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SURGERY

Mytsyk Yu.O., Borys Yu.B., Dutka I.Yu., Datz I.V., Motrynets N.P., Kucher A.R., Pasichnyk S.M., Borys B.Yu., Mytsyk O.I., Matskevych V.M..

APPLICATION OF THE DIFFUSION-WEIGHTED MRI FOR PREDICTION OF THE INTRAOPERATIVE BLOOD LOSS DURING THE NEPHRECTOMY IN PATIENTS WITH RENAL CELL CARCINOMA AFTER EMBOLIZATION OF RENAL ARTERY

Danylo Halytsky Lviv National Medical University, Ukraine

Abstract: During the last decade renal artery embolization (RAE) is used for preparation of patients with renal cell carcinoma (RCC) of large size or significant vascularization to surgical treatment. Currently there is no accurate method of prediction of intraoperative blood loss in patients with RCC after RAE, that may give possibility for more adequate pre- and postoperative patient management and to define the indications for re-embolization. Goal. The goal of the study was to evaluate the possibility of application of diffusion-weighted imaging (DWI) of MRI and of apparent diffusion coefficient (ADC) for the prediction of the estimated intraoperative blood loss (EIBL) during the open radical nephrectomy in patients with RCC and after RAE. **Materials and methods.** The study enrolled 33 patients (main group, 18 males and 15 females) with solid RCC according to clinical and radiologic data and with the indications to selective RAE with subsequent radical nephrectomy. Mean size of the tumor was 8.6±3.8 cm in greatest dimension. In all patients with RCC in the same day or day before RAE and 7 days after the RAE MRI with additional DWI sequence with b-value=0.800 was performed. Subsequent measuring of the ADC over the tumor region was done in all cases. ADC values of the normal renal parenchyma for control were achieved during the examination of 15 healthy volunteers. In all patients with RCC 7-8 days after the RAE open radical nephrectomy with simultaneous EIBL measurement was executed. **Results.** In patients with EIBL less than 500 ml and with no episodes of the hemotransfusions mean ADC value decreased by 18.4-31.9% in comparison with initial ADC value. As opposed to that in patients with EIBL more than 500 ml and in selected cases with hemotransfusions mean ADC value increased by 4.91-65.64% compared to baseline value. Analysis of the acquired data showed significant ($p<0.05$) difference in main group of patients in whom no hemotransfusions were required in post-op period (n=27, 81.82%) in mean ADC values before and after RAE (decrease by 20.25%): 1.63±0.31×10−3 mm2/s vs 1.30±0.19×10−3 mm2/s. In main group patients with hemotransfusions in post-op period this value increased by 28.83%: 1.63±0.31×10−3 mm2/s vs 2.10±0.47×10−3 mm2/s ($p<0.05$). Conclusions. In the result of our study strong direct relationship (correlation coefficient r=0,96) between the volume of EIBL during open radical nephrectomy and ADC values in patients with RCC after RAE was detected. Application of MRI and its imaging biomarkers may be valuable clinical instrument for the prediction of the EIBL volume during the open radical nephrectomy in patients with RCC after RAE and need of the hemotransfusion in post-op period.

KeyWords: renal cell carcinoma, embolization, biomarker, nephrectomy, blood loss, MRI.

INTRODUCTION

Renal cell carcinoma (RCC) accounts for approximately 3.7% of all adult malignancies and more than 90 % of neoplasm arising from kidney. According to National Cancer Registry of Ukraine during last years the indices of morbidity and mortality continued at a stable high level with a tendency to growth.

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In 2014 in Ukraine the indices of morbidity and mortality after RCC accounts for 11.3 (14 men and 9 women) and 5.3 (7.5 men and 3.4 women) and in 2015 11.7 (15.0 men and 8.8 women) та 5.1 (7.3 men and 3.2 women) per 100 thousand of population accordingly [1].

The «gold standard» for localized and locally advanced RCC treatment is nephrectomy in despite of new alternative methods. During the last decade renal artery embolization (RAE) is used as preparation of patients with big or/and hypervascularized RCC's for surgical treatment [2]. The main advantages of RAE before nephrectomy are: re-

duction of tumor clots` size (that facilitate its removal), blood loss reduction during surgery, alleviation of dissection because of edema development. RAE is usually performed 7-10 days before surgery.

The adverse side of RAE is postinfarction syndrome, which appear as pain in the iliac region, nausea and fever, this syndrome occur in ¾ of patients. Some authors observe significant blood loss during nephrectomy after RAE, perhaps it is caused by the incomplete schematization of tumor tissues. Literature data about efficiency of RAE performed as preparation of patients with RCC to nephrectomy are often contradictory, especially concerning estimated intraoperative blood loss (EIBL) volume and amount of blood transfusion, operative time, complications and survival rates [7, 13]. Thus, according to Schwartz and co-authors EIBL volume during radical nephrectomy in patients with RCC after RAE with median tumor size of 11.2 cm was variable and ranged from 100 to 5000 ml [11]. In another new research conducted by Ramaswamy, was established, that blood transfusion volume after radical nephrectomy in patients with RCC after total RAE also was also significantly variable and ranged from 222 to 1050 ml [10]. At the same time, according to Suad and co-authors, medium volumes of EIBL and intraoperative blood transfusion amounted 300 ml and 250 ml accordingly [5].

Currently there is no accurate method of prediction of EIBL volume during nephrectomy in patients with RCC after RAE, that may give possibility for more adequate treatment planning and postoperative patient management and to define the indications for re-embolization. In the last years the attention of researchers was focused on investigation of MRI and its modalities as diffusion weighted imaging (DWI) to estimate the efficiency of arterial embolization in treatment of benign and malignant tumors, as uterine fibroid and leiomyoma, hepatocellular cancer and breast cancer [3, 6, 9, 12]. DWI is the MRI sequence, which uses strong bipolar gradients to enhance sensitivity to thermally induced Brownian motion of hydrogen molecules, that allows to measure molecular diffusion in tissues in vivo and it is important for profound characteristic of neoplasm [4]. However, all conducted studies were directed toward estimating the efficiency of arterial embolization, which was performed as a disease palliation but not during preparation to surgery.

In our previous work we investigated the efficiency of DWI MRI and its quantitative parameter - apparent diffusion coefficient (ADC) as imaging biomarkers in differential diagnostic of solid and cystic RCCs, benign renal tumors, benign cysts and abscesses [8]. We achieved statistically significant difference in mean ADC values of RCC and of the healthy renal parenchyma. We observed significant restriction of diffusion of hydrogen molecules in regions of solid neoplasms and it's increase in renal cysts and in abscesses in comparison with normal kidney parenchyma. Taking into account that tissue density affects diffusion of hydrogen molecules in them and that ADC consists of 3 main components - intracellular (inside the cytoplasm), extracellular (in the interstitial liquid, in blood and lymph vessels) and diffusion between intra- and extracellular enviroments, we suggested that DWI and ADC may be used as imaging biomarkers for evaluation of changes, which occurs in RCC tissues after RAE and for prediction of intraoperative blood volume loss in patients in whom nephrectomy will be executed.

2 PURPOSES, SUBJECTS and METHODS:

2.1 Purpose The goal of the study was to evaluate the possibility of application of DWI of MRI and of ADC for prediction of the EIBL during the open radical nephrectomy in patients with RCC and after RAE.

2.2 Subjects & Methods

The study was allowed by Ethics Committee and conducted on the basis of the Departments of Urology and Radiology of Danylo Halytsky Lviv National Medical University, Department of Interventional Radiology of Lviv Emergency Hospital, Center of Endourology of Lviv Clinical Railway Hospital and at the Medical Center "Euroclinic", Lviv, during 2013-2017 years.

The main group enrolled 33 patients (18 males and 15 females) with solid RCC according to clinical and radiologi-

cal data and with the indications to selective RAE with subsequent radical nephrectomy. The age of the patients ranged from 52 to 70 years old, the mean age was 62±6.3 years. Tumor size ranged from 7.3 to 11.4 cm, the mean size was 8.6±3.8 cm in the greatest dimension. Before surgery males had the level of hematocrit of 38-49% and females of 33-43%.

In all patients with RCC in the same day or day before RAE abdominal MRI using 1.5 T scanner (Signa HDxt, General Electric, USA) and 8-channel coil was performed. In all cases was applied same standardized scanning protocol (General Electric), which additionally included DWI sequence with following parameters: repeat time (TR)=12000 ms, echo time (TE)=90 ms, field of view (FOV)=40x40 cm, matrix=200 x 192, number of excitations (NEX)=3, bandwidth= 250 kHz, diffusion direction= slice, slice thickness= 6,0 mm, interscan gap = 1,0 mm with b-value=0.800 mm2/s, acquisition time=17 sec. DWI was conducted before contrast media administration, using single-shot echo-planar imaging sequence with parallel imaging technique and fat saturation during one breath-hold. The mean duration of MRI examination was 35 minutes.

Selective RAE was performed under fluoroscopic guidance using the following method: under local anesthesia (0.5 % Novocaine) radial artery was punctured by Seldinger and after that introducer (6 Fr) was inserted. Selective angiography of right renal artery was performed using MR-catheter and injection of 80 ml of contrast (Ultratwist-370). After that one COOK spiral and three Gianturco spirals were alternately introduced (all spirals MRI-compatible), achieving total RAE. After RAE puncture area was bandaged with compression for 6 hours. The mean duration of the procedure was 120 minutes. Re-RAE wasn't performed in any case.

In all patients with RCC 7-8 days after selective RAE open radical nephrectomy with further pathologic verification of diagnosis was executed: in 100% of cases RCC diagnosis was confirmed. Measurement of EIBL was performed in all patients according to the following method: it was accepted, that 1 ml of blood weigh is 1 g; swabs saturated with blood were weighted and then the mass of blank swabs from the same lot were subtracted; during weighing capacities for blood the mass of blank capacities were subtracted; blood volume, which got on surgical sheet, under body of the patient was assessed; the volume of the solution for irrigation was taken into account and it was subtracted from the general volume of EIBL.

Control MRI was performed in all patients 7 days after nephrectomy, which on purpose to reduce financial and temporal expense, included only 2 sequences: coronal T2-weighted single shot fast spin echo (SSFSE) and DWI with identical to above mentioned parameters. The mean duration of the examination was 10 minutes.

ADC was measured using ADC-maps, which were generated automatically on the workstation based on DWI and which were used for assessment of diffusion in RCC tissues before and after RAE. Region of interest (ROI) was placed over the tumor region to measure the ADC value whereupon it was measured (image 1).

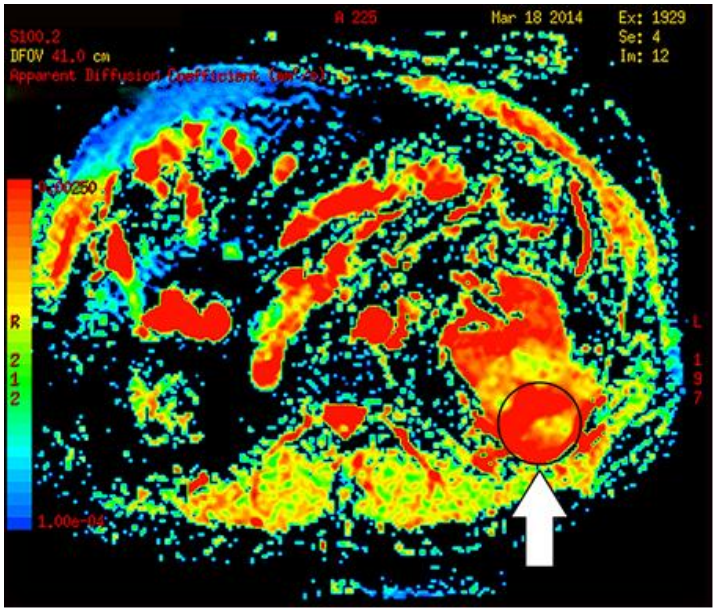

Fig. 1. ADC-map of the patient with RCC of the left kidney before RAE (pointer), ROI is placed over the tumor region, obtained ADC value – 1,62 x10−3 mm2/s.

We obtained ADC values of the healthy renal parenchyma in 15 healthy volunteers without renal pathology (control group) according to data of clinical and imaging research (9 males and 6 females), aged from 38 to 50 years old (with median age 48,2±1,8 years). For assessment of morpho-functional status of kidneys all volunteers passed

through appropriate examination before been included into research: general analysis of blood and urine, biochemical analysis of blood (creatinine, urea, ALT, AST), renal US. MRI was done to all volunteers using 2 sequences: coronal T2-weighted SSFSE and DWI with analogical to the main group parameters. For measurement of ADC values, ROI with median diameter of 3,5 cm was located over renal parenchyma and then registration was performed according to technique described earlier [8].

Antitumor therapy wasn't performed before MRI, RAE and surgery. People with metallic details inside the body weren't included in the study.

Statistics. Microsoft Exel 2016 was used for statistical analysis of obtained data, by Student-Fisher's method and for calculation of Pearson correlation coefficient. Value $p<0.05$ considered as a statistically significant result.

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

During the open nephrectomy in patients with RCC after RAE, EIBL varied from 158 to 1250 ml, the mean volume was 457,3±260,52 ml. Depending on the EIBL all patients of the main group were separated into 4 different categories: I category- EIBL - 0-250 ml (n=9, 27,27%), II category – EIBL 250-500 ml (n=14, 42,42%), III category – EIBL 500-1000 ml (n=8, 24,24%) and IV category - EIBL more than 1000 ml (n=2, 6,06%). Medium values of EIBL for each category were: I – 216,55±34,45 ml, II – 387,29±64,19 ml, III – 669,36±118,23 ml, IV – 1182,5±95,46 ml.

We established, that medium ADC value of the healthy renal parenchyma of volunteers was 2,47±0,12×10−3 mm2/s. In a result of analysis of the obtained ADC data we obtained statistically significant ($p<0.05$) difference between the mean ADC values in the main group before and after RAE and in control group: 1,63±0,31×10−3 mm2/s vs 1,44±0,40×10−3 vs 2,47±0,12×10−3 mm2/s accordingly. We observed direct correlation between the values of EIBL volume and ADC in patients after RAE, Pearson correlation coefficient was r=0,96. In patients of I and II categories with EIBL volume less than 500 ml and with no episodes of hemotransfusions mean ADC value decreased by 18.4-31.9% compared to baseline value, because of bigger infarct areas of the tumor tissues, decrease of microcirculation and as a result the restriction of hydrogen molecules diffusion. As opposed to that in patients of III and IV categories with EIBL volume more than 500 ml and in some cases with hemotransfusions mean ADC value increased by 4.91-65.64% compared to baseline value. The interconnection between the increase of this ADC value and EIBL volume in the IV category of the patients can be explained with expressed oedema of the renal tissue near infarct area, necrotic process and deficient decrease of microcirculation in tumor area. All of this together conduced the increase of molecular diffusion in tumor tissues and possibly as a result of it the increase of bleeding during surgery. The detailed description of EIBL and ADC values of the patients with RCC before and after RAE presented in table 1.

Table **1.**

Mean ADC values of the main group of patients before and after RAE and EIBL volume.

Category of patients/ subgroup	Mean ADC before RAE, $\times10^{-3}$ mm^2/s	Mean ADC after RAE, $\times10^{-3}$ mm^2/s	Mean EIBL, (range), ml
Category I, EIBL 0-250 ml (n=9)	1.63±0.31	1.11±0.10	216.55±34.45 (158-250)
Category II, EIBL 250-500 ml (n=14)		1.33±0.08	387.29±64.19 (275-487)
Category III, EIBL 500-1000 ml (n=8)		1.71±0.12	669.36±118.23 (510-823)
Category IV, EIBL >1000 ml (n=2)		2.70±0.06	1182.5±95.46 (1115-1250)
Patients, in whom no hemo-transfusions were required (n=27)		1.30±0.19*	351.33±119.75 (158-570)
Patients, who had hemotrans-fusion (n=6)		2.10±0.47*	899.0±226.45 (720-1250)

*comparison of groups between each other ($p<0.05$)

Analysis of the acquired data showed significant ($p<0.05$) difference in in mean ADC values in subgroup of patients in whom no hemotransfusions were required in post-op period (n=27, 81.82%) in comparison with baseline mean ADC value: 1.30±0.19×10−3 mm2/s vs 1.63±0.31×10−3 mm2/s accordingly (decrease by 20.25%). In sungroup of patients with hemotransfusions in post-op period (n=6, 18,18 %), the mean ADC value increased by 28.83% compared to baseline value: 1.63±0.31×10−3 mm2/s vs 2.10±0.47×10−3 mm2/s, such difference was statistically significant ($p<0.05$).

CONCLUSIONS

1. In the result of our study strong direct correlation (r=0,96) was observed between the volume of EIBL during open radical nephrectomy and ADC values in patients with RCC after RAE;

2. In patients of the main group that required hemotransfusions in post-op period the mean ADC value increased by 28.83% compared to baseline, in patients in whom hemotransfusion wasn't performed mean ADC value increased by 20.25%: 2.10±0.47×10−3 mm2/s vs 1.30±0.19×10−3 mm2/s ($p<0.05$);

3. The application of MRI and its imaging biomarkers DWI and ADC may be valuable clinical instrument for prediction of the EIBL volume during the open radical nephrectomy in patients with RCC after RAE and need of the hemotransfusion in postoperative period.

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SURGERY

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COMPLICATIONS OF BRONCHIAL BLOCKADE IN THE TREATMENT OF PYOPNEUMOTHORAX (pilote study)

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Abstract: The study deals with the use of bronchial occluders in patients with pyopneumothorax and techniques of the procedure. The authors described complications associated with the employment of bronchial blockade, and methods of their correction.

KeyWords: pyopneumothorax, bronchial occluder, bronchial blockade, bronchial fistula.

INTRODUCTION

Pyopneumothorax occurs in 33.3% of patients with nonspecific infectious lung disease [1, 5]. One of the pathogenic factors of chronic and pyopneumothorax is infringement of lung tissue impermeability, leading to the development of bronchial fistula [2, 4 and 7]. Successful closure of fistula makes surgical treatment of these patients more reliable and less prolonged [3, 6 and 7].

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2 PURPOSES, SUBJECTS and METHODS:

2.1 Purpose goal of the study was to evaluate the complications of bronchial blocade in the treatment of pyopneumothorax by using endoscopic bronchial fistula occlusion

2.2 Subjects & Methods

Our study involved 77 patients who underwent thoracoscopic debridement of pleural cavity and endoscopic bronchial blockade. The indications for endoscopic bronchial fistula occlusion included air in pleural drainages after thoracoscopic operation and X-ray picture of persistent residual cavity. Bronchial blockade was completed in 1- 2 days after operation. We used the endobronchial reverse valve, made of medical rubber compound compatible with human tissues. The valve permits air and bronchial content to move away from residual cavity, during expiration and prevents their reverse motion during inspiration.

Endoscopic bronchial occlusion was performed with imaging procedures: bronchoscope was introduced into the pleural cavity through the drainage of 20 ml of 3% stained hydrogen peroxide solution in a ratio of 10: 1. The procedure was performed under local anesthesia.

After identification of the bronchus associated with fistula, bronchoscope was extracted and the valve of the desired diameter was placed at its end. The diameter of the valve exceeded the diameter of the bronchus by 1.2-

1.5 times. Bronchoscope with a valve was administered orally in the tracheobronchial tree. The valve was fixed by biopsy forceps (inserted through the working channel of bronchoscope) for the jacket installed into the bronchus until it stopped. Then the bronchoscope was removed from the valve, holding the valve by the forceps (figure 1).

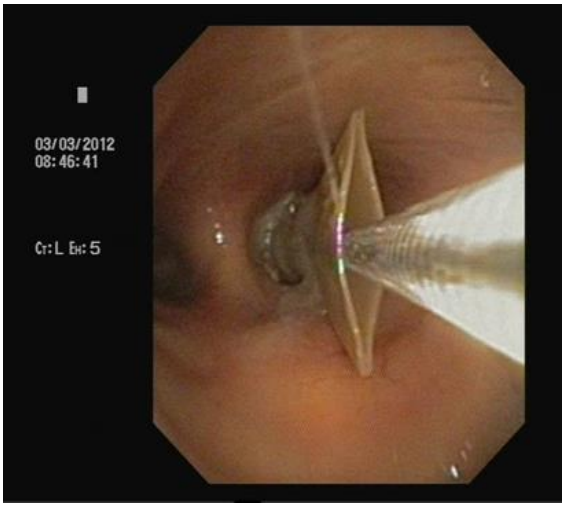

Fig. 1. Installation of the valve

During coughing it can be seen as the valve flaps open and release air (figure 2).

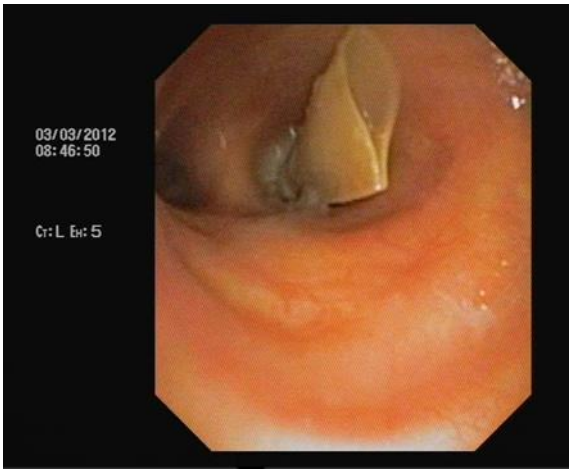

Fig. 2. Valve flaps release air.

In case of adequate valve function, bronchoscope was removed from the bronchial tree.

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

The most common complication was purulent bronchitis, being observed in 15 (19.5%) patients. This problem was due to valve operation features, the purulent contents of pleural cavity freely penetrated tracheobronchial tree through the fistula, but did not get back which resulted in its accumulation in the bronchial tree on the affected side, and sometimes in the opposite lung. This complication was suspected due to patients' presentation, namely difficult breathing, fever combined with increased white blood cell count, scattered dry and moist wheezing on auscultation. The diagnosis was confirmed by X-ray and bronchoscopic examination.

Patients with bronchitis underwent therapeutic bronchoscopy on a regular basis under local anesthesia. Active aspiration of bronchial secretions and irrigation of the tracheobronchial tree with anti-inflammatory, mucolytic and antibacterial agents were carried out under visual control. This procedure was carried out every day.

Inhalations were also performed using nebulizer therapy. We used 2-4 ml 20% -acetyl cysteine solution and 2 - 10 0.02% ml Decasanum solution 2 - 4 times a day. Immediately before inhalation, bronchoscopy sanitation was performed with local administration of 2 ml 2.4% euphyllinum for improving the penetration ability of drugs.

The next commonest complication involved overgrowth of granulation tissue in the area of the valve in 5 (6.5%) patients. The growth of granulation tissue in these patients was detected directly during removal of the valve, clinical manifestations were absent. Granulation tissue was removed endoscopically immediately after valve removal with histological examination. All cases of histological examination showed productive chronic inflammation with the formation of granulations.

In 3 (3.9%) patients the valve migrated into the bronchial tree. This complication was suspected in recovery of discharge air to drain and was confirmed by the chest X-ray examination. We detected the valve that had changed its location (this type of valve has the X-ray contrast element, which allows to identify it by X-ray). This complication was caused by a mismatch in valve size

and bronchus diameter after subsidence of bronchitis and edema of bronchial wall. Only one patient was found to have valve migration into the pleural cavity in partial resection of the lung in artificial respiration after increased oxygen pressure. This complication required re-installation of the valve taking into account new conditions and detailed information provided by intensive care physicians.

CONCLUSIONS

Thus, bronchial blockade is an effective and safe method in the treatment of patients with pyopneumothorax. Possible complications of its use are easy to diagnose and correct, but this method is not widely used and requires further study.

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THERAPY

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LEFT VENTRICULAR REMODELING IN HYPERTENSION: EVOLUTION OF THE APPROACH (review)

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Abstract: Left ventricular remodeling is one of the earliest complications of hypertension that has been proven to be an independent predictor of cardiovascular morbidity and mortality. In this article, the development of approaches to understanding its mechanics and prognostic value are briefly reviewed, with additional focus on patients with obesity and type 2 diabetes mellitus as frequent comorbid pathologies that significantly modify the typical morphological changes of the heart in hypertension. The main intacardiac mechanisms of decrease of left ventricular function constituing the direct pathophysiological and pathomorphological base for development of chronic heart failure are presented.

KeyWords: hypertension, left ventricle, remodeling, dysfunction, chronic heart failure.

Left ventricular (LV) remodeling is one of the earliest complications of hypertension that has been proven to be an independent predictor of cardiovascular morbidity and mortality [1]. In this article, the development of approaches to understanding its mechanics and prognostic value are briefly reviewed, with additional focus on patients with obesity as a frequent comorbid pathology that significantly modifies the typical morphological changes of the heart in hypertension [1-3].

Historically, the development of concentric hypertrophy of the LV was considered the most typical pattern of its structural and functional remodeling in hypertension [4, 5]. An increase in the LV wall thickness allowed the specific tension of the tissue to remain constant [6]. Dilatation of LV, on the other hand, was considered to be a marker of exhaustion of adaptive reserves of the cardiac muscle and a sign of heart failure [5].

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However, this concept was not able to explain the entire range of variations of the LV reaction to prolonged increase in blood pressure.In various studies, the cases of maladaptive (excess) hypertrophy [7], normal LV geometry as well as "paradoxical" findings in patients with eccentric LV hypertrophy in hypertension have been described: some patients of this group were characterized by an increase in cardiac output and minute volume of the heart despite the traditional notions of left ventricular dilatation as a marker of systolic dysfunction [8, 9]. A. Ganau, R.B. Devereux, M.J. Roman et al. should be considered the founders of the modern approach to the LV remodeling classification in hypertension [4, 10]. In 1992, they presented the results of an elegant study in which a comparative analysis of echocardiographic data was performed on 165 untreated patients with essential hypertension and 125 age- and sex-matched healthy subjects. The results obtained in the control group allowed the authors to suggest the relative wall thickness (RWT) of the LV of 0,44 as the upper limit of the normal range (these values were observed in 99% of healthy subjects, of which 98% had normal calculated LV mass). Approximating these results on the patients with essential hypertension, the authors proposed a classification of LV geometry based on the joint accounting of RWT and the presence of LV hypertrophy (Table 1) [4].

Table **1.**

Left ventricular geometry classification by A. Ganau et al. (1992)

LV geometry patterns	LV RWT	LV hypertrophy
Normal	< 0,45	No
Concentric remodeling (CR)	≥ 0,45	No
Concentric hypertrophy (CH)	≥ 0,45	Yes
Eccentric hypertrophy (EH)	< 0,45	Yes

Application of the obtained classification to the cohort of surveyed patients with hypertension has shown somewhat unexpected results. Thus, the presence of concentric hypertrophy of the LV, which was considered typical for patients with hypertension, was found only in 13 (7,9%) of the patients from main group; 21 (12,7%) patients had a concentric remodeling of LV without hypertrophy, 44 (26,7%) - eccentric hypertrophy and 87 (52,7%) - normal LV geometry.

In order to investigate the pathophysiological basis of the obtained results, the authors examined the features of hemodynamics that were typical for the described types of remodeling. It turned out that the patients with concentric patterns of remodeling (CR and CH) were characterized by an increase in the overall peripheral vascular resistance on the background of normal or slightly increased volume load, while patients with eccentric LVH, on the contrary, demonstrated an increase in the cardiac index and circulating blood volume on the background for normal values of peripheral vascular resistance [4, 10].

The study by A. Ganau et al. has caused a considerable interest of specialists in view of the revealed features of the LV structural adaptation among the hemodynamically heterogeneous population of patients with hypertension. In order to more closely investigate the phenomenon described, the studies were conducted to determine the distribution of types of LV remodeling in hypertensive patients with concomitant pathology.

Thus, in [11], a significantly more frequent development of LVH in patients with essential hypertension on the background of type 2 diabetes has been shown (97.0% vs. 63.1%, $p < 0.001$) due to significantly increased rate of patients with concentric hypertrophy (61.2% versus 26.1%, $p = 0.010$), while the share of EH of the LV in both groups was the same (35.8% vs 37.0%; $p > 0.05$). A similar distribution was identified in a study of 400 patients of a Japanese population [12]: the patients with type 2 diabetes had an increased incidence of LV CH (39,4% vs 26.8%, $p < 0.001$); the presence of diabetes mellitus was independently associated with an increase in the LV RWT of ≥ 0.45.

A number of studies have also investigated the features of LV remodeling when combined with obesity. Thus, in [13], a significantly higher incidence of LVH, primarily by concentric type (with an increase in both thickness of the walls and sizes of the LV cavity) in patients with hypertension on the background of abdominal obesity was observed. The author emphasizes the association of LV CH with abdominal obesity due to chronic hyperactivation of renin-angiotensin-aldosterone system (RAAS) and an increase in the level of insulinemia in metabolic syndrome, which results in an increased production of the extracellular matrix components, as well as hypertrophy of cardiomyocytes. The study performed on 1292 male patients has also demonstrated that the presence of obesity was associated with an increased detection of LV hypertrophy; as a note, although CH remained the most common variant of the left ventricular geometry in all groups, an increase in the body mass index (BMI) was clearly related to increased incidence of EH [3].

A number of prospective studies have also been devoted to estimating the prognostic value of LVH and different LV remodeling patterns. It should be noted that the mass and geometry of the LV indices occupy a special place in the definition of cardiovascular risk, being, on one hand, related to the generally accepted risk factors (such as the level of blood pressure, BMI), and on the other hand – having an independent predictive value. Thus, the presence of the LVH (defined by an electrocardiogram and echocardiographic method) was recognized as an independent risk factor for increased cardiovascular and cerebrovascular morbidity and mortality both in the general population and in patients with hypertension in a number of studies with

an odds ratio (OR) of 1.5 to 3.5, weighed OR of 2.3 for all studies [7, 10, 14].

H.M. Krumholz et al. have conducted a comparative analysis of clinical anamnestic data and ultrasound parameters of the LV in 3216 patients with hypertension – participants of the Framingham study [15]. It turned out that the rate of cardiovascular events during 8 years of observation was the highest in the group of patients with concentric hypertrophy, and the lowest – in those with normal LV geometry. This pattern was also maintained after standardizing the frequency of events by the presence of traditional risk factors. The OR of cardiovascular events and death from any cause was 2.1 (1.5-3.1) and 2.1 (1.3-3.4), respectively, for males, and 1.6 (1.0-2.6) and 1.5 (0.9-2.5) for females. An important point was the detection of the highest values of LV myocardial mass in the group of patients with CH; Standardizing the risk levels by myocardial mass significantly weakened the described relations: the mentioned OR were 1,3 (0,8-2,1) and 1,7 (0,8-3,5) for males, 1.2 (0.6-2.3) and 1.1 (0.5-2.3) for females, with no significant differences being revealed in the incidence of cardiovascular events and death for any reason.

The syndrome of chronic heart failure (CHF) is one of the typical complications of the natural current of hypertension. The problem of its diagnosis and treatment remains one of the most pressing in modern cardiology given the high mortality rates among patients with manifest CHF, especially its congestive form [16].

Pathogenetic basis of chronic heart failure is presented by an absolute or relative inability of the heart to supply the cardiac output adequate to needs of the body. At the initial stages of the CHF development, the altered neurohumoral regulation of cardiac activity and vascular tone causes a series of hemodynamic reactions aimed at supporting systemic blood circulation at an adequate level; with the further course of disease, the exhaustion of myocardial adaptation reserves results in the heart's inability to translate the venous pre-load increasing in physical activity to an adequate cardiac output, which is accompanied by clinical manifestations of heart failure. As the functional cardiac failure continues to worsen, the described condition offsets with less physical activity, and then at rest; clinically it is accompanied by the development of congestive heart failure that, along with myocardial infarction and cerebral stroke, classifies the patient to stage III (advanced) hypertension, due to the drastic deterioration in the quality of life and low five-year survival [16].

It should be noted that, similar to the vast majority of nosologies in the internal diseases clinic, CHF was first described as a clinical syndrome, with setting the relations between the characteristics of the objective manifestations of disease and morphological changes of the heart being only possible according to the results of autopsies [17]. This situation naturally contributed to the limitation of CHF syndrome understanding by its congestive form that, on one hand, allowed the physician to diagnose the clinical state, and on the other – was accompanied by the development of severe irreversible changes in the cardiac muscle (primarily, dilation of its cavities) being easily revealed post mortem.

The broad introduction of echocardiography, first to the scientific research and subsequently to the clinical practice, has allowed to confirm the association of severe CHF with the dilatation of the cavities of the heart, especially the LV. In addition, the study of calculated parameters of intracardiac hemodynamics has revealed a significant decrease in LV stroke volume in these patients [18, 19]. This condition was called systolic dysfunction; with the aim of standardizing the values of the stroke volume, the most recognition was obtained by its indexing by the LV end-diastolic volume. The new parameter was called LV ejection fraction (EF). At the same time, it was found that the deterioration of systolic function of the LV compared to healthy persons considerably anticipated the development of manifest CHF. This fact became the basis to the introduction of the concept of asymptomatic left ventricular dysfunction [17, 18].

The studies conducted in patients with hypertension have revealed that the development of latent and, subse-

quently, manifest systolic dysfunction is preceded by a long state of compensatory hyperfunction and LV hypertrophy [4, 10]. Moreover, in a number of studies it was shown that prolonged exposure to neurohumoral effects mediating remodeling of the cardiac muscle leads to a decrease in the LV contractility. These findings complement the concept of natural development of systolic heart failure following LV myocardial hypertrophy after prolonged natural course of hypertension [20-23].

Nevertheless, it did not explain the existence of a large number of patients with advanced clinical symptoms of CHF, in some cases even congestion, in whom the ultrasound examination of the heart revealed the systolic function of the LV to be normal or even elevated. After years of scientific debate, this phenomenon was recognized and a special form of clinical heart failure was defined - heart failure with preserved ejection fraction [16, 17].

Introduction of Doppler analysis of intracardiac blood flow to routine ultrasound examination, and in recent years – analysis of peak velocities of mitral annular motion in the tissue Doppler mode allowed to conduct a careful assessment not only of the systolic function of the ventricles but also state of their diastolic filling; thus, it became possible to directly assess both aspects of ventricular pumping function [16,17]. As a result, studies followed [24] which have revealed that, in the absence of valvular heart disease or tachyarrhythmias which can lead to inefficient hemodynamic, chronic heart failure with preserved ejection fraction of the LV in most cases is caused by impaired diastolic filling of the latter.

Studies conducted in hypertensive patients without systolic dysfunction revealed a strong association between the development of LV hypertrophy, especially its concentric pattern (see Table 1), and type I diastolic dysfunction (impaired relaxation), which did not contradict the concept of active, energy-consuming muscular relaxation mechanism [17]. With further course of disease, extracellular matrix remodeling with development of diffuse myocardial fibrosis leads to restrictive changes of LV diastolic blood filling, causing pseudo-normalization of transmitral blood flow velocities (type II diastolic dysfunction) due to decreased myocardial compliance causing impairment of atrial phase of ventricular filling [17, 24]. This process in separate cases was accompanied by initial manifestations of the LV cavity dilation and, accordingly, the development of its eccentric hypertrophy. Restrictive type of diastolic dysfunction in patients with essential hypertension was rarely observed.

Thus, the development of diastolic and /or systolic myocardial dysfunction in the chronic course of the underlying cardiovascular disease always precedes, and later, with the progression of functional disorders, accompanies clinical manifestation of CHF syndrome. At the same time, the deterioration of the pumping function of the heart may be, to varying degrees, due to decrease in ventricular contractility as well as impairment of their diastolic filling, which should not be considered independent processes in view of their frequent coexistence and, most importantly, common mechanisms of development.

Absolute or relative myocardial energy deficit in the hypertensive heart that may arise both as a result of increased need for oxygen and nutrients (an increase in the myocardial mass and power developed in hypertension) and their reduced delivery (concomitant coronary atherosclerosis) naturally leads to impairment of active relaxation of the LV. Chronic neurohumoral hyperactivation results in development of myocardial hypertrophy, in which an increase in the thickness of the myofibrillae is accompanied by enhanced synthesis of the extracellular matrix components with the development of diffuse fibrosis, resulting in restrictive disorders of LV filling. Both energy deficit and pathological remodeling of the ventricular wall contribute to the development of impaired contractility.

All three components contribute to a decrease in pumping function of the LV, with progression of disease at the later stages usually causing further deterioration of systolic function, which is accompanied by severe structural changes of the myocardium that are difficult and rarely subjected to reverse development even under the influence of adequate treatment [16, 17]. Thus, the possibly

earliest detection of diastolic dysfunction of the heart in patients with hypertension (optimally before the clinical manifestation of CHF) opens the prospects for timely correction of pathophysiological disturbances of circulation and patholigical cardiovascular remodeling, having the potential as the most effective measure for secondary prevention within the commonly recognized strategy of cardiovascular risk factors management.

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THERAPY

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MODERN TRENDS IN THE DIAGNOSIS AND TREATMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE IN OVERWEIGHT SUBJECTS (review)

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is a scourge of the planet's population, especially the developed countries. NAFLD development is due to the global increase in the number of overweight and obese people. NAFLD in its turn is the main factor of cardiovascular risk development. Early detection of the cardiovascular risk development with underlying NAFLD and overweight remains understudied. This article reviews the literature dealing with the diagnosis and treatment of NAFLD in overweight subjects.

KeyWords: non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, hypertension, endothelial lipase, lipid metabolism, overweight.

1.1 Non-alcoholic fatty liver disease: modern understanding and the state of affairs in relation to overweight.

According to one of the leading gastroenterological and hepatological organizations – the World Gastroenterology Organisation (WGO) – non-alcoholic fatty liver disease (NAFLD) usually means a common chronic hepatic disease mainly manifested as accumulation of triglycerides in hepatocytes contributing to the development of subclinical inflammation not associated with alcohol abuse. NAFLD is a complex of pathologies from steatosis to progressive inflammation – non-alcoholic steatohepatitis (NASH) with possible development of hepatic cirrhosis and hepatocellular carcinoma [1]. Morphological criteria of steatosis include macrovesicular lipid storage in more than 5% of hepatocytes [2].

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NAFLD is diagnosed in 20-30% of population in Western European countries and the USA and in 15% of Asian population [7].

The prevalence of NASH as one of NAFLD forms is lower and amounts to 2-3% in general population and 16-37% in overweight groups [8]. NAFLD increases general mortality rates in patients as compared to general population of the same age and sex [9]. The main mortality factor of patients with NAFLD is cardiovascular diseases (25%) [10]. According to G. Marchesini et al. (2001), 67% of patients with NAFLD suffer from overweight; 57% have impaired glucose tolerance, 47% – hypertriglyceridemia, 27% – decreased alpha cholesterol rates, and 17% – hypertension [11].

Manifestations of NAFLD are rather scarce. Common reasons for visiting a doctor include arterial hypertension (AH), diabetes mellitus (DM), coronary artery disease

Steady increase in the incidence of NAFLD correlates with the growing number of overweight patients [3, 4]. For this reason, NAFLD is considered as one of the most common hepatological conditions leading to deteriorated life quality, disability and mortality [5, 6].

(CAD), and cholelithiasis. The majority of patients are females (65-85%) with a mean age of 50 years old. In most cases, BMI is greater than 25 kg/m^2. Clinical symptoms are absent in 50-100% with the most common ones being asthenic syndrome, abdominal discomfort, and heaviness in the right subcostal area [12].

The generalizing NAFLD pathogenesis model was previously believed to be the "theory of two hits", according to which the "first hit" is an increased influx of free fatty acids (FFA) in the liver. The accumulation of fat in hepatocytes is a consequence of FFAs coming from the fatty tissue, their slower oxidation in mitochondria and excessive synthesis of FFAs from acetyl coenzyme A. The increased influx of FFAs and their slower oxidation lead to FFA esterification with excessive formation of triglycerides in hepatocytes and excessive secretion of very-low-density lipoprotein cholesterol (VLDLP CS), which enhances free-radical oxidation of lipids and accumulation of the peroxidation products ("second hit") [13]. Thus, the liver is a target organ in this case; hepatomegaly in patients with overweight or abdominal obesity (AO) is due to the accumulation of fat on account of its expansion from natural sources and to the fact that the hepatocyte apoptosis rate is lower than the proliferation rate [14].

Fibrosis is a result of the steatohepatitis transformation through the merging of fat deposits during hepatocyte breaking with the formation of cysts, inflow of a large amount of lipids from hepatocytes to the interstitial region with an inflammatory reaction and fibrotic changes, mechanical and inflammatory damage of hepatic veins and development of perivenular fibrosis. The development of hepatic cirrhosis is due to the obstruction of hepatic veins, ischemic necroses and collapse of hepatic lobes with the development of connective tissue septa. It is known that the liver performs a number of functions to ensure normal activity of the human body – excretory, digestive, energy, exchange, blood-making, synthetic, deintoxicating, protective, homeostatic, endocrine, and metabolic. The metabolic role consists in the exchange of lipids, proteins, carbohydrates, pigments, enzymes, bioactive substances and microelements. Hepatic metabolic impairments can be divided into primary, which are due to endogenous factors and genetic mutations, and secondary, which are caused by exogenous and endogenous xenobiotics. Thus, primary and secondary hepatic metabolic impairments affect hepatocyte functions disrupting the exchange of bilirubin, biliary acids, proteins, amino acids, glyco- and lipoproteins, porphyrin, copper, iron, mucopolysaccharides, carbohydrates and lipids. Therefore, NAFLD can be both an independent disease and combined with obesity, diabetes mellitus and dyslipidaemia [15].

It has been found in the process of studying lipid exchange that some of its types, especially FFA, have independent toxic action on hepatic cells. It initiated the study of "lipotoxicity" and helped substantiate the relation between the excessive influx of FFAs with food, insulin resistance and the order of events leading to hepatocyte damage. Steatosis ceased to be considered as a precondition for hepatocyte damage. Excessive accumulation of triglycerides (TG) in the cells reflects the adaptive reaction of neutralizing the excess of FFAs [16].

Excessive accumulation of FFAs in hepatocytes plays an important role in NAFLD pathogenesis. The reasons for the "overload" with fatty acids may include:

- excessive influx of FFAs with food (as a result of hydrolysis of food triglycerides) and without it (as a result of active lipolysis in the fatty tissue in case of insulin resistance)
- decreased activity of beta oxidation of fatty acids in hepatocytes in case of insulin resistance (IR)
- impaired export of VLDLPs from hepatocytes in case of impaired synthesis of apoproteins C, E and B.

Unsaturated FFAs are quicker to get bound and prevail in the structure of TGs and phospholipids. Mitochondrial dysfunction combined with NAFLD means structural and functional changes accompanied by membrane damage, disrupted functioning of ion channels and transmembrane potential, decreased synthesis of ATP, pore formation in the membrane, causing an outflow of matrix components

to the cytoplasm. Mitochondrial dysfunction may trigger internal apoptosis and develops at the background of oxidation stress. FFA-initiated cell death is called "lipoapoptosis", and oxidation stress contributes to it [17].

Oxidation stress causes lipid peroxidation, mitochondrial damage and increased secretion of cytokines – TNF-α, IL-6 and IL-8, which leads to inflammation, apoptosis and hepatocyte necrosis further causing fibrosis and hepatic cirrhosis [18].

NAFLD affected by overweight or obesity presupposes impaired secretion of adipokine hormones by the fatty tissue leading to the hyposensitivity of tissues to insulin on account of decreased levels of adiponectin, increased levels of visfatin and resistin and increased levels of chemokines that activate macrophages and contribute to their accumulation in the fatty tissue. Activated macrophages produce cytokines which adversely affect insulin sensitivity [19].

According to I.R. Popova, the prevalence of fatty hepatosis and non-alcoholic steatohepatitis (NASH) is growing with the increase in the body mass index (BMI). In obese patients hepatic steatosis occurs 2.7 times more frequently than in patients with normal BMI, and NASH – eight times more frequently [20].

The notion of abdominal obesity (AO) is introduced which, according to NCEP ATP III, was diagnosed in case of a waist measurement (WM) of more than 102 cm in males and more than 88 cm in females. Abdominal obesity contributes to fast progression of cardiovascular diseases, their severity and incidence of complications. According to INTERHEART study, abdominal obesity is an independent risk factor of myocardial infarction [21].

Abdominal overweight type is an independent risk factor of NAFLD. Progressive weight gain contributes to a faster development of NAFLD; thus, the risk of its development increases by 45% if a person gains 2 kg or more a year. Every 2.5 cm of waist measurement increases blood pressure by 10%, total cholesterol by 8% and triglycerides 18%, and decrease high-density lipoproteids (HDLP) by 15% [12].

For many years, liver biopsy has been the "golden standard" of NAFLD diagnostics. This methods allows assessing pathognomonic morphological symptoms, determine structural organ changes and the degree of connective tissue development [22]. But this method has a number of disadvantages, among which the first is its invasiveness. Biopsy is associated with a risk of complications with the most common being abdominal pain (approximately in 25% of cases). The share of complications requiring hospitalization or extended in-patient care is 1–3%. According to the analysis of the structure and aetiology of biopsy-associated complications, the incidence of complications increases proportionally to the amount of biopsy material and the number of sessions and when biopsy is performed in patients with relative counter-indications [23].

Other limitations include the so called sampling error. It means that, if there is no evidence of pathological processes in the biopsy material, the probability of a hepatic disease cannot be completely ruled out. On the one hand, the possibility of this error is explained by the fact that the morphologist assesses the nature and intensity of hepatic changes based on a fragment of hepatic tissue containing at least 3–4 portal tracts. On the other hand, the sampling error is due to the heterogeneity and different intensity of hepatic changes. In its turn, it results in a low representativeness of biopsy findings [24].

Besides, the interpretation of morphological biopsy findings largely depends on the morphologist's experience so the subjective factor cannot be eliminated, either. Thus, both an underestimation of the existing changes and a hyperdiagnosis of certain liver diseases and fibrosis grades can take place during liver biopsy examinations.

Other limitations include high costs of the procedure, and impossibility to perform frequent repeated biopsies; therefore, this method cannot be used for assessing chronic liver disease progression and therapy efficiency. Furthermore, there is a number of biopsy counter-indications, including coagulopathy, haemangioma or liver echinococcosis [24].

According to 2016 clinical recommendations of EASL-

EASD–EASO for the diagnosis and treatment of NAFLD, the diagnosis of NAFLD is based on the presence of 4 criteria:

1 Liver steatosis according to imaging tests: more clinically applicable ultrasound or MRI, and other techniques such as elastography, MRS, etc.
2 No history of alcohol abuse. According to the past history survey findings of alcohol use and abuse, i.e. more than 21 alcohol units per week for males and more than 14 alcohol units per week for females.
3 No concurrent etiological diseases such as hepatitis C.
4 No other comorbid chronic liver diseases such as virus hepatitis B and C, autoimmune hepatitis, α1-antitrypsin deficiency, Wilson's disease, malignant liver lesions, biliary tract pathology, and drug-induced injury [29].

The most accessible non-invasive diagnostic technique of liver steatosis is abdominal ultrasound scanning. Ultrasound is the preferable technique for the first-line NAFLD diagnosis because it provides additional diagnostic data. It has A1 strength of recommendation [26]. The main diagnostic criteria include increased liver size, increased echogenicity, flattening of the vascular pattern [27]. According to the literature, sensitivity and specificity of this technique is 60–94 and 88–95%, respectively [28].

According to the authors of EASL–EASD–EASO 2016 recommendations for the diagnosis and treatment of NAFLD, the most reliable scales for assessing steatosis are fatty liver index (FLI), SteatoTest and NAFLD liver fat score. Their external validity was confirmed among the general population and among obese patients; they also predict outcomes and mortality rates associated with metabolic, hepatic, and cardiovascular conditions with different degrees of accuracy. These rates are closely linked with IR and reliably predict steatosis [29].

According to S. McPherson et al., the predictive value of the negative AST/ALT test result is 93%, which leads to a conclusion that this index can rule our severe fibrosis in patients with NAFLD with a high degree of confidence [30].

Besides, several authors have offered several other quantitative indices for the estimation of NAFLD and fibrosis risks:

1. HAIR index taking into account hypertension, ALAT > 40 IU/L and IR. It has 80% sensitivity and 89% specificity [31].
2. BAAT index taking into account BMI >28 kg/m^2, age >50 years, ALT and TG increase level [32].
3. Forns index taking into account age, platelet count, GGTP, and cholesterol [33].
4. Bonacini index – platelet count, ALT/AST ratio, INR [34].
5. FIB4 index (ALT, AST, platelet count, age) [35].
6. APRI index (AST to platelet count ratio) [36].

According to Shimada M. et al. (2007), almost all patients with NAFLD and overweight had symptoms of IR assessed through the insulin resistance index HOMA-IR [38]. As a non-invasive marker during the measurement of IR quantitative characteristics at the background of the activity level of the synthesis of adiponectin, HOMA-IR and collagen 4, sensitivity and specificity were 94% and 74% [38].

In an attempt to build an optimum predictive model using clinical and laboratory values for the differentiation of fatty hepatosis from NASH, the complex NashTest system was proposed that encompasses 13 variables such as age, sex, height, weight, TG level, cholesterol, α2-macroglobuline, apolipoprotein A1, haptoglobin, GGT, ALT, AST, and bilirubin. This model demonstrated 33% sensitivity and 66% specificity [39].

Another study used hypertension, DM, AST, ALT, obstructive sleep apnea syndrome, and belonging to a non-black racial group as components of the diagnostic model for determining the NAFLD stage in patients with obesity [40].

Miele et al. proposed a mathematical model based on the analysis of age and the concentration of hyaluronic acid and metalloproteinkinase 1 tissue inhibitor. The sensitivity and specificity of this method during the NASH diagnostics was 86% and 90% respectively; however, these findings need to be confirmed in larger independent studies [41].

The issue of the presence and grade of fibrosis was relevant for diagnosing NAFLD severity and progression. FibroTest was created with the aim to diagnose liver fibrosis in patients with chronic viral hepatitis C. It includes the

following biochemical parameters: α2-globulin, apolipoprotein A1, haptoglobin, total bilirubin and GGT. While testing this method in patients with NAFLD, it was established that the mean FibroTest result grows steadily as the fibrosis stage goes up. Thus, at 0.3 points, FibroTest demonstrated 77% sensitivity and 90% general predictive value for diagnosing fibrosis at F1 and F2 stages [42]. However, similar values at 0.7 points were 98% and 76%, respectively [43], which indicates better results for diagnosing F3 and F4 stage fibrosis.

Another relatively new method for diagnosing fibrosis is FibroScan elastography, which is based on determining liver tissue elasticity by measuring the velocity of ultrasound wave propagation through liver parenchyma. Parenchyma elasticity expressed in kPa is significantly correlated with the liver fibrosis grade [44]. However, according to D.Roulot et al., increased BMI without pronounced liver fibrosis is associated with increased liver density, which means low descriptive value of this method for overweight and obese patients [45].

1.2 NAFLD treatment principles

The pandemic of obesity and diabetes mellitus along with improved control of chronic virus hepatitis caused NAFLD to become a major chronic liver disease and a serious health care issue due to a rise in hepatic and extrahepatic mortality. This shift in the epidemiology of the chronic liver disease in the context of a great number of ambiguous recommendations concerning the diagnostics and treatment of this disease make physicians face many questions because these patients need a multidisciplinary approach in order to take into account all possible modifying factors [46].

It should be taken into account that strict clinical recommendations for the strategic approach of NAFLD treatment has not been developed so far. In 2012, the American Association for the Study of Liver Diseases (AASLD), the American College of Gastroenterology (ACG) and the American Gastroenterological Association (AGA) published the practical guideline “Diagnosis and Treatment of Fatty Liver Disease”, which states that body mass reduction achieved by a hypocaloric or combined diet with increased physical activity leads to a decrease in the intensity of liver steatosis. It is also stated a 3-5% body mass reduction is needed to reduce the intensity of steatosis, but a 10% body mass reduction is needed to reduce the intensity of necrosis and inflammation. Increased physical activity as a monotherapy of NAFLD may reduce the intensity of steatosis, but it still unknown of other histological liver indicators also improve [47].

The recommendations of the World Gastroenterology Organisation (WGO) concerning a number of questions do not comply with the AASLD recommendations. 2012 WGO recommendations maintain that NASH therapy objectives include the improvement of the histological picture, IR and oxidation stress reduction, and normalization of the transaminase rate. It is emphasized that there is currently no medicinal therapy for NAFLD/NASH based on the principles of the evidence-based medicine. It is recommended to change the lifestyle (5-10% body mass reduction, increased physical activity) and correct diabetes, hyperlipidaemia, and cardiovascular risks [1].

Modern approaches to the therapy of NAFLD associated with MS are also based on principles that include body mass reduction, hyperglycaemia correction and hyperlipidaemia with obligatory discontinuation of potentially hepatotoxic agents. Primary activities should include those aimed at decreasing body mass: change of lifestyle, reduction of caloric value, and increase in physical activity [48].

Thus, the treatment of patients with obesity and NAFLD is based on non-medicinal techniques: balanced antiatherogenic nutrition with fat content not exceeding 25–30% of daily caloric value: hypocaloric (with moderate caloric deficit of daily ration of 500–600 kcal) diet at the stage of mass reduction and eucaloric at the maintenance stage [49].

One of the central roles in the treatment of patients with early carbohydrate metabolism impairments is played by metformin. Thus, the Diabetes Prevention Program (DPP), 2002 study convincingly demonstrated that the therapy with metformin decreases the risk of DM by 31% in patients

with impaired glucose tolerance, especially in those with BMI >25 kg/m2 [50]. The data collected over many years caused the American Diabetes Association recommends prescribing metformin to all patients with pre-diabetes (impaired glucose tolerance and impaired fasting glucose), especially those with BMI ≥25 kg/m2, aged below 60 years old and females with prior gestation diabetes [51].

Studies of several authors showed that a significant decrease in IR and hepatic transaminases and improvement of metabolic values were reported at the background of metformin therapy combined with hypocaloric diet for 6 months in patients with metabolic syndrome (MS) and NAFLD [3]. However, in a number of studies, metformin therapy in NASH was not accompanied by histological improvement which caused its role in clinical recommendations to be adjusted [52].

Experts of the American Association for the Study of Liver Diseases use statins and vitamin E for the treatment of NAFLD. The efficiency of antioxidants and cytoprotectors for NASH is questioned [53]; however, the findings of the PIVENS study on the efficiency of vitamin E confirm histological shifts towards decreased intensity of inflammation in hepatocytes in patients with NASH [54]. It was shown that in patients without DM with histologically verified NASH, long-term therapy with vitamin E 800 mg/day has a pronounced antioxidant action and leads to both histological and clinical laboratory improvement. Vitamin E is not recommended for the treatment of NASH in patients with DM, confirmed NAFLD biopsy, steatohepatitis with progression to liver cirrhosis and cryptogenic cirrhosis [48].

According to M. Ekstedt (2007), patients with NAFLD who received statins demonstrated more intensive histologically confirmed reduction of liver steatosis as compared to patients with NAFLD who did not receive statins [55]. According to C. Argo (2008), statins influence the level of gene expression responsible for fibrosis progression and the rate of reparation processes [56].

A decrease in the concentration of TNF-α, IL-6, and c-reactive protein (CRP) served as the mechanism of beneficial effect of statins on the development of NASH. It is believed that statins may affect angiogenesis, growth of tumour cells, apoptosis and metastatic activity, which can prevent the development of hepatocellular carcinoma [57].

According to V.T. Ivashkin (2011), for administration of statins is not dependent on the presence of NAFLD and obesity, elevated liver transaminases observed in 0.5-2% of cases, depending on the dose and drug is transient [57], and in patients with initially increased levels of transaminases (AST>40 IU/l, ALT>35 IU/l) the intake of statins did not produse their increase. According to AGC and AGA recommendations, these drugs cannot be used to specifically treat NASH [48].

Medicinal agents from the incretin group have certain potential for inhibiting NAFLD progression to cirrhosis; however, there is no convincing evidence about their efficiency in patients with NAFLD so far although pre-clinical studies point to their ability to decrease the progression of liver steatosis [59].

Anti-cytokine agents (monoclonal TNF-α inhibitors) for NAFLD were studied in a number of small studies showing that their use decreases the intensity of steatosis, inflammation and intensity of oxidation stress in hepatocytes [60].

Ursodeoxycholic acid (UDCA) is a natural hydrophilic biliary acid, and the immunomodulating and antiapoptic properties of UDCA inhibit the development of NAFLD [61]. The most efficient, accessible and pathogenically justified NAFLD treatment technique (especially in cholestatic forms) is the use of UDCA agents. The therapeutic effect of UDCA is primarily accounted for by the membrane-stabilizing, antioxidant, cholestatic, and immunomodulating action; the drug increases the sensitivity of receptors to insulin, activates the farnesoid X receptor playing a key role in the pathogenesis of NAFLD and lipid pathogenesis. UDCA has an anti-inflammatory effect mediated by a decrease in anti-inflammatory cytokines [62]. However, due to the lack of evidence the Association does not recommend prescribing ursodeoxycholic acid agent to patients with NAFLD and NASH [48].

The liver plays an important role in the development of dyslipidemia and is a target for lipid metabolism impairments being one of the pathogenic stages of NAFLD development, which stimulates the search for medicinal hepatocyte "support" methods. Therefore, agents may be recommended that contain essential polyunsaturated fatty acids. The use of essential phospholipid agents as sources of cell membrane structural elements is pathogenically justified and confirmed by multiple studies. The main active substance is 1,2-dilinoleophosphatidylcholine which is not synthesized in the human body. The presence of two essential fatty acids accounts for the advantage of this special phospholipid form over the endogenous ones [63].

The membrane-stabilizing and hepatoprotective effect of essential phospholipids (EPLs) is achieved by the direct incorporation of their molecules into the phospholipid structure of damaged liver cells, recovery of defects and restoration of the barrier function of the lipid layer. Unsaturated fatty acids of phospholipids contribute to increased activity and fluidity of membranes, activation of phospholipid-dependent enzymes and transport proteins, decreased density of phospholipid structures, and normalization of membrane permeability, which in its turn improves the detoxification and excretory liver potential [64]. In terms of their molecular structure, phospholipids are more similar to triglycerides. The only difference is that one of three fatty acids is substituted by a phosphoric acid ester. Phospholipids differ depending on the nature of the substitute bound to the phosphoric group.

The main therapeutic effect of phospholipids depends on the concentration of phosphatidylcholine, whose molecule has a non-polar (two fatty acid groups) and a polar (phosphoryl choline) part. It is this structure that accounts for the active surface features of phosphoryl choline. Besides, being a good emulsifier, phosphoryl choline increases the bioavailability of nutrients, with which it is injected, decreases the depositing of cholesterol in the liver thus promoting the inhibition of cholesterol acyltransferase by phospholipids. EPLs have antioxidant effect and can slow down the synthesis of collagen by increasing the activity of collagenase [65]. The decreased level of blood cholesterol and its increased excretion with bile is associated with the ability of EPLs to concurrently affect the absorption of cholesterol in the intestine, decrease the membrane concentration of cholesterol and, combined with bile acids, improve its solubility in the bile. The efficiency of polyunsaturated phosphoryl choline in patients with fatty liver disease of different genesis is accounted both by its ability to induce the triglyceride-lipase of hepatocytes promoting the release of fatty acids to the bloodstream. The specific nature of essential phospholipids enable them to substitute for the phospholipids of blood lipoproteins – chylomicrons (ranging up to 80%), very low and low-density lipoproteins (up to 15%), but mainly high-density lipoproteins (80%) and thus to be transported with blood and lymph [63].

Dosages and the duration of EPL treatment are individual and depend on the clinical laboratory and instrumental results. Taking into account the increased activity of lipoprotein lipase that enhances the intravascular splitting of chylomicrons and VLDLPs, the improvement of the function of insulin receptors and the increased activity of lecithin-cholesterol acyltransferase involved in the esterification of HDLP cholesterol, the use of EPLs is pathogenically justified for the treatment of NAFLD, especially if accompanied by metabolic impairments [43].

CONCLUSIONS

Non-alcoholic fatty liver disease is a very common disease among the world's population. Most often, NAFLD is associated with visceral obesity, which leads to an increased risk of cardiovascular disease in these patients. Also, it is clear that in the majority of the patients, NAFLD is characterized by a long, stable asymptomatic course. In turn, the timely diagnosis can significantly improve the patient's quality of life and prevent fatal complications.

Conflict of interests

There is no conflict of interests.

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THERAPY

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MELATONIN AND SOME PARAMETERS OF CELL-MEDIATED IMMUNE RESPONSE IN PATIENTS WITH CHRONIC RHINOSINUSITIS WITHOUT NASAL POLYPS

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Abstract: Chronic rhinosinusitis is a polyetiological disease, the pathogenesis of which is currently being actively studied. It has been known that Th1 type of immune response prevails in chronic rhinosinusitis without nasal polyps. In addition to cytokines that contributes to the activation of cell-mediated immunity, melatonin may be involved in the regulation of the subtype of immune response. Concentrations of IL-8, IL-12 and melatonin in blood serum of patients with exacerbation of chronic rhinosinusitis without nasal polyps (n = 20) and healthy individuals with deviated nasal septum (n = 20), who served as the control group, were determined using the corresponding ELISA kits. Analysis of obtained data was carried out using the Graph Pad Prism 5 application with the help of the Mann-Whitney U test. It was shown that exacerbation of chronic rhinosinusitis without nasal polyps was accompanied by melatonin deficiency against the background of elevated concentrations of Th1-associated proinflammatory cytokines IL-8 and IL-12 in blood serum. Melatonin has been known to affect the expression of IL-8 and IL-12. Thus, melatonin, acting via proinflammatory cytokines, can affect the differentiation of naïve immature CD4+ cells in Th1-cells, promoting realization of the immune response in the cell-mediated direction. Our conclusions about the prevalence of cell-mediated Th1-related immune response in chronic rhinosinusitis without nasal polyps are consistent with the data of other authors. Thus, exacerbation of chronic rhinosinusitis without nasal polyps is accompanied by a twofold increase in serum IL-12 and a threefold increase in serum IL-8 levels against the background of noticeable lack of melatonin, which may indicate the role of melatonin deficiency in the activation of the cell-mediated immunity.

KeyWords: chronic rhinosinusitis, rhinosinusitis without nasal polyps, interleukins, melatonin, inflammation, interleukin-8, interleukin-12.

INTRODUCTION

Chronic rhinosinusitis has been known to be a multifactorial and heterogeneous disease the etiology and pathogenesis of which are being currently studied. Nowadays two histopathologic types of chronic rhinosinusitis are distinguished: rhinosinusitis with and without nasal polyps [1-3]. Both types of chronic rhinosinusitis are characterized by changes in blood serum cytokine profile [4 - 7].

Chronic rhinosinusitis without nasal polyps is characterized by inflammation accompanied by overproduction of type 1 helper T cell (Th1)-associated proinflammatory cytokines, which indicates the prevalence of cell-mediated Th1-subtype of immune response [8].

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However, in addition to cytokines, other factors may contribute to the regulation of immune response subtype. One of such factors is melatonin, which is produced by the pineal gland. It has been reported that this hormone can affect the rate and subtype of inflammation due to its antioxidant properties and via regulation of cytokine synthesis [9, 10]. In particular, melatonin is involved in regulation of IL-12 and IL-8 formation, which are known to promote differentiation of naïve CD4+ cells into Th1-cells [11 - 13].

Thus, the interplay between melatonin and cytokines in the complex response of immune system determines features of the inflammatory process. The role of melatonin in shifting differentiation of naïve T-cells towards Th1- or Th2-cells and effects of this pineal hormone on the mechanism underlying cytokine crosstalk and regulation of the immune response type have not been fully elucidated yet.

2 PURPOSES, SUBJECTS and METHODS:

2.1 Purpose of our research was to study the content of proinflammatory cytokines IL-8 and IL-12 that activate cell-mediated immune response in blood serum and serum melatonin levels, as well as their interplay, in patients with chronic rhinosinusitis without nasal polyps.

2.2 Subjects & Methods

We examined 40 patients of Kharkiv Regional Clinical Hospital. All individuals were randomly subdivided into two equal groups. Group 1 included patients who had been diagnosed with exacerbation of chronic rhinosinusitis without nasal polyps (n=20). Clinical examination, laboratory and instrumental tests in accordance with criteria offered by the WHO expert committee were used to verify their diagnosis. Group 2 served as control and consisted of twenty healthy individuals with deviated nasal septum (n = 20). Patients from group 2 did not show any signs of acute or chronic pathology, endocrine diseases, obesity, oncologic diseases, and hypertension. Pregnant females were excluded. Informed consent was obtained from every patient.

All activities were performed according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) and Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (ETC 164).

Levels of IL-8, IL-12 and melatonin were measured in blood serum samples using enzyme-linked immunosorbent assay kits manufactured by Vector-Best (Russian Federation), Orgenium (Finland), IBL Hamburg (Germany), respectively, in accordance with instructions provided by manufacturers. The Awareness Technology Stat Fax 303 Plus Microstrip Reader was used to register the optical density of solutions.

Statistical analysis of the data obtained in our research was performed using GraphPad Prism 5. Mann-Whitney test was used to detect differences between groups. Values are presented as median and interquartile range (25th – 75th percentage). Difference between groups was considered to be statistically significant at $p < 0.05$.

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

It was found that blood levels of melatonin were over 3 times lower in patients with exacerbation of chronic rhinosinusitis without nasal polyps compared to the control group (Fig. 1). Melationin concentration in group 1 reached 33.24 (29.15; 39.78) pg/ml, while in group 2 its serum levels were 10.30 (9.45; 13.03) pg/ml.

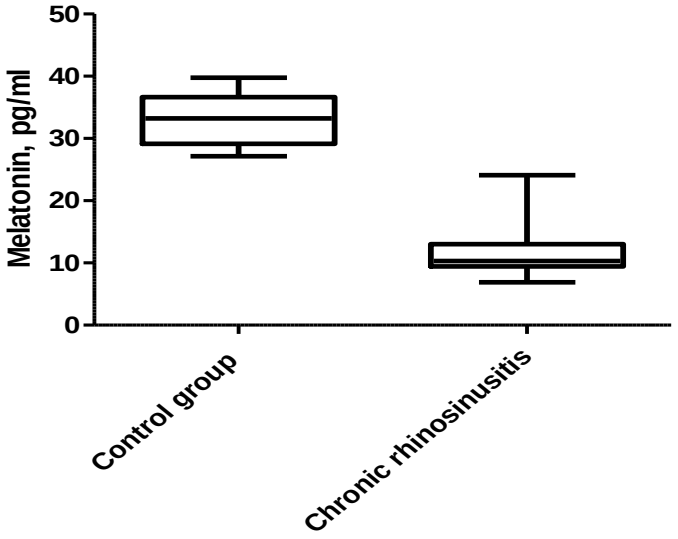

Fig. 1. The content of melatonin in blood serum of patients with chronic rhinosinusitis without nasal polyps

Reduction of melatonin may contribute to the activation of inflammatory process, taking into account its antioxidant properties. Similar changes in blood serum melatonin concentrations were observed in chronic rhinosinusitis with nasal polyps [14]. However, melatonin deficiency was less pronounced in patients with the form of rhinosinusitis mentioned above. It has been reported that melatonin inhibits translocation of NF-κB to the nucleus and its attachment to DNA, which results in downregulation of the expression of certain proinflammatory cytokines [15 - 17]. Thus, we be-

lieve that the lack of melatonin in patients with chronic rhinosinusitis without nasal polyps may make the course of the disease more severe due to upregulation of TNF-α and other proinflammatory cytokines. Nevertheless, while considering the role of the hormone produced by the pineal gland in the pathogenesis of exacerbation of chronic rhinosinusitis without nasal polyps, it is necessary to take into account the interplay of melatonin and cytokines. Melatonin has been known to interact with the immune system at the level of the synthesis of cytokines by immune cells. For example, García-Mauriño S. et al demonstrated that melatonin was able to upregulate IL-12 expression by macrophages [11].

IL-12 blood serum levels in patients from group 1 were found to be 19.45 (14.65; 25.21) ng/ml against 9.43 (9.16; 10.43) ng/ml in group 2.

Despite the fact that the level of IL-12 is twice high in patients with rhinosinusitis without nasal polyps compared to healthy individuals (Fig. 2), its level is lower in comparison with patients with chronic rhinosinusitis with nasal polyps.

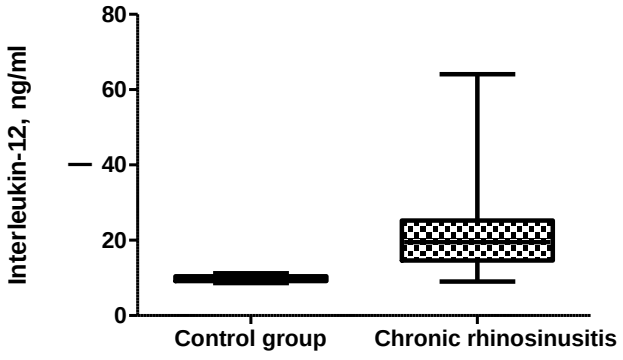

Fig. 2. Levels of IL-12 in blood serum of patients with chronic rhinosinusitis without nasal polyps

Thus, we believe that the more pronounced melatonin deficiency in exacerbation of chronic rhinosinusitis without nasal polyps results in preventing overactivation of IL-12 and maintaining the balance between factors that activate cell-mediated and humoral immune responses. This fact emphasizes the role of interplay between IL-12 and melatonin in regulation of the immune response in chronic rhinosinusitis without nasal polyps.

However, it is worth mentioning that melatonin downregulates IL-8 [12, 13, 18]. Thus, the action of melatonin may contribute to an increase in IL-8 concentrations in patients with chronic rhinosinusitis without nasal polyps. Its levels reach 22.75 (3.8; 47.0) pg/ml and are significantly (p¤0.05) 3.1 times higher than in healthy people from the control group (Fig. 3) whose IL-8 serum concentrations reach 7.25 (3.86; 16.93) pg/ml, while in patients with chronic rhinosinusitis with nasal polyps serum IL-8 levels decrease [19].

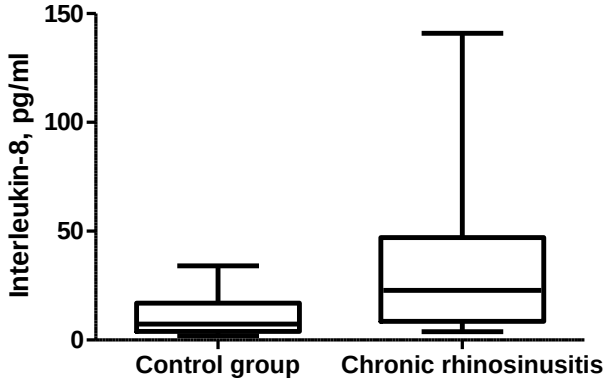

Fig. 3. Concentrations of IL-8 in blood serum of patients with chronic rhinosinusitis without nasal polyps

In general, the slight elevation of IL-12 and the noticeable increase in IL-8 levels may be considered as a sign of the activation of cell-mediated immunity. The data obtained in our research on the predominance of the cell-mediated link of immunity in chronic rhinosinusitis without nasal polyps are consistent with the results of other authors [20, 21].

4 CONCLUSIONS

1. Exacerbation of chronic rhinosinusitis without nasal polyps is characterized by elevation of IL-8 and IL-12 blood serum levels against the background of melatonin deficiency.

2. Melatonin may be involved in regulating the immune response type in chronic rhinosinusitis without nasal polyps via IL-8 and Il-12.

3. Melatonin deficiency promotes the prevalence of cell-mediated immune response in chronic rhinosinusitis without

nasal polyps.

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PEDIATRICS

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RARE CARDIO-RESPIRATORY FINDINGS IN GOLDENHAR SYNDROME (case report)

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Abstract: The Goldenhar Syndrome is the rare congenital abnormalities that include Facio-Auriculo-Vertebral Spectrum, First and Second Branchial Arch Syndrome, Oculo-Auriculo-Vertebral Spectrum, oculo-auriculo-vertebral disorder. Oculo-auriculo-vertebral disorder (OAVD) represents the mildest form of the disorder, while Goldenhar syndrome presents frequently as the most severe form. Hemifacial microstomia appears to be an intermediate form. Goldenhar Syndrome includes patients with facial asymmetry to very severe facial defects (resulting from unilateral facial skeleton hypoplasia) with abnormalities of skeleton and/or internal organs. The most significant are epibulbar dermoids, dacryocystitis, auricular abnormalities, preauricular appendages, preauricular fistulas and hypoplasia of the malar bones, mandible, maxilla and zygomatic arch. Some patients are found to have oculo-auriculo-vertebral disorder, namely low height, delayed psychomotor development, retardation (more frequently seen with cerebral developmental anomalies and microphthalmia), speech disorders (articulation disorders, rhinolalia, different voice disorders, unusual timbre), psycho-social problems, autistic behaviors. The authors describe the clinical case of Goldenhar Syndrome in boy a 3-months-year-old. This case demonstrates a rarely described association of oculo-auriculo-vertebral disorders, malformation of respiratory system (hypoplasia of the lower lobe of the left lung with relaxation of the left cupula of the diaphragm), heart abnormality (atrium septal defect).

KeyWords: Goldenhar Syndrome, children, diagnostic

The multiple congenital malformations (MCM) are defined as a combination of anomalies in the development of two or more body systems. The frequency of diagnostic errors in the MCM structure is high both in Ukraine and Europe. Etiology of MCM is poorly understood, in view of the rare cases, as well as due to to lack of specific laboratory verification of the diagnosis. According to the World Health Organization (WHO), about 7% of infant deaths worldwide are due to congenital pathology, 46% of them are children with congenital malformations who died under one year of age [1].

Goldenhar Syndrome (GS) – Q87.0 (Facio-Auriculo-Vertebral Spectrum (FAV), First and Second Branchial Arch Syndrome, Oculo-Auriculo-Vertebral Spectrum (OAVS), oculo-auriculo-vertebral disorder (OAVD)).

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Goldenhar Syndrome ranks second in incidence after cleft lip and palate [2, 3]. Oculo-auriculo-vertebral disorder (OAVD) represents the mildest form of the disorder, while Goldenhar syndrome presents frequently as the most severe form [4].

Hemifacial microstomia appears to be an intermediate form [4]. Goldenhar Syndrome includes patients with facial asymmetry to very severe facial defects (resulting from unilateral facial skeleton hypoplasia) with abnormalities of skeleton and/or internal organs [5]. The symptoms observed in this syndrome can be divided into groups according to the part of the body. The most significant are epibulbar dermoids, dacryocystitis, auricular abnormalities, preauricular appendages, preauricular fistulas and hypoplasia of the malar bones, mandible, maxilla and zygomatic arch [6]. Some patients are found to have oculo-auriculo-vertebral disorder, namely low height, delayed psychomotor development, retardation (more frequently seen with cerebral developmental anomalies and microphthalmia), speech disorders (articulation disorders, rhinolalia, different voice disorders, unusual timbre), psycho-

social problems, autistic behaviors [7-9]. Approximately 70% of cases are unilateral. In case of bilateral defects one side has the most severe malformations. The right side prevails over the left one with the incidence of 3:2 [5].

CASE REPORT

The child was born from the pregnancy complicated by a respiratory viral infection in the gestation period of 4-5 weeks, suspected congenital heart disease according to the ultrasound data at 28 weeks, clinically and laboratory confirmed lues at 29 weeks. Full-term infant was born by caesarean section with Apgar score 5/7 and birth weight of 3.05 kg. There was no history of trauma to head and neck region or maternal teratogen agents. The child was also found to have bilateral asymmetry at birth. In the early neonatal period he suffered from respiratory failure which was managed by ventilation from the first day of life. Oxygen dependence was maintained up to 3 weeks of life. The child developed according to his age, without mental retardation or impairment of cognitive function.

On examination at 3 months he was found to have asymmetry of the face due to underdevelopment of soft tissues and bones of the facial skeleton on the right auricle: hemifacial (left side) microsomia, hypoplasia and deformation of the auricle, preauricular skin tags visualized from the earlobe (Figure 1), asymmetry of the eye fissures, gothic palate, predominance of the brain skull over the facial; short neck; long toes (Figure 1a); “hammer” deformation of the thumbs of both hands (Figure 1b); polydactyly of the right hand (Figure 1c), short tongue, thickening of the thumb on both hands, left-sided muscular torticollis, bilateral dropsy of testicles.

Body measurements: weight 3700 g, height 53 cm. The condition is severe, which is caused by ventilation disorders. The skin is pale and dry. It is noted that, with concern, marbling with an accent of the lower extremities. Turgor and elasticity of tissues are slow and reduced; subcutaneous fat is not sufficient. The head is hydrocephalic, evenly distributed. Muscular dystonia. The patient has previous history of cyanosis of the nasolabial triangle, dyspnea.

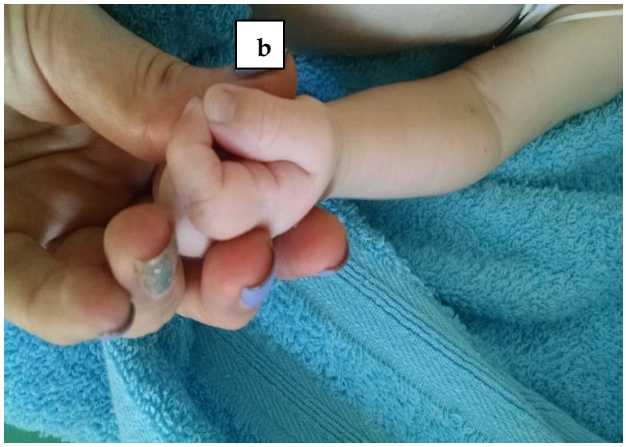

Figure 1. A 3-months-old male patient:
1a - hemifacial (left sided) microsomia, hypoplasia and deformation of the auricle, preauricular skin tags visualized from the earlobe, asymmetry of the eye fissure;
1b - “hammer” deformation of the thumbs of both hands;
1c - polydactyly of the right hand.

Diagnostic imaging findings:

Chest X-ray (Figure 2):

- relaxation of the left side of the diaphragm, hypoplasia of the left lung?
- mild scoliosis
- Butterfly vertebrae (C5 and T1)

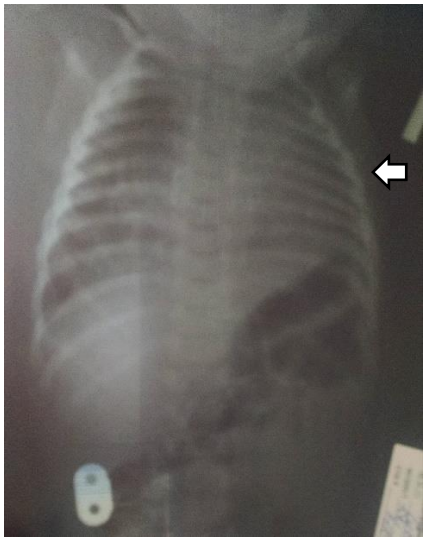

Figure 2. A 3-months-old male patient. Chest's X-ray

ECG: HR 125 min, sinus rhythm, electric axis deflected to the right side, right ventricular dilation and its systolic overload.

Doppler echocardiography (body weight 3700 g):

terminal diastolic diameter of the right ventricle – 14.6 mm (↑);

- diameter of the right atrium – 13.7 mm (↑);
- diameter of the pulmonary artery 12.7 mm (↑);
- mPAP, mean pulmonary artery pressure - 30 mm Hg (↑)

Conclusion: Dilation of the right atrium and ventricle, pulmonary arteria, left-right shunt in the central part of atrium septa, diameter = 4.6 mm. Turbulent flow in the pulmonary artery, pulmonary hypertension.

The patient was found to have developmental defect of the cardiovascular system in the form of a secondary defect of the atrial septum.

High resolution computed tomography of chest organs (spiral pitch of 1 mm) with contrast (Tomoexol 300mg-9ml, IV) (Figure 3).

- Poorly developed lung tissue of the left lung due to lower lobe hypoplasia with a small area of pulmonary tissue infiltration in the lower sections due to residual pneumonia.
- Trachea, main, lobar and segmental bronchi are freely passable throughout the whole length.
- Cupula of the diaphragm with a relaxed left contour.

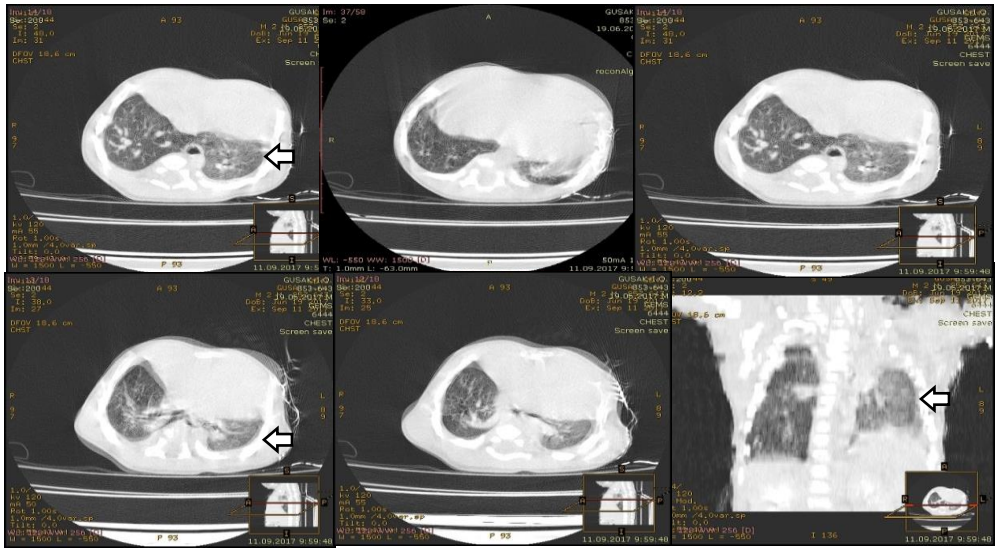

Figure 3. A 3-months-old male patient.
High resolution computed tomography of chest organs (spiral pitch of 1 mm) with contrast: left lung lower lobe hypoplasia, cupula of the diaphragm with a relaxed left contour.

Conclusion: Hypoplasia of the lower lobe of the left lung with relaxation of the left cupula of the diaphragm.

The patient has a combination of rare findings that include:

- hemifacial microsomia (right-sided),
- hypoplasia and deformation of the auricle (right-sided),
- polydactyly of the right hand (right-sided),
- preauricular skin tags (right-sided),
- "hammer" deformation of the thumbs of both hands,
- mild scoliosis,
- Butterfly vertebrae (C5 and T1) hypoplasia of the lower lobe of the left lung with relaxation of the left cupula of the diaphragm,
- Secondary atrial septum defect.

Diagnosis: Goldenhar Syndrome: oculo-auriculo-vertebral disorders, *Hypoplasia of the lower lobe of the left lung with relaxation of the left cupula of the diaphragm.*

DISCUSSION.

Goldenhar Syndrome (GS) has a sporadic cause. Some cases have family history. On the basis of these data, it can be assumed that the disease is more likely to have an autosomal dominant type of inheritance. Researchers suggest that GS may be caused by the interaction of many genes, possibly in combination with environmental factors (multifactorial inheritance). Some scientists think the syndrome reveals mutations of the GSC gene (14th chromosome). The GSC gene defines neural-crest cell-fate specification and contributes to dorsal-ventral patterning. Over activation in Xenopus promotes dorso-anterior migration and dorsalization of mesodermal tissue of the cells along with BMP-4 (Bone morphogenetic protein 4) [10-21].

The significant number of anomalies in the formation of musculoskeletal, nerve elements of the soft facial tissues that are presumably due to a vascular stroke in the region of the I and II gill slits of the embryos, coinciding with the replacement of the source of blood supply in the zone, which leads to pathological transformations of cell proliferation in the above-mentioned zone [22, 23].

Oculo-auriculo-vertebral spectrum represents three rare disorders that are apparent at birth (congenital), and are characterized by a wide spectrum of symptoms and physical features that may vary greatly in range and severity from case to case. However, such abnormalities tend to involve the cheekbones, jaws, mouth, ears, eyes, and/or bones of the spinal column (vertebrae).

Abnormalities in Goldenhar syndrome [24-39]:

- ocular symptoms: epibular dermoids, cleft eyelid, microphtalmia, exophthalmia, anophtalmia, strabismus, eyes asymmetry/dysmorphy, lipodermoids, coloboma, lacrimal duct artresia/stenosis;
- auricular symptomps: dacryocistitis, atresia of the external auditory canal, preauricular appendages, ear dysplasia with or without hearing loss, middle and inner ear abnormalities, anotia ears asymmetry, microtia;
- cranio-facial deformities: abnormalities of the 1st and 2nd pharyngeal arches, facial asymmetry, hypoplasia of the facial skeleton, mandible and/or maxilla, hemifacial macrosomia, malocclusion, cleft face tooth discrepancies, cleft lip agenesis of the 2nd premolars and 3rd molars, cleft palate, supernumerary teeth, macrostomia, malformations of enamel and dentin delay in tooth development;
- skeletal abnormalities: cleft spine, abnormalities of extremities, microcephaly, club foot, dolichocephaly, radial hemimelia, plagiocephaly, thumb abnormalities, vertebral defects;
- internal organs abnormalities, heart: atrial and ventricular septal defects (the most common), Fallot tetralogy, conotruncal defects, persistent truncus arteriosus, aortic arch anomalies, transposition of the great vessels, dextrocardia;
- urogenital anomalies: ectopic kidneys, renal agenesis, fused kidneys, multicystic kidneys, double ureter hydroureter, hydronephrosis;
- central nervous system: diffuse cerebral hypoplasia, hydrocephalus due to aqueduct of sylvius stenosis, dilated lateral cerebral ventricles or asymptomatic hydrocephalus, corpus callosum lipoma, asymmetric lateral ventricles, absence of septum pellucidum, corpus callosum dysgenesis, diffuse cerebral hypodensity, frontal hypodensities, facial palsy, microcephaly, tri-

geminal anesthesia encephalocele, developmental delay, spine deformities, holoprosencephaly, arnold-chiari malformation, hypothalamic hamartoma, aplasia/hypoplasia of temporomandibular joints;

- gastrointestinal tract: rectal atresia trachea-esophageal fistula, esophageal atresia;
- respiratory system: abnormal anatomy of larynx and pharynx, disorder of lobular anatomy of lungs.

There are several classifications that reflect the degree of its severity. The most complete is OMENS [1]. It identifies three stages of the severity of the lesion of each of the malformation objects in hemifacial microsomy: the eye (orbit), mandible, ear, facial nerve and skeletal bones. Since defects are multiple and each structure is usually affected in different degrees, it looks like this: O2M3E3N2S1 *. The asterisk reflects the presence of additional defects of non-skeletal-facial objects.

Symptoms of the following disorders may be similar to those of Goldenhar syndrome. Comparisons may be useful for a differential diagnosis with Treacher Collins, **CHARGE,** Townes-Brocks syndromes and VACTERL association. *Treacher Collins syndrome* (TCS) is an extremely rare genetic disorder (mutations in the *TCOF* gene) characterized by distinctive abnormalities of the craniofacial area due to underdevelopment (hypoplasia) of certain portions of the skull (e.g., supraorbital rims and zygomatic arches) and lower jaw [40]. Opposite GS infants with TCS may also have hypoplastic and/or microtic outer ears with blind ending or atresia of external ear canals, conductive hearing loss. Infants with the disorder may have colobomas. *CHARGE* syndrome stands for coloboma, heart defect, atresia choanae (also known as choanal atresia), restricted growth and development, genital abnormality, and ear abnormality.[1] Signs and symptoms vary among people with this condition; however, infants often have multiple life-threatening medical conditions. The diagnosis of *CHARGE syndrome* is based on a combination of major and minor characteristics. In more than half of all cases, mutations in the *CHD7* gene cause CHARGE syndrome [41]. *VACTERL association*, a rare disorder resulting from fetal development defects, is characterized by congenital abnormalities affecting several organ systems. VACTERL is an acronym representing (V)ertebral abnormalities (like GS) including hemivertebrae and malformation of the lower vertebrae (sacrum); (A)nal atresia, a condition in which there is absence of the anal opening; (C)ardiac defects, particularly ventricular septal defects; (T)racheo(E)sophageal fistula; (R)enal abnormalities including absence of the kidney and hydronephrosis; and improper development of one of the forearm bones (radial dysplasia) and other (L)imb defects. *Townes-Brocks syndrome* associated with abnormalities tend to involve the face (hemifacial microsomia), ears (malformation of the outer ears, preauricular tags and/or pits, sensorineural hearing loss)), arms (polydactyly, syndactyly) and legs (limbs), gastrointestinal system (rectovaginal or rectoperineal fistula), and kidneys (renal hypoplasia; vesicoureteral reflux). Diagnosis of Goldenhar, Treacher Collins, **CHARGE,** Townes-Brocks **syndromes** and VACTERL association are based on anomalies in the structure of the facial skull, but the combinations of anomalies found in this case are more in favor of Goldenhar syndrome, which was established after the refining examinations and differential diagnosis.

CONCLUSIONS

This case demonstrates a rarely described association of oculo-auriculo-vertebral disorders, malformation of respiratory system (hypoplasia of the lower lobe of the left lung with relaxation of the left cupula of the diaphragm), heart abnormality (atrium septal defect).

CONFLICT OF INTERESTS

There is no conflict of interests.

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PEDIATRICS

Sirenko T. V.[1], Plakhotnaya O. N. [1], Ashcheulov O.M. [1], Kukuruza A.V.[2]

PSYCHOLOGICAL ASPECTS IN TREATMENT AND CARE FOR NEWBORNS WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY AT THE ACUTE STAGE OF DISEASE

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State Institution "Institute for Children and Adolescent Care of the National Academy of Medical Sciences", Department of Psychological Development [2]

Abstract: the article presents the experience of using medical-psychological aspects of treatment of hypoxic-ischemic encephalopathy in newborns in acute period of the disease. The elements of early influence on the development of the child's psychomotor activity were used on the basis of modern medical technology of treatment. These elements included sensor stimulation: (vestibular, proprioceptor, tactile), development of motility, hearing and sight perception. The study showed positive changes in neurological symptoms in children, resulting in a reduced duration of the hospital stay.

KeyWords: newborn, hypoxic-ischemic encephalopathy, medical-psychological treatment.

INTRODUCTION

Hypoxic-ischemic impairment of the nervous system, namely hypoxic-ischemic encephalopathy in newborns remains to be one of the serious medical and social problems due to high incidence, severity and unfavorable impact on further development of children [1, 2, 3, 6]. Deficient contact of newborn with mother during hospital stay after delivery has negative influence on adaptation, emotional and psychophysical condition and reduces effectivity of rehabilitation measures [8, 15, 19, 20].

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2 PURPOSES, SUBJECTS AND METHODS:

2.1 Purpose of the study was to improve non-invasive treatment of hypoxic-ischemic encephalopathy in newborns, to improve the elements of early influence on the development of their psychomotor activity by increasing participation of mothers in the process of management.

2.2 Subjects & Methods

The study was carried out in Kharkiv Regional Clinical Children Hospital No.1 at the Department of Neonatal Anesthesiology and Intensive Therapy and the Department of Neonatal Pathology. The study was conducted from January 2015 till June 2016. The study involved 297 newborns with hypoxic-ischemic encephalopathy, 91.8 % were observed at the Department of Neonatal Anesthesiology and Intensive Therapy and 68.4 % at the Department of Neonatal Pathology.

Inclusion criteria:

1. Full-term newborns with asphyxia at birth
2. Premature newborns at gestational age of not less than 32 weeks with asphyxia at birth

Exclusion criteria:

1. Manifestations of infection
2. Congenital defects of development

Patients were divided into 2 groups according to the age of gestation, namely 177 full-term newborns and 120 preterm newborns.

Examination of newborns comprised clinical and laboratory-instrumental methods. Clinical examination was carried out with traditional methods of examination of newborns, assessment of condition of the nervous system implied determination of pathological symptoms and syndromes. Laboratory-instrumental examination included blood test, urinalysis, arterial blood gas test, electrolytes imbalance blood test, X-ray examination, ECG, ultrasound examination of the brain and internal organs, computed tomography. TORCH screen and the immunologic tests were made on an as-needed basis.

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

Assessment of mothers' health, duration of pregnancy and delivery showed impairments in 42.2 % of Group 1 mothers and 100 % of Group 2 mothers. Somatic pathology was observed in 45.2 % mothers of Group 1 newborns and 83.3 % mothers of Group 2 newborns. Gestational toxicosis and threat of preterm delivery were registered in 30.5 % mothers of Group 1 newborns and 100 % mothers of Group 2 newborns. Chronic placental insufficiency and chronic intrauterine hypoxia of fetus were diagnosed in 49.5 % mothers of Group 1 newborns and 83.3% mothers of Group 2 newborns.

All the newborns were born with asphyxia. General condition after birth was severe and complicated by neurological disorders and cardiorespiratory insufficiency. Due to severity of condition the newborns were transferred from maternity clinic to the Department of Neonatal Anesthesiology and Intensive Therapy of Kharkiv Regional Clinical Children Hospital No.1.

At admission to the hospital the condition of all the newborns remained severe due to depression of the nervous system, respiratory and cardiovascular insufficiency and metabolic disorders. Severe asphyxia was diagnosed in 59.9 % of newborns, moderate form in 40.1 % of Group 1 newborns, in 71.7 % and 29.3 % of Group 2 newborns.

Examination of newborns comprised clinical and laboratory-instrumental methods. Clinical examination was carried out with traditional methods of examination of newborns, assessment of condition of the nervous system implied determination of pathological symptoms and syndromes. Laboratory-instrumental examination included blood test, urinalysis, arterial blood gas test, electrolytes imbalance blood test, X-ray examination, ECG, ultrasound examination of the brain and internal organs, computed tomography. TORCH screen and the immunologic tests were made on an as-needed basis.

Sonography showed different signs of CNS involvement. They depended on the severity of CNS involvement, stage of maturity of newborns and periods of the disease. During the first days of the disease full-term newborns had a diffuse increase in echogenicity of the brain, compression of ventricles, decreased vessels pulsation, smooth pattern of convolutions and furrow.

Premature newborns had increased echogenicity in the periventricular zone, enlargment of the ventricles system of the brain, asymmetry of ventricles, compression of the choroidal plexus, cysts in periventricular zone. These sonography findings conform to the literature data [7, 16].

Taking into consideration medical history, clinical symptoms, severity of neurological disorders, laboratory and instrumental findings, hypoxic-ischemic encephalopathy was diagnosed in all the newborns. Symptoms of the central nervous suppression or hyperactivity, metabolic acidosis, hypoxic lesions of the heart and kidneys, and coagulation were diagnosed in 25 % of newborns.

Clinical neurological symptomatic and laboratory-instrumental findings gave information on abnormal processes in the brain (hypoxia, decreased blood circulation, edema of the brain) and were used for diagnosis of hypoxic-ischemic encephalopathy and administration of treatment.

The treatment was prescribed according to the current guidelines (13, 14, 16)

During the early neonatal period background therapy included oxygen therapy, magnesium sulfate, vitamin C, calcium gluconate. In increased neuroreflector activity the patients were administered Seduxen and Relanium. Vitamins B_6, B_{12} and nootropics were used in severe suppression of nervous system.

Psychological influence on the newborn with hypoxic-ischemic encephalopathy started from the first days of life. The advantage of this method bases on information represented in the medical literature on favorable action of psychological influence on parents and children in some severe diseases preceding the pathology under investigation (5, 9, 10, 11, 12, 17, 19).

Assessment of the interview with parents after delivery of children with severe asphyxia showed high incidence (78 %) of depression in parents resulting from uncertainty of parents in favorable outcome of the disease, possible consequence such as retardation of psychomotor development, the ability to receive education and choose a profession. In these circumstances physicians had to provide a qualified and non-biased explanation of the disease pathogenesis, options for diagnosis and treatment of this disorder, measures for prevention of possible complications and comments on the importance of a qualified follow-up after the neonatal period.

Our study showed the importance of participation of parents in care for children at early stages of hypoxic-ischemic encephalopathy.

Parents were permitted to visit children in the intensive care department, to take their newborn in the arms, to take part in carrying out certain procedures of care. The staff of the department pointed out positive changes in the children's condition.

After improvement of the condition the newborns were transferred to the department of neonatal pathology, where they stayed together with mother.

The ward for joint stay of the newborns with their mothers had sufficient space and equipment necessary to maintain sanitation and epidemiological regime.

In these conditions early non-invasive influence on the child's development was conducted in combination with medical treatment. Non-invasive methods were based on stimulation of the children's sensor sensitivity.

Medical staff of the neonatal department instructed mothers how to have the contact with the child, to give the child posture for psychological hypertonia of the flexor muscle, to fix the look, to fix the sound eruption

The common movement activity was stimulated for the development of vestibular, proprioceptive, tactic sensitivity, encouragement was done to such actions as the attempt to hold the head, to change the body position and practice to put the child on the abdomen was used. Massage of the hands and fingers was used for stimulation of fine movements.

The demonstration of bright objects stimulated the sight perception. It was recommended to speak with a child for stimulation of sound perception. Musical influence by classical music was used for 3-4 hours per day at the department.

Medical staff of neonatal department thoroughly inspected the mothers' care for newborns, provided corrections, and pointed out successful results, such as an increase in spontaneous motility, improvement of muscular tone, activation of congenital reflexes.

In normal epidemiological situation and favorable weather the mothers with newborns were permitted to walk in the hospital park and to meet with members of their family.

Physicians of the neonatal department consulted the mothers on care for children, rational feeding, advantages of natural feeding, prevention of diseases and importance of prophylactic vaccination. They were also given leaflets with information on care and feeding.

Effectivity of medico-psychological aspects of treat-

ment of newborns with hypoxic-ischemic encephalopathy was evaluated by comparing the results of the treatment of this population of patients in 2015 and 2005.

Groups of patients with severe forms of hypoxic-ischemic encephalopathy and moderate severity forms were selected in these two periods. Groups of newborns were similar by gestational age, body mass index, neurological symptoms and medication. The assessment showed that the term of stay in the hospital of patients with HIE decreased from 38.3 ± 5 days in 2005 to 20.7 ± 4 days in 2015. This fact gives reason to consider this method effective, which means it should be introduced into practice.

It is known that structural defects of the brain are manifested by esentially new neurological symptoms (16). They can include syndrome of an increased excitability of the nervous reflexes, hydrocephalic syndrome, convulsive syndrome, epileptic syndrome, syndrome of vegetative dysfunctions, syndrome movement disorders (spastic paresis, pyramidal insufficiency, pseudobulbar syndrome, muscular hypo- and hypertonia, syndrome of retardation of psychological and pre-speech development, cerebro-asthenic syndrome).

Before discharging the newborns from the hospital the parents should be consulted on management during restoration period. The child should undergo follow-up observation by pediatricians, neurologists, ophthalmologists and other specialists.

Assessment of psychomotor, social and emotional development of the child is a component of medical-psychological management of children with HIE. It involves consultations for parents, observation by neurologists, psychologists and other specialist; it is necessary for prognosis of further development, including speech and cognitive abilities.

CONCLUSIONS

1. Combination of medical and psychological aspects of treatment promotes a decrease in duration of neurological disorders in newborns with hypoxic-ischemic encephalopathy.
2. Active participation of mothers in care and rehabilitation is effective in prevention of disorders and is the basis for formation for harmonic children-parents relationship.

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PEDIATRICS

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APPLICATION OF PAIN ASSESSMENT SCALES IN PEDIATRICS (pilote study)

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Abstract: The International Association for the Study of Pain, IASP, has long proven that pain and its effects on the child can lead to pathological reactions resulting in behavioral changes in the form of functional and vegetative disorders, emotional responses such as loss of skills, sleep disorders, or child's irritability, enuresis and others. There are currently a number of pain assessment scales for children of all ages that include many different parameters (cardiovascular and respiratory systems, changes in behavior, autonomic reactions in the form of changes in the function of the eyes, skin color, etc.) using a quantitative estimate to evaluate those signs. In pediatric and family practice, it is important to choose from a range of pain scales that are simple and accessible to all healthcare providers and other caregivers. We compared scales that are used most often and are recommended by the medical community. As well as investigating their use in practice, FLACC conducted a study of children with organic lesions of the central nervous system that had episodes of acute pain. Because this scale, in combination with the definition of autonomous regulation parameters, allows to provide non-biased assessment of the degree of pain experienced by the patient.

KeyWords: Pain, pain assessment, pain relief, pain scales, palliative medicine, pediatrics

INTRODUCTION

International Association for the Study of Pain, IASP defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage” [1]. Additional note concerns the pain experienced by children: “The inability to verbal communication does not deny the possibility that the individual feels pain and require analgesic treatment” [1].

It is known that the pain and its impact on the child can lead to pathological reactions resulting in behavioral changes in the form of functional and vegetative disorders, emotional responses such as loss of earlier obtained skills, sleep disorders or irritability of the child, enuresis and others.

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It has long been known that the inability to localize pain in young children in hospital may have a systemic response such as compensatory systems disorders that are rapidly depleted. In-patients are most commonly found to have cardiovascular and respiratory disorders, in the form of protective physiological adaptations to stress (increased cardiac and respiratory rate, pulse, muscular tone, oxygen demand). These physiological responses to pain as well as changings in the child's behavior with the appearance of emotional reactions such as crying, grimacing on face, and others were used as a base for a number of evaluation scales of pain in children.

Children express vegetative reactions in the form of functional changes of the eyes, skin color and thermoregulation, accompanied by activation of endocrine system (thyroid gland, adrenaline, insulin and pituitary hormones).

Apgar scale used worldwide after the birth of a child includes assessment of vegetative (cardiac and respiratory rate, skin color), movement (muscular tone) and emotional (grimace or cry) components.

Thus, in pediatric and family practice it is important to use simple scales for pain assessment, accessible for all healthcare providers and child-caregivers.

At the moment there is a number of scales for pain assessment in children of different ages. Thus, there are scales for children under one year, such as the Neonatal Infant Pain Scale. It is behavioral pain assessment involving the following parameters: facial expression, crying, character of respiration, motor activity and tone of the legs, state of irritation [2].

For children under 3 years there are FLACC scale and TVP Scale. Behavioral FLACC scale scores from 0 to 2 such parameters as facial expression, motor activity and tone of the legs, common activity, crying, possibility to calm the child [3].

FLACC scale and The Pain Indicator for Communicatively Impaired Children can be used in children with mental disabilities [4].

In accordance with the WHO guidelines regarding pharmacological treatment of resistant pain in children with medical diseases, most common are tools for pain measurement based on the idea of counting and recommended by the Ped-IMMPACT and SPP-ATF [4]:

- Faces Pain Scale-Revised
- Poker Chip Tool
- Visual analogue scale (VAS)
- Oucher photographic scale
- Numeric rating scale

Faces Pain Scale-Revised. This scale represents the schematic drawing without the ethnic features that range from a neutral expression to an expression of severe pain. The majority of three-year-old children are able to use this scale. Besides, according to the opinion of children and their parents this scale is the most convenient [5].

Poker Chip Tool. Four red poker chips are used to assess the level of pain, the child is asked to choose chips to describe the pain. One piece corresponds to mild pain, while all four mean very severe pain. This scale can be used in children over the age of three years. It is important to disinfect the chips before use, and there are only four gradations of pain. This scale is easy to use but, according to the assessment of children and their parents, less convenient than the FPS-R [6].

The visual analogue scale (VAS). Using a horizontal segment with the length of 10 cm, marked “no pain” at one end and “very severe pain” at the other. There are also “mild pain”, “average pain” and “severe pain” marks at equal intervals on the scale. The child is asked to draw a vertical line on the scale indicating the level of pain. This scale requires a high level of abstraction, suitable for children over the age of eight years [4].

Oucher photographic scale. This scale consists of two vertical scales: numeric marks from 0 to 100 and six photos of children's faces, expressing a growing level of pain. There are four versions: African-American, Asian, European and Spanish children populations. It can be used in children over the age of three years and it requires color printing [4].

Numeric rating scale. A horizontal segment with the length of 10 cm, one end means “no pain” and “very severe pain”, with marks from 0 to 10. It is used in children over the age of 7-8 years. Besides, it may be used verbally [4].

A special group of patients are children with persistent pain. Persistent pain is a long-term pain associated with somatic diseases lasting more than three months. New WHO guidelines recommend two-stage analgesia. At the first stage patients with mild pain are mostly administered ibuprofen and paracetamol. At the second stage moderate and severe pain is managed by morphine. The use of opioids has a number of features. First, opioids should be administered in equal intervals of time, not “on demand”. Second, opioids are administered orally to patients who can swallow. Morphine dose should increase gradually, and the maximum dose should correspond to individual needs of the child. In case of the so-called “breakthrough pain” the selected doses of morphine are accompanied by additional small doses [7].

The dependency syndrome in patients with pain is rare, and the risk of its development should not be a reason for the refusal from adequate analgesia with morphine. It is currently impossible to give recommendations for use of additional drugs such as tricyclic antidepressants, anticonvulsants, ketamine, benzodiazepine and baclofen [6].

It is important to pay attention to the fact that all of the tools to assess the pain were developed for acute pain,

which was associated with diagnostic medical procedures [8].

It is clear that the measurement of pain in conditions of persistent pain in young children and children with cognitive impairment requires further study and continuous dynamic monitoring of a particular child [10].

2 PURPOSES, SUBJECTS and METHODS:

2.1 Purpose was to present experience of using pain assessment scales in pediatrics.

2.2 Subjects & Methods

The study involved 10 patients with episodes of acute pain aged from 3 to 8 years with organic lesions of the central nervous system, treated in Kharkiv Regional Children Clinical Hospital.

FLACC scale was used in patients with organic lesions of the central nervous system experiencing acute pain, in the complex with parameters of autonomic regulation including cardiac and respiratory rate as well as skin color (Table 1).

Table **1.**

FLACC scale

Criteria	Score
Face	
No particular expression or smile	0
Occasional grimace or frown, withdrawn, uninterested	1
Frequent to constant quivering chin, clenched jaw	2
Legs	
Normal position or relaxed	0
Uneasy, restless, tense	1
Kicking, or legs drawn up	2
Activity	
Lying quietly, normal position, moves easily	0
Squirming, shifting, back and forth, tense	1
Arched, rigid	2
Cry	
No cry (awake or asleep)	0
Moans or whimpers; occasional complaint	1
Crying steadily, screams or sobs, frequent complaints	2
Consolability	
Content, relaxed	0
Reassured by occasional touching, hugging or being talked to, distractible	1
Difficult to console or comfort	2

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

It is obvious that such scales as Faces Pain Scale-Revised and the Oucher photographic scale may not be used in this patient population because of the peculiarities of facial expressions and reaction to acute pain. The visual analogue scale, numeric rating scale and Poker chips tool require high levels of abstract thinking in the child that sometimes does not achieve the validity and reliability of the results. However, FLACC scale parameters do not require verbal skills or the development of abstract thinking in the child. Such parameters as facial expression (options: calm face, the expression of concern or extreme suffering), motor activity and tone of the legs, common activity, crying, possibility to calm the child, are relevant for patients of various age groups and possible for use in patients with different severity of neurological lesion. But it is necessary to evaluate not only the indices of the emotional sphere and motor component. A comprehensive assessment of the level of acute pain is impossible without taking into account vital signs, such as cardiac and respiratory rate and skin color, which can signal the stress, and, eventually, exhaustion of homeostasis and vegetative component. These parameters should be measured quite often for adjustment to the child's condition.

The study showed that FLACC score in 80% of patients was 8-10 points (severe pain). However, despite the severity of the organic lesion of the central nervous system and severity of general condition, pain assessment may not be accurate. We propose to focus also on the parameters of cardiac and respiratory rate in the absence of somatic pathology of the cardiovascular and respiratory systems in this group of patients, since our study showed high variability of these indices (growth more than 30%).

CONCLUSIONS

FLACC is a valid scale for the assessment of pain in pediatric patients with somatic diseases. High FLACC score and variability of autonomic indices are effective for the pain control.

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PSYCHIATRICS & MEDICAL PSYCHOLOGY

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CLINICAL-PSYCHOPATHOLOGICAL FEATURES OF PATIENTS WITH DEMENTIA IN ALZHEIMER'S DISEASE WITH HIGH RISK OF SUICIDE

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Abstract: The study involved comprehensive clinical-anamnestic, neuroimaging and pathopsychological examination of 75 patients with dementia in Alzheimer's disease. The patients were divided into main group with a high risk of suicide (36 patients), group without signs of suicidal behavior (SB) and control group (39 patients). Patients with SB were found to have the damage of basal nuclei and alba; expansion of the cerebral fissures; subcortical damage in insular, frontal, occipital, parietal regions; hippocampal atrophy. The following factors of suicide risk in patients with Alzheimer's disease were determined: psychic trauma (life-threatening condition, loss of work or money); communicative (lack of emotional, financial and communicative support); anamnestic (suicidal attempts and depressive episodes in past); personal (physical, verbal and indirect aggression, irritability, susceptibility, negativism, suspicion and feeling of guilt); clinical (hallucinatory-paranoid syndrome). Specified predictors serve as target symptoms for psychoprophylaxis.

KeyWords: dementia in Alzheimer's disease, suicidal risk, predictors of suicide, clinical and psychopathological peculiarities.

INTRODUCTION

The relevance of the study is stipulated by a significant increase in the level of dementia worldwide. Dementia is the main cause of disability after cancer and spinal cord injury. Main causes of dementia include neurodegenerative and cerebrovascular diseases [1-3]. Dementia in Alzheimer's disease is the most common among all types of dementia, amounting for about 60-80% [4-7]. Pathogenesis of Alzheimer's disease plays a leading role in the accumulation of beta amyloid in the thymus, occipital regions and around the vessels; cerebral cortex and hippocampal atrophy, axonal transport disorder and acetylcholine deficiency and as a consequence, a decrease in neuroplasticity. As early as at the initial stage of Alzheimer's disease patients are found to have congestive neurotic conditions, depressive episodes, chronic paranoids with ideas of jealousy and damage, transient psychoses [8,9,4,5].

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It is known that mental diseases greatly increase the risk of suicidal behavior; moreover, pathoanatomical examination of persons, who committed suicide shows alterations similar to those of Alzheimer's disease [10-12]. We consider it reasonable to provide a comprehensive study of suicidal behavior in this type of dementia and its patterns of formation to determine the predictors and for further development of treatment methods and prevention techniques.

2 PURPOSES, SUBJECTS and METHODS:

2.1 Purpose of the study was the search of pathoanatomical, clinical-anamnestic, psychopathological predictors of suicidal behaviour in patients with dementia in Alzheimer's disease.

2.2 Subjects & Methods

The study involved examination of 75 patients with dementia in Alzheimer's disease; 36 of them had suicidal manifestations (antivital phrases, suicidal thoughts, intentions, attempts) and they comprised the basic group. The control group included 39 patients without signs of suicidal behavior (SB) during a clinical and anamnestic examination. All patients or their relatives gave informed consent

to participate in the study. The average age of patients was 69.4 years. The interval between the onset of the disease and the examination was 6.2 years. The diagnosis was established according to ICD-10 criteria based on data of objective research methods. The following research methods were used: neuroimaging, clinical-anamnestic, psychodiagnostic: MMSE scale; Clinical Dementia Rating Scale (CDR), Suicidal Risk Assessment Scale, Death Self-Awareness Assessment Scale, Hamilton Depression Rating Scale, Barthel Index, Bass-Darky Questionnaire; method of statistical processing of results.

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

Structural and anatomical changes in the main group were characterized by atrophy of the convolutions of the brain and cerebellum in 71.4% of the examined, by expansion of the convexital surfaces of the cerebral hemispheres in 85.7%; with the same frequency there was expansion of the ventricular system of the brain, deepening of the fissures, perioventricular leukoaraiosis, damage of basal nuclei and alba in 28.6%.

Patients without SB were found to have expansion of convexital surfaces of the cerebral hemispheres in 100% of cases, atrophy of the brain convolutions mainly in the projection of parietal and temporal areas in 88.8%; damage of basal nuclei and alba in 66.6%; expansion of fissures of the brain in 44% of patients; expansion of the brain ventricles in 22.3%; subcortical damage in insular, frontal, occipital, parietal regions in 22.3%.

Concomitant disorders were as follows: hypertonic disease was observed in 33.3% of patients, ischemic heart disease and diffuse cardiosclerosis in 44.4%, 2nd-3rd degree DEP in 33%, chronic cerebrovascular disorder of the ischemic type in 11% of the examined, chronic obstructive pulmonary disease in 11%, type 2 diabetes mellitus, age-related cataracts, arthroses of large joints in 11% of patients.

Family history of alcohol addiction was found in 11% of patients; 22% of patients had relatives with moderate or severe cognitive impairment; 11% of patients had family history of schizophrenia. Suicidal history was observed in the families of 13.5% of patients.

Accompanying psychopathological symptoms included visual and auditory hallucinations in 66.2% of patients, 44% of patients had delusion of theft, self-abasement, persecution; 22% had comorbid depression; 55.6% of patients had volitional disorders in the form of apathy and inactivity; 30.7% of the examined felt fear, tension, irritability; 44.8% had sleep disorders.

Clinical presentation of SB was characterized by the predominance of external behavioral forms over ideational ones. Thus, the main group comprised 55.5% of patients, 22.3% of them had real suicidal intentions, showed antivital mood, 33.4% of them expressed and made suicidal attempts in the past.

The study implied the assessment of factors of suicidal behaviour, severity and specificity of cognitive impairment, social functioning, peculiarities of depressive symptomatology and manifestations of aggression.

Thus, all the patients of the main group with Alzheimer's disease had high SR (100%), there were no patients with low SR in the main group.

In the comparison group 83.33% of patients had low SR, and 16.67% had high SR. Statistical analysis of data showed the prevalence of SR in patients of the main group ($p \leq 0.0001$, DK = 7.78, MI = 3.24) and low SR among the patients in the comparison group ($p \leq 0.0001$).

The analysis of death self-awareness indices demonstrated that 77.78% of patients with AIDS had a low level of death awareness, and 22.22% had a high one.

Patients without SB were found to have a low level of death awareness (91.67 ± 7.56%). There were no probable differences in the death rate between the main and control groups.

It is known that a low level of death awareness indirectly indicates a tendency to commit autodestructive actions. So, the obtained data suggest that dementia in

Alzheimer's disease is a vulnerable form of dementia as to committing self-destructive actions.

A detailed analysis of the factors influencing the increase in SR among patients with dementia was performed to determine the factors causing the increase in SB. Thus, the total SR score in the main group was 101.67 points, which corresponded to the high level, while in the comparison group, the total SR score was 38.00 points, which corresponded to the average level (Table 1).

The study showed probable differences reflecting the prevalence of SR in the main group of patients (t = 13.158, p≤0.0001). The analysis of the factors influencing the increase in SR among patients with Alzheimer's disease demonstrated the predominance of depression symptoms (sleep disturbance, weight loss, depressed mood) (8.89 ± 0.32 points) in patients of the main group compared to the patients of the control group (6.00 ± 2.38 points), (t = 7.207, p≤ 0.0001).

Table **1.**

Factors of suicidal risk in patients with Alzheimer's disease.

Indices	Main group	Comparison group	t-value	p
	m ± σ			
1	2	3	4	5
Symptoms				
Deep depression	**8.89 ± 0.32**	**6.00 ± 2.38**	**7.207**	**0.000**
Feeling of hopelessness, helplessness and exhaustion	**5.00 ± 4.54**	**1.69 ± 2.96**	**3.769**	**0.000**
Disorganization, disorientation, illusion, hallucinations	6.11 ± 3.40	5.08 ± 2.88	1.426	0.158
Alcoholism, drug use, homosexual relationships, participation in risky events	**2.22 ± 3.37**	**0.31 ± 1.08**	**3.364**	**0.001**
Excitement, tension, anxiety	3.33 ± 3.02	2.23 ± 2.11	1.843	0.069
Guilt	**2.56 ± 2.91**	**0.69 ± 1.66**	**3.439**	**0.001**
Feeling of hostility, annoyance, suspicion	**4.56 ± 2.49**	**0.77 ± 1.87**	**7.484**	**0.000**
Weak impulsive control, insufficient prudence	2.56 ± 2.91	1.92 ± 2.12	1.081	0.283
Chronic weakening illnesses	2.78 ± 3.27	1.92 ± 2.46	1.285	0.203
Repeated experiences of troubles associated with the treatment of doctors and psychotherapists	0.78 ± 1.90	0.31 ± 1.08	1.331	0.187
Stress				
Loss of a beloved person caused by death or breakup	3.89 ± 4.42	3.77 ± 3.79	0.126	0.900
Loss of work, money, status	**3.67 ± 4.20**	**1.00 ± 2.38**	**3.418**	**0.001**
A life-threatening disease	**5.67 ± 4.17**	**1.85 ± 2.83**	**4.674**	**0.000**
Threat of judicial implementation	**1.00 ± 2.87**	**0**	**2.178**	**0.033**
Changes in life or environment	1.67 ± 3.17	0.69 ± 1.75	1.664	0.100
Acute and accidental symptoms of stress	**2.78 ± 3.99**	**1.15 ± 2.24**	**2.193**	**0.031**
Occasionally recurring symptoms of stress	**1.11 ± 2.85**	**0**	**2.439**	**0.017**
Increase of stress symptoms	**0.11 ± 1.47**	**0**	**2.178**	**0.033**
Suicidal behaviour in the past and current plans				
Danger to life in previous suicidal attempts	**2.89 ±4.15**	**0**	**4.347**	**0.000**
Repeated threats and depressions in the past	**4.67 ± 4.28**	**0.38 ± 1.35**	**5.944**	**0.000**
Peculiarities related to intentions, and mortal threat of planned methods	**3.00 ± 4.21**	**0**	**4.357**	**0.000**
Sufficiency of planned methods and features related to the choice of time	**1.00 ± 2.87**	**0**	**2.178**	**0.033**
Opportunities, communicative aspects and answers of the persons important for the patient				
Lack of financial support	**4.67 ± 4.06**	**0.69 ± 1.66**	**5.633**	**0.000**
Lack of emotional support from family and friends	**5.67 ± 3.88**	**1.15 ± 2.81**	**5.801**	**0.000**
Violation of relations accompanied by refusal from attempts to restore them	**4.22 ± 4.22**	**0.77 ± 1.83**	**4.664**	**0.000**
Relations have an internally directed goal	**1.56 ± 3.24**	**0**	**3.133**	**0.002**
Communicative relations have an interpersonal orientation	**1.78 ± 3.41**	**0.46 ± 1.92**	**2.163**	**0.034**
Reaction of persons important for the patient				
Denying the need for help	**2.22 ± 3.34**	**0**	**4.159**	**0.000**
Lack of care for the patient, lack of understanding	0.89 ± 2.55	0.31 ± 1.08	1.303	0.197
Indecisive or changing position	1.00 ± 2.87	0.38 ± 1.35	1.204	0.233
Total amount	**101.67 ±26.89**	**38.00 ± 13.27**	**13.158**	**0.000**

Moreover, the patients had the feeling of hopelessness, helplessness and exhaustion (5.00 ± 4.54 points), symptoms of disorganization and disorientation (6.11 ± 3.40 points), feeling of hostility, irritation, suspicion (4.56 ± 2, 49 points), anxiety (3.33 ± 3.02 points) and guilt (2.56 ± 2.91 points). Patients of the comparison group were also found to have symptoms of disorganization and disorientation (5.08 ± 2.88 points), as well as chronic weakening diseases (1.92 ± 2.46 points) and low prudence (1.92 ± 2.12 points). Such symptoms as the feeling of hopelessness (t = 3.769, p≤ 0.0001) and irritability (t = 7.448, p≤ 0.0001), excessive consumption of alcohol (t = 3,364, p≤0,01) and feelings of guilt (t = 3,439, p≤0,001) were shown to have a likely influence on the increase of SR in the main group. The study demonstrated a cause-effect relationship between the effect of stress factors and the increased risk of suicide in Alzheimer's disease.

In the main group stress factors predominantly included life-threatening diseases (5.67 ± 4.17 points), loss of a beloved person (3.89 ± 4.42 points), work and money (3.67 ± 4.20 points). Among the patients in the comparison group, the severity of the stress factors was lower, but the severity of feelings regarding the loss of a beloved person (3.77 ± 3.79 points) was higher. It should be noted that life-threatening diseases (t = 4.674, p≤0.0001), loss of work, money or status (t = 3,418, p≤0,001), the threat of judicial implementation (t = 2.178, p≤0.05) were typical for patients with high SR as compared to the patients of the comparison group. It also should be noted that the character of impact of a stressful event was of an essential significance. Thus, acute and sudden onset of stress factor (2.78 ± 3.99 points), its repetition and increase ((1.11 ± 2.85 points) and (0.11 ± 1.47 points) respectively) likely increased the level of SR in the main group in Alzheimer's disease ((t = 2.193, p≤0.05), (t = 2.439, p≤0.01) and (t = 2.178, p≤0.05) respectively).

The factor “Suicidal behaviour in the past and current plans” was a significant factor influencing the SR. Suicidal intentions (3.00 ± 4.21 points) and their seriousness (1.00 ± 2.87 points), as well as previous suicidal attempts (2.89 ± 4.15 points), were typical for patients of the main group as compared to the patients of the comparison group, who did not have the abovementioned factors ((t = =4.357, p≤0.0001), (t = 2.178, p≤0.05) and (t = 4.347, p≤0.0001) respectively). Also, patients of the main group had more depressive episodes in the past (4.67 ± 4.28 points), (t = 5.944, p≤ 0.0001).

Communicative impairments were also found to influence SR. Thus, assessment of factors “Opportunities, communicative aspects and responses of important people” and “Reaction of important people” showed that the lack of financial sources (4.67 ± 4.06 points), (t = 5.633, p≤0.0001), and emotional support (5.67 ± 3.88 points), (t = 5.801, p≤ 0.0001), abscence of interpersonal relations (4.22 ± 4.22 points), (t = 4.664, p≤0, 0001), feelings of guilt, on the one hand, and hostility, on the other, ((1.56 ± 3.24) and (1.78 ± 3.41) points respectively), ((t = 3.133, p≤0. 0025) and (t = 2.163, p≤0.05) respectively), as well as denial of need for help (2.22 ± 3.34 points), (t = 4.159, p≤ 0.0001) were more manifested among the patients with high SR.

Evaluation of clinical-psychopathological structure of depression in patients with dementia showed that patients with SR in the main group predominantly had “inhibitory depression’ (43.18 ± 14.62)% manifested in inhibition, torpidity of mental processes and motor reactions, as well “agitated depression” (39.20 ± 15.28)%, manifested in anxiety and tension. Phobic (34.38 ± 15.95)% and somatized (31.25 ± 17.17)% forms of depression were less expressed.

In the group of patients without SB, the “inhibitory” (37.59 ± 8.60)% and “agitated depression” (32.69 ± 11.53)% were the predominant types of depressive disorder, but a statistical analysis of the results allowed to establish that these rates of depressive disorders were significantly higher in patients with high SR ((t = 2.039, p≤0.05) and (t = 2.091, p≤0.05) respectively).

A more detailed analysis of depression symptoms in patients of the main and control group helped to understand the influence of depressive symptoms on SR. Thus,

the overall level of depression was higher in the main group (31.44 ± 9.83 points) than in the control group (26.77 ± 7.86 points), while the differences were at the level t = 2.283, p≤0.025, which indicates that depressive disorders in Alzheimer's disease are a factor resulting in an increase in SR (Table 2).

Table 2.

Depression Symptoms in Patients with Alzheimer's Disease (by the Hamilton scale)

Indices	Main group	Comparison group	t-value	P
	m ± σ			
1	2	3	4	5
Depressive mood *	**1.78 ± 1.24**	**1.15 ± 1.11**	**2.292**	**0.025**
Feeling of guilt	1.33 ± 1.26	1.38 ± 1.71	-0.137	0.884
Suicide intentions *	**2.56 ± 1.27**	**0.15 ± 0.37**	**11.279**	**0.000**
Early insomnia	1.22 ± 0.80	1,00 ± 0,79	1.208	0.231
Moderate insomnia	**1.22 ± 0.80**	**0.77 ± 0.81**	**2.439**	**0.017**
Severe insomnia	1.00 ± 0.83	1.00 ± 0.69	0.000	1.000
Work and activities	3.67 ± 0.68	3.69 ± 0.61	-0.172	0.864
Inhibition	2.22 ± 1.15	2.46 ± 0.85	-1.029	0.307
Restlessness	2.33 ± 1.51	2.15 ± 1.31	0.551	0.583
Mental anxiety	1.89 ± 1.39	1.54 ± 1.17	1.186	0.239
Somatic anxiety	1.44 ± 1.18	1.62 ± 0.63	-0.789	0.433
Digestive somatic disorders *	**1.00 ± 0.83**	**0.62 ± 0.63**	**2.270**	**0.026**
General somatic symptoms *	**1.33 ± 0.48**	**1.00 ± 0.56**	**2.755**	**0.007**
Genital symptoms *	**1.00 ± 0.96**	**0.31 ± 0.73**	**3.539**	**0.001**
Hypochondria	0.89 ± 1.21	1.15 ± 1.53	-0.826	0.412
Weight loss	0.78 ± 0.93	1.00 ± 0.97	-1.009	0.316
Weight Loss (actual)	0.33 ± 0.68	0.54 ± 0.85	-1.147	0.255
Criticality *	**1.00 ± 0.68**	**1.69 ± 0.61**	**-4.649**	**0.000**
Daily fluctuations	0.78 ± 0.93	0.62 ± 0.75	0.837	0.405
Daily fluctuations (degree)	0.67 ± 0.83	0.69 ± 0.83	-0.134	0.894
Depersonalization/derealization	1.33 ± 1.43	0.77 ± 1.27	1.809	0.075
Paranoid symptoms	1.33 ± 1.35	1.23 ± 1.74	0.283	0.778
Obsessive and compulsive symptoms	0.33 ± 0.68	0.23 ± 0.58	0.705	0.483
Total score *	**31.44 ±9.83**	**26.77 ± 7.86**	**2,283**	**0,025**
Symbols: * – the difference is probable at p ≤ 0.05				

As can be seen from the Table 2, patients of the main group with Alzheimer's disease were predominantly found to have a decrease in activity and work productivity (3.67 ± 0.68 points), suicidal intentions (2.56 ± 1.27 points), excitement (2, 33 ± 1.51 points), inhibition (2.22 ± 1.15 points), mental and somatic anxiety ((1.89 ± 1.39) and (1.44 ± 1.18) points respectively), depressive mood (1.78 ± 1.24 points), feelings of guilt (1.33 ± 1.26 points), suspicion (1.33 ± 1.35 points), symptoms of derealization/ depersonalization (1.33 ± 1.43 points) and general somatic symptoms (1.33 ± 0.48 points), and sleep disturbance (1.22 ± 0.80 points). Obsessive and compulsive symptoms (0.33 ± 0.68 points), weight loss (0.33 ± 0.68 points) and hypochondria (0.89 ± 1.21 points) were not typical for patients with SB.

Such depressive symptoms as decreased productivity in work and activity (3.69 ± 0.61 points), symptoms of excitement and inhibition ((2.15 ± 1.31) and (2.46 ± 0.85) points respectively), decrease in disease criticality (1.69 ± 0.61 points), somatic and mental anxiety ((1.62 ± 0.63) and (1.54 ± 1.17) points respectively), suspicion (1.23 ± 1.74) and hypochondria (1.15 ± 1.53 points) prevailed in the comparison group. Suicide intentions, obsessive-compulsive symptoms (0.23 ± 0.58 points), genital symptoms (0.31 ± 0.73 points) and digestive somatic disturbances (0.62 ± 0.63 points), actual weight loss (0.54 ± 0.85 points) and daily fluctuations (0.62 ± 0.75 points) were the least expressed in the comparison group (0.15 ± 0.37 points).

Comparison of the results between the main and comparison group showed that in Alzheimer's disease in patients with high SR the depressive mood (t = 2.292, p≤0.025), suicidal intentions (t = 11.279, p≤0.0001), frequent waking up in the middle of the night (t = 2.439, p≤0.01), digestive, general somatic and genital disturbances ((t = 2.270, p≤0.025), (t = 2.755, p≤0.01) and (t = 3.539, p≤ 0.001) respectively) prevailed, and the patients had more conservative criticism as to their own illness (t = -4.649, p≤ 0.0001).

Cognitive impairments at different types of dementia and their impact on SR severity were evaluated with MMSE, CDR and Barthel Index. The assessment showed moderate (33.33 ± 7.77)% and mild (33.33 ± 7.77)% cognitive deficiency in the majority of patients of the main group, severe dementia in 22.22% of patients, and separate signs of a cognitive deficiency in 11.11%. In the comparison group, the majority of patients had a severe degree of dementia (53.85 ± 9.62%), 38.46% had moderate and 7.69% of patients had mild dementia.

Statistical analysis of the results allowed to determine that in patients with Alzheimer's disease, high SR prevailed in the groups with mild cognitive deficiency and its indi-

vidual characteristics ((p≤0.005, DK = 6.37, MI = 0.82) and (p≤0.05)) respectively), and low SR in the group with severe degree of dementia (p≤0.005, DK = 3.84, MI = 0.61).

Evaluation of character of separate cognitive functions showed the following features: patients with high SR were found to have a reduction in short-term memory (14.66 ± 0.04)%, praxis (22.00 ± 0.06)% and time orientation violations (33.40 ± 0. 09)%, decreased concentration and ability to count (37.80 ± 0.10)% (Fig.3.24). These indicers had the lowest values and reflected the deficit specificity of this category of patients.

Patients in the comparison group had praxis (8.00 ± 0.02%), memorizing (12.66 ± 0.03%), concentration of attention (7.60 ± 0.01%), time and place orientation violations ((12,40 ± 0,03)% and (35,40 ± 0,09)% respectively).

The statistical analysis of data showed that patients with high SR in Alzheimer's disease had less severe speech disorders (t = 3.893, p≤0.0001), time and place orientation (t = 3.718, p≤ 0.0001) and (t = 4.921, p≤ 0.0001) respectively), concentration of attention (t = 3.984, p≤0.0001). For assessment of the specificity of cognitive impairments, the Clinical Dementia Rating (CDR) was used to determine the depth of dementia process, based on qualitative changes in such indices as the degree of memory impairment, orientation, thinking, qualitative disorders of social and household interaction and the degree of preservation of self-service skills.

Thus, the study showed that the patients of the main group typically had narrowing of interests (2.22 ± 0.86 points), moderate memory impairments (2.17 ± 0.89 points), moderate difficulties in solving problems (2.11 ± 0 , 89 points), loss of independence outside the home (2.22 ± 0.80 points), difficulties with self-service (2.00 ± 0.956 points) and disorientation (1.94 ± 0.91 points).

The comparison group was characterized by severe violations in intellectual and mnestic spheres ((2.11 ± 0.48 points) and (2.50 ± 0.65 points) respectively), narrowing the range of interests and communication (2.46 ± 0.86 points), moderate loss of self-dependence outside the home and when doing hygiene procedures ((2.33 ± 0.76 points) and (2.08 ± 0.77 points) respectively), as well as severe disorientation (2.58 ± 0.65 points).

Statistical analysis showed probable differences in patients with Alzheimer's disease in comparison group, who had more severe violations in the intellectual sphere and orientation process ((t = 3.307, p≤ 0.001) and (t = 3.433, p≤ 0.001) respectively).

Moreover, the study implied evaluation of peculiaritiess of aggression (as a reaction developing as a result of negative feelings and negative assessments of people and events). Thus, in the main group there was a predominance of feelings of guilt (77.78 ± 22.53)%, grievance (58.33 ± 22.92)%, suspicion (56.67 ± 18.34)%, negativism (53.33 ± 22.59%) and irritability (51.52 ± 16.67%), which was manifested in the remorse along with the feeling of hatred towards the others expressed in most cases by irritability.

In the comparison group, the level of aggression was significantly lower and was manifested in grievance (48.44 ± 9.97)%, guilt (48.61 ± 7.90)% and indirect aggression (30.56 ± 15.80)%. There were significant differences between the main group and the comparison group in all the indices of aggression: indices of physical, verbal and indirect aggression ((t = 2.6772, p≤ 0.01), (t = 3.674, p≤ 0.001)) and (t = 2.155, p≤0.05) respectively), irritation, grievance, negativism and suspicion ((t = 5.407, p≤ 0.0001), (t = 1.940, p≤0.05), (t = 4.165, p≤ 0.0001) and (t = 6.088, p≤ 0.0001) respectively) were typical for patients with high SR and the feeling of guilt was significantly expressed (t = 5.736, p≤ 0.0001)

Correlation analysis showed that the intensity of cognitive deficit (r = -0.542), perception violation (r = -0.542), decrease in concentration of attention (r = -0.589), praxis violation (r = -0.671), linguistic functions (r = -0.401), reading ability (r = -0.390), and performing commands (r = -0.592) were associated with a low suicide rate, and decreased mnestic functions (r = 0.542), feeling of decision-making difficulties (r = 0.720) and disorientation (r = 0,311) were associated with a high suicidal risk. In other words, the risk of suicide was less in the more severe cognitive impairments manifested in decreasing concentration of attention, praxis, linguistic functions, ability to read

and perform commands, but mnestic violations, disability to make decisions and disorientation were a risk factor for suicidal behaviour in patients with Alzheimer's disease.

Thus, high suicide risk was associated with the severity of depression (r = 0.505), first of all of age-related type (r = 0.664), and depressive episodes in the past (r = 0.605); suicidal intentions (r = 0.887), feelings of guilt (r = 0.694) and sleep disturbances (early insomnia (r = 0.659), moderate insomnia (r = 0.710) or severe insomnia (r = 0.586)) and also apathy (r = 0.598), inhibition (r = 0.492), mental anxiety (r = 0.321) and symptoms of derealization (r = 0.451).

“Loss of work, money or status” (r = 0.616), life-threatening diseases (r = 0.727), acute and accidental onset of the stress factor (r = 0.304) and their occasional recurrence (r = 0.539) in patients with Alzheimer's disease were associated with a high risk of suicide among the factors of psychiatric traumatism.

Features of interpersonal communications also included the correlates of suicidal risk. Thus, in Alzheimer's disease, feelings of guilt, worthlessness and self-accusation (r = 0.759), as well as violations of interpersonal relations (r = -0.326) were associated with a high suicidal risk.

Assessment of personal features identified correlates of suicidal risk for different types of dementia. Thus, the severity of irritability (r = 0.367), grievance (r = 0.452) and feelings of guilt (r = 0.438) in Alzheimer's disease were associated with a high suicidal risk.

CONCLUSIONS

Thus, determinants of suicidal behaviour risk in dementia in Alzheimer's disease are as follows:

- Damage of basal nuclei and alba of the cerebral hemispheres; expansion of the cerebral fissures; subcortical damage in insular, frontal, occipital, parietal regions; hippocampal atrophy (p≤0.05). Leukoaraiosis is a factor of suicide anti-risk (p≤ 0.001)
- Visual and auditory hallucinations and delusions of theft, self-abasement, persecution (p≤0,05)
- Mild dementia (p≤0.005, DK = 6.37, MI = 0.82) and certain signs of cognitive deficiency (p≤0.05)
- The prevalence of inhibitory (37.59%, t = 2.039, p≤0.05) and agitated depression (32.69%, t = 2.091, p≤0.05)
- The severity of depression (t = 2.283, p≤0.025), feeling of hopelessness and helplessness (t = 3.769, p≤0.0001), irritability (t = 7.484, p≤0.0001) and guilt (t = 3.439, p ≤0.001), suicidal intentions (t = 11.279, p≤0.0001); frequent waking up in the middle of the night (t = 2.439, p≤0.01), digestive, general somatic and genital disturbances ((t = 2.270, p≤0.025), (t = 2.755, p≤0.01) and (t = 3.539, p≤0.001), respectively), and more conservative criticism of own illness (t = 4.649, p≤0.0001)
- Anamnestic data: previous suicidal attempts in the history (t = 4.347, p ≤ 0.0001) and depressive episodes in the past (t = 5.944, p≤0.0001)
- Factors of mental traumatization: “life-threatening disease” (t = 4.674, p≤0.0001) and “loss of work, money or status” (t = 3.418, p≤0.001)
- Acute and accidental onset of the stress factor (t = 2.193, p≤0.005) and its recurrence (t = 2.439, p≤0.01)
- Lack of emotional (t = 5.801, p≤ 0.0001), financial (t = 5.633, p≤ 0.0001) and communicative (t = 4.664, p≤ 0.0001) support
- Peculiarities of social functioning: prevalence of little dependence on others (p ≤ 0.0005, DK = 7.27, MI = 1.31) and complete dependence on others (p ≤ 0.025)
- Personal features: predominance of physical, verbal and indirect aggression ((t = 2.6772, p≤ 0.01), (t = 3.6774, p≤ 0.001) and (t = 2.155, p≤0.05) respectively), irritability, grievance, negativism and suspicion ((t = 5.407, p≤ 0.0001), (t = 1.940, p≤0.05), (t = 4.165, p≤ 0.0001) and (t = 6.088, p≤ 0.0001) respectively), and the intensity of guilt (t = 5.736, p≤ 0.0001).

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DENTISTRY

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ANALYSIS OF VNTR POLYMORPHISM OF MUC5B GENE IN CONNECTION WITH CERTAIN PHYSICO-CHEMICAL PROPERTIES OF ORAL LIQUID IN CHILDREN WITH DOWN SYNDROME

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Abstract: Protective function of oral fluid is evident in maintaining constant saliva volume, moisturizing mucous membranes of the oral cavity, teeth enamel, preventing the defeat of soft and hard tissues of the oral cavity by pathogenic microorganisms. A number of factors, called "barriers of colonization", specifically and nonspecifically manage the process. What matters most is "mucous block", which characterizes the set of mechanical, humoral, nonspecific factors of protecting mucous membranes against microorganisms. Mucin proteins which are the main glycoprotein saliva components affect the creation and selection of biofilm microflora, facilitating or inhibiting the adhesion of microorganisms and maintaining healthy microbial environment in the oral cavity. The dominant mucin of submucosa glands is MUC5B, which is encoded by the same gene, located in a short shoulder of segment 15.5 of chromosome 11. Changes of the basic physical and chemical properties of non-stimulated saliva in children with Down syndrome, namely, reduction of pH level and increasing oral fluid viscosity, is certainly an important prerequisite for formation of cariogenic situation.

KeyWords: Down syndrome, MUC5B, oral cavity, saliva.

INTRODUCTION

The study of molecular-genetic bases of multifactorial diseases including diseases of the oral cavity refers to one of the challenges of modern genetics. Identification of genetic factors predisposing to the development of the disease has a prognostic value and can be used in presymptomatic diagnosis, that is, before the appearance of any clinical or biochemical disease symptoms. The intensity of pathological processes in children's oral cavity is directly related to the composition and properties of oral liquid [1].

Acid-base status and saliva viscosity indicator are one of the most important indices of oral cavity homeostasis [2; 3].

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Changes in physical and chemical properties of oral liquid have a significant impact on the structural properties and stability of oral liquid as a colloidal system, leading to destabilization of its micellar state and causing further changes in organs and tissues of the oral cavity, namely disorder of remineralization process of tooth enamel, the change of a state of periodontal tissues and mucous membranes of the oral cavity [4].

Protective function of oral liquid is evident in maintaining frequent volume of saliva, moisturizing mucous membranes of the oral cavity, tooth enamel. All this is essential for keeping the oral cavity organs in a functionally active state, as well as for prevention of soft and hard tissues of the oral cavity from the damage by pathogenic microorganisms. Immunoglobulins, lysozyme, mucin, lactoferrin, nucleases, proteases, myeloperoxidase, salivary peroxidase play the most important role in maintaining this feature [5]. Despite the fact that oral liquid contains only 0.2-0.4% of protein and not more than 2% of other substances, it has high internal structuredness due to the presence of mi-

celles based on calcium phosphate. The presence of acid proteins, rich in proline, in mixed saliva, gives it viscosity and ductility [6]. Mucin proteins, which are the main glycoprotein components of saliva, affect the creation and selection of microflora in biofilm, facilitating or preventing from the adhesion of microorganisms and maintaining healthy microbial environment in the oral cavity [6].

Every person has 11 families of mucins encoded by non-allelic genes. Genes' expression is found in the buccal salivary glands, mucosa of respiratory tract and cholecyst. All mucins are glycoproteins with high amount of carbohydrates. Dominant mucin of submucosa glands is MUC5B [7] which is encoded by the homonymous gene, located in a short shoulder of segment 15.5 of chromosome 11.

2 PURPOSES, SUBJECTS and METHODS:

2.1 Purpose of this work is to study VNTR polymorphism (variable number of tandem repeats) of MUC5B gene and to search for connection of this gene's polymorphism with level pH and viscosity of oral liquid in children living in Kharkiv. Selected for the study the option of VNTR polymorphism of MUC5B gene is connected with including in the gene similar fragments from 59 base pairs in introne 36 [8]. Analysis of polymorphism associated with the satellite regions of the gene is informative enough, because it allows to identify the diversity of allelic forms of a gene in a population.

2.2 Subjects & Methods

The study was conducted at the University Dental Center of Kharkiv National Medical University. Total number of examined patients was 43 children aged from 2 to 17 years. The main group comprised 9 children with Down syndrome. The control group included 34 children without chromosomal pathology. To compare the main and control groups adequately, we divided the control group into 2 age categories: the first for children from 2 to 8 years (9 patients – control 1) and the second is for children from 9 to 17 years (25 patients – control 2). All the examined children and their parents have been informed of the aim of the study and the methods to be applied. Parents have given written consent to participate in the study.

Determination of pH in oral liquid. Determination of pH of mixed saliva was carried out with the help of test strip indicator ("SPOFA", The Czech Republic). In patients with Down syndrome we collected non-stimulated oral liquid on glass. Test strips for determining pH were sunk in a drop of oral liquid for 10 seconds, then the color of the test strips was compared with the table from a set [9; 10; 11].

Viscosity determination method (gradation level) of oral liquid [4] implied that thin threads were extruded from saliva accumulated in the sublingual region within 2 minutes with the help of dental forceps. Cutting threads happened at some level which was the basis for detecting four gradations of viscosity test, designated by points from 1 to 4. 1 – sharply negative (cutting threads at the level of the central teeth of the upper jaw or upper lip); 2 – negative (cutting threads at the level of the wings or nose tip); 3 – positive (cutting thread at the brow level); 4 – sharply positive test (cutting threads at the scalp level and above) [12] [10; 11].

To conduct genotyping buccal epithelial cells were used. Selection of material for the study was conducted during dental examination by using sterile disposable urogenital probe in an individual container marked in accordance with the method [13]. DNA was isolated by using a commercial set Diatom ™ DNA Prep 100 (Russia) in accordance with the manufacturer's instructions [14]. Typing VNTR polymorphism in introne 36 of MUC5B gene was conducted by using polymerase chain reaction (PCR) with detection of amplificated fragments in agarose gel. For amplification such primers were used: MUC5BF - 5'- AGTGTGCAGTGACTGGCGAG-3' and MUC5BR - 5'-CTAGAGTTGCAGGTGGCAGG-3' [15]. Automatic thermocycler "Tercik" (Russia) and commercial sets of reagents GenPak ™ PCR Core (0.5 ml) (Russia) were used for PCR of alleles of MUC5B gene in accordance with the manufacturer's instructions. PCR conditions: denaturation for 3 min at 95°C; 30 cycles consisting of denaturation for 30 s at 95°C, annealing of primers for 30 s at 95°C, elongation for 45 s at 72°C; final elongation for 7 min at 72°C [15]. Detection

of PCR results was conducted by dividing the amplification products in 2% agarose gel at constant voltage 70V within an hour. Commercial sets ELA-50 ("Neogene", Ukraine) were used for electrophoresis. Visualization of the fragments was conducted by processing gel with ethidium bromide and subsequent analysis on transilluminator in ultraviolet light. The size of the fragments was determined in comparison with a molecular weight of pUC19 DNA/Mspl (HpaII) Marker, 23 (Thermo Fisher Scientific Inc.).

Statistical analysis of the results. The difference between the control and the main groups of the alleles in introne 36 of MUC5B gene was established by using the Kraskell-Wallace criterion. The difference between the control and the main groups on quantitative criterion (oral liquid pH level), as well as the dependence of quantitative indication from allele in introne 36 of MUC5B gene was established by using variance analysis (ANOVA). Reliability of differences was assessed by Student's t-test. Statistical processing of data and mathematical analysis was carried out by using BioStat 2008 Professional, frequency of MUC5B gene alleles was calculated by using GenoMprofessional.

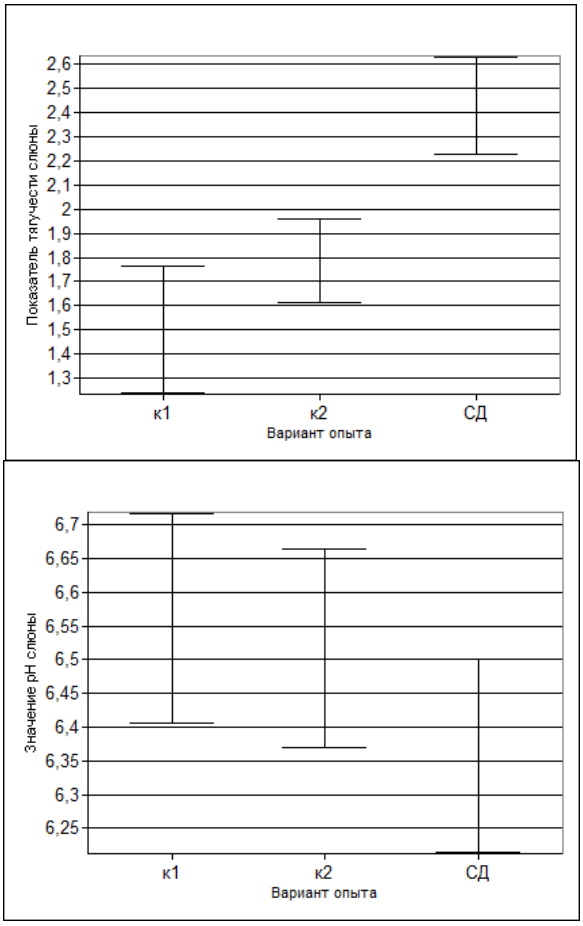

Fig. 1. The differences in level of viscosity and acid-base status of non-stimulated oral liquid in main (DS - children diagnosed with Down syndrome) and control (K1 and K2) groups.

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

The results of the study on indices of acidity and viscosity of non-stimulated oral liquid in children living in Kharkiv region are shown in Figure 1.

Children with Down syndrome, in comparison with the control group children, were shown to have a positive change in viscosity index of saliva – cutting salivary threads was carried out at the brow or the scalp level. The results of the variance analysis revealed a significant difference of this indicator in the main and the control group 1 ($F = 6.05$; $p<0.05$ for K1 and $F = 4.58$; $p<0.05$ for K2). Perhaps this is because children with Down syndrome have a decrease in the secretion of the salivary parotid (Spec Care Dentist).

Rheological properties of saliva, which include viscosity and ducility, characterize the overall functional state of the body. This is quite a sensitive indicator, and even short-term and slight chemical and metabolic disorders, accompanying generally somatic pathologies, can change it [16; 17; 18]. The results of our study showed a downward tendency in pH level of oral liquid in children with Down syndrome in comparison with the children of both authors [19]. The variance analysis showed no reliable difference of this indicator in the main and control groups ($F = 0.9$; $p>0.05$ for K1 and $F = 0.28$; $p>0.05$ for K2).

Changes of the basic physical and chemical properties of non-stimulated saliva in children with Down syndrome, namely reduction of pH level and increasing oral liquid

viscosity, is certainly an important precondition for the formation of a cariogenic situation.

The main factors of destabilization of the physical and chemical properties of the oral cavity in norm and pathology are meal and metabolic activity of microorganisms. Oral cavity is colonized by representatives of different taxonomic groups of microbes that enter into biochemical, immunological and other interactions with macroorganism and with each other, forming microbiocenosis of the oral cavity [20; 21]. Colonization of conditionally pathogenic microbes of the oral cavity in low resistance of teeth tissues to caries creates conditions for

There is a number of factors called "barriers of colonization", specifically and non-specifically governing this process [22]. The greatest value has the “mucous block” which characterizes the set of mechanical, humoral, non-specific factors of mucosal protection against microorganisms [23].

The main protective protein that stabilizes minerals in saliva, supporting its micellar composition, is mucin which is encoded by MUC5B gene. Gene MUC5B is included in 4-genic cluster located in segment p 15.5 of short shoulder of chromosome 11. Gene in a human genome has 40 triphosphopyridine nucleotides, their encoding sequence having 10713 nucleotides [24].

The results of the genetic analysis have showed that in the population of Kharkiv there are 8 types of alleles differing from each other by the number of tandem repeats in introne 36 of MUC5B gene. Among the examined the most frequent ones have been homozygotes (21 people – 48.8% of all the examined), their alleles having 2, 8, 5, 7 and 6 repeats, respectively, 10, 4, 3, 3 and 1 people. The less presented ones have been heterozygotes – 8 people (18.6% of all the examined). The most commonly detected individuals are ones with options 7/9 (3 people), also there are combinations of alleles of 3/7, 8/9, 2/7, 2/3, 6/9 (1 examined).

Checking by Kraskell-Wallace criterion has not shown significant differences in the frequency of alleles of MUC5B gene with different number of repeats in the control and main group ($p = 0.1821$). The results of the study of 14 examined (32.6%) have shown the presence of three allelic MUC5B genes in genotype. In 3-allelic genotype combinations there are dominant options of alleles with three (25.5%) and eight (21.6%) repeats. Extreme options – 2 and 9 repeats – by 3.9%, respectively, are less presented.

It is interesting to note that the control and main groups are significantly different in the frequency of individuals with VNTR alleles – 22.2% and 37.5%, respectively. The presence of three alleles in one genotype can be explained by the high frequency of recombination, which demonstrates, in our opinion, an increased level of genome instability in the main group of children (diagnosed with Down syndrome). It has been previously shown that trisomy of chromosome 21 affects the transcription of genes of the chromosome involved in numerical chromosomal anomaly and the frequency of unequal X-inactivation [25].

Basic recombination mechanisms may be unequal chromosomal crossing-over, gene conversion and exchange of sister chromatids. Increased frequency of recombinations is directly connected with the epigenetic mechanism and may be the result of non-methylated cytosine in satellite repeats. It is expected that methylation prevents from undesired recombinations between homologous satellites in shifted positions and helps stabilize the tandemly located units in the cell nucleus [26]. DNA methylation is a stable epigenetic modification changing the pattern of gene expression. However, throughout the life of the individual DNA methylation profile changes can occur. These changes are often connected with any pathological process, such as oncogenic transformation, cell aging or hereditary diseases [27]. This is confirmed by the results obtained in our work – children with Down syndrome have an increased frequency of 3-allelic combinations of MUC5B gene.

It is obvious that genetic instability in children diagnosed with Down syndrome, shown by us on the example of MUC5B gene, is the reason causing the change of physical and chemical properties of oral liquid. Thus, the increase in a viscosity indicator of oral liquid in children with Down syndrome, as shown in our work, can

be explained by the increase in the concentration of mucin in saliva.

The analysis of VNTR polymorphism in introne 36 of MUC5B gene has shown no significant association with acidity and viscosity of oral liquid of the examined children. The results of the variance analysis (ANOVA) when identifying the contribution of each of the alleles are shown in Table 1.

Table **1.**

The contribution of alleles with different numbers of repeats in introne 36 of MUC5B gene in indices of acidity and viscosity of oral liquid (ANOVA results)

VNTR polymorphism in introne 36 of *MUC5B* gene	Salivary viscosity			Salivary pH		
	MS	F	Significance level	MS	F	Significance level
2 repeats	0.91	0.34	0.52	0.35	0.83	0.36
3 repeats	0.05	0.07	0.79	0.39	0.92	0.33
4 repeats	0.12	0.15	0.69	0.15	0.39	0.53
5 repeats	0.17	0.22	0.64	0.81	2.01	0.16
6 repeats	0.32	0.42	0.52	0.04	0.10	0.75
7 repeats	1.36	1.89	0.18	0.02	0.04	0.84
8 repeats	0.14	0.18	0.67	0.36	0.90	0.34
9 repeats	0.02	0.02	0.88	0.15	0.36	0.55

CONCLUSIONS

Thus, the results have led to the following conclusions:

1. Analysis of acid-base balance of the oral cavity has revealed that children with Down syndrome, compared to the children of control age groups, have a decrease in oral liquid pH level ($F = 0.9$; $p>0.05$ for K1 and $F = 0.28$; $p>0.05$ to K2) and an increase in viscosity of non-stimulated saliva ($F = 6.05$; $p<0,05$ for K1 and $F = 4,58$; $p<0,05$ for K2).

2. The results of VNTR polymorphism in introne 36 of MUC5B gene have detected that in the children population of Kharkiv there are alleles with two (0.379), seven (0.190) and eight (0.155) repeats. 32.6% of the examined have 3-allelic combinations of MUC5B gene in genotype. The control and main groups differ in frequency of individuals with VNTR alleles – 22.2% and 37.5%, respectively.

3. Significant associations of VNTR polymorphism in introne 36 of MUC5B gene with acidity of the oral cavity and saliva viscosity indices of the examined children are not shown.

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