not contradict the available literature data, and in some cases complement them [2, 4, 10].

Forensic assessment of clinical observations was performed using a morpho-clinical approach, which must be applied in accordance with current

state regulations [11, 12]. At the same time, according to these documents, the presence of ARF of any severity, including mild, already allows to determine the presence of a life-threatening condition in the victim. Therefore, in the forensic assessment of CBCT established the presence of ARF, which was mostly mild on the set of clinical signs.

Thus, according to the results of forensic assessment of clinical observations of CBCT we have established the following degree of severity of injuries. Serious bodily injuries according to the criterion of "danger to life" item 2.1.3 of item "O" of the "Rules…" were found in 13 (18.3%) patients of the third group with CBCT, who had signs of acute respiratory failure. At the same time, patients had intrapleural complications and injuries: in 1 (1.4%) case of hemothorax, in 2 (2.8%) cases of folding hemothorax, in 3 (4.2%) cases of pneumothorax, in 6 (8.5%) cases of hemopneumothorax, in 1 (1.4%) case bronchopleurothoracic fistula.

We also found serious bodily injuries in 1 (3.2%) case in a patient with CBCT of the second group according to the criterion "health disorder associated with permanent disability of at least one third" of paragraph 2.1.6 of the "Rules…". At the same time the patient had multiple rib fractures, hematoma, contusion of the lung, pulmonary hemorrhage, performed surgery – "Resection of the lower lobe of the lung".

Moderate injuries that caused a long-term health disorder lasting more than 3 weeks (more than 21 days), paragraph 2.2.1 "c" of the "Rules…", were found in patients of the first group: 16 (22.5%) cases of CBCT with fractures of the ribs, without complications; 41 (57.7%) cases of CBCT with rib fractures, intrapleural complications and combined injuries, of which 9 (12.2%) cases of hemothorax, 5 (7.1%) cases of collapsed hemothorax, 8 (11.3%) cases of pneumothorax, 15 (21.1%) cases of hemopneumothorax, 2 (2.8%) cases of encapsulated pleurisy, 1 (1.4%) case of chondroma of the ribs, 1 (1.4%) case of sternal fracture, pneumonia.

The morpho-functional approach used by us in the forensic assessment of clinical observations in determining the severity of CBCT with damage to the costal skeleton, showed a sufficient level of justification for the established severity of injuries. At the same time, if we applied a purely morphological approach, which is proposed by some experts [10, 13, 14], the number of unreasonably established by us serious injuries would be greater. This, in turn, could lead to incorrect classification of the gravity of the crime and the establishment of a degree of legal responsibility for its commission.

Conclusions

1. Our findings suggest that intrapleural injuries and complications occurred in 43.7% of patients with CBCT with rib fractures undergoing inpatient treatment. Injuries with signs of danger to life account for 18.3% of cases.

2. In the structure of forensic assessment of CBCT with rib fractures, according to clinical observations, severe injuries account for 19.7% of cases. They are established mainly in the event of acute respiratory failure. 80.3% of CBCT cases with rib fractures, in the absence of danger to life, were classified as moderate injuries.

3. The available scientific and methodological literature does not contain clear diagnostic morphological and clinical criteria for qualitative forensic assessment and prediction of the final results of CBCT.

4. When applying the morphological and clinical approach, the additional diagnostic criteria for CBCT assessment should be considered when determining the severity of injuries: the dynamics and duration of recovery of post-traumatic morpho-functional changes of injured organs, loss of part or all of the respiratory organ (lungs), the emergence of life-threatening phenomena.

The prospect of further research is to conduct research to study all possible morphological and clinical manifestations of the studied injury, followed by development of a clear algorithm for forensic expert research in these cases.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

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MODERN VIEW ON CHRONIC RESPIRATORY DISEASES IN PREGNANT (review)

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Abstract

Disorders in the fetoplacental complex of pregnant women with chronic respiratory diseases (CRD) include a wide range of problems in modern obstetrics in medical, economic and social aspects. Respiratory diseases in the context of disorders of the fetoplacental complex (FPC) may be a comorbid process, a background to the abnormal pregnancy, or a premorbid condition that contributes to the development of placental dysfunction (PD) or even initiates it; morphophysiological changes characteristic of pregnancy also affect the state of the respiratory system, moderating the course of bronchoobstructive diseases. Respiratory diseases in women, the impact of its treatment and features of the course and medical support of pregnancy in these conditions affect the condition of both the woman and the fetus, and physical and neuropsychological development of the child in future.

Keywords: *angiogenesis factors, bronchial asthma, chronic bronchitis, placental dysfunction, respiratory diseases.*

INTRODUCTION

Extragenital diseases negatively affect the course of pregnancy and childbirth, constantly increasing the rates of maternal and perinatal morbidity. Chronic respiratory diseases of pregnant (bronchial asthma, chronic bronchitis) have recently spread among women of childbearing age and can complicate the course of pregnancy and childbirth.

Respiratory diseases in the context of fetoplacental complex disorders may be a comorbid process, underlie abnormal pregnancy or a premorbid condition that contributes to the development of fetoplacental insufficiency or even initiate it; morphophysiological changes typical for pregnancy also affect the state of the respiratory system, moderating the course of bronchoobstructive diseases [1, 2]. Respiratory disorders in women, the action of agents for its treatment and features of the course and medical support of pregnancy under these conditions are reflected in the condition of the woman and fetus, as well as physical and neuropsychological development of the child in the future [3, 4].

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The aim of the study was to analyze the modern literature on the problem of the influence of chronic respiratory disorders on the course of pregnancy and childbirth, the condition of the mother and newborn.

Physiological changes in the body of a pregnant woman may increase the risk and severity of extragenital diseases, including the respiratory system [5–7]. The processes that take place in a woman's body during pregnancy are aimed at forming reserves and compensating for the energy needs of the pregnant woman and the fetus [8].

Mehta et al. [7] indicate that the physiological and anatomical features of the mother's body during pregnancy affect the respiratory system, sometimes changing the presentation of respiratory disorders. Adaptive changes in the respiratory system in pregnant women, emphasized by researchers [9–11], begin after fertilization under hormonal stimulation, continue throughout pregnancy and can sometimes mask the pathological process or, conversely, be misinterpreted as a disease. Thus, Mehta et al. (2015) believe that vasodilation due to the action of progesterone results in the development of edema and increased vascularization of mucous membranes, which can cause rhinitis and nosebleeds. At the same time, normal partial pressure of CO_2 should be interpreted as the development of respiratory failure, as pregnancy is characterized by a decrease in $PaCO_2$ [7].

Bronchial asthma (BA) is one of the most common pulmonary abnormalities in the general population is [11, 13]. Asthma, by definition, is a chronic recurrent disease that involves airway hypersensitivity, leading to recurrent symptoms, including wheezing, coughing, and shortness of breath [14]. Tamasi et al. [15] add that asthma is an allergic disease caused by the activity of T-helpers type 2 (Th2), which leads to bronchial hyperreactivity, inflammation, respiratory obstruction and remodeling of respiratory tissues. Traditionally, asthma is diagnosed in existing airway obstruction during spirometry with its reversibility (increase in forced expiratory capacity for 1 second by at least 12%) [16].

Asthma symptoms are not specific, and are in particular predominated by cough and shortness of breath, which can result in a misdiagnosis of "shortness of breath of pregnant". In addition, the development of respiratory complications is determined in 8% of cases of mild asthma, 48% of moderate asthma and 65% of severe asthma [17].

In pregnant women, BA is the most common disease of the pulmonary system, the incidence of which varies from 1 to 4% [12, 18]. According to Meakin et al. (2017), in Australia, BA is detected in 12% of pregnant women annually, with an increase in the frequency in pregnant from socially disadvantaged categories [19].

In Europe, the incidence of asthma in pregnant women is estimated at 8% and becomes one of the most common comorbidities during gestation.

According to [20], BA is detected in 15% of pregnant women. According to a study by Alqalyoobi [21], the incidence of asthma among pregnant in the United States is up to 8.8%, with 1–4% developing especially severe pregnancy complications caused by asthma.

BA is known to affect the course of pregnancy with the development of complications, and pregnancy can significantly worsen the course of BA. Alqalyoobi et al. [21] add that BA symptoms peak in the late second and early third trimesters. In 3.7–8.4% of cases, BA complicates the course of pregnancy [22]. Pregnant women with asthma per 500 pregnancies develop severe complications such as preeclampsia, fetal growth retardation (FGR), premature birth, fetal distress and neonatal asphyxia, etc., which determine high rates of perinatal morbidity and mortality and can have long-term consequences, including neurological and other chronic disorders [23].

During pregnancy, depending on the severity, almost 30–50% of pregnant women are found to have complications of BA, which is a significant medical problem [24, 25]. A retrospective study conducted by Ali et al. [12] determined a relationship between asthma complications and pregnancy. Asthma has been shown to be associated with both pregnancy complications and adverse effects on the fetus [1].

Inflammation, hypoxia and stress reactions are the most common pathological processes in BA. Thus, in the case of uncontrolled recurrent course and exacerbations of BA during pregnancy, the level of systemic inflammation, oxidative stress and the development of fetal hypoxia increases. Exacerbations of asthma cause the development of alkalosis in the mother, which impairs uterine blood flow and, consequently, oxygenation of the fetus, leading to hypoxia and hypercapnia, in particularly severe cases, the possible development of acidotic conditions [7].

Pregnant with BA, according to the authors, develop pregnancy complications more commonly, including preeclampsia, gestational diabetes, placenta previa, premature rupture of membranes, postpartum hemorrhage, miscarriage, low birth weight, etc. The course of BA in almost a third of pregnant women deteriorates during pregnancy [26, 27]. Almost 10% of women are diagnosed with exacerbations of asthma during childbirth [28].

Studies on asthma as a risk factor for caesarean section, prematurity, low Apgar score, low gestational age, or intrauterine growth retardation remain relevant today [2, 29]. It should be added that conflicting data from different researchers may be related to differences in research structure, diagnostic aspects and multifactorial nature of the disease, including the impact of asthma on pregnancy and child health [29].

A study [30] showed that women with insufficient asthma control (69.8%) had significantly more ($p<0.001$) pregnancies compared to pregnant women with sufficient asthma control (56.3%). There was no significant difference between the frequency of smoking and alcohol consumption between the groups [30]. At the same time, the social status was higher than the average in 77.9% of women with adequate asthma control than with insufficient (62.5%), $p<0.001$. Significantly ($p=0.01$) higher average daily dosage of inhaled corticosteroids was registered in pregnant women with insufficient asthma control (481.1± ±88.0 ng) than with sufficient (399.3±314.0 ng) [31]. Decreased disease control may be due to a number of factors, including low adherence or

reduced response to treatment, increased severity of pathology, or incorrect choice of treatment strategy. According to the authors [30], insufficient control of asthma and higher frequency of hospitalizations due to asthma were observed in patients with higher doses of glucocorticosteroids (GCS). At the same time, higher doses of corticosteroids in such patients may mean a reduced response to their action or insufficient effect in patients with more severe conditions.

During the treatment of BA in pregnant women, some difficulties are caused by restrictions on the use of certain groups of drugs for the treatment of pulmonary diseases. Bacterial infections can significantly complicate pregnancy. Thus, the treatment of infectious diseases (chronic bronchitis, pneumonia, etc.) is carried out taking into account the systemic effects of antibiotics. The use of mechanical ventilation requires an accurate assessment of the state of oxygenation and hemodynamics of the mother with the support of saturation at the level of at least 95.0% [7].

The authors [22, 32] add that insufficient control of BA significantly increases the risk of fetal growth disorders. In addition, existing obesity has also been associated with an increased incidence of asthma complications during pregnancy and, as a consequence, with a risk of developing other respiratory diseases, including nocturnal apnea [33]. The latter can be explained by the fact that the decrease in immune reactivity during pregnancy may be impaired in obese patients, as the latter detects a greater number of young T cells.

A significant increase in the number of peripheral cells that produce interferon-γ (IF-γ) has been identified [15]. Subsequent assessment found a negative correlation with the number of these IF-γ-positive T cells and the weight of the newborn, which may indicate that intrauterine growth retardation may be associated with immune responses mediated by asthma.

At the same time, Tamasi et al. [15] state that approximately one third of pregnant women with BA are found to have an improvement in the course of asthma, one third are diagnosed with worsening symptoms and one third of pregnant women have a stable course of the condition. In the literature, such features are described as the "rule of thirds" [22]. It was also determined that pregnant women with female fetuses were more often diagnosed with exacerbation of the course of asthma and a higher frequency of fetal growth retardation.

Meakin et al. [19] also emphasize the difference in the adaptation of the placenta to asthma-associated changes depending on the sex of the fetus, with a higher risk of complications in male fetuses, especially in the presence of exacerbations of asthma. The authors note the presence of a gender-specific difference in fetal development and the chances of its survival in pregnant women with asthma. Thus, females are more likely to be underweight at birth (<2500 g) and underweight at gestational age (<10 centiles), while males are more likely to be born prematurely (<37 weeks gestation) and have a higher risk of stillbirth [19].

Pregnant with BA are more likely to require regular medication during pregnancy [11]. In the first trimester, exacerbation of BA increases the risk of congenital malformations. Garne et al. [11] emphasize the increased risk of congenital malformations due to exposure to BA drugs (β-2 agonists, corticosteroids), despite the inhalation route of administration, which reduces the systemic effects on the body. The authors [11] also add that it is currently unknown how the risk of fetal malformations is affected by etiological factors of asthma (systemic chronic inflammation and hypoxia), the use of drugs for its treatment (corticosteroids [31], short β2-agonists, etc.) or a combination of these factors.

In addition, restoring control of BA as early as possible during pregnancy reduces the risk of exacerbations. Thus, according to the authors [34], in an 8-year study 1208 pregnant women were diagnosed with 468 exacerbations of BA, of which 273 (58%) were moderate and 195 (42%) severe. Moreover, 33 (3%) pregnant had more than one complication. It was also determined that the risk of exacerbations of BA was influenced by the age of the mother (OR=1.05; 95.0% CI [1.02–1.08] p=0.003), the absence of a history of childbirth (OR=0.59; 95.0% CI [0.44–0.81] p<0.001), gestational weight gain during the first trimester (OR=1.22; 95.0% CI [1.17–1.28] p<0.01) and total weight gain throughout pregnancy (OR=1.11; 95.0% CI [1.06–1.17] p<0.001). The authors add that these indicators of increasing risk in relation to maternal age and gestational weight gain are, respectively, calculated for each year and kilogram. Moreover, the age of the mother significantly (p=0.02) affects the severity of complications: OR=1.05; 95.0% CI [1.00–1.10]. A similar trend also persists with respect to gestational weight gain during the 1st trimester (OR=1.20; 95.0% CI [1.13–1.27] p<0.001) and total gesta-

tional weight gain (OR=1.11; 95.0% CI [1.06–1.17] p<0.001) [34].

Chronic bronchitis is also one of the common diseases of the respiratory system, which complicates the course of pregnancy and can lead to fatal consequences. The incidence of complications of chronic bronchitis in pregnancy is 1:1000 in the United States and 6.7:1000 in Taiwan. An important aspect that complicates timely diagnosis is the potential teratogenic effects of radiography and therapeutic agents [35]. Physiological changes in immunity, including T-lymphocyte counts, decreased lung volume, and increased oxygen demand may be important risk factors for complications in pregnant [7, 35]. In addition, it is noted that pregnant women have an increased risk of complications of childbirth compared to pregnant without the mentioned pathology [7]. According to the authors [36], chronic bronchitis during pregnancy increases the risk of prematurity, young children of gestational age, and such patients in most cases have a lower average weight of children.

The findings of Chen et al. [36] indicate a higher incidence of pregnancy complications. Thus, according to the obtained data, patients with chronic bronchitis significantly (p<0.001) more often had children with lower birth weight than in the control group: 9.8% and 5.9%, respectively (OR=1.73 [95.0% CI 1.41–2.12], p<0.001). Premature births were almost twice as common: 12.3% and 7.1% in control (p<0.001) (OR=1.71 [95.0% CI 1.42–2.05], p<0.001). The number of children younger in gestational age than in the control group also prevailed: 20.7% and 16.2%, respectively, p<0.001 (OR=1.35 [95.0% CI 1.17–1.56], p<0.001). Besides, caesarean section was performed significantly (p<0.001) more often in pregnant women with respiratory diseases than in the control group: 55.5% and 40.6%, respectively (OR=1.77 [95.0% CI 1.58–1.98], p<0.001). The development of preeclampsia in such pregnant women was observed almost three times more often, the researchers diagnosed the development of preeclampsia: 2.7% compared to 0.8% in the control group (p<0.001) (OR=3.05 [95.0% CI 2.01–4.63], p<0.001) [35]. The authors emphasize that pregnant with respiratory diseases typically have a high risk of pre- and eclampsia.

It should also be added that Tang et al. [35] among diseases of the respiratory system pay special attention not only to chronic bronchitis, but also the development of pneumonia and its course in pregnant. According to the study, 2 patients out of 12 pregnant with pneumonia died of progressive respiratory failure. The main complications of the process were: septic shock (42%), respiratory distress syndrome in adults (42%), liver and kidney failure (33%, respectively), severe preeclampsia (25%) and stress ulcers (17%). At the same time, intrauterine fetal death was determined in 17% of pregnant women, premature birth in 86%, neonatal fetal death in 14%, cesarean section was employed in 71% of cases.

The pathophysiological mechanism of pregnancy complications in women with chronic respiratory disorders involves severe systemic hypoxia, which naturally leads to placental dysfunction. The primary aspect of the development of hypoxia is the physiological elevation of the diaphragm by almost 4 cm with a natural decrease in lung function and increased by 40% oxygen demand [36].

Placental hypoxia is considered to be a trigger for the expression of antiangiogenic and inflammatory factors that adversely affect the maternal endothelium, provoking the development of endothelial dysfunction, hypertension and target organ damage. In addition, the development of pulmonary edema in this case significantly complicates the course of pregnancy due to a significant deterioration in oxygenation [35].

At the same time, there are some contradictions about the exact mechanisms that shape the development of complications during pregnancy [36]. One of the proposed mechanisms is infection of the placenta from the pulmonary focus and subsequent infection of the fetus. The latter is realized through the umbilical vein or aspiration of the affected amniotic fluid [36].

Hung et al. [37] studied the effect of respiratory failure (RF) on pregnancy. The authors determined that the most common obstetric cause of acute respiratory failure is hemorrhage and hypertensive disorders. At the same time, non-obstetric causes include sepsis and pneumonia. According to the data obtained, respiratory failure was diagnosed in 33.3% of bacterial and 22.2% of viral pneumonia in pregnant women. At the same time, acute fetal distress, meconium aspiration and the incidence of sepsis significantly (p=0.001) prevailed in women with non-obstetric RF (33.3%). Unfortunately, there are very few reliable data on the course of pregnancy, the impact on the condition of the fetus and newborn in pregnant women with chronic bronchitis.

Placental dysfunction (PD) is a significant medical and social problem, which is found in 17–35%

of pregnant women. Almost 70% of miscarriage and 35% of preeclampsia are caused by the development of PD. Up to 60% of cases of PD are detected in pregnant women who have suffered a bacterial or viral infection and up to 45% in the presence of other extragenital conditions. A number of extragenital disorders have been identified that increase the risks of developing PD, including pulmonary, endocrine, cardiovascular, infectious, etc. [22].

Disorders in the fetoplacental complex can significantly complicate the course of pregnancy, significantly affecting perinatal morbidity and mortality [38]. Timely determination of fetoplacental insufficiency markers, especially in women with extragenital disorders, still remains an urgent medical problem.

Decreased uteroplacental circulation and placental ischemia may stimulate the release of TNF-α and IL-6 into the mother's systemic bloodstream, leading to generalized endothelial dysfunction and hypertension.

According to Tamasi et al. [15], one of the mechanisms of development of pregnancy disorders is physiological changes in immunity. Physiological immunosuppression during pregnancy is known to protect the fetus from the mother's immune response to antigens expressed in her body. In addition, the activity of type 2 T-helpers predominates during pregnancy. There is also an increase in regulatory T cells, which depends on the trimester. Tamasi et al. [15] add that a decrease in regulatory T cells may lead to fetal rejection due to maternal immunological activity. In addition, inhibition of natural killer cells (NK cells) by regulatory T cells may cause hypersensitivity to viral infections.

Shah et al. [40] add that during physiological pregnancy, the development of the decidual membrane involves remodeling the stromal vessels of the uterus into epithelium-like decidual tissues. It was determined that the degree of decidualization correlates with the concentration of decidual natural killers, which under the influence of IL-15 produce interferon-γ, which is a necessary substance for remodeling of spiral arteries.

During physiological pregnancy, type 2 T-helpers begin to produce IL-10. Its predominant amount is determined in the trophoblast, and anti-inflammatory action helps in the formation of "mother-fetus" tolerance, provides the development of vascular network of the placenta for sufficient blood supply to the fetus and reduces the activity of MMPs. On the other hand, NK cells induce the development of tolerance to the fetus by the main histocompatibility complex type 1 (GCG-1) [40]. Intrauterine NK cells have been shown to produce angiogenesis factors, including vasculoendothelial growth factor, placental growth factor, and tissue growth factor-β. Their activation of GCG-1 partner causes the production of VEGF, which further affects the middle and extravillous trophoblast, causing its adequate invasion.

The authors [41] consider that the activation of the immune response during pregnancy is accompanied by elevated plasma levels of plasminogen activation inhibitor type 1 (IAP-1), tumor necrosis factor-α (FPN-α) and C-reactive protein (PSA). Due to placental ischemia in preeclampsia, the production of tumor necrosis factor-α, interleukin IL-6 and IL-8 is determined. Under the action of TNF-α, vasodilation is reduced and endothelin-1 (ET-1) production is induced by endothelial cells. Further release of free oxygen forms and activation of lipid peroxidation are also caused by the action of TNF-α.

ET-1, which has a potent vasoconstrictor effect, also plays a significant role in the pathogenesis of obstetric complications of preeclampsia. In addition to hypoxia, ET-1 production is affected by a number of cytokines and the presence of autoantibodies to the angiotensin receptor type 1 (AT1-AA) [40]. Concentrations of ET-1 increase up to 2-3 times in plasma, 4-8 times in the umbilical cord and kidney tissue. However, its levels quickly return to normal after delivery [40, 41].

According to [41], the levels of TNF-α and IL-6 increase 2-3 times in patients with preeclampsia as compared with normal pregnancy in the 3rd trimester. It should be added that hypoxia may be a trigger for the production of endothelin-1, a vasoconstrictor synthesized by endothelial cells. It is noted that the concentration of ET-1 in the umbilical cord of pregnant with preeclampsia is approximately 4-8 times higher than in normal pregnancy. Moreover, the concentration of ET-1 is normalized within 48 hours after childbirth in pregnant with preeclampsia [40, 41].

Shah et al. [40] note that pregnancy is characterized by excessive oxidative stress, and reactive oxygen species (ROS) (O2, superoxide, hydrogen peroxide H2O2 and hydroxyl ion OH) produced by the placenta are compensated by the antioxidant system (AOS) [18]. Hypoxia leads to a decrease in the activity of AOS (superoxide dismutase, glutathione peroxidase, catalase, etc.). This causes an imbalance in the direction of oxidative activity, which stimulates lipid peroxida-

tion and loss of glutathione peroxidase activity of the placenta.

Shah et al. [40] add that in obstetric complications, including hypertensive disorders, overexpression of IL-6 alters the differentiation of monocytes into macrophages. The latter produce TNF-α, which alters the activity of placental vascular adhesion molecules, induces metalloproteinase (MMP) production, trophoblast apoptosis, and expression of the plasminogen-1 activator inhibitor. This mechanism inhibits trophoblast invasion, which in turn leads to placental ischemia [40].

Normal placental function is associated with VEGF and PIGF activity. Their sufficient number in mid-pregnancy is associated with normal placental weight [8]. The development of PD is associated with the existing imbalance of antiangiogenic and proangiogenic factors [38, 40, 42]. The former includes soluble receptor type 1 vasculoendothelial growth factor (sVEGFR-1), the latter is placental growth factor. They have been identified as direct markers of changes associated with PD. Tomimatsu et al. [165] emphasize that excessive activity of these antiangiogenic factors reduces the function of VEGF and PIGF, which stimulates the development of generalized endothelial dysfunction in pregnant with obstetric disorders [42].

However, the authors [38] add that although the influence of these factors is a direct trigger in the development of PD and can serve as valuable diagnostic markers, other clinical, laboratory and instrumental indicators remain the gold standard of diagnosis, including hypertension and proteinuria in the second half of pregnancy. Moreover, the latter are not always the cause of complications of PD and cannot serve as a reliable prognostic criterion. At the same time, the diagnosis of FGR is based on Doppler ultrasound (US) data, although these methods do not allow for accurate differential diagnosis with other conditions, such as constitutionally small fetuses.

Herraiz et al. [38] emphasize that all the outlined factors significantly complicate the preliminary diagnosis of PD, and this leads to its detection at a later stage, which worsens the prognosis and complicates the selection of optimal treatment. Other authors add that such diagnostic limitations actualize the study of the vascular factor system, in particular the sVEGFR-1/PIGF ratio, which may be important in the early diagnosis of PD.

Another element of the development of PD is that vascular endothelial disorders are triggered by placental insufficiency. As a result, it causes peripheral vasoconstriction, with a compensatory increase in the systemic blood pressure of the mother to enhance the flow of oxygenated blood to the intervillous space, which further causes the development of preeclampsia [38]. Shah et al. [40] added that an experiment on the pathogenesis of preeclampsia suggested that decreased uteroplacental perfusion pressure, subsequent hypoxia, and placental ischemia contribute to the release of biologically active substances that cause generalized vascular dysfunction and hypertension.

Shah et al. [40] emphasize that in placental ischemia, the action of ROS reduces the amount of L-arginine in platelets, while the activation of arginase stimulates the transition of L-arginine to urea, not nitric oxide. The latter may explain the decrease in nitric oxide concentrations in pregnant with obstetric abnormalities (preeclampsia) [40]. Interaction of nitric oxide with superoxide ion forms peroxynitrite, which reduces NO activity and inhibits trophoblast growth and stimulates endothelial dysfunction in pregnant. Also, according to the authors [40], the action of nitric oxide increases the concentrations of VEGF and PIGF by reducing the activity of sVEGFR-1 in ischemic trophoblast cells. The pathophysiological mechanism is severe systemic hypoxia, in particular pneumonia, which naturally leads to placental hypoxia. It is noted that placental hypoxia is a trigger for the expression of antiangiogenic and inflammatory factors that adversely affect the maternal endothelium, provoking the development of endothelial dysfunction, hypertension and target organ damage. Endothelial dysfunction is a complex process associated with disruption of homeostasis of constrictive and dilated mechanisms, factors of vasculogenesis and their inhibitors, etc. Thus, extragenital pathology, in particular chronic respiratory diseases, can cause impaired remodeling of the spiral arteries, leading to changes in perfusion in the feto-placental complex and placental dysfunction.

Thus, extragenital diseases negatively affect the course of pregnancy and childbirth, constantly increasing the rates of maternal and perinatal morbidity. Chronic respiratory diseases of pregnant (bronchial asthma, chronic bronchitis) have recently spread among women of childbearing age and can complicate the course of pregnancy and childbirth.

Manifestations of BA symptoms reach their apogee in the late second and early third trimesters, leading to complications that manifest themselves in the form of increased systemic in-

flammation, oxidative stress and the development of hypoxia, especially in the case of uncontrolled recurrence. Exacerbations of BA cause the development of alkalosis in the mother, which impairs uterine blood flow and, consequently, oxygenation of the fetus, which leads to hypoxia and hypercapnia, the possible development of acidotic conditions. This develops such severe obstetric complications as preeclampsia, fetal growth retardation, prematurity, increased frequency of surgical delivery. Patients with chronic bronchitis were significantly more likely to have children with a lower birth weight, younger gestational age and FGR [43, 44].

The course of a normal pregnancy causes significant remodeling of the vascular system to ensure metabolism and adequate metabolism in the "mother-placenta-fetus" system.

Conclusion

Timely identification of placental dysfunction markers in women with chronic respiratory disorders remains an urgent task and requires a comprehensive approach with further research to identify specific markers of pathological changes that can predict placental dysfunction.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

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THE ROLE OF "MICROBIAL FACTOR" IN THE DEVELOPMENT OF ADENOMYOSIS (review)

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Abstract

Endometriosis is a multifactorial disease that affects mainly women of reproductive age. The exact pathogenesis of this disease is still a mystery. The analysis of modern etiology concepts and mechanisms of adenomyosis development was carried out. The review includes data from foreign articles published in the PubMed, UpToDate databases over the past ten years. A number of works presenting studies of the uterus microbiota and its influence on the disease development were analyzed. The possibilities of cultural and molecular genetic diagnostic methods, in particular 16S rRNA, in studying the state of the uterine cavity microbiota are described. The modern paradigm of the development and progression of adenomyosis provides for the presence of endometrium bacterial contamination which, in turn, is a trigger for cell modifications activating a vicious circle of pathology.

Keywords: *pathogenesis of adenomyosis, risk factors, endometrial microbiota*.

Adenomyosis is one of the main problems of modern gynecology, leading to significant violations of reproductive and menstrual functions, disability of patients, dysfunction of adjacent organs, decreased performance and quality of life of women [1]. All of the above determines the relevance of studying the problem and requires the search for new approaches to the diagnosis, tactics of treatment of the disease.

For the first time adenomyosis was defined in 1972 by C. Bird et al. as "benign invasion of the endometrium into the myometrium, leading to a diffuse enlargement of the uterus, which is microscopically represented by ectopic, non-neoplastic endometrial glands and a stroma surrounded by hypertrophied and hyperplastic myometrium" [2].

A search of literature sources in the databases MEDLINE (2016–2021), PubMed (2016–2021) and Science Citation Index Expanded (2016–2021) in order to identify risk factors for the development of adenomyosis showed that a genetic predisposition, inflammation, hormonal changes, extracellular matrix enzymes, and the influence of immunological factors play an important role in the onset and development of adenomyosis and explain the clinical picture of the disease.

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Throughout the history of the study of endometriosis, scientists and criticists put forward numerous theories of its origin. Currently, there are seven main theories and mechanisms for the development of endometriosis. One of the first was the embryonic (dysontogenetic) theory proposed by Recklinghausen (1896) suggesting that cells of the ectopic endometrium could develop from the cells of the Müllerian duct remaining in other tissues after migration [35].

Another theory was developed by N. S. Ivanov (1897), R. Meyer (1903), J. A. Sampson (1921), K. P. Ulezko-Stroganov (1925). It is the implantation and metaplastic concept of the endometriosis origin, according to which adenomyosis can develop as a result of metaplasia from de novo ectopic intramyometrial tissue of the endometrium [25].

Among modern theories, we pay attention to the genetic and epigenetic theory, suggesting the presence of genetic and epigenetic defects in cells, as well as the possibility of hereditary transmission of these defects [26].

The concept of Crain D. A. et al. (2008) [37] is also worth mentioning. It indicates that the development of endometriosis is based on the reprogramming of normal endometrioid cells under the influence of any external stimuli (chemicals, endocrine factors, and changes in the immune status).

To explain the reasons for the development of endometriosis, hormonal and immune theories were formed.

Discussing individual theories of the genital endometriosis pathogenesis, we should submit that none of them can reveal the main pathophysiological mechanism underlying the development of adenomyosis, namely, the penetration of the basal layer of the endometrium into the adjacent myometrium.

Noteworthy is the hypothesis put forward by Bergeron C. et al. (2006) in which adenomyosis is defined as invagination of the basal endometrium in the myometrium due to violations or absence of the uterine "connecting zone", or "transition zone" [11]. The exact trigger for intussusception is not known.

Endometrial invasion can occur during trauma at the border of the endometrium and myometrium [15].

When studying the immunological aspects of adenomyosis, we note that an important role in the disease pathogenesis is played by cytokines, mediators of intercellular interactions involved in proliferation, cell differentiation, tissue repair and remodeling, as well as in the regulation of the immune response [3–5, 8].

Recent publications increasingly discuss the role of infections in the etiology of diseases of the female genital organs [10]. This problem is most relevant in women of reproductive age. It is the reproductive age that is active in terms of sex life, pregnancy, childbirth, and the use of contraceptives.

Chen C. et al. (2017) reported the existence of various bacterial communities throughout the female reproductive tract and their influence on diseases of the uterus [16].

At the turn of the 20th century, Henry Tissier (Tissier, 1900) expressed the paradigm of the sterile uterus, which is one of the stable dogmas [18]. Sterility is maintained by cervical mucus, which provides a barrier to bacteria from entering the vagina [14, 48, 54]. However, the endocervical barrier can be disrupted, which is confirmed by the study by Kunz Getal (1997), demonstrating how radioactively labeled macrospheres reach the uterine cavity within a few minutes after their insertion into the external cervical canal [14].

The subject of research in recent years is the study of the uterus microbiota from the viewpoint of reproductology [17]. Microbiota is a collection of microorganisms living in a separate human biotope, which are in symbiosis with the host organism. Despite the fact that these symbiotic relationships have developed evolutionarily, our understanding of the physiological and pathophysiological role of the microbiota remains largely insufficient [31]. In 2007 the staff of the National Institute of Health (USA), using highly sensitive molecular genetic methods, demonstrated the importance of the physiological role of the microbiota of various biopsies in healthy female volunteers. Samples of biomaterial discharge from the vagina and aspirate from the uterine cavity were studied. To determine the species composition of the microbiota, they used the method of sequencing the 16S ribosomal RNA (rRNA) subunit, which is unique for each bacterium. The obtained data showed that such organs of the human body as the uterine cavity and placenta, which had been previously considered sterile, were colonized by their unique microflora [21, 22, 29, 31].

It should be noted that the researchers paid the most attention to the study of the vaginal biotope microbiota. Normally, the vaginal microbiota of a healthy woman is dominated by lactobacilli, although this indicator is characterized by significant variability and depends on many factors, such as age, hormonal status, age of the first sexual intercourse, pregnancy and childbirth of the menstrual cycle. In addition, sedentary lifestyles, contraceptives, and late pregnancy, common in modern life, can affect the microbiota of the female reproductive system. [7, 27, 42, 51].

Baker J. M. (2018) [14] examined the uterus microbiota in healthy women and identified the following types of bacteria: *Firmicutes, Bacteriodetes*, *Proteobacteria* and *Actinobacteria*.

Chen C. et al. (2017) [16] conducted a systematic study of microbiota samples in 95 women of reproductive age using culture methods. The material was obtained from the lower third of the vagina, posterior fornix, cervical mucus, endometrium, fallopian tubes, and peritoneal fluid. The study showed that genus *Lactobacillus* with low diversity was dominant in the lower third of the vagina and posterior fornix. These samples contained *L. crispatus*, *L. iners*, and another *Lactobacillus* spp. The obtained results are similar to those of other researchers [19, 23, 34]. Cervical mucus samples contain lower amounts of *Lactobacillus* than vaginal samples. *Lactobacillus* was not dominant in the endometrium, and bacteria such as *Pseudomonas, Acinetobacter, Vagococcus*, and *Sphingobium* made up a significant proportion of the microbiota. The content of these bacteria increased in the fallopian tubes, and the average relative abundance of *Lactobacillus* was 1.69%. *Lactobacillus* was absent in peritoneal fluid samples, but contained a diverse microbiota other than the endometrium.

Baker J. M. (2018) [14] also presents possible transmission routes of bacteria (hematogenous, oral, intestinal, canalicular, iatrogenic (during assisted reproductive technologies), intrauterine contraceptive administration. In addition, bacterial colonization of the uterus is associated with adverse reproductive health outcomes, including premature birth, chorioamnionitis, and endometritis [16, 20, 22, 24, 28, 30, 38–41, 44, 49, 50].

The microbiota of the reproductive tract is mainly studied by two methods: cultural and molecular genetics.

Cultural methods have some limitations: the duration and complexity of the study, the need to provide microbiological laboratories with special equipment, strict requirements for the storage and transportation of biomaterials [9, 12–14, 45, 53]. Moreover, a new approach to the study of the microbiota of the reproductive tract, in particular, the uterine cavity, using molecular genetic research methods has recently appeared. Most studies of endometrial microbiota have been carried out using the next generation sequencing method (NGS sequencing), an expensive approach which is poorly adapted for practical health care system [14, 31–33]. The most suitable for every day research is the molecular genetic method (Polymerase chain reaction – PCR) in real time. Currently, the use of molecular genetic research methods allows identifying associations of difficult to cultivate and uncultured microorganisms on the surface of the endometrium in women of reproductive age [30, 46, 47, 52].

Hilier S. et al. (2013) [24] conducted a study of 136 women with chronic pelvic pain who underwent pipel biopsy of the endometrium, followed by histological examination and microbiological assessment of the endometrium using PCR. In 55 (40%) women with clinical signs of chronic pelvic pain, endometritis was histologically confirmed. A wide spectrum of bacteria was obtained from 53 endometrial samples, represented by 63 different species, including 8 species of opportunistic microorganisms. The presence of true pathogens such as *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis* in endometrial specimens was associated with endometritis (29% vs. 6%, $p<0.001$). Among opportunistic microorganisms with histologically confirmed endometritis, G. vaginalis (35% versus 16%, $p=0.01$) and *A. vaginae* (22% versus 3%, $p<0.001$) were significantly more often detected.

Cicinelli E. et. al (2012) [43] assessed the uterine cavity microbiota in women of reproductive age with infertility and miscarriage by PCR. *Lactobacillus spp.* was detected in 86.1% of cases, opportunistic microorganisms were identified in 36.1% of the samples, including 22.2% in combination with lactobacilli and in 13.9% without lactobacilli.

Swidsinski et. al (2013) [36] used the FISH fluorescent hybridization probes to detect *G. vaginalis, A. vaginae, Lactobacillus, Bacteroides, Prevotella, Enterobacteriaceae,* and *Eubacteria*. The study showed that the microbiological environment of the endometrium differs from that of the vagina.

Mitchell M. et al. (2015) [29] studied uterus samples of 58 women. Material for research was obtained from the upper part of the endocervix and the body of the uterus after opening it under sterile conditions. Vaginal discharge was collected before surgery. The analysis was performed using the 16S rRNA sequencing method. Microbial contamination of the uterine cavity was detected in 55 (95%) patients, 52 of them had only 1 type of microorganisms. The most common species were: *Lactobacillus iners (L. iners)* (45% of women had it in the uterine cavity and 61% of women – in the vagina), *Prevotella spp.* (33% of patients had it in the uterine cavity, and 76% – in the vagina), *Lactobacillis crispatus (L. crispatus)* (33% – in the uterine cavity, 56% – in the vagina). *G. vaginalis, A. vaginae* and *Lactobacillus jensenii* (L. jensenii) were found in the vagina in more than 40% of women, but much less frequently in the uterine cavity (*G. vaginalis* in 19% of women, *A. vaginae* in 10% of women, and *L. jensenii* – in 20%). The uterine cavity colonization by microorganisms was significantly lower than that of the vagina. The endometrium inflammation markers did not significantly differ in women who did not have microorganisms in the uterine cavity compared to those who had only lactobacilli or microbes associated with bacterial vaginosis.

Verstraelen H. et al. (2016) [40] studied the composition of the endometrial microbiota using 16S rRNA sequencing in 19 patients with implantation failures and miscarriage. To obtain material in order to exclude contamination with the vaginal microflora, the authors used a Tao Brush cytobrush surrounded by a transparent casing that protects the sample taken from the endocervical and vaginal discharge. As a result of the study, 15 types of microorganisms were represented in all samples. 90% of patients had a similar composition of the endometrial microbiota, where *Bacteriodes xylanisolvens, B. thetaiotaomicron,* and

B. fragilis predominated. In 6 women, *L. crispatus* or *L. iners* predominated in the presence of *Bacteriodes*. The results of this study are consistent with previous evidence of dysbiotic shifts in the endometrial microbiota in the absence of a predominance of lactobacilli, and such disorders are most common in the sub fertile population.

Franasiak J. M. et al. (2016) [22] studied 33 patients admitted for embryo transfer into the uterine cavity. Analysis of the uterine cavity microbiota was performed using the 16S rRNA sequencing method. As a result, 35 samples of biomaterial were received: 33 samples were obtained from patients and 2 control samples containing *Escherichia coli*. Pregnancy occurred in 18 women, and did not occur in 15 patients. In total, the presence of 278 different genes of microorganisms was registered in the samples under study. The uterine cavity microbiota during embryo transfer in both groups was characterized by the predominance of lactobacilli.

Moreno I. et al. (2016) [30] carried out a comparative analysis of the microbiota of paired samples of endometrial aspirate and vaginal discharge in 13 fertile women. As a result, *Lactobacillus* was identified in 71.1%, *Gardnerella* was detected in 12.6%, *Bifidobacterium* – in 3.7%, and *Prevotella* – in 0.9% of women. Patients, depending on the microbial composition of the endometrium, were divided into categories with a predominance of *Lactobacillus* (more than 90%) and without a predominance of *Lactobacillus* (more than 10% of bacteria other than *Lactobacillus*, such as A. *vaginae, G. vaginalis,* species of the *genera Clostridium, Megasphaera, Parvimonas, Prevotella, Sphingomonas* or *Sneathela*). 18 out of 26 women showed stable microbiota profiles, 12 of them were assigned to the *Lactobacillus*-dominated group, 6 women – to the *Lactobacillus*-free group. Thus, the composition of the bacterial community in most healthy fertile women was relatively stable.

The endometrial microbiome in infertile patients was assessed in a study conducted by Tao X. et al. (2017) [38]. The study included samples of endometrial microbiota obtained from 70 patients who underwent the *in vitro* fertilization (IVF) program. 33 samples contained more than 90% of lactobacilli and 50 samples contained 70% of lactobacilli. In addition to lactobacilli, opportunistic pathogens were identified: *Corynebacterium spp.* was detected in 40 women, *Bifidobacterium spp.* was identified in 15 patients, *Staphylococcus spp*. was in 38, and *Streptococcus spp.* was in 38 women.

Thus, molecular genetic research methods allow assessing the relationship between the endometrial microbiota and the frequency of embryo implantation in the IVF program. The uterus microbiota study is extremely important in reproductology.

Noteworthy are reports of differences between microbiome profiles in healthy women and women with endometrial polyps and chronic endometritis. Fang et. al [20] examined women in three groups: group I included healthy women, group II consisted of women with endometrial polyps, and group III had patients with endometrial polyps on the background of chronic endometritis. As a result, the found statistically significant content of *Firmicutes, Lactobacillus, Gardnerella, Bifidobacterium, Streptococcus,* and *Alteromonas* in the vagina and uterus samples of groups II and III compared with the group of healthy women. The detection of *Lactobacillus* more than 3.0 times in the uterine microbiome of patients in groups II and III, compared with healthy controls, may indicate the growth of vaginal bacteria.

The surveyed women without uterine leiomyoma had a higher number of *Lactobacillus spp.* in vaginal and cervical secretions, while women with uterine leiomyoma had abundant *L. iners* in cervical mucus [6].

Thus, there are more and more new data indicating that the microbiota of the female genital tract is important for women's health. Today the outdated concept of uterine sterility can be argued about, although the determination of the true uterus microbiota in normal conditions and in adenomyosis requires further detailed research.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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SIMULATION OF EPIDEMIC PROCESSES: A REVIEW OF MODERN METHODS, MODELS AND APPROACHES

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Abstract
The paper is devoted to an overview of the current state of research on the modeling of epidemic processes. The classification of mathematical and simulation models of epidemic processes is carried out. The disadvantages of classical models are revealed. Specific characteristics inherent in epidemic processes have been determined, which must be taken into account when constructing mathematical and simulation models. A review of deterministic compartment models is carried out. Various methods and approaches to the construction of statistical models of epidemic processes are considered. The types of problems that are solved using machine learning are analyzed.
Keywords: *epidemics, forecasting, infected organism, infections, mathematical modeling, simulation modeling, susceptible organism*.

INTRODUCTION

Epidemics and pandemics of infectious diseases have accompanied the entire history of mankind. New emergent infections continue to appear, while old infections, which humanity has already learned to fight, return. The spread of the SARS-CoV-2 pathogen, first identified in December 2021, has led to a pandemic that has been going on for almost two years. The current COVID-19 pandemic has affected all aspects of human life and reiterated the importance and need for tools to prevent, prepare, detect and respond to epidemics and pandemics. Therefore, it is necessary to be able to predict epidemics, i.e., to determine the probability of occurrence, scale of development of epidemics and their consequences in order to develop and justify measures to prevent the spread of infectious diseases among the population and eliminate the socio-economic consequences caused by epidemics. An epidemic is a progressive spread of an infectious disease among humans that significantly exceeds the usual incidence rate in the area and can cause an emergency. However, the basis of any epidemic is an epidemic process – the continuous transmission of an infectious disease agent among humans – from the source of the infectious agent through transmission mechanisms to the susceptible organism.

Simulation of the epidemic process is a tool that is used to study the mechanisms of the spread of diseases at the population level, predict a possible increase in the development of an outbreak, and assess the feasibility and rationality of strategies to combat the epidemic.

The aim of the review paper is to classify models of epidemic processes and to analyze current state of researches in field.

1. Classification of epidemic process models

The following types of models of the epidemic process are distinguished:

- a stochastic model is a tool for estimating the probability distributions of potential outcomes by allowing for random variation in one or more inputs over time. Stochastic models depend on random variations in the risk of disease occurrence and spread;
- when working with large populations, deterministic mathematical models are often used. In the deterministic model, individuals in a population are divided into different subgroups, any of which represents a specific stage of the epidemic;

The coefficients of transition from one class to another are mathematically defined by derivatives, and the model is described by the corresponding differential equations. When creating such models, it is assumed that the function of population change is differentiated over time, and the epidemic process is deterministic. In other words,

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population change is calculated using only the background history used to estimate the model parameters.

The simplest definition of epidemic dynamics considers the total population in the system as a fixed one, consisting of N individuals and ignoring any other demographic process (migration, birth, etc.). One of the simplest possible compartments is the SIS model with two possible transitions: the first labeled S → I, occurs when a susceptible individual interacts with an infected individual and becomes infected. The second transition, designated I → S, occurs when an infectious individual recovers from illness and returns to the susceptible pool.

The SIS model suggests that disease is not immune and people can be infected over and over again by undergoing the S → I → S cycle, which under certain conditions can be sustained forever.

Another basic model is the classic three-state SIR model. In the SIR model, the I → S transition of the SIS process is replaced by I → R, which occurs when an infectious individual recovers from an illness and is considered to have acquired permanent immunity or has been removed (e.g., died).

2. Formalization of epidemic processes

Classic epidemic models do not take into account many factors, which reduces the accuracy of modeling and the reliability of the dynamics of the epidemic process under consideration.

Among these factors are the following:

- vertical transmission. In the case of certain diseases, such as HIV infection and hepatitis B, the offspring of the parents may be born infected. This transmission of disease from the ancestor is called vertical transmission. The appearance of additional members in the category of infected can be considered within the framework of the model, including the proportion of newborn members in the infected cell of the environment [1];

- vector transmission. Diseases transmitted from person or animal to person through a vector, that is, the spread of malaria by mosquitoes or Lyme disease through ixodid ticks, are transmitted through a vector. In these cases, the infection is transmitted from person to vector, and the epidemic model should include both, usually requiring much more properties than the direct transmission model [2];

- population heterogeneity;

- age groups of the population;

- variable infectivity. As a result of seasonality or other influencing factors;

- heterogeneity of the environment;

- immunity acquired through vaccination.

To eliminate this disadvantage and take into account the above factors, it is proposed to use an intelligent multi-agent approach to modeling the epidemic process of the population dynamics system. For this, it is necessary to distinguish a class of models of the epidemic process among the models of population dynamics.

The following characteristics are specific of the epidemic process:

- *cyclicity (periodicity)* of the epidemic process is an increase or decrease in the incidence of the population, regularly repeating in long-term dynamics. Most of the manifestations of cyclicality are explained by the infectious-immunological relations of populations. An increase in the susceptible layer due to fertility and migration determines the formation of a pathogen with higher epidemic potential and an increase in morbidity. The activation of the epidemic process, in turn, is accompanied by an increase in the layer of immune cells, which reduces the epidemic potential of the pathogen and determines the decline in the incidence even before the layer of susceptible ones is exhausted. Identification of long-term cyclicality is important for the development of forecasts of morbidity and the development of rational preventive measures;

- *irregular ups* and downs in morbidity in long-term dynamics arise in connection with episodic changes in social and natural factors. These changes contribute to the formation of pathogens with a high epidemic potential, which leads to the development of epidemic outbreaks or epidemics. Epidemics develop in times of war, after natural disasters, with omissions in the conduct of highly effective anti-epidemic measures. Any pronounced migration processes are accompanied by the development of epidemics.

The annual dynamics of the incidence is characterized by regular increases and decreases in the incidence, the so-called seasonal and off-season periods. The interval that includes the first month of a seasonal increase in incidence in one year and the month preceding a new seasonal increase in incidence the next year is called an epidemic year. When considering different epidemic processes, different seasonality stands out. For example, they usually talk about the winter seasonality of aerosol infections and the summer seasonality of intestinal infections, when considering viral marketing on the Internet, autumn-spring seasonality is observed, etc. However, the specific manifestations

of seasonality are individual for different forms of the epidemic process, and within individual forms, they are individual for different territories and for separate groups of the population in the same territory. Seasonality is one of the most intelligible manifestations of the phasic nature of the development of the epidemic process. It is here that interepidemic and epidemic periods associated with the phases of reservation of the pathogen and its epidemic spread are easily detected.

In the annual dynamics of morbidity, in addition to periodically recurring seasonal epidemics, epidemics develop that do not have a regular recurrence or are characterized by one or another relatively local manifestation. The limits between these concepts are relative since the seasonal epidemic is accompanied by an increase in the number of affected populations in each of them.

When forecasting, there are three types of forecasts: short-, medium- and long-term. At the same time, experts studying the epidemic process are only interested in forecasting an epidemic outbreak, that is, the period of "epidemic-recession" [3]. This is because the dynamics of the process can change the distribution rules after an outbreak as a result of the anti-epidemic measures taken, as well as the natural extinction of the infected population.

3. Deterministic compartment models

The most popular approach to modeling epidemic processes is deterministic models based on the use of systems of differential equations and the use of compartments that characterize the state of objects in the population. The first such type of models was applied by W. Kermack and A. McKendrick [4–6], who expanded the model of R. Ross and H. Hudson [7], and built a model based on the types S (susceptible), I (infected) and R (recovered) to study the epidemic nature of infectious diseases. The first to study the models of epidemic actions on the terrain of Ukraine and the USSR were L.A. Rvachev and O.V. Baroyan, who used this approach to model the incidence of influenza [8–9].

Compartment models are popular now, and many modern studies are based on their extension [10]. M.B. Trawicki [11] considers the extension of the model, adding the state E (incubation period) and additional transitions between the compartments. The proposed model takes into account the dynamics of life activity with unequal fertility and mortality rates, vaccination of infants and non-newborns, as well as temporary immunity from an infectious disease. In this case, the recovered have only temporary immunity from an infectious disease and can potentially go back to the susceptible class. The study authors [12] add states H (hospitalized) and D (dead). The model explored policies that included large-scale quarantine, tight travel controls, monitoring of suspected cases, and social distancing.

The study [13] considers the possibility of close contact and the latent course of morbidity. The model included four compartments based on COVID-19 incidence data in Wuhan and was supported by data collected from Italy, the United Kingdom, and the United States. The model reflects the effectiveness of various disease containment measures through three changing factors: contact ratio per capita, which can be reduced by social distancing; the likelihood of infection through contact with infectious individuals, which can be reduced by wearing face masks, maintaining personal hygiene, etc.; and populations of infectious individuals in contact with susceptible populations, which can be reduced through quarantine. The model was used to predict the best approach to breaking out of lockdown.

In [14] the classical model is extended by the state Q (quarantine) and V (vaccinated), and the spread of coronavirus disease in Saudi Arabia is investigated. The mathematical analysis illustrates the inalienability, limitation, epidemic equilibrium, the existence and uniqueness of endemic equilibrium, as well as the basic reproduction number of the proposed model. To improve the classical model and find little-known parameters, the authors applied a data assimilation structure based on Kalman filters to estimate state parameters to improve the prediction parameters of the model.

The authors of [15] use step-by-step modeling, taking into account the edges, which eliminates the assumption that all people have the same frequency of contacts, and partnerships are fleeting, having a classical model. The authors derive models of simple ordinary differential equations that reflect social heterogeneity (heterogeneity of contacts) and take into account the effect of the duration of the partnership. The paper also provides a graphical interpretation that makes it easy to derive and communicate the model and apply the technique with different assumptions about how the frequency of contacts is distributed and how long the partnership lasts.

Study [16] proposes a non-linear model of the COVID-19 epidemic that simulates the spread of coronavirus influenced by social distancing caused

by government measures to halt the spread of coronavirus. At the same time, the study focuses specifically on the impact of public policies aimed at containing the pandemic. [17] investigated the effect of travel from other US states on common infections in the destination state and found a strong inverse correlation of 0.98 between the index of contagiousness and the compartment of social awareness, that is, people who are no longer susceptible to infection. This study uses a compartmental metapopulation model to represent the correlation between exposure and mobility indices and the likelihood of susceptibility to infection. A wealth of cellular data has made it possible to study many aspects of user mobility, including their travel, contact, and residence patterns.

In [18], a model structure is proposed for predicting new outbreaks of tuberculosis based on compartment models that include properties such as, for example, the immigration of infected people from countries with a high prevalence. In addition, the aspect of trained immunity is taken into account in the model. Using a mathematical approach, a system of ordinary differential equations that can be developed for several points in time, different levels of infection or attack were obtained, which led to different effects of vaccination, depending on the setting of certain parameters and initial values in the vaccine compartments.

[19] examines compartment models of epidemic processes from the point of view of economics. It proposes three distinct areas in which economists could contribute or provide information to the epidemiological literature: modeling the heterogeneity of susceptible populations in different dimensions, taking into account the endogeneity of the parameters governing the spread of disease, and helping to understand the importance of political economy in disease control. Case and death projections based on these models are discussed, which went not so much with the early projections, but how they adapted to the current COVID-19 pandemic.

In [20], the spread of the incidence of COVID-19 in Ukraine is investigated using a compartment model extended by the F (lethal) state, and [21] compares this model with regression methods for the territory of Ukraine before vaccination.

Despite the high popularity of compartment models, they have several disadvantages [22], the main of which are low accuracy and the complexity of introducing changes into the model. In particular, the dynamics of the epidemic process lead to an increase in the virulence of the infection, which changes the behavior of its spread. This requires changes in the model, and in the case of using systems of differential equations, it leads to restructuring and calibration of the model from the very beginning.

To improve the accuracy of models of epidemic action, some studies are trying to combine traditional models with other approaches. For example, the study [23] describes a parametric bootstrap approach for generating simulated dynamic system data for quantifying uncertainty and identifying parameters. The confidence intervals and root-mean-square errors of the distributions of the estimated parameters were calculated to assess the identification of the parameters. To demonstrate this approach, it is applied to a low complexity SEIR model that corresponds to pandemic influenza, Ebola, and Zika virus applications. In [24], deep learning methods were applied as an alternative with less dependence on data to estimate the transmission parameters of an individualized compartment model to model the dynamics of the coronavirus disease epidemic in the United States and predict further development. As a result, a comparative model was built and a multi-stage deep learning methodology was developed to estimate the transmission parameters of the model. Then the estimated transmission parameters are loaded into the model to anticipate the development of the COVID-19 epidemic in the United States for 35 and 42 days.

4. Statistical models

More accurate models of epidemic actions are statistical methods for studying time series [25]. Let's consider some applications of such approaches to real problems.

The most popular statistical method used to model epidemic dynamics is the moving average method and its derivatives. Thus, the simple moving average method is applied to modeling COVID-19 in Iraq [26], USA [27], Pakistan [28], Italy and Spain [29], China [30], etc. Also popular are such derivatives of the method as the exponential moving average [31–33], ARIMA (autoregressive moving average) [34–36], and SARIMA (seasonal autoregressive moving average) models [37–39].

Statistical analysis is also used to quickly study the epidemic process of emerging diseases or new outbreaks of already known diseases [40], which is gaining new relevance with the rapid spread of COVID-19. Examples of such models have been applied to SARS [41–42], H1N1 influenza [43–44],

Ebola [45], foot and mouth disease [46], COVID-19 [47–48], etc. The main difference from previous tasks is that outbreak assessments are needed to determine effective control measures. It is impossible to wait for the outbreak to end and to use the definitive data on infectious disease virulence parameters and associated parameters. Instead, it is necessary to conclude early in the outbreak's growth. In addition to less data, this also poses the risk of biased assessments because people infected in the early stages of an outbreak are usually not presented to the community as a whole.

5. Machine learning models

Methods for studying epidemic processes based on machine learning are now most accurate. Among them are the following tasks.

Regression. Regression is a predictive approach for examining the relationship between the dependent and independent variables. Separately, parametric equations can be evaluated taking into account all data. These parameters include morbidity and weather [49], the level of population heterogeneity [50], the mortality rate [51], vaccination [52], restrictive measures [53], etc. Regression analysis models are used to show or anticipate the relationship between a process and what the process might trigger. However, such a correlation does not always show causality, so the interpretation of such correlations is another important task. Linear regression [54], logistic regression [55], polynomial regression [56], Ridge regression [57], Lasso regression [58], and others are used, depending on the types of data and the tasks set.

Classification. The classification task is aimed at separating objects according to predefined classes. In the study of epidemic processes, classification methods are applied to various objects: determination of population groups by behavior [59], determination of Spatio-temporal features of the epidemic process [60], distribution of available information about outbreaks [61], identification of climatic zones affecting morbidity [62], determination of geographic zones depending on various infectious diseases [63], etc.

Clustering. The clustering task is aimed at separating objects in the case when the classes are not predefined, and the clusters must be formed according to the similarity of certain characteristics of the elements. In this case, the number of clusters can be determined by the researcher in advance, or by the model itself. Also, the researcher can determine the features according to which the sample needs to be divided independently, or the model will do it on its own. Investigating epidemic processes, models, and methods of clustering are used to solve such applied problems as the determination of geographic territories based on similar signs of the epidemic process [64], the determination of epidemic outbreaks [65], the determination of groups of carriers of infection [66], the determination of the phylogenetic characteristics of individuals of the population [67], determination of patterns of infection spread [68], etc.

Dimension reduction. This is the reduction of a larger number of features to a smaller one for the convenience of their further use. In epidemiological diagnostics, this is an extremely urgent task, since the data collected by institutions and government centers does not depend on their importance for modeling. Therefore, dimensionality reduction methods help to discard unnecessary data on morbidity [69], reduce computational complexity [70], and identify informative signs [71] and factors influencing the epidemic process [72].

Identification of anomalies. The anomaly detection task is designed to detect abnormal deviations from normal cases. The task is akin to classification, but it has a significant difference: anomalies are a rare phenomenon, so there are either very few samples on which a model can be taught, or there are none at all. Therefore, other methods are used for this. In the study of epidemic processes, such methods are used to process morbidity data in real-time [73], monitor trends in the flow of morbidity data [74], and identify epidemic outbreaks [75].

Forecasting. The forecasting problem is most common in the analysis of epidemic processes. Forecasts are calculated taking into account the social dynamics of the processes [76], the nature of the outbreak [77], geographic features [78], trends in epidemic processes in the early stages of the outbreak development [79], the impact on the population [80], data from social networks (Twitter [81], Facebook [82], etc.), queries from search services [83], data from mobile operators [84], and many other factors.

The main disadvantage of machine learning methods in the study of epidemic processes is the interpretation of the results. It is usually impossible to identify factors influencing the dynamics of morbidity and conduct experiments on the effectiveness of preventive measures.

6. Further development of epidemic processes simulation

To study epidemic processes and assess the influence of factors and the effectiveness of va-

rious measures, it is advisable to use agent-based simulation. In this approach, objects of the population, that is, people, act as agents. Each agent is characterized by many states and characteristics. The transition between states occurs through events, which can be interaction with other agents, with the external environment, etc. The interaction between agents and changes in their states affect the overall system, so the introduction of changes into the model and its research is much easier than using other approaches.

The disadvantage of the agent-based approach to modeling epidemic processes is the low accuracy of the model. Therefore, a promising area is the combination of agent-based models and machine learning [85].

CONCLUSIONS

The analysis of models and methods for studying epidemic processes carried out in the article showed that different modeling approaches are used for various tasks. At the same time, the greatest number of shortcomings has the classical deterministic compartment models. The greatest accuracy is shown by machine learning methods, but they do not allow conducting experimental studies with epidemic processes to identify factors influencing the epidemic process. Therefore, the most effective is the combined use of an agent-based approach to simulating epidemic processes with machine learning methods.

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Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

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PREDICTING THE EFFECTIVENESS OF MYOPIA CONTROL WHEN USING ORTHOKERATOLOGICAL LENSES BASED ON INDIVIDUAL EYE PARAMETERS

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ABSTRACT

Introduction. The prevalence and progressive course of myopia is one of the most important medical and social problems worldwide. In recent years, in our country and abroad a tendency to increase the incidence of myopia, becoming in some countries an epidemic, is observed. In recent years, the most common method of effective control of myopia is the method of refractive therapy with orthokeratological lenses. **Aim:** to develop criteria for predicting the effectiveness of myopia control using orthokeratological lenses based on individual eye parameters. **Object and methods.** A total of 60 children (117 eyes), who were selected by OKL of combined design, SkyOptix, licensed by KATT Design Group (Canada), were included in the clinical study. The average age was 11, from 7 to 14 years, of which 37 were females (61.7%), 23 males (38.3%). Ophthalmological examination consisted of visometry without correction and with optical correction, autorefractometry on the narrow pupil and in the state of drug cycloplegia, biomicroscopy, biometry, ophthalmoscopy of the central and peripheral fundus, keratotopography of the horns, pupilometry. **Results.** Among the studied people, the average refractive index at the beginning of the study was –2.25 [–3; –1.5] diopters. The initial diameter of the pupils was determined from 2.78 to 6.30 mm according to the pupilometry performed on the topograph. The average values of eccentricity (Ex) studied in the flat meridian averaged 0.51 [0.47; 0.58], in the steep – 0.53 [0.43; 0.59] at the beginning of the study. In our study, the keratometry of the cornea averaged 43.5 at the beginning of the study [42.7; 44.4]. **Conclusions.** Our findings show that when examining a child with progressive myopia, it is important to pay attention to the diameter of the pupil in photographic conditions, because it can be a predictor of progression and influence the choice of correction individually. The smaller the value of keratometry before the appointment of refractive therapy, the greater the value of the size of the APS, so this factor can be indicated as prognostic. The differential topographic force of the cornea along the peripheral ring corresponding to the reverse zone of the lens is a prognostic practical factor. Taking into account the primary parameters of the eye allow to customize the approach to each child with myopia, improving the individual design of orthokeratological lenses.

Keywords: *myopia, axial size of the eye, orthokeratology lenses, pupil size*.

INTRODUCTION

The prevalence and progressive course of myopia is one of the most important medical and social problems worldwide. In recent years, in our country and abroad, a tendency to increase the incidence of myopia, becoming in some countries an epidemic, is observed. [1, 2]. According to statistical forecasts, the number of nearsighted people is expected to reach 5 billion by 2050, which will be almost 50% of the world's population (up to 50.4% in Eastern Europe). Moreover, the number of patients with a high degree of myopia will increase from 2.7% to 9.8% [2]. In the structure of ophthalmic pathology of Ukraine among the 18-year-old population and older myopia is 12.38%, ranking second among diseases of the visual organ [3]. Statistics for 2014–2017 show that among children 0-6 years the frequency of registration of myopia is 3.68 per 1000 children of the correspond-

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ding age, in 7–14 year-olds 10 times higher (35.57 per 1000 children of the corresponding age), in adolescents aged 15–17 years – 23 times higher (84.86 per 1000 adolescents of the appropriate age) [4]. In addition, progressive myopia is one of the most common diseases in the structure of childhood disability, accounting for 80% of all detected eye pathology and 32.7% in the structure of childhood blindness in Ukraine [4–8]. Therefore, myopia is not only a medical and social but also an economic problem: in developed countries, significant financial resources are spent on measures to prevent and treat this disease.

For today, in the arsenal of practical work of an ophthalmologist there are a huge number of methods of hardware, conservative and surgical treatment. It was previously thought that incomplete correction may slow the progression of myopia, but randomized trials have shown that the anteroposterior size of the eyeball increases more. Various designs of optical correction with bifocal, progressive glasses, multifocal contact lenses are offered, numerous results are obtained, which testify both to and against the mechanism of myopia stabilization. [9–19].

In recent years, the most common method of effective control of myopia is the method of refractive therapy with orthokeratological lenses (OKL) [9, 10, 20–26]. It has been proved that the change in the curvature of the outer surface of the cornea and, as a consequence, the change in the refraction of the eye under the influence of OKL is due to changes in the architecture of the corneal epithelium. The hydrodynamic forces arising in the lacrimal layer under the OKL affect the epithelium. Because of the action of OKL, myopic design there is a flattening of the epithelium in the central zone and its thickening (zone of increased curvature) on the middle periphery of the cornea. As a result, the optical zone of the cornea is formed, which provides high visual acuity during the day. Experimental evidence has been obtained that effective inhibition of myopia progression by OKL is based on a change in the nature of peripheral refraction. A number of authors describe the development of peripheral myopic defocus, which slows the growth of the anteroposterior size of the eyeball [15, 24, 27–31]. Other researchers believe that the use of OKL changes corneal aberrations and increases accommodation reserves, and because of these changes, the progression of myopia stabilizes. [32–36].

In addition, the effectiveness of the method varies from patient to patient; the question arises as to why, and on what factors it depends. The study of these indicators may shed light on the mechanisms of slowing the progression of myopia in children and adolescents using refractive therapy OKL. The above information is due to the need to develop criteria for predicting the course of myopia when using OKL, taking into account the individual parameters of the patient's eye.

Purpose, subjects and methods:

1. The purpose of the study was to develop the criteria for predicting the effectiveness of myopia control using orthokeratological lenses based on individual eye parameters.

2. Subjects & methods

Research design. The study was performed at the Department of Ophthalmology of Kharkiv National Medical University, on the basis of the Pediatric Ophthalmology Center "Raduzhka", Kramatorsk, with which a contract was concluded. All studies were conducted in compliance with basic bioethical norms and requirements of the Declaration of Helsinki adopted by the General Assembly of the World Medical Association, the Council of Europe Convention on Human Rights and Biomedicine (1977), the relevant WHO regulations, the International Council of Medical Societies, the International Code of Medical Ethics) and the Order of the Ministry of Health of Ukraine No.690 on September 23, 2009. The study was initiated after obtaining the patient's consent and informed consent of one of his parents to participate in a clinical examination in accordance with the UN Convention on the Rights of the Child.

A simple cohort case study was initiated after the patient's consent and the informed consent of one of the parents to participate in a clinical trial in accordance with the provisions of the UN Convention on the Rights of the Child.

Obtaining formal consent to participate in the study.

a. The research doctor received the document of consent from the participants of this study.

b. Consent to participate in the study was given by the parents of the sick child in writing at the medical center after diagnosis.

Sixty children (117 eyes), who were selected by OKL of combined design, SkyOptix, licensed by KATT Design Group (Canada), were included in the clinical study. The average age was 11 [10; 13], from 7 to 14 years, of which 37 were females (61.7%), 23 males (38.3%). According to the degree of myopia, the patients of the main group were distributed as follows:

• patients with mild myopia – 15 patients (27 eyes – 23.1%);

• patients with moderate myopia – 45 patients (90 eyes – 76.9%).

Criteria for inclusion in the study:

• children aged 7 to 14;

• diagnosis of uncomplicated mild to moderate myopia;

• informed consent of the patient and his/her parents.

Verification of the diagnosis of refractive error (myopia), its type and degree were performed based on the history data, the results of general and instrumental ophthalmological examination according to the order of the Ministry of Health of Ukraine No.827 on December 08, 2015. Therefore, the patients underwent a standard examination of a child with myopia, which included determining complaints, detailed history (duration of the causative disease, treatment methods used), heredity, growth rate of the child, comorbidities.

The general ophthalmological examination consisted of visometry without correction and with optical correction, autorefractometry on the narrow pupil and in the state of drug cycloplegia, biomicroscopy, and biometry, ophthalmoscopy of the central and peripheral fundus.

The standard ophthalmological examination did not involve a detailed study of corneal asphericity, thus we additionally performed corneal corneotopography (Keratotopograph, Easygraph, Germany), pupilometry, determination of peripheral refraction of the eye with calculation of algebraic refractive index (Optorefractometer).

Biometric investigations were performed using an ultrasound scanner (A-scan, PIROP, Poland). Before starting the work, the device was calibrated according to the instructions for use. The data of 10-fold automatic registration was displayed on the screen with the final average value, which was taken into account as a result. Indications were recorded in mm with an accuracy of 0.01 mm. Biometrics were performed before the start of refractive therapy or the appointment of glasses and every 6 months of follow-up.

Determination of the anterior surface of the cornea, namely the eccentricity of the flat and steep meridians, keratometry, corneal astigmatism was performed using corneal corneotopography (Keratotopograph, Oculus Easygraph, Germany).

Pupilometry was performed using the keratotopograph mentioned above. The lighting conditions were the same; the red light corresponded to the photographic conditions of the survey. This corneotopograph has a large bell, so in close contact with the patient's face, the effect of ambient lighting is negligible. For the reliability of the obtained data, pupilometry was performed each time during a scheduled visit of patients. Namely, pupil diameter was measured at 1, 7, 14 days, 1 month and every three months to obtain a mean numerical value for each patient. In our study, we studied exactly those parameters of the anterior segment of the eye, which we believe may affect the results of effective use of OKL. The method of constructing logistic regression models was used to analyze the probability (increase in APS for two years).

OKL were selected according to the selection protocol with mandatory lens centering. Patients used lenses every day during night sleep. The duration of sleep was agreed by the parents and should have been lasted at least 8 hours.

Statistical data processing was performed using Statistica 10.0 software. The conformity of the analyzed parameters of the law of normal distribution was evaluated by the values of Kolmogorov-Smirnov, Lilliefors and W-criteria Shapiro-Wilk tests. Since in most cases the distribution did not comply with the law of normal distribution, the data are presented in the form of the number of observations in the group, the median and the interquartile range. Assessment of the statistical significance of differences in indicators in the compared groups was performed using a non-parametric criterion for independent groups - Mann-Whitney rank test. The significance level p was assumed to be 0.05, which meets the criteria adopted in biomedical studies. If the value of p was less than 0.001, then p was indicated in the format $p<0.001$.

Results & Discussion

The average refractive indices at the beginning of the study were –2.25 [–3; –1.5] diopters; after a year of observation, this figure did not change significantly. At the beginning of the study, the eye length of the APS was 24.33 [23.72; 24.65] mm, after 2 years of observation 24.37 [23.79; 24.82] mm.

The initial diameter of the pupils was determined from 2.78 to 6.30 mm according to the pupilometry performed on the topograph. The average pupil diameter was 4.52 [4.07; 5.02] mm. To assess the influence of the patient's pupil diameter on the rate of myopia progression, a correlation analysis was performed, which showed that with a weak degree of myopia, the base pupil diameter had an inverse correlation (–0.53, $p=0.001$) with the annual progression gradient (AGP). That is, the larger the pupil, the

less progress of myopia, because the growth of APS is slower.

The average values of eccentricity (Ex) studied in the flat meridian averaged 0.51 [0.47; 0.58], in the steep – 0.53 [0.43; 0.59] at the beginning of the study. The following results were obtained when evaluating the correlation between baseline Ex and the size of the APS before refractive therapy. With a weak degree of myopia, there is a direct strong correlation between the value of Ex, both in the flat and steep meridians, and the size of the APS at the beginning of therapy, which is equal to 0.28 at a significance level of p=0.011. In our study, the keratometry of the cornea averaged 43.5 at the beginning of the study [42.7; 44.4].

Evaluating the correlation between the initial value of keratometry and the size of APS, a negative correlation was found –0.69 in the group with mild myopia (p<0.001) and –0.67 – among children with moderate myopia (p <0.001).

Statistical processing showed a negative correlation of –0.2 (p=0.03) and –0.22 (p=0.019) between the differential strength of the cornea in the reverse 6 mm zone and the peripheral refraction in the corresponding peripheral refraction of 20–25°C temporal and nasal sides, respectively.

A positive correlation with a correlation coefficient of 0.21 (p=0.026) was received between the defocus in the temporal part and the gradient of myopia progression for the year. While in the nasal part the same result was obtained with a correlation coefficient of 0.2 (p=0.036), that is, the more positive the defocus (namely, the smaller the myopic defocus) is the greater the gradient of progression or increase in APS during the study period.

Considering that a certain number of studies by different authors revealed a correlational dependence between the baseline state of corneal Ex and the annual gradient of myopia progression, we assumed that we would get the same results. Thus, when determining the correlation between the average Ex and the initial refraction of patients, the obtained correlations were 0.08 and 0.04 (p=0.444, p=0.861) for mild and moderate degrees, respectively, but the level of significance was unreliable. Bingjie Wang reported that corneal eccentricity had a statistically significant relationship with axial length change in univariate but not multivariate analysis. The authors found that a greater value of corneal eccentricity is associated with a greater change in axial length. A more elongated corneal periphery results in greater peripheral hyperopic defocusing of the retina, which is thought to stimulate increased axial length [37]. In our study, we obtained a strong direct relationship between the initial Ex value and the difference in refraction (Δ R) after 24 months of follow-up, which was 0.32 (p=0.001) with a weak degree of myopia. Consequently, we can assume that the greater the value of Ex of the cornea, the greater the change in refraction we can expect, however given the above-mentioned lack of connection with changes in APS, it is necessary to differentiate refractive myopia from axial myopia.

As a result of probability analysis (increase in APS for two years), the method of construction of logistic regression models was used and we selected five factor features (X):

1. *Initial refraction* (X1) of the patient before the appointment of orthokeratological lenses.
2. *Pupil diameter* (X2).

As follows, the size of the pupil determines how much light actually enters the eye, but it mostly blocks peripheral light rays when narrowed. Past studies have shown that OKLs cause a shift of the peripheral defocus to the myopic side in the more remote periphery. From this point of view, the size of the pupil may affect the relative contribution of peripheral myopic defocus to the effectiveness of refractive therapy OKL.

Given that in our previous studies the base pupil diameter was inversely correlated with the annual progression gradient, that is, the smaller the pupil diameter is, the greater is the value in the difference in APS for the observation period we received. And vice versa, the more pupil size, the less progression of myopia, so this factor should be used as a prognostic factor.

Thus, Zhi Chen and co-authors also evaluated the influence of pupil size, but in scotopic conditions, because according to the authors, the size was more stable. In other cases, the photopic state imitates the conditions of everyday life, so scientists conducted research under such conditions. According to the researchers, a larger pupil diameter increases the effectiveness of OKL and slows down the axial growth of the eye length in myopia. The authors also believe that this occurs due to changes in peripheral defocus in the myopic direction [38]. In the comparison group of the study, patients used regular daily lenses as a correction, but no correlation was found between the base pupil area and the increase in axial size, as in our study in the comparison group, where glasses were used, such a relationship was not noted.

3. *Keratometry* (X3)

According to the literature, in some cases of

progression of myopia due to the fact that a flatter cornea causes more hyperopic defocus on the border of the small pupil, and with flattening of the cornea OKL practitioner may start a vicious circle of greater progression of myopia. During ortho-k treatment, the degree of myopia is reduced by flattening the central cornea. This central flattened zone is referred to as the treatment zone. It has been hypothesised that a reduced treatment zone following ortho-k creates increased peripheral refractive power and higher spherical aberration, which may further retard myopia progression. [39–41]. Therefore, the question of the effectiveness of OKL in all patients with myopia is ambiguous. The author also suggests that patients who have a greater change in the strength of the cornea on the periphery, progress less. Therefore, if the eccentricity of the cornea is too low, the potential correction of myopia will be limited due to insufficient peripheral defocus.

According to our study, the lower the value of keratometry before refractive therapy, the greater the value of the size of the APS, so this factor was also included as a prognostic.

4. *Peripheral force of the cornea in the ring of the reverse zone* (X4 and X5)

Taking in account the differential strength of the cornea on the periphery, we can predict the results of peripheral refraction in OKL users, assuming that the greater the strength of the cornea in the reverse 6 mm zone, the more pronounced myopic refraction in the retinal projection zone on the periphery. It is very important for orthokeratologists to have an idea of the peripheral refraction of a patient with progressive myopia with the use of OKL to control myopia, but it should be noted that not everyone in practice has the ability to make such calculations. Therefore, knowing the differential topographic strength of the cornea in the peripheral ring corresponding to the reverse zone of the lens, according to our study, we can say that this is a prognostic practical factor.

Similarly, it is determined in the literature that the greater the degree of myopia, the more pronounces the curvature of the middle periphery of the cornea changes (increases), inducing at the same time more significant peripheral myopic defocus and positive spherical aberrations. A relationship between the changes in periphery refraction on the keratotopogram after OKL and the rate of growth of APS was found [42]. Recently, a new method of analyzing the relative refraction of the cornea based on the keratotopogram in children using OKL was developed, and its relationship-with the control of myopia was shown. The value of the maximum relative refraction of the cornea greater than 4.5 dptre demonstrated a high probability of the effect of slowing the progression of myopia [43].

5. *The diameter of the cornea*

Axial length of the eye (APS) at the time of treatment (initial) allows determining the type of myopia axial or refractive, and is one of the main prognostic factors in the progression of myopia. This correspondence is noted by V.I. Pospelov, in whose work the axial size of the eyeball from 24 to 26 mm, is called axial uncomplicated, which also characterizes the patients included in the group of our study [44].

The regression coefficients of the factor features are given in *Table 1*.

Table 1. Signs included in the model for predicting the likelihood of progression of myopia when using OKL

The name of the sign	The level of the sign	Regression coefficient	Standard error
Initial refraction	X1	–0.33	0.24
Pupil diameter	X2	–1.702	0.463
Keratometry	X3	–0.119	0.294
Peripheral force of the cornea on the ring of the rotary zone *temporally*	X4	0.137	0.347
Peripheral force of the cornea on the ring of the rotary zone *nasally*	X5	–0.403	0.358
Corneal diameter	X6	–0.498	1.001
APS initial	X7	0.059	0.495
	Const	15.239	24.98

The logistic regression equation of the model for predicting the probability of progression of myopia on the background of the use of OKL had the form:

$$p = \frac{1}{1+e^{-(15.239-0.33*X1-1.702*X2-0.119*X3+0.137*X4-0.403*X5-0.498*X_6+0.059*X_7)}} \quad (1)$$

The classification ability of the model was determined according to the training sample and amounted to 79.5%. The probability of a true positive result (increase in APS less than 0.3) when using this model was 91.9%, and the probability of a true negative result – 38.5%. (*Table 2*).

Evaluation of the quality of the model using ROC-analysis (*Fig. 1*) showed the following: the area under the ROC-curve (AUC) was equal to 0.86 ($p<0.001$), which characterizes the good quality of the classification of traits. The sensitivity of the model was 82.6%, specificity – 73.1%.

Table 2. Classification table of the calculated probability of myopia progression when using OKL

Observation groups	Predicted cases		Percentage of correct indicators
	APS<0.3	APS>0.3	
	n=95	n=17	
APS<0.3	79	7	91.9%
APS>0.3	16	10	38.5%
total percentage	–	–	79.5%

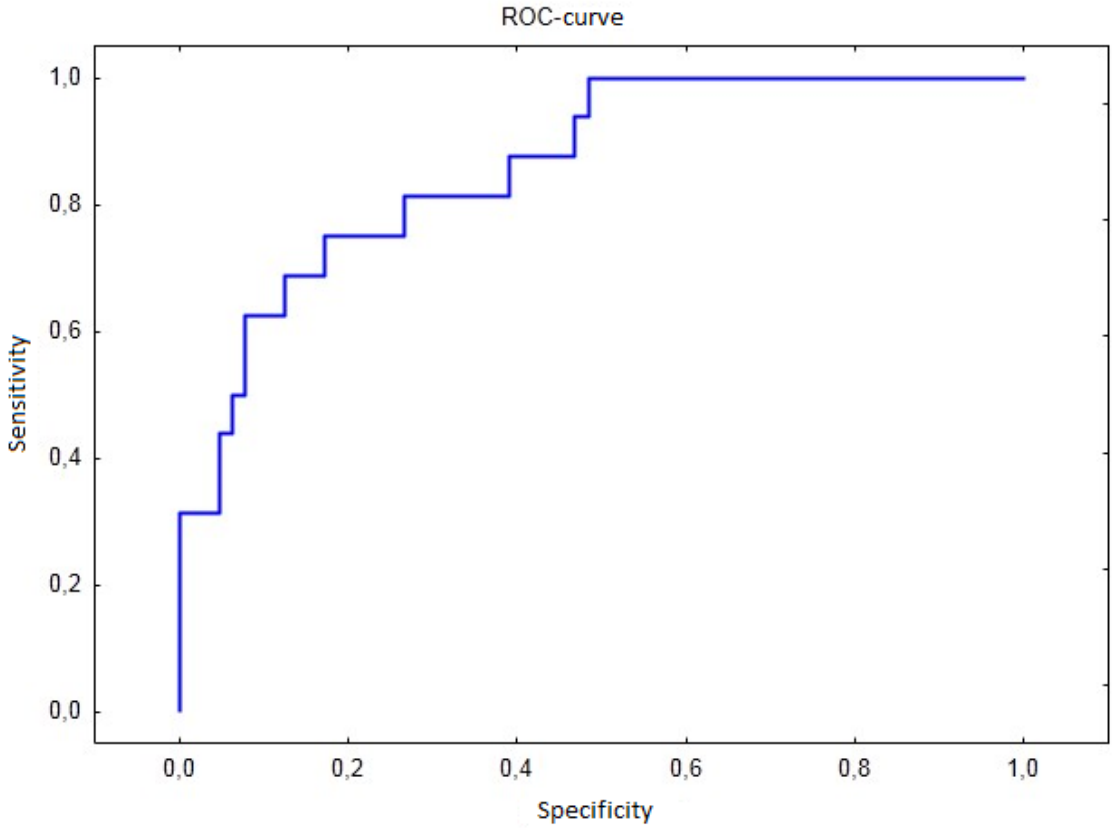

Fig. 1. The ROC-analysis data.

Clinical case 1

Patient A., aged 12, was brought with complaints of decreased vision. Hereditary history was unremarkable. The data of the primary ophthalmological examination were as follows.

Visual acuity OD=0.15, OS=0.15 Refraction OD= =–2.25 diopters, OS=–2.5 diopters (X1), pupil diameter (X2) – OD=6.09 mm, OS=5.25 mm, refractive power of the cornea (keratometry – X3) OD=46 diopters, OS=46.12 diopters; axial eye length OD=23.62 mm, OS=23.46 mm (sign X7); diameter of the cornea OD=11.5 mm, OS=11.51 mm (sign X6); orthokeratological lenses were selected for the child. When performing a corneotopography in the process of use, the difference (change between the initial cornea and under the influence of OKL) was determined on the refractive map. Refraction in the middle periphery of the cornea, corresponding to the rotational zone of the OKL on the temporal and nasal sides, was assessed. Thus, on the temporal side (X4) – OD=0.16, OS=0.19, on the nasal side (X5) this figure was – OD=0.8, OS=0.7.

Using the equation of binary logistic regression, calculate the sum of the values:
p(OD)=1/(1+exp(-(15.239-0.33*(-2.25)-1.702*6.09-0.119*46+0.137*0.16-0.403*0.8-0.498*11.5+0.059*23.62)))=0.01
p(OS)=1/(1+exp(-(15.239-0.33*(-2.5)-1.702*5.25-0.119*46.12+0.137*0.19-0.403*0.7-0.498*11.51+0.059*23.46)))=0.05

Conclusion. The patient is not at risk of myopia progression (estimated value of "p" less than 0.5). Re-examination of the child 12 months after the end of the term using lenses showed no progression of myopia and an increase in axial length within normal limits.

Clinical case 2

Patient L., aged 11, presented with complaints of decreased vision. Hereditary history was unremarkable. The data of the primary ophthalmological examination were as follows.

Visual acuity OD=0.1, OS=0.1. Refraction OD= =–1.75 diopters, OS=–2.5 diopters (X1), pupil diameter (X2) – OD=3.58 mm, OS=3.27 mm, refractive power of the cornea (keratometry – X3) OD=42.1 diopter, OS=41.9 diopter; axial eye length OD=24.96 mm, OS=25.17 mm (sign X7); diameter of the cornea OD= =12.16 mm, OS=12.23 mm (sign X6); orthokeratological lenses were selected for the child. When performing a corneotopogram in the process of use, the difference (change between the initial cornea and under the influence of OKL) was determined on the refractive map. Refraction in the middle periphery of the cornea, corresponding to the rotational zone of the OKL on the temporal and nasal sides, was assessed. Thus, on the temporal side (X4) – OD=–0.52, OS=–1.06, on the nasal side (X5) this figure was – OD=–0.57, OS=1.69.

Using the equation of binary logistic regression, calculate the sum of the values:
p(OD)=1/(1+exp(-(15.239-0.33*(-1.75)-1.702*3.58-0.119*42.1+0.137*(-0.52)-0.403*(-0.57)-0.498*12.16+0.059*24.96)))=0.57
p(OS)=1/(1+exp(-(15.239-0.33*(-2.5)-1.702*3.26-0.119*41.9+0.137*(-1.06)-0.403*1.69-0.498*12.23+0.059*25.17)))=0.52

Conclusion. The patient is at risk of myopia progression (estimated value of "p" greater than 0.5), so he changed the design of OKL with a smaller optical zone for more effective control of myopia.

As a part of the research and mathematical calculations, an interactive WEB application was also developed, which can be accessed from various types of devices connected to the World Wide Web. Modern solutions and approaches used in IT (Information Technology) were used for development.

The following technologies were used as tools for development:

• HTML 5 (HyperText Markup Language, version 5), CSS 3 (Cascading Style Sheets) and JavaScript to create a frontend part;

• PHP programming language version 7.4 for developing backend parts.

The developed WEB-application was hosted using the Salesforce [https://developer.salesforce.com/]. This service allows hosting the applications on the Internet for free and provides developers with all the necessary tools. The program code of the application is located in the Github [https://github. com/] repository, which is intended for storing various versions of the application, which are created during its development. Link to the application: [https://ophtalmologyequation.herokuapp.com].

Creating a page layout to place all the necessary fields and their captions with units of measurement was done using the modern Bootstrap framework [https://www.amazon.com/Full-Stack-Web-Development-Beginners/dp/B092P76L9Y], which allows making a stylized and adaptive layout for WEB-applications. The structure of the WEB application page has a standard look with the rules of their creation: <head>, <footer> and <body> tags in between. Between the <head> </head> tags is the necessary information about the page and the CDN link to the Bootstrap framework. Between the <footer> </footer> tags, information was placed about the affiliation of the developed application. The main content together with the formula is located between the <body> </body> tags. The form for calculating the Logistic Regression Equation is a set of fields with captions and default field values (*Fig. 2*) and shows a fragment of the form with the title, instructions and fields for data entry.

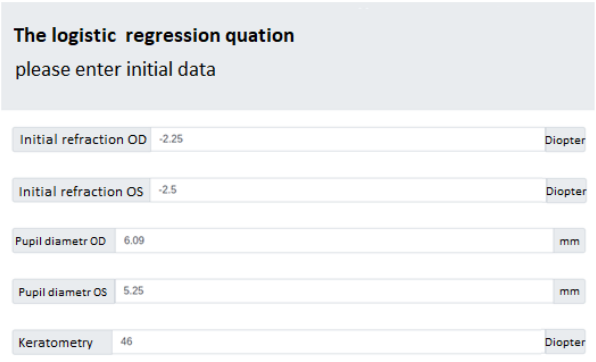

Fig. 2. Fragment of the form for calculating the values of the logistic regression equation.

$$(p\frac{1}{1+e^{-(15.239-0.33*X1-1.702*X2-0.119*X3+0.137*X4-0.403*X5-0.498*X_6+0.059*X_7)}})$$

All parameters used in the form are taken from our previous research results. After entering all the necessary parameters and checking them, click on the "Calculate" button. The result of the calculation is displayed below (*Fig. 3*). As follows from Fig. 3, the obtained pointers have different colors depending on the values.

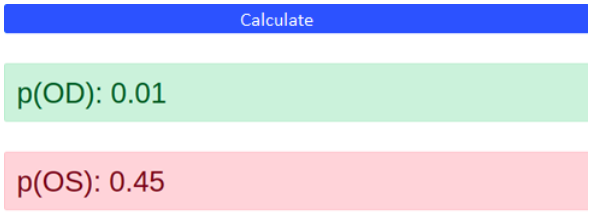

Fig. 3. Results of the calculation of the logistic regression equation.

If the value is in the range 0 ... 0.5, the field with the answer is highlighted in green. However, if the value obtained is not within these limits, it is highlighted in red to attract attention. In the example shown in Fig. 3, the values for the eye OD fall in the range of 0.5, so this value is good, but the value for the eye OS should be noted. Thus, changing the values of the parameters can determine the rate of progression of myopia on the background of the use of OKL, taking into account the individual parameters of the anterior segment of the eye of each patient.

Conclusions

1. It is determined that when examining a child with progressive myopia, it is important to pay attention to the diameter of the pupil in photographic conditions, because it can be a predictor of progression and influence the choice of correction individually. The children with a pupil diameter smaller than the average have a greater tendency to increase APS and, accordingly, to the progression of myopia. With the progressive form of myopia and the basic pupil size less than 4.52 mm, the most effective method of control is the appointment of refractive therapy with orthokeratological lenses.

2. It is established that the basic value of the corneal Ex has a direct correlation between the axial size of the eye before the appointment of OKL in mild myopia. However, there is no correlation between the baseline Ex and the annual gradient of myopia progression with the use of OKL because the profile of the cornea changes and there are other factors influencing the dynamics of changes in APS.

3. It is determined that the smaller the value of keratometry before the appointment of refractive therapy, the greater the value of the size of the APS, so this factor can be indicated as prognostic.

4. It is found that the differential topographic strength of the cornea along the peripheral ring corresponding to the reverse zone of the lens is a prognostic practical factor.

5. Criteria for predicting the progression of myopia have been developed in patients using OKL based on a mathematical prognostic model, taking into account the primary parameters of the eye, which allows customizing the approach to each child with myopia, improving individual design of orthokeratological lenses.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

The authors have no potential conflicts of interest to disclosure. The authors declare that the review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Data Transparency

The data can be requested from the authors.

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THE EFFECT OF DIFFERENT TYPES OF HYPOXIA ON THE MORPHO-FUNCTIONAL STATE OF THE KIDNEYS OF FETUSES AND NEWBORNS: THE RESULTS OF OWN LONG-TERM EXPERIMENTAL STUDIES

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ABSTRACT

Introduction. The vast majority of kidney diseases in children and adults have their origins in the antenatal, intranatal or postnatal periods of development. Poor health of women of childbearing potential, complications during pregnancy and delivery often lead to the development of various types of hypoxia (chronic intrauterine hypoxia (CIH), acute intranatal hypoxia, acute postnatal hypoxia (APH), mixed hypoxia (MH)). The latter are a common cause of fetal and neonatal disorders, leading to damage of various organs and systems, including the kidneys.

The purpose is to highlight the main results of own long-term experimental studies aimed at determining the effect of different types of hypoxia (CIH, APH, MH) on the morpho-functional state of the kidneys in fetuses and newborns.

Materials and methods. In this study, the author modelled alpine hypoxia using a sealed pressure chamber from which air was pumped out, creating conditions for a sharp decrease in atmospheric pressure. Histological, histochemical, immunohistochemical, morphometric and statistical research method were used.

Results. Acute postnatal, chronic intrauterine and mixed hypoxia stimulate fibroblastic cells in the kidneys, and chronic intrauterine and mixed hypoxia also induce epithelial-mesenchymal transformation, causing the development of sclerosis, induce apoptosis, proliferation, leading to an imbalance between them due to the prevaling proliferation in acute postnatal and chronic intrauterine hypoxia and apoptosis in mixed hypoxia.

Conclusions. Identified morphological changes in the kidneys of fetuses and newborns developed under the influence of acute postnatal, chronic intrauterine and mixed hypoxia, given the unity of structure and function, will lead to functional changes in these organs in subsequent postnatal ontogenesis in such children and the emergence of different nephrological pathology. This study actualizes the implementation of preventive measures among persons of reproductive age, dictates the need for quality pre-pregnancy training, which should be aimed at timely detection and treatment of genital and extragenital pathology in women.

Keywords: *acute postnatal hypoxia, chronic intrauterine hypoxia, fetus, kidneys, mixed hypoxia, morphology, newborn.*

INTRODUCTION

Nephropathology remains an urgent problem in modern pediatrics due to high prevalence of this pathology in the pediatric population, low efficiency of treatment, high risk of complications, frequent disability [1].

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In recent years, we have seen the atypical clinical picture of kidney disease in children, predominance of both chronic, latent forms, and manifest, aggressive, severe forms [2]. Doctors very often diagnose kidney diseases in children when they already develop irreversible changes and chronicity of the pathological process. The prevalence of pediatric chronic kidney disease in the world ranges from 15 to 74.7 cases per 1 million children. Mortality among children who progress to end-stage kidney disease is 30 to 50 times higher compared to that in general population [3]. In Ukraine congenital malformations and dysplasia of the kidneys, including polycystic kidney disease, glome-

rulonephritis, interstitial nephritis as a consequence of acute kidney damage, hereditary nephritis are common causes of chronic kidney disease in children [4].

The vast majority of kidney diseases in children and adults have their origins in the antenatal, intranatal or postnatal periods of development, as evidenced by numerous epidemiological studies [5, 6].

Poor health of women of childbearing potential [7], complications during pregnancy and delivery often lead to the development of various types of hypoxia (chronic intrauterine hypoxia (CIH), acute intranatal hypoxia, acute postnatal hypoxia (APH), mixed hypoxia (MH)). The latter are a common cause of fetal and neonatal disorders, leading to damage of various organs and systems, including the kidneys.

Purpose, subjects and methods:

1.The purpose of the study is to highlight the main results of own long-term experimental studies aimed at determining the effect of different types of hypoxia (CIH, APH, MH) on the morphofunctional state of the kidneys in fetuses and newborns.

2. Materials & ᴍethods

In this study, the author modelled alpine hypoxia using a sealed pressure chamber from which air was pumped out, creating conditions for a sharp decrease in atmospheric pressure. Every day, we placed mature female rats with an average weight of 220–250 g in conditions corresponding to 7,500 meters hight at 287 mm Hg for 20 minutes at the same time. During the experiment, we ranked the animals into four groups: group 1 – control group – pregnant female rats (n=3) that did not experience alpine hypoxia, some females were removed from the experiment in late gestation to remove fetuses (n=7). The offsprings (n=11) received from the rest of the females, were removed from the experiment on the first day after birth. Group 2 – a study group with simulation of CIH – pregnant female rats (n=4) throughout pregnancy (21 days) were subjected to daily alpine hypoxia, with some females removed from the experiment in late gestation to remove fetuses (n=6). Offsprings (n=10) from the rest of the females were removed from the experiment on the first days of life. Group 3 – a study group with modeling of APH – pregnant female rats (n=2) were not subjected to alpine hypoxia, but their offsprings (n=8) on the first day of life were subjected to alpine hypoxia once for 15 minutes, and then they were removed from the experiment. Group 4 – a study group for modeling MH – pregnant female rats (n=3) throughout pregnancy were subjected to daily alpine hypoxia, then the offsprings obtained from them (n=8) on the first day of life were subjected to alpine hypoxia once for 15 minutes and removed from the experiment.

The study material was the kidneys of fetuses and newborns. The material was fixed in a 10% solution of neutral formalin (pH 7.4) for 24-48 hours according to the generally accepted technique and embedded in paraffin. Serial sections 2 μm thick were made from paraffin blocks and stained with hematoxylin and eosin, according to Mallory, Rego method. The author conducted immunohistochemical studies using monoclonal antibodies (MCA) to CD20 (clone L26), CD4 (clone 4B12), CD68 (clone KP1), CD8 (clone SP16), p53 (clone SP5), Ki-67 (clone SP6), vimentin (clone V9), smooth muscle actin (clone 1A4), desmin (clone D33), cytokeratin 18 (clone CY-90), cytokeratin 19 (clone A53-B/A2.26), CD3. MCA to smooth muscle actin was manufactured by "DAKO" (Denmark), to CD20, CD4, CD68, CD8, p53, Ki-67, vimentin, desmin, cytokeratins 18 and 19 – "Thermo Fisher Scientific" (USA), to CD3 – "Novocastra Laboratories Ltd." (Great Britain). The microspecimens were examined on microscopes "Olympus BX-41" (Japan), "Axioskop 40" (Germany).

Morphometric study was performed on a microscope "Olympus BX-41" (Japan) using the program "Olympus DP-soft version 3.1".

The obtained digital data were statistically processed using the statistical package of the licensed program Statistica 6.0 and Microsoft Excel 2003. The author checked distribution of digital indicators normality in groups. To compare the parameters in the groups, we used parametric and nonparametric methods: Student's t-test, χ^2 test, Mann-Whitney U-test. Differences in groups were significant at $p<0.05$.

Results and discussion

In the renal cortex of newborns exposed to APH, the nephrogenic zone was normal. However, in fetuses and newborns exposed to CIH, and in newborns exposed to MH, the nephrogenic zone was thinned and had a reduced number and density of rudiments of both glomerular and tubular components of the nephron, indicating a delay in nephrogenesis. In newborns, compared with fetuses exposed to CIH, the thickness of the nephrogenic zone decreased. The nephrogenic zone regresses, undergoes sclerosis, and becomes mitotically active [8].

In the cortical substance of the kidneys of newborns in APH renal corpuscles were characterized by a uniform location, while in fetuses and newborns in CIH, in newborns in MH was found uneven location of glomeruli with reduced number and the presence of agglomeral areas.

The number of functioning nephrons in the kidneys plays an important role in the long-term functioning of this organ – their total number is from 300 thousand to 1 million (average 600 thousand) [9].

Deficiency of nephrons is a factor that causes the development of hypertension, kidney disease, increases the susceptibility of the kidneys to their secondary damage in such children in future [5, 10].

The number of nephrons in rats increases not only in intrauterine period, but also in the first days – weeks of postnatal life, and then changes slightly [11]. There are no clear data in the literature on the postnatal formation and development of nephrons in humans. Some publications have noted that nephron formation in humans ends at about 32–34 weeks of gestation, and therefore any nephron deficiency that occurs at birth cannot be compensated by increased nephrogenesis after birth [12, 13]. At the same time, there is scientifically proven data that in premature infants nephrogenesis is observed up to 40 days after birth [12, 14].

Scientists note that given the possibility of the evolutionary reserve of maturation of morphological structures, with timely prediction of pathology (immediately after birth), studying obstetric and gynecological history of the mother, using modern medical technology, may create conditions for maturation of immature structures in nephrogenic tissue and, consequently, prevent the disease [15].

In APH, the shape of mature renal corpuscles was normal, but in CIH and MH, these structures took the wrong shape in places. The size of the renal corpuscles in APH was normal, but in CIH and MH renal corpuscles were small [16].

In fetuses and newborns in CIH, in newborns in MH, renal corpuscles were with reduced or absent of glomerular capillaries. In the renal corpuscles there were signs of circulatory disorders, the severity of which increased from fetus to newborn, and they were minimal in APH, maximum in MH and moderate in CIH. The latter were manifested by plethora and stasis of the glomerular capillaries, thrombus formation, hemorrhage into the renal corpuscle cavity, edema of the basement membranes of the capillaries and the parietal leaf of the Bowman's capsule. In CIH and MH in the basement membranes, edematous changes often combined with sclerosis, as a result of which the basement membrane had an uneven thickness due to the areas of thickening and loosening. Dystrophic, necrotic and desquamative changes of capillary endotheliocytes and epitheliocytes of the outer leaf of the Bowman's capsule developed in the areas where the basement membrane changed. The described changes in the basement membranes with adjacent cellular elements increased from fetus to newborn, were minimal in APH, moderate in CIH and pronounced in MH [16]. The structural changes found in the basement membranes were described in children with some glomerulopathies, characterized by the development of nephrotic syndrome [17].

The author defined the accumulation of fibrin threads in the expanded urinary space of a part of renal corpuscles between the capillaries and the capsule which are known to be the initial manifestations of glomerulosclerosis [18]. Prolonged action of the damaging factor, presented by CIH, led to an increase in the severity of the detected general pathological processes in the glomerular apparatus in newborns compared with fetuses.

When modeling different types of hypoxia in the kidneys of fetuses and newborns, it was found morphological changes not only in the glomerular apparatus of the nephron, but also in the tubular. Although the tubular apparatus of the nephron in comparison with the glomerular apparatus is better protected from the damaging effects of various exogenous and endogenous factors [19], in our study the tubular apparatus was characterized by pronounced changes.

Morphological changes in the tubular component of the nephron were characterized by dystrophic, necrotic and desquamative changes of nephrocytes. In the tubular basement membrane, the basement membrane of the collecting tubules, edematous changes developed in APH, and in CIH and MH edematous and sclerotic changes, in consequence of which the basement membrane looked focally thickened, swollen in places, and thinned in part of the visual field. Desquamated cells and cellular detritus filled the lumens of the tubules and collecting tubules. Structural changes in the tubular system and collecting tubules were minimal in APH, moderate in CIH and maximum in MH, while in CIH they increased from fetus to newborn. Under the action of APH the maximum structural changes were in the proximal tubules compared to other parts of the tubular system and collecting tubules, and in CIH and MH they were

equally pronounced in all tubules and collecting tubules of the cortex and medulla substance of the kidneys.

The involvement sequence of different nephron parts in the pathological process is due to their morpho-functional heterogeneity caused by the complexity and scope of functions, the sequence of functional activities [19]. The maximum changes in the proximal tubules indicated in APH are most likely due to the fact that this part of the tubular system is characterized by greater functional activity, respectively, it is more prone to structural changes.

The immature tubules and ducts in the kidneys of fetuses and newborns of all groups were found. In the control group and in APH they localized mainly in the cortex while in CIH and MH, they were in the cortex and medulla substance of the kidneys. In CIH and MH, the number of immature structures was higher compared to the control and APH, which indicated an inhibition of the maturation of these structures. In CIH immature structures increased in number at newborns in comparison with fetuses.

Glomerular and tubular cysts were found in the cortex and medulla substance of the kidneys in fetuses and newborns in CIH, in newborns in MH. The number of cysts increased in newborns in comparison with fetuses in CIH.

In fetuses and newborns in the renal stroma developed hemodynamic disorders, characterized by stasis of blood cells, predominance of dilated and full-blooded vessels over spasms, thrombosis, hemorrhage and edema. They were minimal in APH, moderate in CIH and severe in MH. In CIH, the severity of these changes increased from fetus to newborn.

A number of scientists noted various hemodynamic disorders in the organs in response to hypoxia. They noticed slowing of blood flow in the vascular bed of organs, sludge of formed blood elements, their sequestration with local increase of blood coagulation potential and development of local microthrombus formation. This, in turn, impairs gas exchange in tissues, increases hypoxia, disrupts vascularization of organs, creates a situation of their microcirculatory blockade with subsequent dysfunction [20].

Signs of circulatory disorders always combine with structural changes in the vascular walls of stroma, manifested by dystrophic, necrotic and desquamative changes of endothelial cells, as well as edema and sclerosis of the layers. Moreover, if damage of endothelial cells and edema of the layers of vascular walls was in all types of hypoxia, in CIH and MH CHV sclerosis was in the wall layers. These vascular endothelial cell lesions were minimal in APH, severe in MH and moderate in CIH, increasing from fetus to newborn. Interestingly, the detected hemodynamic disorders in the renal stroma in all types of experimental hypoxia were more pronounced in the cortex compared to medulla substance. Similar signs of circulatory disorders with structural changes in the vascular walls were also found in the fat capsule of the kidneys of fetuses and newborns, and the degree of their severity was similar to that already described above in the stromal component of the kidneys.

There was a balance between apoptosis and proliferation in the kidneys of fetuses and newborns of the control group, corresponding to the physiological norm [21]. However, in the studied groups, it was determine an imbalance characterized by the predominance of proliferative processes over apoptotic in APH and CIH, and the predominance of apoptosis over proliferation in MH [22].

In the simulation of CIH and MH, it was noticed a decrease of the specific volume of parenchyma and an increase of the specific volume of stroma due to the development of sclerotic changes. Our analysis of immunohistochemical reactions with vimentin, desmin, smooth muscle actin, cytokeratins 18 and 19 revealed several important points explaining the mechanism of sclerosis in the kidneys of fetuses and newborns, developed under different types of hypoxia. First, APH led to an increase in the number and activate the morpho-functional state of fibroblastic cells, including myofibroblasts. Second, CIH and MH led not only to an increase in the number of fibroblastic differon cells, including myofibroblasts, but also to the induction of epithelial-mesenchymal transformation [23]. The author understand the latter as a process of disruption of cell-cell and cell-matrix adhesion of epithelial cells, freeing them from interconnection and their loss of polarity (essential properties of epithelial tissue), reorganization of the cytoskeleton and the acquisition of fibroblast-like cells, enabling movement, remodeling of the extracellular matrix and maintenance of the mesenchymal phenotype [24]. This phenomenon has been demonstrated not only during embryonic development, but also in the postnatal period during tissue repair, as well as in the process of carcinogenesis [25]. At the heart of this process is a change in the transcription program involving many signaling pathways at different

levels of their regulation, ranging from extracellular signals affecting cytoplasmic effects and then nuclear transcriptional regulators [26].

In fetuses and neonates exposed to various types of hypoxia, cellular infiltration was in the capsules and stroma of the kidneys, represented mainly by fibroblastic cells and immune cells. This cellular infiltration increased compared to control and was minimal in APH, moderate in CIH and severe in MH. Among the immune cells it was found CD3-, CD4-, CD8-, CD20- and CD68-positive cells. Interestingly, local immune responses in the kidneys were different, determined by the type of experimental hypoxia. Thus, APH did not affect the number of CD3-, CD4-, CD8-, CD20-cells, i.e. T-cell and B-cell immunity, but led to an increase the number of CD68-cells, i.e. activated the macrophage system. CIH led to a decrease the number of CD3-, CD4-, CD8-, CD20-cells and an increase the number of CD68-cells. MH led to a decrease the number of CD3-, CD4-, CD8-, CD20-cells and an increase the number of CD68-cells. When comparing different types of hypoxia, there was a maximum increase in the number of CD68-cells in MH, a minimum in APH and moderate in CIH. In MH compared with CIH, there were no differences in the number of CD3-, CD4-, CD8-, CD20-cells, but the number of CD68-cells was greater. In CIH, as well as in control, it was found age increase the number of all immune cells [27].

Thus, a comprehensive morphological study of the experimental material allowed to prove the damaging effect of acute postnatal hypoxia, chronic intrauterine hypoxia and mixed hypoxia on the kidneys of fetuses and newborns based on the identified morpho-functional changes.

CONCLUSIONS

1. Acute postnatal, chronic intrauterine and mixed hypoxia lead to the development of respectively minimal, moderate and pronounced morphological changes in the capsules, parenchymal and stromal-vascular components of the kidneys, primarily damaging the vessels of the stroma and parenchyma, where more pronounced changes occur in the tubules, collecting tubules, and in chronic intrauterine hypoxia these changes increase in newborns compared to fetuses. Experimental hypoxia causes the development of hemodynamic disorders, degenerative-desquamative changes of vascular endotheliocytes, epitheliocytes of Bowman's capsules, tubules, collecting tubules, and the latter in acute postnatal hypoxia are observed mainly in the proximal tubules, and in chronic intrauterine and mixed hypoxia in all parts of the tubular system and collecting tubules. Chronic intrauterine and mixed hypoxia promote cyst formation, delay the processes of glomerulogenesis and tubulogenesis. Acute postnatal, chronic intrauterine and mixed hypoxia stimulate fibroblastic cells in the kidneys, and chronic intrauterine and mixed hypoxia also induce epithelial-mesenchymal transformation, causing the development of sclerosis. Acute postnatal, chronic intrauterine and mixed hypoxia induce apoptosis, proliferation, leading to an imbalance between them due to the prevaling proliferation in acute postnatal and chronic intrauterine hypoxia and apoptosis in mixed hypoxia.

2. Identified morphological changes in the kidneys of fetuses and newborns developed under the influence of acute postnatal, chronic intrauterine and mixed hypoxia, given the unity of structure and function, will lead to functional changes in these organs in subsequent postnatal ontogenesis in such children and the emergence of different nephrological pathology.

3. This study actualizes the implementation of preventive measures among persons of reproductive age, dictates the need for quality pre-pregnancy training, which should be aimed at timely detection and treatment of genital and extragenital pathology in women.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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CREATION OF ANTI-INFLAMMATORY PHARMACEUTICAL COMPOSITIONS (review)

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Abstract

The review article summarizes the data of literature and our own research on the creation of more effective and safe domestic combined drugs with a pronounced anti-inflammatory and analgesic effect. Untimely treatment of inflammatory processes often leads to long-term incapacity and even disability, so today there is an active search for new effective and safe domestic combination drugs with pronounced anti-inflammatory and analgesic effects. Due to the constant search for innovative drugs the treatment of a significant number of diseases and pathological conditions with existing drugs remains ineffective or causes addiction and a large number of side effects. As a result, inflammatory processes can be accompanied by severe pain, spasms, increased convulsive activity of the brain, other disorders, so non-narcotic analgesics and nonsteroidal anti-inflammatory drugs are combined with other agents that can cause complementary effects or potentiate each other's effects. Caffeine is a useful adjuvant of nonsteroidal anti-inflammatory drugs of various chemical structures in terms of anti-inflammatory and analgesic effects. The article presents a literature review of the current state of treatment of inflammation and pain, conducting research to expand the therapeutic capabilities of known pharmaceuticals, the creation of pharmaceutical compositions of nonsteroidal anti-inflammatory drugs with caffeine.

Keywords: *pain, inflammation, caffeine, non-narcotic analgesics, nonsteroidal anti-inflammatory drugs, pharmaceutical composition.*

The efficiency and continuous development of medical science involves the search for new drugs, as well as research to expand the therapeutic potential of known pharmaceuticals. An urgent problem at the present stage of development of medicine and pharmacy is the creation of new domestic combined drugs, the pharmacological effects of which are achieved through a rational combination of ingredients. The pharmaceutical composition of several components in one drug expands its pharmacological spectrum [1–4].

The combination of ingredients in multicomponent pharmaceutical compositions mutually enhances their pharmacological effects. Clinical studies have confirmed the advantages of combined drugs over single drugs for the pharmacotherapy of pain syndromes, inflammatory processes as they are more effective than each individual component [2]. Such pharmaceutical compositions make it possible to add to the composition of drugs active pharmaceutical ingredients in smaller doses, reducing toxicity and adverse side effects [3].

Recently, the world has seen an increase in the incidence of diseases accompanied by inflammatory processes, and their level continues to grow every year. Inflammation underlies most pathological processes and is the basis of more than 70% of known human diseases [1–4]. Inflammation as a typical pathological process developed in the process of evolution and occurs in response to any damage to body tissues. It is aimed at localization, destruction and removal from the body of pathogenic factors that cause inflammation, as well as to eliminate the consequences of their action. The cause of inflammation can be any factor or factor that can cause tissue damage (inflammatory agent or phlogogen (Greek *Phlogogen*, a substance, causing inflammation)) [5]. Classification of causes of inflammation depends on the nature and origin of phlogogenic factors [6].

Inflammatory processes can be accompanied by severe pain, spasms, increased convulsive

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activity of the brain, other disorders of the body, so non-narcotic analgesics (NNAs) and nonsteroidal anti-inflammatory drugs (NSAIDs) are combined with other drugs that can cause complementary effects or potentiate each other's effects.

Pharmacological regulation of pain is one of the most pressing problems of modern medicine [7]. Prevention and treatment of pain requires the use of drugs mainly from the group of analgesics. Pain is a clinical manifestation of inflammation and the main complaint of the patient with various inflammatory diseases.

In addition, long-term and combined use of non-narcotic analgesics and NSAIDs increases the incidence of complications of anti-inflammatory pharmacotherapy. Side effects of NSAIDs necessitate the search for safer anti-inflammatory drugs. Selective cyclooxogenase-2 (COX-2) inhibitors developed in recent years, which were supposed to solve the problem of NSAID side effects on the gastrointestinal tract, did not live up to the expectations of pharmacologists. Doses in which selectivity is maintained do not show sufficient clinical effect, and increasing the dose leads to loss of selectivity and gastrotoxicity.

Currently, there is an arsenal of modern NSAIDs, which are used to treat many diseases, ranging from fever to severe autoimmune processes [8]. But the current wide range of anti-inflammatory drugs does not solve the problem of successful treatment of inflammatory diseases and their recurrence, the frequency of which after discontinuation of drugs occurs in almost 100% of cases and whose use is complicated by many side effects [9].

Therefore, despite the saturation of the pharmaceutical market of Ukraine with NSAIDs, the search for new safe domestic drugs for the treatment of inflammatory processes that would be more effective and less toxic today remains relevant.

The group of traditional NSAIDs includes drugs that have different chemical structure and differ in the intensity of analgesic, antipyretic and anti-inflammatory effects and the frequency of side effects. General availability, fast and tangible analgesic effect, ease of use and the availability of a large number of dosage forms allows patients to use drugs without consulting a doctor, and this leads to their uncontrolled use [10]. Therefore, treatment with known non-narcotic analgesics or NSAIDs can worsen the quality of life of patients, and the lack of treatment of inflammatory processes, which is often accompanied by pain, leads to long-term disability and even disability [11].

NSAIDs are among the most commonly used symptomatic drugs. They are used to treat rheumatic diseases, deforming osteoarthritis, neuralgia and myalgia, osteochondrosis, cardiovascular disease, febrile conditions of infectious-inflammatory origin, headache and toothache and many other pathological conditions accompanied by inflammation and pain [12]. The dominant pharmacological effect of drugs in this group is anti-inflammatory action, which in its level is close to the action of anti-inflammatory drugs of steroidal structure (glucocorticoids and their synthetic analogues).

According to modern ideas, non-narcotic analgesics and NSAIDs have a complex mechanism of influence on various parts of the inflammatory response. The vast majority of non-narcotic analgesics and NSAIDs have been shown to indiscriminately inhibit the activity of COX-1 and COX-2 cyclooxygenase enzymes, which catalyze the production of prostaglandins from arachidonic acid and thus reduce inflammation and relieve pain. The therapeutic effect of NSAIDs is realized by inhibiting the activity of COX-2, and the main side effects occur due to inhibition of COX-1 [13, 14].

Simultaneously with the study of the clinical efficacy of the first NSAID salicylic acid, the first reports of its adverse effects on the gastrointestinal tract began to appear. Gastrointestinal complications are among the most common and dangerous side effects of NSAIDs [13]. The term "NSAID-gastropathy", introduced in 1986 by S.H. Roth, means erosive-ulcerative gastrointestinal lesions on the background of NSAIDs. NSAID gastropathies have some features: the appearance of ulcers on the background of NSAIDs, acute multiple erosions or ulcers, localization of ulcers in the antrum of the stomach, little or no asymptomatic course, frequent manifestations, disappearance after discontinuation of the drug. There are several factors that increase the risk of NSAID gastropathy: age (over 60 years), history of gastrointestinal pathology, high doses or concomitant use of multiple NSAIDs, concomitant use with glucocorticoids, long-term use of NSAIDs (over 3 months), therapy anticoagulants and/or antiplatelet agents [14–18]. NSAIDs are also characterized by a number of other side effects: sodium and water retention and, as a consequence, increased blood pressure; tendency to bleed; allergic reactions (bronchospasm, anaphylactic shock, Quincke's edema), etc. It is also known that renal dysfunction may be due to both the inhibitory effect of NSAIDs on the synthesis of vasodilators

prostaglandins and their nephrotoxic effect [16]. Liver damage from NSAIDs can be caused by both immune processes and their toxic effects. Almost all NSAIDs are able to change the dynamics of drugs of other pharmacological groups and are themselves under no less active influence from them. The use of NSAIDs should take into account the possibility of their interaction with other drugs, especially with indirect anticoagulants, antiplatelet agents (bleeding tendency), diuretics, antihypertensive drugs (reduced effectiveness of antihypertensive drugs) [17]. It is known that in patients with chronic heart failure NSAIDs may increase the incidence of decompensation due to reduced therapeutic effect of angiotensin-converting enzyme inhibitors and diuretics [18–20].

There is no doubt about the need for widespread use of NSAIDs, which is largely due to the rejuvenation of rheumatic diseases, increasing the number of patients with cardiovascular risks who take acetylsalicylic acid in low doses for a long time and others. In addition, these drugs are available, mostly available without a prescription. The clinical effect provides reliable compliance. The problem of eliminating the side effects of NSAIDs remains unresolved. In recent years, the mechanisms of gastroprotection and in particular the mechanisms of adaptation of the mucous membrane to the adverse effects of NSAIDs have been actively studied. Scientific research is aimed at studying the state of blood circulation, mechanisms of angiogenesis, balance of proliferation and apoptosis of gastric epitheliocytes, epidermal and transforming growth factors. However, despite the constant interest in studying the nature and characteristics of gastropathies, the problem of safe use of NSAIDs remains relevant, will require further research and new research, which will allow in the near future to create new effective and safe drugs that will improve patients' quality of life, safe even with long-term, if necessary, lifelong use. Modern ways to reduce the ulcerogenicity of NSAIDs (changes in tactics, combination with prostaglandins` analogues, H2-histamine blockers, proton pump inhibitors, drugs with antihypoxant and antioxidant activity) do not solve this problem. Therefore, the search for reliable and safe drugs remains an urgent problem.

Scientists have studied COX and found that this enzyme, which is detected in different tissues, usually shows a different spectrum of sensitivity, which allowed us to make assumptions about the existence of isoforms of the enzyme [21]. In the early 1990s a group of scientists led by J. Wayne found two isoforms of the enzyme COX in the body at the same time. The constructive isoform of COX-1 is responsible for the synthesis of prostaglandins, which are involved in the protection of the gastrointestinal mucosa, regulation of platelet function and renal blood flow, i.e., performs important physiological functions in the body [22]. The researchers also found COX-1 to be mostly in the cytoplasm or associated with the endoplasmic reticulum. Induced COX-2 is involved in the synthesis of prostaglandins in inflammation [23–30].

Depending on the nature of COX blockade, NSAIDs are divided into selective and non-selective COX inhibitors. Classification of NSAIDs depending on their ability in therapeutic doses to selectively block the activity of COX-1, COX-2 and COX-3 [30] is presented in *Table 1*.

Table 1. Classification by the mechanism of inhibition of COX activity [30].

Group of drugs	Drugs
Selective COX-1	Low doses of acetylsalicylic acid
Non-selective COX-1 and COX 2 inhibitors	Diclofenac, ketorolac, ibuprofen, naproxen, ketoprofen, indomethacin, etodolac, aspirin, paracetamol, metamizole, piroxicam and most other modern NSAIDs
Selective COX-2 inhibitors	Lornoxicam, meloxicam, nabumetone and nimesulide
Highly selective COX-2 inhibitors	Parecoxib, rofecoxib, celecoxib, etoricoxib
COX-3 inhibitors	Paracetamol

Traditional NSAIDs include non-selective COX-1 and COX-2 inhibitors. Selective COX-2 inhibitors have less effect on COX-1. Highly selective COX-2 inhibitors (coxibs) have virtually no effect on COX-1. Modern views on the mechanism of action of paracetamol are based on the fact that it is not a typical NSAID due to the fact that it blocks COX-3, which is synthesized in the CNS [31–37]. In addition to affecting prostaglandins synthesis, NSAIDs affect various links in the pathogenetic chain of inflammation – inhibit lipid peroxidation, stabilize lysosomal membranes, etc. [38].

Literature sources analysis showed that combined anti-inflammatory and analgesic drugs often include 1,3,7-trimethylxanthine – caffeine, $C_8H_{10}N_4O_2$ (*Fig. 1*) [39–40].

Caffeine is an alkaloid of the purine series, colorless or white bitter crystals. The structural struc-

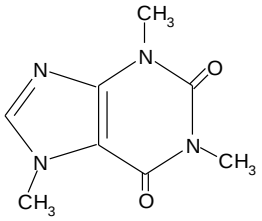

Fig. 1. Caffeine (1,3,7-trimethylxanthine).

ture is a heterocyclic alkaloid of the purine series. It was first extracted from coffee extract in 1821. It is known to be a psychostimulant, found in coffee, tea, energy and many beverages. Caffeine is found in plants such as coffee, tea, cocoa and others [41–52].

According to the literature, caffeine is known to have a positive effect on the bioavailability of NSAIDs and non-narcotic analgesics. As a result, the anti-inflammatory and analgesic effects of these drugs increase [41, 42]. Enhancement of the analgesic effect of non-narcotic analgesics is associated with caffeine induction of central cholinergic analgesia [43, 45], structural similarity of adenosine and caffeine molecules, which contributes to the neurochemical mechanism of action of the latter in the form of blocking P1 "purine" brain receptors [47].

At the Department of Medical and Bioorganic Chemistry of KhNMU the created pharmaceutical compositions of NSAIDs of different chemical structure (analben (potassium salt of 2,4-dichlorobenzoic acid), acetic acid derivative – diclofenac-sodium (2-[(2,6-Dichlorophenyl)amino]benzeneacetic acid sodium salt), a derivative of propionic acid – ibuprofen (((±)-2(4-isobutylphenyl)-propionic acid), a derivative of amino-phenol – paracetamol (N-(4-hydroxyphenyl)acetamide), oxycams – NSAIDs are derivatives of pyridin-2-ylamide 3-carboxylic acid: meloxicam (4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide) and piroxicam (4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide), a secondary analgesic – carbamazepine (5H-dibenz-[b, f]-azepine-5-carboxamide)) and others with caffeine were studied. Our long-term research has shown that caffeine enhances the pharmacological effects of NSAIDs of different chemical structure and analgesics [41–52], so we believe that caffeine is a suitable adjuvant of NSAIDs of different chemical structure in terms of anti-inflammatory and analgesic effects [41–52]. Due to the fact that pharmaceutical compositions with coxibs are not available on the pharmaceutical market, we decided to create such pharmaceutical compositions and investigate the above types of biological activity.

The main advantage of coxib drugs compared to other NSAIDs is their much smaller negative impact on the gastrointestinal tract. The objects of our research in the framework of the departmental initiative research work "Chemical-pharmaceutical substantiation of biologically active compounds, conjugates and drug compositions with anti-inflammatory and analgesic activities", we chose celecoxib and rofecoxib [19, 22]. These drugs belong to the group of highly selective inhibitors of COX-2 and their pharmacological action is to inhibit the biosynthesis of prostaglandins – mediators of pain and inflammation [19]. Since COX-2 is the main trigger of inflammation and neoangiogenesis, these signs accompany a wide range of pathological conditions and diseases [22]. Therefore, the therapeutic properties of celecoxib, which is very widely used, in particular, have been studied in all areas of medical practice [18]. Celecoxib has shown a high therapeutic effect in the treatment of acute pathologies of the musculoskeletal system, surgery, post-traumatic stress disorder [18, 19]. This drug is used in rheumatology [22] and even as an urgent analgesic. Positive results have been obtained in the treatment of celecoxib even schizophrenia [53]. Due to the antitumor effect the drug is used in oncology. Celecoxib is one of the few NSAIDs that can be taken for a long time [53–72] because it is well tolerated and virtually free from the risk of complications from both the gastrointestinal tract and the cardiovascular system [22].

The chemical structure of coxibs is based on the structure of benzenesulfonic acid. Celecoxib is 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide (*Fig. 2*):

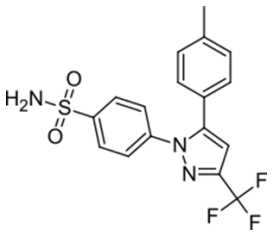

Fig. 2. Celecoxib (4-(5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide), $C_{17}H_{14}F_3N_3O_2S$.

Rofecoxib [[4-[4-(Methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone]) is an NSAID, a synthetic drug of the coxib group. In its structure it contains a side sulfone chain (*Fig. 3*):

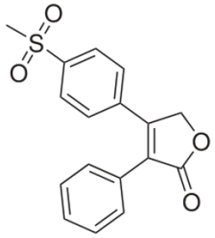

Fig. 3. Rofecoxib ([4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5H)-furanone]), $C_{17}H_{14}O_4S$.

Rofecoxib is also widely used in clinical practice in Ukraine [58]. The mechanism of its pharmacological action is identical to highly selective NSAIDs [22]. The drug is highly effective as an analgesic and antipyretic in the treatment of rheumatic conditions, dysmenorrhea [58] and migraine [59]. In the treatment of musculoskeletal disorders, it shows not only anti-inflammatory and analgesic, but also chondroprotective properties [59–61]. Oncologists note the positive effect of rofecoxib on the course of familial pollinosis, colon cancer in combination therapy of these diseases. Rofecoxib has a positive effect on the course of neurodegenerative processes [61]. There are no combined drugs based on celecoxib and rofecoxib in the arsenal of pharmaceuticals, so we have created such pharmaceutical compositions and are conducting research on them.

Studies of domestic and foreign scientists in recent years indicate the prospects for the search for biologically active molecules among the derivatives of 1,2,4-triazole [62–72]. It should be noted that drugs derived from 1,2,4-triazole are among the 200 drugs with the highest sales (alprozalam, trazodone, fluconazole).

The viability of this heterocyclic system is due to many factors: high reactivity, low toxicity, availability of reagents for synthesis, solubility in most solvents and, especially, a wide range of biological activity. Amino derivatives of 1,2,4-triazole and 1,2,4-triazole-3-thione provide great opportunities for the search for biologically active compounds [62–72].

Compounds of this class have proven to be low-toxic and highly effective substances [62–72]. Relying on data on the high pharmacological activity of various 4-R-3-thio-1,2,4-triazoles and their derivatives with analgesic, antibacterial, antiviral, antifungal, anti-inflammatory, antitumor, anticonvulsant, antituberculous effects, which is covered in the works of O. I. Panasenko, E. Knysha, J. B. Polya, A. Kumar, etc., and taking into account the insufficient amount of data on the pharmacological properties of 3,4,5-trisubstituted 1,2,4-triazole, we considered it appropriate to obtain substances with improved pharmacological activity and increased safety, which would be the basis for the creation of original drugs. Thus, the heterocyclic system of 1,2,4-triazole is a promising fragment for the synthesis of new biologically active substances with different types of pharmacological action, in particular with anti-inflammatory and analgesic effects [62–72].

The study in the framework of PhD dissertation of the senior lecturer of the Department of Medical and Bioorganic Chemistry of KhNMU N. M. Chalenko involved an assessment of literature sources on the synthetic and biological potential of 4-amino-3-thio-5-R-4H-1,2,4-triazoles and selection of the basic structures that are the most promising for modification and production of new biologically active substances, determination of the optimal directions of structural modification and elaboration of new derivatives on the grounds of basic structures, for which virtual screening was performed and compounds with the highest predicted anti-inflammatory activity were selected for synthetic studies, a synthesis of starting compounds 4-amino-3-thio-5-R-4H-1,2,4-triazoles, proposal of optimal conditions for their alkylation with chloroacetic anilides to obtain a number of 4-amino-(1H-pyrrol-1-yl)-5-R-4H-1,2,4-triazol-3-ylthio-acetanilide, a modification of the amino group in the synthesized substances; modeling of structures and purposeful synthesis of new surfactants based on 4-amino-3-thio-5-R-4H-1,2,4-triazoles along with a study of their reactivity, physicochemical, anti-inflammatory and analgesic properties with a search of structures with the most promising compounds of anti-inflammatory and analgesic action [62, 63].

According to the results of pharmacological screening, 15 "hit compounds" with anti-inflammatory activity from the group of 4-amino-(1H-pyrrol-1-yl)-5-R-4H-1,2,4-triazol-3-ylthio-acetanilide, for which the laws of connection "chemical structure-antiexudative and analgesic activity" in a number of synthesized derivatives were established.

Based on SAR-analysis, recommendations for the rational design of NSAIDs in the group of 4-amino-(1H-pyrrol-1-yl)-5-R-4H-1,2,4-triazole-3-ylthio-acetanilides [62, 63]. According to the results of pharmacological screening of analgesic and antiexudative activities [64], 3 new "leader compounds" were selected for further experimental studies (*Fig. 4–6*):

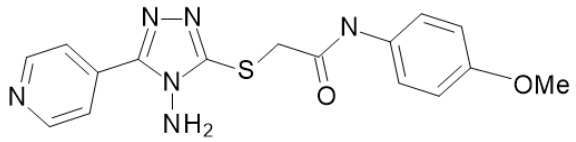

Fig. 4. N-(4-methoxyphenyl) -2- [4-amino-5-(pyridin-4-yl) -4H-1,2,4-triazol-3-ylthio] acetamide (Compound 1).

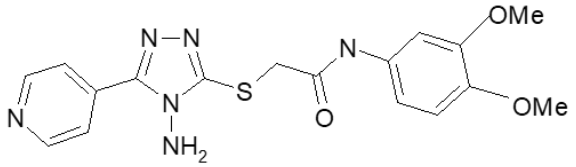

Fig. 5. N-(3,4-dimethoxyphenyl) -2- [4-amino-5-(pyridin-4-yl) -4H-1,2,4-triazol-3-ylthio] acetamide (Compound 2).

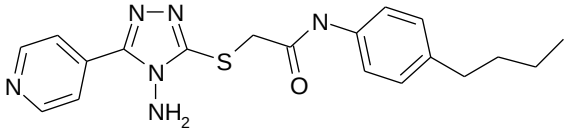

Fig. 6. N-(4-butylphenyl)-2- [4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio] acetamide (Compound 3).

Based on these 3 new "leader compounds", we have also created pharmaceutical compositions with caffeine adjuvant. We see the prospect of increasing efficiency, reducing toxicity and side effects in the development of pharmaceutical compositions based on coxibs (rofecoxib and celeco¬xib) and caffeine and derivatives of 4-amino-(1H-pyrrol-1-yl)-5-R-4H-1, 2,4-triazole-3-ylthio-acetanilides and caffeine, which is what the staff of the Department of Medical and Bioorganic Chemistry [62–63].

Thus, the review of literature sources, including our own experimental studies, indicate the prospects for the development of two-component pharmaceutical compositions based on NSAIDs of different chemical structure and derivatives of 4-amino-5-(pyridin-4-yl)-1,2,4-triazole(4H)-3-yl-thioacetanilides. As an adjuvant, it is advisable to choose 1,3,7-trimethylxanthine (caffeine). This is the basis for the creation of new domestic two-component pharmaceutical compositions with anti- inflammatory and analgesic effects.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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Data Transparency

The data can be requested from the authors.

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