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INTERNATIONAL JOURNAL OF BIOMEDICINE

BRIEF REVIEW

Radiology

The Imaging of Legg-Calve-Perthes Disease

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Abstract

Diagnosis of hip joint pathology remains a serious problem in childhood and adolescence. A wide range of pathologies—including dysplastic, dystrophic, inflammatory, oncological and post-traumatic diseases of the musculoskeletal system—leads to the advancement of imaging methods and techniques. Legg-Calve-Perthes disease (LCPD) is a common cause of hip pain in children that may be initially clinically and radiographically difficult to diagnose Radiography of the pelvis in two views (anteroposterior and Lauenstein) is the main method of diagnosing aseptic necrosis. Destructive changes of the femoral head and neck of the femur are clearly determined. However, the presence of X-ray negative patterns at the first stage of the disease and the impossibility of visualizing all the anatomical structures induce us to improve the diagnostic algorithm of this difficult pathological process. (**International Journal of Biomedicine. 2019;9(1):9-12.**)

Key Words: Legg-Calve-Perthes disease • osteochondropathy • hip pain • radiography • MRI

Musculoskeletal diseases are a prevalent modern health problem that leads to social maladjustment. Among the widespread disabling orthopedic diseases, there is a group of nosologies accompanied by aseptic osteonecrosis. The imaging of the femoral head is the most important topic due to its significant influence on the quality of life. According to the severity of the impairment, one out of every 11 patients with a hip disorder eventually is disabled, whereas in cases of other diseases of the musculoskeletal system, only one out of every 100 patients becomes handicapped.[1]

LCPD is an osteochondropathy, with the lesions of the proximal femur and hip joint caused by disruption of the blood supply to the cartilage, resulting in necrosis of the femoral head.[2] According to Russian state statistics, the incidence of LCPD among the hip joint diseases within the pediatric population is 15%.[3] The disease has been known for over 100 years; however, studies concerning its causes, development, diagnosis and treatment are still in progress. In the early 20th century, this disease was mentioned in reviews as casuistic observations; in the 1990s, its frequency increased 10 times. The proportion of aseptic necrosis of the femoral head is about 15% among other orthopedic diseases.[4]

The etiology of the disease has not been finally established. Because neither of the theories could answer the questions about its origin, LCPD should be considered as a multifactorial disorder with congenital and acquired causes.[5,6]

The diagnosis of hip joint dysplasia and LCPD in children remains an urgent problem due to social consequences of unsatisfactory results of treatment. It should be mentioned that the patient's quality of life depends on when the disorder is detected.[3]

The data of imaging modalities for hip pathology have increased in recent years. The well-known methods have been essentially improved and become more informative; the minimally invasive techniques have also been upgraded. Despite all existing methods of diagnosis, the early detection of aseptic femoral head necrosis in the Russian Federation is estimated to be about 15%.[7] It is difficult to determine the risk groups for this disease; there are no pathognomonic

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clinical signs, so the patients often seek medical attention at the clinical stage with considerable bone destruction.[8]

The onset of the disease usually occurs at the age of 4 to 12, with the highest incidence in 4- to 8-year-old children, though it may happen in adolescence. Boys suffer 3–5 times more often than girls. The frequency of bilateral LCPD amounts 10%, the disease lasts from 2 to 6–8 years, and it is difficult to date the onset of the disease. The first clinical manifestations are pain in the hip joint radiating to the femur and the knee, limping, and early fatigue during physical exercises. The pain appears in the moment of movement and disappears at rest. LCPD is a common cause of hip pain in children that may be initially clinically and radiographically difficult to diagnose.[9]

The clinical examination demonstrates the painful restriction of hip joint movement in abduction and rotation. There is a hypotrophy of gluteus and femoral muscles, a positive Trendelenburg's symptom, and feet of different lengths although the general condition of children does not suffer. The results of general analyses are normal, but they should be done to exclude other pathology.[5,6]

In the International Statistical Classification of Diseases and Related Health Problems (ICD 10), LCPD is referred to the group of chondropathies and called «Juvenile osteochondrosis of head of femur».

There are several classifications of LCPD; the most popular are those of RP Ficat & J. Arlet (1980), Steinberg (1995), and DC Mitchell (1987). The classification by RP Ficat & J. Arlet [10] is more useful and widespread: it includes the clinical data, and radiographic and MRI findings, for the aseptic necrosis staging:

Stage 0

Radiography: no pathology

MRI: no pathology

Clinical picture: absent

Stage I

Radiography: no pathology or minimal osteopenia

MRI: edema

Clinical findings: pain irradiating to the inguinal region, knee

Stage II

Radiography: mixed osteopenia and/or subchondral sclerosis and/or subchondral cysts without subchondral lucencies (Figure 1).

MRI: geographic pattern

Clinical findings: painful restriction of hip joint movement

Stage III

Radiography: crescent (sickle) sign and possibly collapse of the subchondral bone (Figure.2)

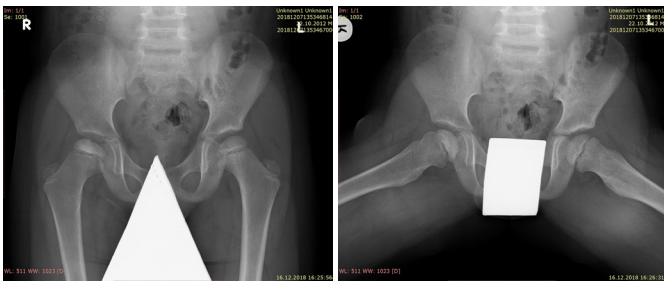
MRI: crescent (sickle) sign and possibly collapse of the subchondral bone

Clinical findings: painful restriction of hip joint movement and/or pain irradiating to the knee joint, limp

Stage IV

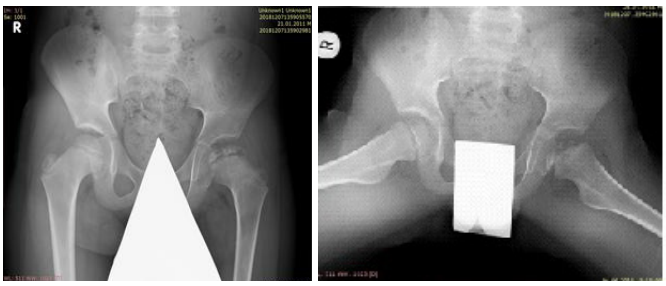
Radiography: final stage with significant secondary degenerative changes (Figure.3).

MRI: similar to the radiography



(a) (b)

Fig. 1. *Radiographs of the pelvis in the anteroposterior (a) and Lauenstein (b) views: LCPD, right side, stage II.*



(a) (b)

Fig. 2. *Radiographs of the pelvis in the anteroposterior (a) and Lauenstein (b) views: LCPD, right side, stage III.*

(a) (b)

Fig. 3. *Radiographs of the pelvis in the anteroposterior (a) and Lauenstein (b) views: LCPD, right side, stage IV.*

Radiography remains the most useful imaging technique for LCPD. However, this method is non-informative at an early stage; this fact often results in late diagnosis.[11]

Radiologically there are 4 stages of aseptic necrosis[12] (G. Axhausen, 1923):

1.The stage of primary subchondral necrosis of spongeous bone and marrow of the femoral head.

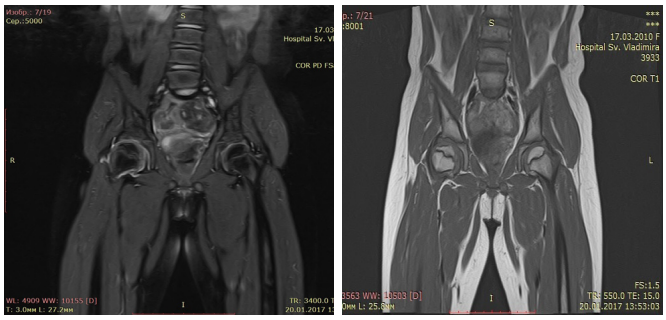
2.The stage of impression fracture. Necrotic trabeculae are compressed into bone powder. The strong elements of the connective tissue dissect the crushed head into sequesters; there are cysts with gigantic cells or adipose accumulations.

3. The stage of epiphysis fragmentation. This degenerative and dystrophic process can go on for years and transforms into the next stage of reparative osteochondrogenesis.

4. Stage of reparation

At the first stage, the reliability of diagnosis using minimal clinical findings and radiographic signs is only 8%–10%.[13] Radiography shows an insignificant widening of the articular space and a decrease of the epiphysis height compared to the healthy side. Ultrasound and MRI are recommended to

visualize the synovial membrane, the articular effusion, and cartilage lesions. MRI is a method of choice for diagnosing LCPD at its early stages. Exudative synovitis and soft tissue edema are the most frequent signs. The presence of reactive trabecular edema of the marrow of the femoral head and neck is a characteristic of the early stage of aseptic osteonecrosis [9,14] (Figure 4).



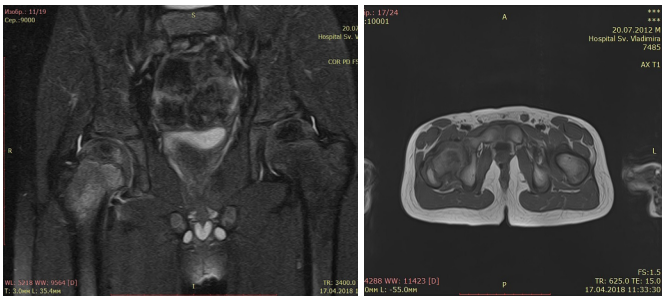
(a) (b)

Fig. 4. *MRI, PD Fat Sat (a), T1 (b), frontal view: LCPD, right side, stage I.*

The pain syndrome in the debut of LCPD is an indication for ultrasound (US) examination of the hip joints.[15] A longitudinal scanning is traditionally performed along the frontal surface of the joint. The neck-to-capsule distance (from 3 mm in newborns to 8 mm in adolescents), the capsule margin (normally, concave, more or less parallel to the neck), the width of the synovial membrane (about 2 mm), and the effusion should be assessed.[16] Evaluation of the bone margin fragments of the femoral head and metaepiphyseal area is obligatory (normally, margins are well defined but could be irregular due to metaepiphyseal ossification). An examination of the contralateral joint must also be performed. In the debut of LCPD, it is typical to find US signs of coxitis, such as synovial membrane thickening up to 2–4 mm, a trace or small volume of effusion, deformed capsule outlines, and an increase in the neck-to-capsule distance (reliably – 2mm or more compared to the unaltered contralateral side). The decrease in the head height with irregular epiphyseal margins cannot always be visualized with certainty with US, so it is an absolute indication for using other imaging modalities.[17]

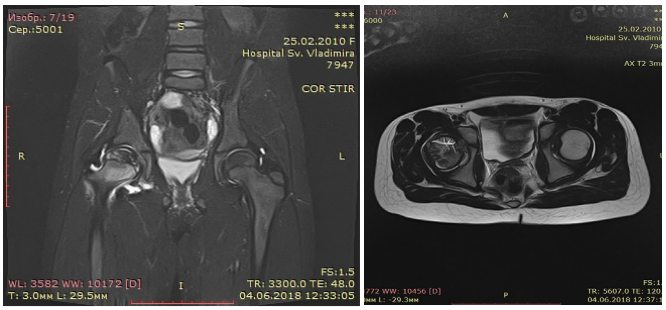
At the second stage, the head is devoid of a structural pattern; its density increases and it becomes homogeneous. A thin lucent line appears around the denser part of the epiphysis while the epiphyseal height continues to decrease. At this moment the structural changes in the head can be visualized using radiography. On MRI, there is a zone of necrosis in the epiphysis clearly limited by a double line along the peripheral area (the outer layer of ossification and the inner layer of hypervascularized tissue). The secondary changes are the exudative hypertrophic synovitis, thinning of the articular cartilage and ligament impairment (Figure 5).

At the third stage, radiological signs are best expressed. It becomes possible to visualize the resorption of the necrotized zone, articular space enlargement in all the segments, and a decrease in the epiphyseal height. The epiphysis is more flattened, displaced upwards and laterally from the tear and crescent figures; it loses its structural pattern, breaks into sequester-like structureless zones of various configuration, and the neck becomes shorter and thicker [3] (Figure 6) .



(a) (b)

Fig. 5. *MRI, PD Fat Sat, frontal view (a), T1, axial view (b): LCPD, right side, stage II.*



(a) (b)

Fig. 6. *MRI, STIR, frontal view (a), T2, axial view (b) – LCPD, right side, stage III.*

At the fourth stage, there is a distinct epiphyseal lamina, the trabecular structure is restored and the sequester-like bone fragments disappear. The structure in the zone of former necrosis and in adjacent parts becomes more homogeneous, the epiphyseal height increases, although the structural pattern of the head remains rough, and the trabecula are disorderly directed. The width of the articular space decreases due to normalization of endosteal and enchondral osteogenesis.

At the stage of the outcome of aseptic necrosis, MRI shows the depressed epiphyseal fracture and empty necrotic place without a free fragment. There are some secondary degenerative changes in the joint (i.e. focal cartilage thinning and ligament degeneration).[14]

Thus, the most frequent and constant radiological signs are enlargement of the articular space (epiphyseal displacement upwards and laterally), epiphysis flattening and sclerosis, soft tissue thickening, subepiphyseal osteoporosis, narrowing of the femoral neck and loosening of epiphyseal lamina.

Introduction of multislice computer tomography (MSCT) into clinical practice permits detection of the signs inaccessible to radiography because of polyprojectional visualization of the articular elements and absence of summation for planning surgical treatment. It is necessary to emphasize the role of MSCT at the final stages of aseptic necrosis: the transformation of the fragmentation stage into the stages of reparation and outcome. It is very important

due to the necessity to resolve on the weight bearing for the limb of interest. MSCT is a more informative method than radiography for necrosis staging at early times; it is possible to estimate a correlation in the joint and to exclude any pathological changes in the femoral neck and acetabular roof. However, the presence of an X-ray negative pattern in the first stage and in the beginning of the second one leads to use of the imaging modality that could show the femoral head at the stage of bone marrow edema—MRI. In addition, MRI can provide assessment of the chondral and soft elements, the synovium, and the volume of ischemic zone in the proximal part of the femur.

US of the hip joints is widely used, absolutely harmless unlike radiography and CT and can be repeatedly performed to assess these X-ray negative structures, such as muscles, tendons and capsules. US permits visualization of the transitory synovitis and detection of an irregularity of articular surfaces before any bone changes. It also reveals, or arouses suspicion of, LCPD at the early stage when radiologic signs are not to be seen. Doppler US successfully demonstrates the microcirculation in the hip joint area before and after surgery as a control for osteogeneous reparative processes as the presence and dynamics of interfragmental blood supply reflect the efficiency of treatment.[7]

In recent studies there are references to digital tomosynthesis of bones and joints for the assessment of structural changes. Additionally, the subchondral changes at the initial stages of the diseases can also be visualized.[18] The information value is higher than radiography and linear tomography but lower than MSCT.[19]

In conclusion, early diagnosis and treatment of LCPD are the key points of the prevention of childhood disability. The need to develop early diagnosis of hip joint diseases in children is well understood: the implementation of diagnosis and treatment standards, the precise examination algorithm, and the modern imaging modalities. Despite the great number of publications concerning early diagnosis of LCPD, the number of children with late stages of this disease is still high.

Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Cardiology

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Results of Off-Pump Coronary Artery Bypass Surgery in Patients with EuroSCORE≥5

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Abstract

Background: The lack of reliable data on the possibility, safety and results of OPCABG in patients with high surgical risk hinders the further wide introduction into clinical practice of such operations. In this regard, conducting a comparative evaluation of the results of OPCABG in patients with low and high surgical risk seems to us a worthwhile project.

Materials and Methods: During the period between 2015 and 2017, 310 OPCABG operations were performed. Patients were divided into 2 groups depending on the EuroSCORE risk calculator value. Group 1 consisted of 130 (41.9%) patients with a high surgical risk (EuroSCORE≥5), and Group 2 consisted of 180 (58.1%) patients with a low surgical risk (EuroSCORE<5).

Results: We could not find between the two groups significant differences in the number of mean grafts per patients (3.12 in Group 1 and 3.13 in Group 2), in operation times, or in the level of morbidity and mortality (1.5% in Group 1 and 1.2% in Group 2). All intraoperative conversions to on-pump CABG (5 cases or 3.8%) occurred in patients of Group 1 (P=0.008).

Conclusion: The OPCABG operation in patients of high-risk group is a safe method and can be performed without compromising the completeness of myocardial revascularization with the same low mortality as in low-risk patients. The most common type of complication in high-risk patients is on-pump conversion, which at earlier and planned implementation is not reflected significantly at the level of hospital mortality. (**International Journal of Biomedicine. 2019;9(1):13-18.**)

Key Words: off-pump CABG • high-risk patients • EuroSCORE • myocardial revascularization

Abbreviations

AF, atrial fibrillation; **CABG**, coronary artery bypass grafting; **LAD**, left anterior descending artery; **LIMA**, left internal mammary artery; **LVEF**, left ventricular ejection fraction; **LMS**, left main stem; **OPCABG**, off-pump CABG.

Introduction

The first two decades of the 21st century were characterized by the introduction into clinical practice, and distribution, of OPCABG surgery without artificial blood circulation. Most of the studies carried out to date have been based on the study of the results of OPCABG in patients with low surgical risk in comparison with similar patients who had a conventional on-pump CABG. The lack of reliable data on the possibility, safety and results of OPCABG in patients with high surgical risk hinders the further wide introduction into clinical practice of such operations. In this regard, conducting a comparative evaluation of the results of OPCABG in patients with low and high surgical risk seems to us a worthwhile project.

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Material and Methods

In the Department of Cardiac Surgery of the Republican Specialized Center of Cardiology, during the period between 2015 and 2017, 310 OPCABG operations were performed. Patients were divided into 2 groups depending on the EuroSCORE risk calculator value. Group 1 consisted of 130 (41.9%) patients with a high surgical risk (EuroSCORE≥5),

and Group 2 consisted of 180 (58.1%) patients with a low surgical risk (EuroSCORE<5).

Examination of clinical and demographic data in the groups showed (Table 1) that the mean age of patients in Group 1 was significantly higher than in Group 2 (63.9 against 57.8 years, respectively). In Group 1, the percentage of women was 2 times higher than in Group 2. About one-third of patients in each group had concomitant pathology, like obesity (31.5% and 38.8%) and diabetes (34.6% and 27.4%), and their distribution in both groups was relatively homogeneous. There was a statistically significant difference in the number of patients with chronic lung pathology (35.4% in Group 1 versus 22.8% in Group 2) and chronic renal pathology (16.9% in Group 1 versus 8.9% in Group 2). About two-thirds of patients in each group had a previous history of MI. Acute coronary syndrome (ACS) was found in 34.1% of cases in Group 1 vs. 5.6% of cases in Group 2 (P<0.001). The majority of patients in both groups (93% and 97.2%) had angina pectoris.

Table 1.

Preoperative demographic and clinical data

Variable	Group 1	Group 2	P-value
Women	30 (23.1%)	20 (11.1%)	0.005
Age, years	63.98±6.8	57.84±6.1	<0.001
Obesity	41 (31.5%)	68 (38.0%)	0.489
COPD	46 (35.4%)	41 (22.8%)	0.015
DM	45 (34.6%)	49 (27.4%)	0.245
Chronic renal disease	22 (16.9%)	16 (8.9%)	0.033
History of MI	101 (77.7%)	113 (62.8%)	0.015
ACS	44 (34.1%)	10 (5.6%)	<0.001
Symptoms: angina dyspnea	 120 (93.0%) 76 (59.4%)	 173 (97.2%) 67 (37.6%)	 0.084 <0.001
Single-vessel disease Two-vessel disease Three-vessel disease	3 (2,5%) 17 (14.2%) 100 (83.3%)	4 (2,4%) 20 (12.1%) 141 (85.5%)	 0.877
LMS disease (or its equivalent)	56 (47.1%)	74 (45.1%)	0.747
Carotid artery lesion >50%: One-sided Two-sided	 27 (22.9%) 11 (9.3%)	 24 (14.3%) 10 (6.0%)	 0.072
LVEDD, mm	57.7±10,9	55.1±6.3	0.009
LVEDV, ml	174.8±66.2	146.9±39.7	<0.001
LVEF, %	51.9±11.2	56.8±8.5	<0.001
LVEF <45%	40 (30.8%)	23 (12.8%)	<0.001
NYHA class III-IV	123 (95.3%)	154 (85.5%)	0.020
Angina class III CCS	27 (20.8%)	109 (60.6%)	<0.001
Angina class IV CCS	102 (78.5%)	69 (38.3%)	<0.001
EuroScore add, points	6.5±1.5	2.4±1,3	<0.001
EuroScore log, %	7.29±4.39	1.93±0.73	<0.001

COPD – chronic obstructive pulmonary disease; DM – diabetes mellitus; MI – myocardial infarction; ACS – acute coronary syndrome; CCS – Canadian Cardiovascular Society

On coronarography, the vast majority of patients had a three-vessel disease (83.3% and 85.5% respectively).

Left main stem (LMS) or equivalent coronary artery disease was found in 47.1% of cases in Group 1 and 45.1% of cases in Group 2 (P>0.05). According to the echocardiography findings, the changes in LV volumes and LVEF were more pronounced in Group 1: LVEF <45% was found in 30.8% of cases in Group I versus 12.8% of cases in Group 2 (P<0.001).

The majority of patients in both groups were in NYHA functional classes III and IV (95.3% in Group 1 and 85.5% in Group 2). Group 1 included more patients with angina class CCA IV (78.5% compared to 38.3% in Group 2, P<0.001). The risk of mortality, calculated with a EuroSCORE risk calculator, was greater in patients of Group 1 - 6.5 (7.29%), while in Group 2 it was 2.4 (1.93%), P<0.001.

Surgical Technique

We described the surgical technique in detail earlier.[1] All operations were performed through the median sternotomy. In all patients, the left internal thoracic (or mammary) artery (LIMA) was harvested. In parallel, the great saphenous vein (GSV) was harvested. In a part of patients (70 patients), GSV was harvested endoscopically (Carl Storz, Germany). The pericardium was opened in a standard inverted "T" fashion. Heparin was administered at a dose of 1 to 1.5 mg/kg of patient weight (until ACT value>300 sec).

The positioning of the heart was carried out with the help of deep pericardial stay suture technique, as well as the use of the vacuum heart positioner (Starfish, Medtronic, Minneapolis, MN). To stabilize the myocardium of the heart in the field of anastomosis, we used commercially available tissue stabilizers (Octopus 4 Tissue Stabilizer, Medtronic, Minneapolis, MN). Temporal occlusion of the coronary artery was performed by applying a silicone vessel loop to the proximal part of artery. After performing an arteriotomy, always, if possible, intra-coronary shunts were used (ClearView Intracoronary Shunt; Medtronic).

The sequence of revascularization of coronary arteries differed depending on the presence or absence of occluded arteries, the degree of stenosis, the need to use complex surgical techniques, preferences of the operating surgeon and reaction of hemodynamics to the positioning of the heart and other manipulations.

Revascularization was initiated from an occluded recipient artery. Usually it was either LAD or RCA (or PDA). In cases where it was LAD, first LIMA-LAD anastomosis was constructed and then continued in the usual manner. If it was an RCA (or PDA), then at first, a distal anastomosis with the vein was constructed. Then all proximal anastomoses were constructed on the ascending aorta using a side-bite clamp, and blood supply was restored to the RCA region. This was followed by revascularization of the LAD region. Arteries of the lateral side of the heart were revascularized only after the arteries of the anterior surface (LAD and RCA regions) had been revascularized and received a blood supply. If there were no occluded arteries, revascularization was always initiated with the LAD artery.

If the use of the "aorta-no-touch" technique was planned, first we constructed a T- or Y-graft by anastomosing of the radial artery (or venous conduite) to LIMA. With this technique, the

sequence of anastomoses looked as follows: diagonal branch (Dx) (LIMA side-to-side anastomosis)–LAD (LIMA end-to-side); followed by the circumflex artery and RCA branches (all side-to-side anastomosis and the last one end-to-side). With this technique, after each completed distal anastomosis, the blood flow to the recipient artery was launched.

After the completion of the construction of all grafts, flowmetry (Transit-time flow measurement) was performed to assess the quality of anastomoses (MiraQ, Medistim, Norway). If there was no excess bleeding we neutralized only half the dose of heparin.

Statistical analysis was performed using the statistical software «Statistica». (v6.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of $P<0.05$ was considered statistically significant.

Results

Between the two groups, the mean duration of the operation did not differ significantly (232.2 minutes in Group I and 228.0 minutes in Group 2) (Table 2).

Table 2.

Intraoperative data

Variable	Group 1	Group 2	*P*-value
Duration of operation, min	232.2±61,1	228.0±50.3	0.505
Internal thoracic artery usage (left or right)	130 (100%)	180 (100%)	0.977
Mean number of grafts per patient	3.12±0.7	3.13±0.8	0.956
≥4 grafts	30 (23.0%)	53 (29.5%)	0.346
Sequential technique	15 (11.5%)	29 (16.2%)	0.259
Composite grafts	11 (8.5%)	11 (6.1%)	0.389
«LAD first» technique	98 (76.6%)	129 (72.1%)	0.376
Combined grafting technique	39 (30.0%)	67 (37.2%)	0.420
Mean blood loss, ml	527.6±261.2 (200-1500)	483.6±185.7 (100-1200)	0.087
Intraoperative complications: No complications PVC Group PVC AF, VT, VF, AV-block Need for inotropic support Bleeding Technical difficulties, intramyocardial coronary arteries, calcified vessels (or aorta), etc	 107 (82,3%) 1 (0.8%) 1 (0.8%) 5 (3.8%) 7 (5.4%) 2 (1.5%) 7 (5.4%)	 156 (86.7%) 0 (0.0%) 0 (0.0%) 6 (3.3%) 3 (1.7%) 4 (2.2%) 11 (6.1%)	0.372
Intraoperative defibrillations	4 (3.1%)	4 (2.2%)	0.348
On-pump conversion	5 (3.8%)	0 (0.0%)	0.008

PVC – premature ventricular contraction; VT – ventricular tachycardia; VF – ventricular fibrillation; AV- atrioventricular

In both groups, LIMA (or RIMA) was used in all patients.The average number of grafts in both groups was more than 3 per patient (3.12 and 3.13 grafts per patient in Group 1 and Group 2, respectively). In 23% of patients of Group 1 and 29.5% of patients of Group 2, 4 and more grafts were constructed.

In about 20-23% of patients in both groups, we used a complex surgical technique in the form of sequential and composite grafts. In Group 2, the sequential grafting technique was used more frequently (16.2% versus 11.5% in Group 1) (P=0.259), while in Group 1 we constructed more composite grafts (8.5% versus 6.1% in Group 1) (P=0.389).

As we indicated earlier, the sequence of grafting varied depending on the presence or absence of occluded arteries. In accordance with this, initiation of revascularization with the LAD artery accounted for 76.6% in Group 1 and 72.1% in Group 2 (P=0.376). In the remaining cases, grafting began from other territories (as a rule, it was the RCA region). Mean blood loss in both groups did not differ and amounted to 527.6 ml and 483.6 ml, respectively.

The frequency of intraoperative complications was 17.7% in Group I and 13.3% in Group 2 (P=0.372). As a rule, life-threatening arrhythmias were the prevailing complications (3.8% in Group 1 and 3.3% Group 2). About 5.4% of patients in Group 1 and 1.7% of patients in Group 2 needed inotropic support until full restoration of normal hemodynamics.

In 5.4% of cases in Group I and 6.1% of cases in Group 2, the surgical team had technical difficulties in conducting the procedure (such as calcified target coronary vessels, intramyocardial coronary arteries, etc.). In addition, there were cases with very calcified aorta (porcelain aorta), which required finding other places to place proximal anastomoses or changing the technique of operation in favor of the "aorta-no-touch" technique; in two cases adhesive pericarditis was found, which required the conduction of cardiolysis.

We found that all intraoperative conversions to on-pump CABG (5 cases or 3.8%) occurred in patients of Group 1 (P=0.008). The causes of the on-pump conversion were life-threatening uncontrolled arrhythmia, critical hemodynamic disturbances, and severe adhesive pericarditis (in one case), which required on-pump support to perform complete cardiolysis. In the immediate postoperative period (Table 3), 51.5% of patients in Group 1 and 26.8% of patients in Group 2 needed inotropic support until full restoration of normal hemodynamics (P<0.001) with duration between 6.6 hours in Group 1 and 2.6 hours in Group 2 (P<0.001). The average duration of ventilation support was 6.8 hours in Group 1 and 5.0 hours in Group 2.

ICU drainage blood losses were 352.2 ml in Group 1 and 371.8 ml in Group 2. Patients of Groups 1 and 2 received on average 329 ml and 376 ml of erythrocyte mass, respectively (P=0.758), as well as on average 308ml and 280.5 ml per patient of fresh frozen plasma, respectively (P=0.432).

The incidence of nonfatal complications in postoperative period was 18.5% in Group I and 7.2% in Group 2 (P=0,074). Perioperative MI was noted in two cases (1.2%) in Group 2. Paroxysms of AF were noted in 3.1% of patients in Group 1 and in 2.8%of cases in Group 2. Neurologic complications

(TIA) were noted in one case (0.8%) in Group 1. Acute graft thrombosis was noted in one case (0.8%) in Group 1. Respiratory complications were observed in two patients (2.3%) in Group 1 and in one patient (0.6%) in Group 2.

Table 3.

Postoperative data

Variable	Group 1	Group 2	*P*-value
Need for inotropic support	67 (51.5%)	49 (26.8%)	<0.001
Duration of inotropic support, hours	6.6 ± 10.9	2.6±6.4	<0.001
Ventilation time, hours	6.8 ± 4.1	5.0 ± 2,2	<0.001
Drainage blood loss, ml	352.2±179.0	371.8±177.0	0.343
RBC transfusion, ml	329±118.9	376±136.4	0.758
FFP transfusion, ml	300.8±244.3	280.5±205.7	0.432
Complications:			
No complication	106 (81.5%)	167 (92.8%)	0.074
Bleeding, incl. surgical hemostasis	3 (2.3%)	2 (1.2%)	0.074
Perioperative MI	0 (0%)	2 (1.2%)	0.074
Atrial fibrillation	4 (3.1%)	5 (2.8%)	0.074
Neurologic complications	1 (0.8%)	0 (0%)	0.074
Acute renal failure	1 (0.8%)	1 (0.6%)	0.074
Acute graft failure (thrombosis)	1 (0.8%)	0 (0%)	0.074
Respiratory complications	3 (2.3%)	1 (0.6%)	0.074
Superficial wound infection	5 (3.8%)	1 (0.6%)	0.074
Mediastinitis	0 (0.0%)	1 (0.6%)	0.074
Mortality	2 (1.5%)	2 (1.2%)	0.763
ICU stay, hours	49.2±23.4	46.6±18.8	0.287
Length of stay (after surgery)	8.2±2.9	6.8±1.9	<0.001

RBC – red blood cells; FFP – fresh frozen plasma; MI – myocardial infarction; TIA – transitory ischemic attack; ICU – intensive care unit

In Groups 1 and 2, superficial wound infection was noted in 3.8% and 0.6% of cases, respectively *(P*=0.074). In Group 2, one patient (0.6%) underwent re-exploration of the mediastinum for hemostasis.

Hospital mortality amounted to 1.5% in Group 1 and 1.2% in Group 2 (*P*=0.763). The average ICU stay was 49.2 hours in Group 1 and 46.6 hours in Group 2. Patients of Group 1 were discharged home, on average, in 8.2 days, and patients of Group 2 - in 6.8 days (*P*<0.001).

Discussion

Literature data present various criteria of high surgical risk in patients undergoing CABG surgery. Among them are age >70 years, low LVEF, the presence of left main stem disease, EuroSCORE ≥5 points, anemia, etc. However, the most common criterion of high surgical risk is EuroSCORE ≥5 points.[2-7] It is known that in general,[8-11] in the initial period of popularization of OPCABG techniques, patients of low surgical risk comprised "typical patient profile."

The expanding of indications on patients of high surgical risk was slowed down by a number of factors, among which, in our opinion, was the lack of information on the results of operations in this group of patients. Numerous studies have compared the results of OPCABG and on-pump CABG, mainly in patients with low surgical risk. In general, these studies could not convincingly demonstrate the advantages of OPCABG in comparison with the conventional on-pump CABG.[2,3,12,13] At the same time, first reports on results of OPCABG surgery in patients of high risk started appearing in the literature.[10] Puskas et al.[5] convincingly demonstrated the advantages of OPCABG over on-pump CABG surgery in a high-risk group of patients. For the first time the advantages of off-pump techniques could be demonstrated in patients grouped by a preoperative risk-stratification index.

In this paper, we present a consecutive series of patients subjected to an isolated OPCABG procedure, which reflects the daily surgical practice of our group. The comparison of the initial preoperative data shows that patients of the high surgical risk group are much older, with a more widespread atherosclerotic process, a higher class of angina and chronic heart failure, among them more persons in the acute phase of MI. In addition, according to ECHO findings, the LV sizes and volumes of Group 1 patients were larger and LVEF was lower. In Group 1, the risk of surgery, calculated with the EuroSCORE calculator, was much higher than in patients of Group 2. Thus, the risk of mortality by EuroSCORE was nearly 4 times higher in Group 1 compared to Group 2 (7.29% versus 1.93%). The difference by all these indicators was statistically significant.

In our series of 310 operations with isolated CABG, we managed to perform them off-pump in 305(98.4%) cases. This rate is one of the criteria of the effectiveness of OPCABG operations. According to different reports, this rate varies between 49% and 99%.[2,5,8,11-15] Leading groups in this field, such as J.Puskas, P.Sergeant and others, perform isolated OPCABG in more than 99% of cases. In addition, one of the indicators is the level of intraoperative conversions to on-pump. This rate varies by different authors from 1% to 15%.[10,15-17] In our series of operations, this rate amounted to 1.6%, and it is also within the values reached by other leading groups.

In some studies, it was demonstrated that the mean number of grafts per patient was less in OPCABG groups than in on-pump CABG groups. Such data may indicate selection bias or highly selected cases (when OPCABG was performed in patients with one-and two-vessel disease), or incomplete revascularization. Thus, according to some authors, this figure varies from 1.9 to 3.7 grafts per patient.[8,14,18] The mean number of grafts in our series amounted to 3.12 and 3.13 in both groups, respectively, which may indicate an absence of selection and a high level of completeness of revascularization (patients with two- and three-vessel disease in our series). All patients received at least one arterial conduit. In more than 20% of the cases in both groups, we used complex surgical techniques in the form of constructing sequential and composite grafts.

The level of intraoperative complications was roughly the same in both groups despite the heavier and unstable contingent of patients in Group I. However, we found that all conversions (5 for the whole series) occurred only in patients of Group 1. In the literature available to us, we only managed to find several reports devoted to the analysis of conversion causes.[16,17,19] However, a high surgical risk is not highlighted as a conversion risk factor. In the above-mentioned papers, the presence of AF prior to the surgery and history of coronary artery stenting are

identified as risk factors of conversion.[16] Other risk factors are the presence of left main stem disease, as well as low LVEF.[19] Pullan and co-authors[17] have shown that the planned transition to on-pump operation (conversion) before the occurrence of emergency situations does not increase significantly the rate of complications and mortality in these patients in comparison with patients without conversion. It seems to us that the improvement of results and reduction of the level of complications after on-pump conversions lies in the plane of lowering the threshold to the on-pump transition in patients with high surgical risk (i.e. earlier, planned transition with still stable hemodynamics), and the use of heart positioners, intracoronary shunts, and other surgical and anesthetic maneuvers.

As we mentioned above, the peculiarity of the immediate postoperative period in patients of Group 1 was a bigger need for inotropic support, a longer duration time of it, as well as a 1-hour longer duration of respiratory support. For all other basic parameters, we have not identified statistically significant differences between the two groups. The frequency of rhythm disturbances in the groups amounted to 3.9% and 1.5%, respectively, and did not differ significantly. As is well known, the frequency of atrial fibrillation after conventional on-pump CABG varies between 30% and 45%. According to different authors, after OPCABG operations, atrial fibrillation happens in 4% to 26% of cases.[20] Thus, our results are on the lower edge of the given spectrum. In addition, we did not identify any significant difference in the frequency of nonfatal complications (perioperative MI, neurological disorders, bleeding, etc.) between the groups.

Hospital mortality constituted 1.5% and 1.2% respectively. At the same time, the risk of surgical intervention calculated by the EuroSCORE calculator was 7.29% for patients of Group 1 and 1.93% for patients of Group 2. Thus, this result demonstrates a peculiarity of OPCABG surgery: actual mortality does not increase with the increase in surgical risk by EuroSCORE. This is confirmed by other research that studied the performance of EuroSCORE risk calculator on patients undergoing OPCABG surgery.[4,5,7]

Conclusion

The OPCABG operation in patients of high-risk group is a safe method and can be performed without compromising the completeness of myocardial revascularization with the same low mortality as in low-risk patients. The most common type of complication in high-risk patients is on-pump conversion, which at earlier and planned implementation is not reflected significantly at the level of hospital mortality.

Study limitations

The retrospective nature of this study makes it difficult for the authors to draw any definite conclusions. In addition, the number of patients in both groups is relatively small, which may reduce the statistical reliability of the results obtained, or a possible difference is not apparent due to the small number of cases. Nevertheless, the results we obtained clearly demonstrate the safety of performing OPCABG surgery in patients with high surgical risk.

Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Psychophysiological Characteristics of Male Survivors of Myocardial Infarction

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Abstract

The objective of this study was to analyze the psychophysiological personality characteristics of male survivors of myocardial infarction (MI) in order to identify the risks of recurrent cardiovascular events.

***Material and Methods*:** We observed 60 male patients (mean age of 46.5 [38.7; 54.3]) diagnosed with ischemic heart disease (IHD) during outpatient and inpatient treatment. In the past (from one to three years ago), all of them suffered Q-wave MI (75% - with localization in the posterior wall of the left ventricle, 25% - in the anteroseptal area). The psychological study included the following techniques and methods: the abridged MMMPI, the Spielberger-Khanin test, the TAS in the variant modified at V.M. Bekhterev Research Institute, S. Subbotin's STQ, E. Heim's technique for revealing individual coping strategies, and the projective psycho-geometric test.

Results: The discovered characteristics applicable to the psychological portrait of males with a history of MI (elevated levels of neuroticism and anxiety with elements of demonstrative behavior, complications in the expression of feelings and emotions) are a reflection of the psychosomatic radical component of IHD. This finding enables us to recommend including methods of psychological correction of discovered intrapersonal changes in the measures for preventing recurrent cardiovascular events in the studied cohort of patients.(**International Journal of Biomedicine. 2019;9(1):19-22.**)

Key Words: male patients • myocardial infarction • cardiovascular events • stress tolerance • coping behavior

Abbreviations

IHD, ischemic heart disease; **MI**, myocardial infarction; **MMPI**, Minnesota Multiphasic Personality Inventory; **STAI**, State-Trait Anxiety Inventory; **STQ**, Stress Tolerance Questionnaire; **TAS**, the Toronto Alexithymia Scale.

Introduction

According to the 2014 survey data, half of all fatal cases in the Russian Federation occurred due to cardiovascular diseases, while more than 80% of such cases are associated with IHD episodes, primarily MI.[(1)] Among the numerous markers of increased risk of occurrence and complicated course of coronary pathology, the male gender is recognized as an independent factor.[(2)] According to the Russian Federal Statistics Service, in 2016, the number of deaths caused by IHD per 100,000 of the working age population amounted to 123.6 men and 15.3 women.[(3)]

Currently, multicentric prospective studies have established the following independent risk factors for IHD and its complications: acute and chronic stress, hostility, heightened anxiety, depression, and alexithymia.[(4-6)]

Despite the significant number of works devoted to the study of the psychological portrait of MI survivors, research has

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not been conducted into their personal response characteristics in terms of protective and compensatory behavior with differentiation by spheres in combination with factors of stress tolerance, anxiety, alexithymia and classification of patient personalities.

In this regard, the objective of this study was to analyze the psychophysiological personality characteristics of male survivors of MI in order to identify the risks of recurrent cardiovascular events.

Material and Methods

We observed 60 male patients (mean age of 46.5 [38.7; 54.3]) diagnosed with IHD during outpatient and inpatient treatment. In the past (from one to three years ago), all of them suffered Q-wave MI (75% - with localization in the posterior wall of the left ventricle, 25% - in the anteroseptal area), they received a full course of inpatient treatment and passed all stages of rehabilitation.

Inclusion criteria:

- •Male gender
- •Age from 30 to 55
- •Confirmed IHD (stable angina CCS class I-III) in patients with uncomplicated Q-MI

Exclusion criteria:

- •Female gender
- •Age over 55
- •Cognitive impairments of various origins
- •History of psychiatric treatment and observation
- •History of psychoactive substances consumption
- •Psychosomatic diseases as comorbid or competing pathologies
- •Acute IHD forms registered in the last 90 days
- •Untreated or uncontrolled arterial hypertension
- •Chronic cardiac insufficiency stages II-III
- •Acute forms of diseases
- •Surgery in the last 90 days
- •Oncological pathology
- •Chronic diseases in the acute stage

The observed participants were in scheduled inpatient treatment in the cardiology department of the V.I. Razumovsky Saratov City Clinical Hospital №2, Saratov, Russia. The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013). Written informed consent was obtained from all participants.

Along with the clinical and physiological examinations, which included a list of mandatory methods of research (in accordance with the standards), the patients participated in a psychological study, which employed the following techniques and methods:

- •The abridged MMMPI[7,8] for determining basic personality characteristics
- •The Spielberger-Khanin test [9,10]
- •The TAS in the variant modified at V.M. Bekhterev Research Institute [11]
- •S. Subbotin's STQ
- •E. Heim's technique for revealing individual coping strategies with differentiation by cognitive, emotional, and behavioral spheres [12,13]
- •The projective psycho-geometric test (S. Dellinger's test) modified by A.A Alekseyev and L.A. Gromova [14] for express personality type evaluation.

Statistical analysis was performed using StatGraph Plus for Windows 6.0 software package. The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. For descriptive analysis, results are presented as mean ± standard deviation (SD), median, interquartile range (IQR; 25th to 75th percentiles). Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney *U*-test. The Wilcoxon criterion was used to compare the differences between the paired samples. Categorical variables were analyzed using the Chi-square test. A probability value of $P \leq 0.05$ was considered statistically significant.

Results

Results of the abridged MMPI are shown in Figure 1. The averaged MMPI scales profile shows a relative and peaked increase in T-cores on Hypochondriasis (1), Hysteria (3) and a peak tendency on Psychastenia (7) scales. Wherein, the lowest points of the profile are on the Depression (2), Psychopatic Deviate (4), and Hypomania scales (9). The "neuroticism triad" scale set transitions into the "conversion pentad."

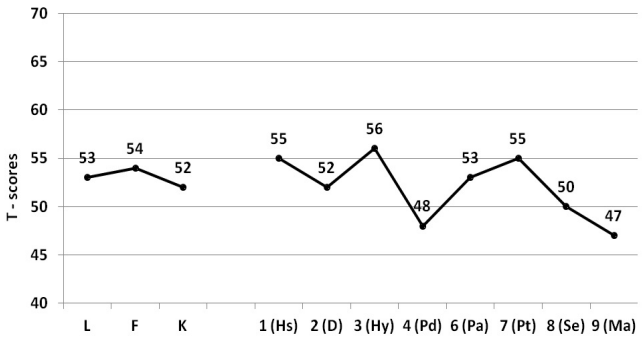

Fig. 1. Averaged profile of MMPI scales (T-Scores)

L – Lie Scale, F – Infrequency or Feeling Bad Scale, K – Correction Scale, 1 - Hypochondriasis, 2 - Depression, 3 - Hysteria, 4 - Psychopatic Deviate, 6 - Paranoia, 7 - Psychastenia, 8 - Schizophrenia, 9 – Hypomania

Results of the Spilberger-Khanin test are shown in Figure 2. The state anxiety level in patients was at a low value (22.0 points), while trait anxiety, although average as judged by absolute values, still approached high levels (45.1 points).

The TAS results showed that the average level of alexithymia amounted to the score of 64.2 points, which belongs to the risk-band evaluation.

When determining the stress tolerance levels according to STQ (Fig.3), the majority of participants (61%) showed average levels, while only 3% of examined individuals had low stress tolerance values (P=0.04).

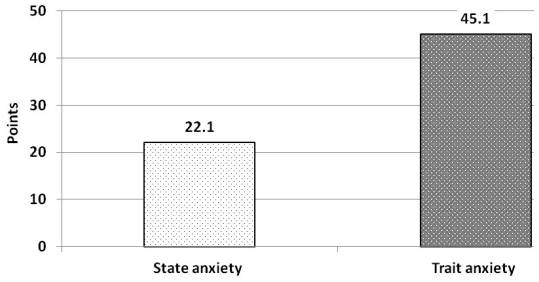

Fig. 2. *Levels of state and trait anxiety according to the Spielberger-Khanin test (in point scores).*

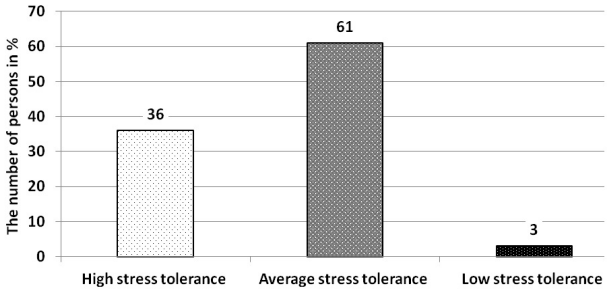

Fig. 3. *Distribution of stress tolerance levels (%).*

Results of the Heim test are shown in Fig. 4. Thus, the adaptive reactions were dominant in all spheres of coping behavior ($P \leq 0.05$). When further detailing the data of strategies for protective and compensatory behavior, it was found that problem analysis (50%) was the dominant coping style in the cognitive sphere, while submission was the prevalent non-adaptive style. Optimism (82%), among the adaptive coping reactions, dominated in the emotional sphere, while emotional suppression and self-incrimination were equally (40%) prevalent among the non-adaptive approaches. When building the actual coping behavior patterns in participants, our attention was drawn to the prevalence of cooperation (56%) as the adaptive coping form, while active avoidance (86%) ($P \leq 0.05$) was dominant among the non-adaptive reactions.

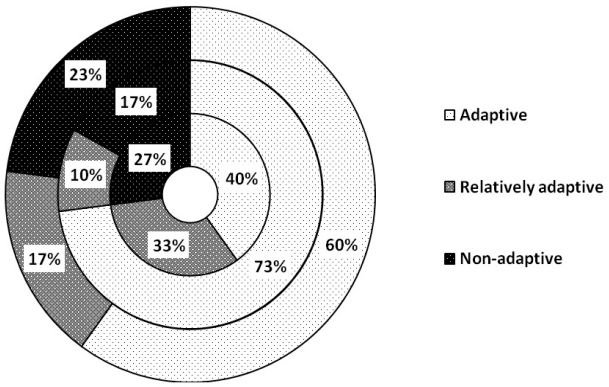

Fig. 4. *Coping behavior strategies with differentiation by spheres. Inner ring - cognitive sphere, medium ring - emotional sphere, and outer ring - behavioral sphere.*

According to the psycho-geometric test results with the stimulus material (Fig.5), the participants preferred the circle to other geometric shapes and rejected the zigzag figure with the same frequency (47%) ($P \leq 0.05$).

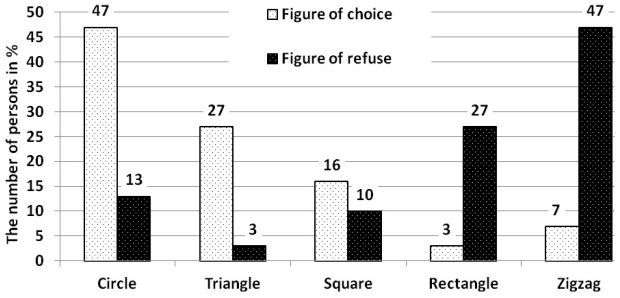

Fig. 5. *Results of the psycho-geometric test.*

Discussion

Based on the results of psychophysiological examination of male patients with a history of IM, they can be characterized as individuals with a tendency for increased levels of neuroticism and anxiety. In addition, the participants were characterized by conformity, strict adherence to social norms of behavior, a tendency to impose high standards on self and others combined with pessimistic assessment of their own capabilities and outcome of current events.

As a method of psychological protection, IHD patients with IM history tend to employ rationalization and intellectualization with elements of demonstrative behavior showing desire to acquaint others with their invented logical structures, which provides a possibility to force out, although not entirely, the oppressing anxiety and fear of recurrent acute cardiovascular events into the subconscious (relative increase on the MMPI Hypochondriasis and Hysteria scales with the transition of the "neuroticism triad" set of scales into the "conversion pentad" while retaining the tendency for peaked increase on the Psychastenia scale and having the lowest profile point on the Hypomania scale.

A combination of high levels of trait and low levels of state anxiety by the Spielberger-Khanin test may indicate active repression of high anxiety in order to "appear in positive light." This is supported by not only the characteristic tendency for demonstrative behavior (relative increase in MMPI T-scores on the Hysteria scale), but also by difficulties in understanding and expressing emotions and feelings (which is typical for alexithymia, by the observed levels of which the surveyed patients belong to the risk band).

The above is confirmed by the fact that, despite the preferential choice of adaptive forms of coping strategies in all spheres of stress-overcoming behavior (cognitive, emotional, and behavioral), almost two-thirds of the participants showed some decrease in stress tolerance (against the background of prevalence of adaptive coping styles in all three spheres by *E. Heim's* technique and the corresponding distribution

of stress tolerance levels by the Subbotin Questionnaire). We would like to note that the obtained results on elevated neuroticism and anxiety levels in IHD patients are in line with the results of earlier studies.[15] However, the available literature does not provide a detailed analysis of protective behavior in male survivors of MI (outlining the positive and negative sides of subsequent psychophysiological coping behavior patterns).

Conclusion

The discovered characteristics applicable to the psychological portrait of males with a history of MI (elevated levels of neuroticism and anxiety with elements of demonstrative behavior, complications in the expression of feelings and emotions) are a reflection of the psychosomatic radical component of IHD.

This finding enables us to recommend including methods of psychological correction of discovered intrapersonal changes in the measures for preventing recurrent cardiovascular events in the studied cohort of patients.

Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Ophthalmology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Analyzing Trace Elements in the Structures of Glaucomatous Eyes

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Abstract

Trace elements (TE) were analyzed in sclera biopsy specimens, samples of anterior chamber aqueous humor, and tear fluid of 12 patients aged between 53 and 79 years with various stages of primary open-angle glaucoma (POAG), using an atomic absorption spectrometer AS-3, inductively coupled plasma-mass spectrometer ELAN 6100-ICP-MS (Perkin-Elmer), and a Thermo Scientific plasma spectrometer iCAP-7000 (USA). Detected irregularities in the content of some TE (magnesium, iron, zinc, etc.) in anterior chamber aqueous humor of POAG patients may be a serious pathogenetic factor that violates the viscosity, and hence, the hydrodynamics, of intraocular fluid. An imbalance of TE in the tissues and media of glaucomatous eyes can contribute to the development of glaucomatous damage. (**International Journal of Biomedicine. 2019;9(1):23-25.**)

Key Words: sclera • anterior chamber • aqueous humor • trace elements • glaucoma

Abbreviations

ACAH, anterior chamber aqueous humor; **POAG**, primary open-angle glaucoma; **TE**, trace elements.

Introduction

Recent studies have shown that biomechanical disorders of the corneoscleral shell are an important factor in the pathogenesis of POAG.[1-6] It has been found that with POAG, excessive intramolecular and intermolecular cross-links are formed in the collagen structures of the sclera, contributing to an increase in its rigidity.[4,7] At the same time, it is known that some TE (zinc, copper, aluminum, silicon, etc.) involved in collagen biosynthesis and fibril formation, as well as in the antioxidant protection mechanism, play a key role in the post-translational modifications of collagen and in the formation of cross-links.[8-11] Changes in the content of these TE may contribute to the development of ophthalmic pathology. In particular, it was shown that weakened support function of the sclera, a key factor in the progression of myopia, is largely associated with a certain imbalance of TE, leading to a decrease in the number of cross-links stabilizing the sclera.[3]

The significance of TE, a large group of chemicals whose content in the body does not exceed 10^{-9}-10^{-12} mass percent, is extremely high and diverse in the life of cells and in maintaining the internal environment of the body, and is not inferior to the role of vitamins.[8,9,12] An essential feature of essential TE in their optimal doses is that each of them activates a more or less extensive group of enzymes (although the activation mechanisms may be different). In this regard, a considerable amount of attention has been paid to the study of the TE participation in the physiological and pathological processes in the eye.[10,12,13] However, studies aimed at the role of TE in the pathogenesis of glaucoma are practically absent. Individual studies focusing on trace element analysis of ACAH in patients with glaucoma indicate the promise of further development in this scientific direction.[13-15]

The aim of our work was to study the content of TE in the tissues and media of the glaucomatous eye involved in the metabolism of connective tissue and in the functioning of the antioxidant defense system.

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Materials and Methods

Scleral biopsy samples, ACAH samples (obtained during antiglaucomatous interventions) and tear fluid samples were collected from 12 patients (8 women and 4 men) aged between 53 and 79 years with different stages of POAG (the initial stage [I-II] in 6 patients and the advanced stage [III] in 6 patients). All samples were subjected to trace element analysis. Samples of ACAH, obtained during the extraction of immature senile cataract, and tear fluid samples, obtained from 18 patients (10 women and 8 men) aged between 54 and 70 years without ophthalmic pathology other than immature cataracts served as the control. Eight sclera samples were taken from the eyes of patients without glaucoma, which had to be removed due to severe eye injury.

For analysis, 100 µl of ACAH sample were taken with a microdispenser and placed in a Teflon autoclave, to which 0.5ml of 70% HNO_3 and 0.1ml of 30% H_2O_2 were added. Then, the autoclave was placed in a microwave sample preparation system similar to MD-2000 (CEM, USA) to attain a higher pressure and the boiling point temperature of HNO_3. The samples were decomposed within 15 minutes in three heating-cooling cycles. After cooling for 10–15 min, the resulting solutions were diluted 5 times with double-distilled and deionized water. Separately, a control solution was prepared with the content of HNO_3, H_2O_2, and H_2O (double-distilled and deionized) in proportions identical to the ones used in the studied samples. As an internal standard, indium was introduced into the solutions at a concentration of 25 µg/L. Calibration solutions were prepared from standard VTRC solutions with the known content of TE in the range of 5–1000 µg/L. Analysis of TE content (Zn, Cu, Fe, Al, Ba Cr, Si, Mg, Mn, Ti) was carried out using an AS-3 atomic absorption spectrometer and a mass spectrometer with an ion source in the form of inductively coupled plasma ELAN 6100-ICP-MS (Perkin-Elmer). The working power of the microwave generator was set at 1.3 kW, the flow rate of the plasma gas (argon) at 14 L/min, and the flow rate of the carrier gas at 0.89 ml/min.

Three exposures of each sample were conducted with the integration time of the signal set at 60 seconds. The results of the control analysis were automatically subtracted in the analysis. Samples of the sclera were degreased with ether, dried, weighed on an analytical balance and then placed in centrifuge tubes; 2 ml of nitric acid were added to the tubes and heated in a sand bath for 20–25 minutes until a clear solution was obtained. The resulting solutions were brought to a volume of 10 ml with double-distilled water. The analysis was performed by atomic emission spectral analysis using an iCAP-7000 plasma spectrometer (Thermo Science H, USA). As the excitation source,[16] the inductively coupled plasma was used with a generator power of 1.5 kW, a temperature of 8000 °K, and a working flame height of 15 mm. The units of the studied elements were µg/L (ppb) and mg/kg.

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). Values are presented as mean ± standard deviation (SD). The Mann-Whitney U Test was used to compare the differences between the two independent groups. A probability value of $P<0.05$ was considered statistically significant.

This study was performed in accordance with the Declaration of Helsinki and was approved by Local Committee of Biomedical Ethics of the Moscow Helmholtz Research Institute of Eye Diseases. Written informed consent was obtained from all participants.

Results

The following results indicate significant changes in the level of TE in ACAH during the development of the glaucomatous process (Table 1).

Table 1.

The content of TE (mg/L) in ACAH of patients with different stages of POAG

Element	Glaucoma stage		Control group
	I-II	III	
Al	0.035±0.017	0.012±0.006*	0.0311±0.007
Ba	0.0067±0.0023	0.0057±0.003*	0.0079±0.0028
Cu	0.0150±0.0011*	0.0090±0.006*	0.0210±0.009
Fe	0.146±0.051*	0.220±0.058*	0.053±0.014
Mg	5.9±0.51	3.0±0.43*	6.70±0.94
Mn	0.0044±0.0006*	0.0009±0.00008*	0.0120±0.0035
Si	1.8±1.2	0.34±0.21*	1.9±1.3
Ti	0.005±0.007	0.006±0.009	0.0058±0.0017
Zn	0.124±0.06	0.090±0.04*	0.139±0.03
Cr	0.0045±0.001*	0.0073±0.002*	0.0284±0.006

** $P<0.05$ - differences between Control group and Glaucoma stage*

As can be seen from the data presented in Table 1, as the glaucomatous process progressed in the anterior chamber, the level of Fe in ACAH increased and the content of Zn, Cu, Mg, and Cr decreased. Primarily in the level of Zn, Mg, and Fe, these differences indicated an increased viscosity of the intraocular fluid in glaucoma, since the nature of the imbalance was very similar to the deviations found in the trace element content of POAG patients' blood.[15,18] According to J. Ge,[18] with POAG there is a disturbance in the rheological properties of blood (hyperviscosity syndrome) associated with an abnormal level of the listed TE, which contributes to the deterioration of the blood supply to the glaucomatous eye and a decrease in visual function. It should be noted that the decreased Zn level in ACAH in patients with glaucoma has also been detected by N. Akyol et al.[14]

The detected elevated content of Fe, also known to have a prooxidant effect, in ACAH of POAG patients correlates with a high level of iron-containing transferrin protein in the intraocular fluid of an exfoliative glaucomatous eye.[17] This elevated content of Fe undoubtedly indicates the increase of peroxidation processes amongst weakening antioxidant protection in the media of glaucomatous eyes. In eyes with

POAG, we also detected a decrease in intraocular fluid resistance to oxidative stress correlated to a decrease in the Mg level (Table 1), which is involved in suppressing peroxidation processes.

With POAG, the sclera, as well as the intraocular fluid, was characterized by an abnormal balance of the studied microelements (Table 2). First, there was a decrease in the copper content (from 1.2 mg/kg in the initial stage to 0.77 mg/kg in the advanced stage of POAG). A decrease in zinc level and an increase in Fe level were also detected. This observation was consistent with the dynamics of the microelement levels in ACAH. Notably, our study of the Mg level showed a significant decrease with the progression of glaucomatous lesions. The Mg concentration in eye sclera samples was 19.3±1.8 mg/kg in POAG stage I-II and 17.5±1.2 mg/kg in POAG stage III. This measurement was significantly lower than the Mg level in normal scleral tissue of the anterior region—29.0±6.0 mg/kg (P<0.001).

Table 2.

The content of TE (mg/kg) in the scleral tissue of patients with POAG

Element	Glaucoma stage		Control group
	I - II	III	
Cu	1.2±0.13	0.77±0.12*	1.38±0.11
Fe	48.03±3.2	68.03±4.3*	42.03±4.0
Mg	19.3±1.8*	17.5±1.2*	29.0±6.0
Zn	68.0±3.9	39.0±4.1*	69.0±4.2

** P<0.05 - differences between Control group and Glaucoma stage*

According to the literature, the concentration of Mg in the tear fluid of healthy eyes is comparable with (or slightly lower than) its level in blood serum (i.e., for the older age group, it is 0.66–0.99 mmol/L).[10] At the same time, the results of our research on the trace element analysis of tear fluid showed that the Mg level in this biological fluid of POAG patients was detected in trace amounts.

In conclusion, the trace element analysis of glaucomatous eyes and eye tissues revealed an imbalance in TE that contributes to the formation of excessive cross-links in the collagen structures of the sclera. Specifically, we observed a deficiency in magnesium, an element that is necessary for regulating the metabolism of pathologically changed connective tissue structures of glaucomatous eyes. Detected irregularities in the content of some TE (magnesium, iron, zinc, etc.) in ACAH of POAG patients may be a serious pathogenetic factor that violates the viscosity, and hence, the hydrodynamics, of intraocular fluid.

Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Radiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

A Non-Invasive Method for the Diagnosis of Sjogren's Syndrome with the Evaluation of the Capacity of Salivary Glands

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Abstract

This study describes a non-invasive method for diagnosing Sjogren's syndrome (SS) with MR sialography, which is based on the contrasting of the ductal system with its own secretion and the possibility of simultaneous assessment of the secretory function of parotid salivary glands (PSG).

The objective of this study was to evaluate the effectiveness of MR sialography in the diagnosis of SS and chronic parenchymal sialadenitis.

Methods and Results: The study included 60 patients aged between 25 and 70 years with PSG impairments. Inclusion criteria: patients suffering from dry mouth and enlarged PSG, with an established diagnosis of SS and chronic parenchymal sialadenitis. The sensitivity and diagnostic accuracy of MR sialography was 96.6% and 95.2% in the diagnosis of SS, and 94.4% and 93.3% in the diagnosis of chronic parenchymal sialadenitis. Specificity of the method was 91.6%.

Conclusion: MR sialography, highly effective and identical to sialography, allows detecting typical disorders in parotid salivary glands that are inherent to Sjogren's syndrome and chronic parenchymal sialadenitis. (**International Journal of Biomedicine. 2019;9(1):26-30.**)

Key Words: magnetic resonance imaging • Sjogren's syndrome • parotid salivary glands • parenchymal sialadenitis

Abbreviations

CPS, chronic parenchymal sialadenitis; **PSG**, parotid salivary glands; **US**, ultrasound scan; **MRI**, magnetic resonance imaging; **SS**, Sjogren's syndrome.

Introduction

Among many dental diseases, the prevalence rate of the diseases of the major salivary glands reaches 24%. They include sialolithiasis (36%), chronic sialadenitis (26%), and sialadenosis (18%).[1] Sjogren's syndrome (SS) takes a special place in this line with a prevalence of 0.1% to 3.3%.[2] SS is an autoimmune disease characterized by lymphocytic infiltration of salivary and lacrimal exocrine glands with the development of xerostomia and xerophthalmia. SS can be found in 5%-25% of patients with systemic diseases of connective tissues, and in 50%-75% of patients with the following autoimmune diseases of liver and thyroid gland: chronic active hepatitis, primary biliary cirrhosis, and Hashimoto thyroiditis.[2,3]

According to the criteria classification of SS, introduced by the American-European Consensus Group in 2002, the diagnosis is based on the clinical implications, serological and radiological studies (including sialometry and a Schirmer test), and a biopsy of the minor salivary glands.[4] Additionally, the following examinations can be done: computed tomography, ultrasound scan, and MRI.[5] The most reliable methods recognized to detect typical changes in the glandular tissue

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during SS are salivary gland biopsy and sialograph.[6]

However, performing a sialography is often impossible in patients with allergic reactions to the iodine medications and in cases of thyroid diseases, as the administration of the iodine-containing contrast agent is required to perform it. Also, during sialography, PSG are examined only from the one side as performing a dual-sided examination causes additional radiation exposure to the patient. Another important condition that prevents performing the sialography is duct obstruction.[4,7]

In these cases, US of the salivary glands is used as a non-invasive method. The main diagnostic criterion of SS during US is parenchymal heterogeneity. This is shown on sonograms in the form of hypoechoic areas surrounded by a hyperechoic line or spot, resembling a reticulated image.[5] At the same time, this complex of symptoms can correspond either to the accumulation of the lymphoid cells or to the fine-focal lymphomas and can be mistakenly interpreted.[8]

The important data about structural changes of PSG and about the presence and distribution of the adipose tissue can be obtained with an MRI. This is a non-invasive method without radiation exposure, which can also be performed during the recrudescence of sialadenitis. It is usually performed according to the traditional method with a power of 1.0T and 1.5T without visualization of the ductal system.[3]

The objective of this study was to evaluate the effectiveness of MR sialography in the diagnosis of SS and chronic parenchymal sialadenitis (CPS).

Materials and Methods

The study included 60 patients aged between 25 and 70 years with PSG impairments. Inclusion criteria: patients suffering from dry mouth and enlarged PSG, with an established diagnosis of SS and chronic PS. Exclusion criteria: patients having a pacemaker, metal implants, a salivary gland tumor, or claustrophobia.

Randomization of study groups:

Group 1 included 30 people diagnosed with SS and, depending on the SS stage, was divided into subgroups (Group 1a - 17 patients with a clinically-apparent stage; Group 1b - 13 patients with an advanced stage of a disease).

Group 2 included 18 patients with CPS.

The control group (CG) consisted of 12 volunteers without any salivary gland impairments.

All participants underwent a complex examination to diagnose diseases of PSG.

The secretory function of PSG was evaluated using sialometry. A secretion stimulated by a 2% ascorbic acid solution was collected for 5 minutes in a graduated tube using a Leshley-Yushchenko-Krasnogorsky capsule.

For sialography, a contrast agent Omnipack-350 was used, which was administered in the ductal system of PSG with the help of the syringe and intravenous plastic catheter, followed by radiography in the anteroposterior and lateral view. MR sialography was performed on a 3.0T GE Signa system.

In the dental office, a duct of PSG was examined with a bougie, followed by obturation with a disposable plastic catheter. After that, stimulation of the salivary discharge with a 2% ascorbic acid solution was performed for 15 minutes. MRI T1-weighted and T2-weighted scans (cor/sag/ax) and T2- with suppression of the signal from adipose tissue and in FIESTA mode were performed. The slice thickness was 1-5 mm.

The effectiveness of the method was evaluated using the values of diagnostic sensitivity (Se), accuracy (Ac) and specificity (Sp) according to the following formulas:

$$Se = \frac{TP}{TP+FN} \times 100\%, \text{ where}$$

TP - a number of true positive test results in the patients who are known to be ill

FN - a number of false negative results in the patients who are known to be ill

$$Ac = \frac{TP+TN}{TP+TN+FP+FN} \times 100\%, \text{ where}$$

TN - a number of true negative results in patients who are known to be healthy

FP - a number of false negative results in patients who are known to be healthy

$$Sp = \frac{TN}{TN+FP} \times 100\%$$

Sialography was a referent method.

The quality of the duct visualization on an MR sialogram depends on the amount of salivary secretion in the ducts. To count the volume of the liquid in the ductal system, a formula for calculating the volume of the vessel (Vm^3) of a cylindrical shape was used. As ducts are visualized on an MR sialogram due to the presence of natural secretion in them, the volume of the vessel (duct) and the volume of liquid in it will be equal.

To find the volume of the vessel, it is necessary to measure its length and radius, while the radius must be constant. Considering that the ductal system of PSG has a different diameter on different sections, first, the volume of the section of the duct with the even radius was determined. For this purpose, the length of the fragment of the duct was measured in the sagittal plane (ΔXi) and its radius (r^2) for this length was measured in the coronal plane.

The volume of the section of the duct (ΔVi) was calculated by the following formula:

$\Delta Vi = \pi r^2 \times \Delta Xi$, where

ΔVi - the volume of the section of the duct

r^2 - the radius of the duct

ΔXi - the length of the section of the duct

Next, the volume of the duct sections (ΔVi) was summed up and the result was the total volume of the vessel $Vi(m)^3$

$Vi = \Delta Vi_1 + \Delta Vi_2 + \Delta Vi_3 \ldots$

Next, this data was converted into milliliters (Vml), which corresponded to the volume of the liquid in the duct.

In the case of parenchymal parotitis, to obtain reliable data about the amount of the secretion, it is necessary to consider the volume of the liquid, not only in the duct but also in the intraglandular cysts, which are also filled with natural secretion. The following formula for calculatingof the volume of the sphere (cyst) was used:

$Vs = \frac{4}{3}\pi r^3$, where

Vs - the volume of the cyst
r^3 - the radius of the cyst

Obtained results of the volume of ducts and volume of cysts were summed, and the result was a total volume of the glandular secretion.

To measure the size of PSG, the special program for calculating the area of figures (Scm^2) of arbitrary shape IpSquare 5 for Windows was used. The MR-image of PSG in the axial projection was uploaded into the program; the outlines of the parenchyma were traced with the mouse cursor, followed by painting over the gland. The obtained data were transferred to a computer, processed, and the value of the area of the organ appeared at the bottom of the program window.

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. Inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

Results

During the examination of CG, the vermilion border had no abnormal elements, PSG were non-palpable. The mucous membrane epithelium of the mouth had a light pink color, moderately moist. With the massage, ducts of PSG released a clear transparent secretion. Sialometry was within a normal range (4±0.5 ml). There were no abnormal changes detected on sialograms. Parenchyma and ducts were clearly detected, homogeneously filled with a radiopaque contrast agent (Fig.1a). There were no disturbed topographic anatomical criteria on the MR sialogram. PSG were normally positioned and not enlarged (S=5.5±0.5 cm^2), and had typical shape and structure. Glandular tissue was in a normal condition. Ducts did not have any abnormal changes. Only contours of the ductal system without secretion were visualized without acidic stimulation. After acidic stimulation, it was possible to clearly visualize the ductal system up to second-order ducts. The main duct and first- and second-order ducts with clear even contours were evenly filled with natural secretion along their entire length. The specificity of MR sialography was 91.6% (Fig.1b).

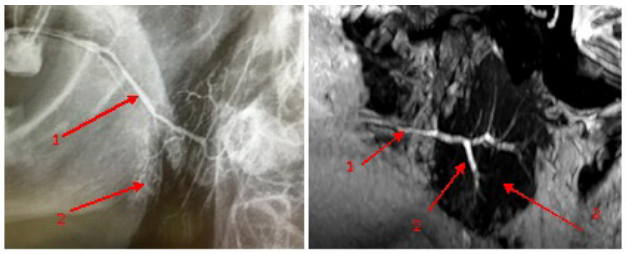

Fig. 1. *A 26-year-old patient. Parotid salivary glands. Normal condition.*
(1a). Radiographic sialogram of PSG on the righ. *(1b). MR sialogram of PSG on the right.*
Arrows: 1 – main duct; 2 – ducts of first- and second-order; 3 - parenchyma of the gland

Patients of Group 1 complained about dry mouth, the inability to chew food without pre-wetting the mouth with water, about a periodic bilateral enlargement of parotid salivary glands, dry eyes, and joint pain. During a clinical examination, the PSG were found enlarged and painless during palpation; the mucous membrane epithelium of the mouth was dry, hyperemic, free saliva was foaming and was present in low amounts, and multiple non-carious lesions of hard tissue in the area of the necks of the teeth were present.

Patients of Group 1a had xerostomia degree II (sialometry of 1.7±0.1 ml). The spotted filling of the parenchyma of the gland with a radiopaque contrast agent was visualized on the sialogram presented in Figure 2a. On an MR sialogram, PSG were enlarged (S = 8.3±0.3 cm^2), parenchyma structure was heterogeneous due to interstitial fibrotic changes, and there was an occurrence of fatty involution and edema. The main duct was clearly and evenly filled with its own secretion along its entire length but only before bifurcation as the secretory function in Group 1a patients was lowered by 2.3 times compared to CC (Fig.2b).

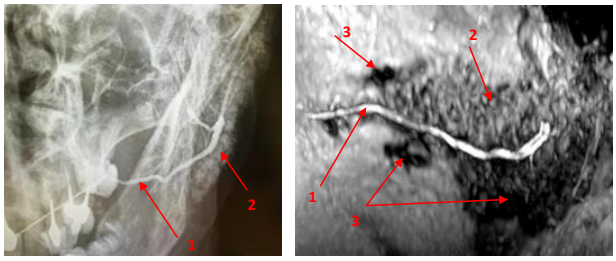

Fig. 2. *A 62-year-old patient. SS: clinically-apparent stage.*
(2a). Radiographic sialogram of PSG on the left. *(2b). MR-sialogram of PSG on the left. Loss of MR signal.*
Arrows: 1 – main duct; 2 – parenchyma of the gland, 3 - interstitial fibrotic changes.

In Group 1b, xerostomia was significantly pronounced, free saliva was absent, and sialometry was 0.7±0.1 ml (xerostomia degree III). Cyst-like cavities in the form of "clouds" with fuzzy blurred outlines were found on the sialogram presented in Figure 2, which indicates an increased permeability of the gland tissue. The third- and fourth-order ducts cannot be tracked, local sections of strictures and ectasias of first- and second-order ducts were present (Fig.3a).

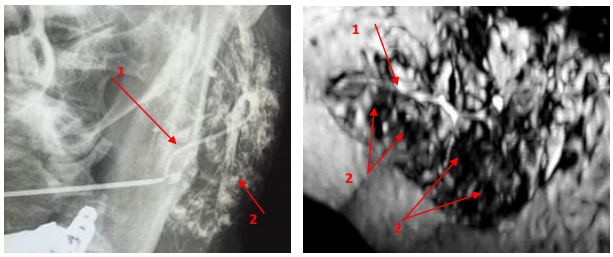

Fig. 3. *A 65-year-old patient. The advanced stage of SS.*
(3a). Radiographic sialogram of PSG on the left. *(3b). MR-sialogram of PSG on the left. Loss of MR signal.*
Arrows: 1 – main duct; 2 – parenchyma of the gland, 3- fibro-cystic adipose changes.

On the MR sialograms of the advanced stage of SS, PSG were found to be enlarged (S=6.6±0.1 $см^2$), and parenchyma

had diffuse cellular restructuring due to the pronounced fibrocystic adipose changes (the consequences of destructive changes). The ductal system can be traced only partially, which is indicated by a sharp decrease in the PSG secretion. The MR image corresponds to structural changes in the salivary glands (Fig. 3b). The sensitivity and diagnostic accuracy of MR sialography in the diagnosis of SS were 96.6% and 95.2%, respectively.

In Group 2, PSG were enlarged on both sides, soft and elastic in consistency under palpation, and painless. During the examination of the oral cavity, a mucous membrane was found to be poorly moistened, wedge-shaped defects of teeth were found, and ducts released cloudy secretion. Sialometry was 2.2±0.2 ml, which corresponds to xerostomia stage I. The sialogram presented in Figure 4a shows signs of CPS in the form of cystic cavities filled with a radiopaque contrast agent more than 1mm in diameter. The MR sialogram presented in Figure 4b shows bilateral enlargement of PSG (S=6.7±0.1 cm^2) with numerous small intraglandular cysts of different sizes, filled with natural secretion, and also fibrosis changes of the interstitial tissue of the gland. The sensitivity and diagnostic accuracy of MR sialography in the diagnosis of CPS were 94.4% and 93.3%, respectively.

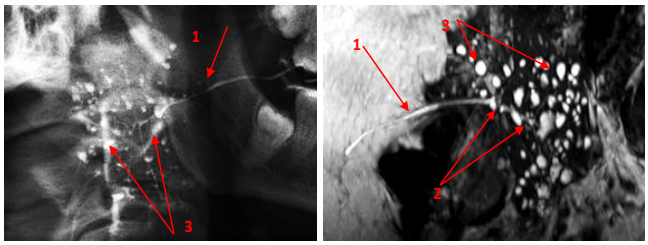

Fig. 4. *A 38-year-old patient. Chronic bilateral parenchymal sialadenitis.*
(4a). Radiographic sialogram of PSG on the right. *(4b). MR sialogram of PSG on the right.*
Arrows: 1 – main duct; 2 – ducts of the II order; 3 – cystic-shaped cavities.

Results and Discussion

During analysis of MR sialograms and data received from sialometries, it was possible to determine a verifiable relation between the secretion level of PSG and the visualization degree of the ductal system of glands during MR sialography after acidic stimulation for salivary discharge. As salivation decreased, the volume of secretion in the ducts also decreased, which led to a worse tracing of the architectonics of the salivary gland ducts (Table 1).

Using the program for calculating the area of figures of arbitrary shape, IpSquare 5 for Windows, allowed us to determine the true size of parotid salivary glands in their normal condition and when affected by different diseases. It was established that PSG in the clinically-apparent stage of SS are enlarged by 1.5 times. With the advanced stage and PS, PSG are enlarged 1.2 times compared to the normal condition (P<0.05). This may be caused by the pronounced destructive processes in the form of fibrous involution of glandular tissue (Fig.5).

Table 1.

Data of MR sialograms and sialometry in the examined groups

Group	Sialometry (amount of saliva, ml)	MR sialogram (volume of secretion in the duct, V ml)
CG	4.0±0.5	2.1±0.3
Group 2	2.2±0.2* xerostomia degree I	1.2±0.2*
Group 1a	1.7±0,1* xerostomia degree II	0.5±0.1*
Group 1b	0.7±0.1* xerostomia degree III	0.2±0.1*

**-P<0.05*

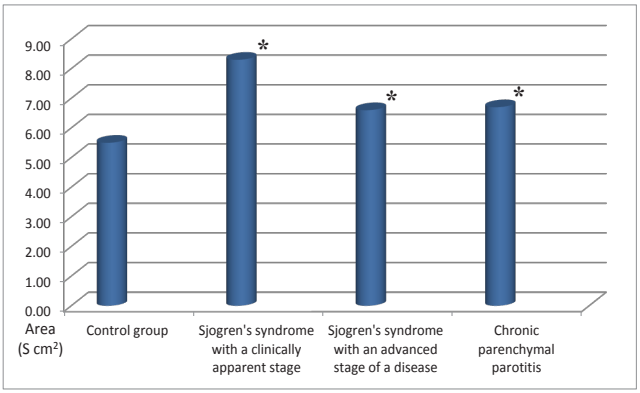

Fig. 5*. Diagram of the area (S cm^2) of PSG affected by different diseases (*- P<0.05).*

Conclusions:

•MR sialography, highly effective and identical to sialography, allows detecting typical disorders in PSG that are inherent to SS and CPS.

•The sensitivity and diagnostic accuracy of MR sialography was 96.6% and 95.2% in the diagnosis of SS, and 94.4% and 93.3% in the diagnosis of CPS. Specificity of the method was 91.6%.

•Direct relations between the secretion level of PSG and the visualization of the ductal system on MR sialograms were established, which allows using MR sialography as a functional method of evaluating the secretory function of PSG

•To determine the sizes of PSG, it is recommended to measure their area in axial projection using the program IpSquare 5 for Windows.

Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Male Reproductive Health

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Features of the Processes of Lipoperoxidation and Antioxidant Protection in the Pathogenesis of Infertility in Men of Different Ethnic Groups, Carriers of Nonfunctional Polymorphisms of the *GSTT1* and *GSTM1* Genes

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Abstract

A comparative analysis was carried out of the parameters of lipoperoxidation and antioxidant protection in fertile and infertile men of two ethnic groups (Caucasians and Mongoloids), who were carriers of different genotypes of xenobiotic biotransformation genes (*GSTT1* and *GSTM1)*. In serum and ejaculate, we determined the content of TBA-active products, α-tocopherol, reduced glutathione (GSH) and oxidized glutathione (GSSG); the activity of glutathione-S-transferase (GST), glutathione peroxidase (GPO) and glutathione reductase (GR); and total antioxidant activity (AOA). It was found that in Caucasian fertile men, carriers of genotypes *GSTT1(*0/*0)/GSTM1(*0/*0)*, there was a decrease in GST activity in serum and ejaculate with an increase in AOA in these substrates in comparison with similar indicators in carriers of genotypes *GSTT1(1+/1+)/GSTM1(1+/1+)*. In Mongoloid fertile men, carriers of genotypes *GSTT1(*0/*0)/GSTM1(*0/*0)*, in comparison with similar indices in carriers of genotypes *GSTT1(1+/1+)/GSTM1(1+/1+*), we found an increase in serum content of α-tocopherol and activity of GPO, a decrease in GST activity, and a decrease in the level of α-tocopherol in the ejaculate. In Caucasians with infertility, carriers of genotypes *GSTT1(*0/*0)/GSTM1(0*/*0)*, we found decreased activity of GST in the blood and ejaculate. In this group, we also registered a decrease in the GSH level, with an increase in the level TBA-active products and GPO activity. In infertile Mongoloids, carriers of genotypes *GSTT1(*0/*0)/GSTM1(*0/*0)*, we found an increase in the level TBA-active products and GPO activity and a decrease in serum α-tocopherol level.(**International Journal of Biomedicine. 2019;9(1):31-34.**)

Key Words: oxidative stress • male infertility • ethnicity • *GSTT1* • *GSTM1*

Introduction

Modern negative trends, which characterize the reproductive health of the population, cause great concern.[1-3] Russia has one of the highest rates of marital infertility, reaching 19%–20%.[4,5] The proportion of the "male" factor in the structure of family infertility has reached 50%.[2,6,7] As a rule, male infertility is the result of various pathological processes in the body that have an adverse effect on the endocrine system, including sex glands. Professor Eduardo Ruiz-Castañé (Director of the Andrology Department of the Fundació Puigvert Clinic, Barcelona) noted, in order to identify the possible causes of reproductive disorders, factors associated with "subfertility, potential correction of anomalies, changes in genetic aspects" should be considered.[8] Male fertility is affected by various metabolic, toxic, immune and vascular disorders, congenital and acquired diseases, inflammatory processes, and even mental disorders.[1-3,9,10]

Genetic factors play a special role in the pathogenesis of male infertility.[3,11] Different types of chromosomal and gene disorders affect the male reproductive system at different levels: from the normal formation of the male genital organs and spermatogenesis to ejaculation and fertilization of the

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egg.[3,11,12] The gene system plays a key role in the processes of ensuring and maintaining the normal vital activity of cells, including spermatozoa. This is primarily because the genes that are part of this system encode enzymes that catalyze various types of chemical transformations in the cell.[12] The *GST* gene family plays a primary role, taking part in the second phase of detoxification of xenobiotics and in protecting cells against oxidative stress.[11,13-15]

The purpose of this study was to investigate the parameters of the lipid peroxidation and antioxidant protection system associated with polymorphisms of biotransformation genes in fertile and infertile men of different ethnic groups.

Materials and Methods

A retrospective analysis of the results of a survey of Mongolian men (using the example of the Buryats; n=143; mean age of 31.6±5.9 years) and Caucasians (using the Russian example; n=222; mean age of 29.9±5.3 years) of infertile couples who applied to the Republican Perinatal Center, the city of Ulan-Ude. Two control groups of 53 Buriats and 104 Russians (mean age of 31.9±7.5 and 30.2±3.6 years, respectively) were formed, consisting of healthy men with realized reproductive function. All men had a laboratory and clinical examination by an andrologist, including an ultrasonic scan of scrotum and prostate. Macroscopic and microscopic examination of ejaculate was performed in accordance with with the WHO recommendations (2010). The study was conducted in accordance with ethical principles of the Declaration of Helsinki (2000; revised October 2013, Fortaleza, Brazil). Written informed consent was obtained from all participants.

In serum and ejaculate of the examined men, the content of thiobarbituric acid (TBA)-active products (TBA-AP) was determined by the method of V.B. Gavrilova et al.(1984), α-tocopherol - by the method of R.Ch.Chernyauskene et al. (1984), total antioxidant activity (AOA) according to GI Klebanov et al. (1988). The content of reduced glutathione (GSH) and oxidized glutathione (GSSG) was determined by the method of P.Y. Hissin (1976). The activity of glutathione-S-transferase (GST), glutathione peroxidase (GPO) and glutathione reductase (GR) was estimated by the method of A.I.Karpishchenko (2002). The measurements were performed using a Shimadzu RF-1501 spectrofluorophotometer.

DNA samples were genotyped for polymorphisms in the genes *GSTT1* and *GSTM1*. DNA was isolated from venous blood samples using the sorbent method with the certified reagent kit DNA-Sorb-B (Central Research Institute of Epidemiology, Moscow, Russia). Genetic polymorphism of insertion/deletion in the *GSTT1* and *GSTM1* genes was determined by PCR in the automatic thermocycler Tercyc using the reagent kit of Central Research Institute of Epidemiology (Moscow, Russia).

Amplification products were detected in a 3% agarose gel; the electrophoresis results were registered and documented with the help of the system of computer gel documentation GelDoc. *GSTM1+, GSTT1+* (wild type) and *GSTM1*0* and *GSTT1*0* homozygotes (null genotype) were analyzed.

Patients with genetic causes of infertility (AZF deletions, CFTR mutations, mutational changes in the number of CAG repeats controlled by androgen receptors) were excluded from the study.

The statistical analysis was performed using the software package Statistica 6.1 (StatSoft, USA) and Biostat. Deviation from Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed by $\chi 2$- test and Fisher's exact test. Two-tailed *P* values <0.05 were considered statistically significant.

Results and Discussion

In fertile Caucasian men who carried nonfunctional genotypes *GSTT1 (*0/*0)/GSTM1(*0/*0)*, a statistically significant decrease in GST activity by 50% in blood plasma and 35% in ejaculate was found, with an increase in total serum AOA by 22% and ejaculate by 32% when compared with similar indicators in carriers of functional genotypes *GSTT1(1+/1+)/GSTM1(1+/1+)* (Table 1).

Table 1.

Components of the lipid peroxidation and antioxidant protection system in fertile Caucasian men, carriers of different polymorphisms of xenobiotic biotransformation genes

Variable	GSTT1(*0/*0)/ GSTM1(*0/*0)	GSTT1(1+/1+)/ GSTM1(1+/1+)
Total AOA (serum)	18.62±4.75	14.52±4.72*
GST (serum)	560.45±357.67	1122.19±607.66*
Total AOA (ejaculate)	4.5±3.59	3.08±2.52*
GST (ejaculate)	378.32±304.13	585.53±554.93*

** - P <0.05 between two groups.*

The increased level of total AOA, which characterizes the total activity of enzymatic and non-enzymatic inhibitors of free radical oxidation in fertile Caucasians, is a reliable protection of cell membranes, including spermatozoa.

In fertile Mongoloid men who carried genotypes *GSTT1 (*0/*0)/GSTM1(*0/*0)*, we found a statistically significant decrease in blood GST activity by 45% and an increase in blood GPO activity by 21% (Table 2).

Table 2.

Components of the lipid peroxidation and antioxidant protection system in fertile Mongoloid men, carriers of different polymorphisms of xenobiotic biotransformation genes

Variable	GSTT1(*0/*0)/ GSTM1(*0*/0)	GSTT1(1+/1+)/ GSTM1(1+/1+)
α- tocopherol (serum)	9.36±3.20	7.77±2.32*
GPO (serum)	566.7±167.2	467.5±223.1*
GST (serum)	642.6±566.8	1170.0±944.4*
α- tocopherol (ejaculate)	2.68±1.19	4.76±3.05*

** - P <0.05 between two groups.*

In infertile Caucasians, carriers of genotypes *GSTT1 (*0/*0)/GSTM1(*0/*0)*, the activity of GST was reduced by 23% in the blood and 32% in the ejaculate. They also registered a decrease in the GSH content by 6% and an increase in the TBA-AP content by 32% and blood GPO activity by 17% (Table 3).

Table 3.

Components of the lipid peroxidation and antioxidant protection system in infertile Caucasian men, carriers of different polymorphisms of xenobiotic biotransformation genes

Variable	GSTT1(*0/*0)/ GSTM1(*0/*0)	GSTT1(1+/1+)/ GSTM1(1+/1+)
TBA-AP (serum)	1.21±0.66	0.82±0.54*
GSH (serum)	1.71±0.22	1.82±0.23*
GPO (serum)	175.89±113.62	145.29±59.76*
GST (serum)	794.73±667.08	1033.39±773.46*
GST (ejaculate)	550.26±510.36	806.55±686.26*

** - P <0.05 between two groups.*

In infertile Mongoloids, carriers of genotypes *GSTT1(*0/*0)/GSTM1(*0/*0)*, we found an increase in blood content of TBA-AP by 18% and blood GPO activity by 25%, with a decrease in serum α-tocopherol level by 13%. The ejaculate showed a decrease in α-tocopherol level by 12%, GSSG level by 43% and GST activity by 46% (Table 4).

Table 4.

Components of the lipid peroxidation and antioxidant protection system in infertile Mongoloid men, carriers of different polymorphisms of xenobiotic biotransformation genes

Variable	GSTT1(*0/*0)/ GSTM1(*0/*0)	GSTT1(1+/1+)/ GSTM1(1+/1+)
TBA-AP (serum)	1.16±0.48	0.94±0.53*
α- tocopherol (serum)	8.52±2.75	9.74±3.19*
GPO (serum)	213.7±141.5	159.8±71.7*
α- tocopherol (ejaculate)	2.91±1.06	3.33±1.32*
GSSG (ejaculate)	1.8±1.17	3.16±2.05*
GST (ejaculate)	615.8±525.2	1141.9±789.5*

** - P <0.05 between two groups.*

The study found that men with infertility, both Caucasians and Mongoloids, produce potentially damaging factors (TBA-AP), which exceed the capacity of the body's natural antioxidant defense, leading to cell damage and, consequently, oxidative stress. A genetically determined imbalance in the system of glutathione-dependent antioxidant protection determines the activation of lipid peroxidation and contributes to a significant weakening of the metabolic and detoxifying functions of the body in infertile men, both Caucasians and Mongoloids. As a result, the susceptibility of cells to the damaging effects of xenobiotics, which adversely affect any changes in the external and internal constants of the germ cell formation process, increases significantly.

Thus, genetic factors determine the change in the oxidative-antioxidant balance, both at the system and at the local level, leading to dysfunctions of the male reproductive system and, as a consequence, to a decrease in fertility. Identification of carriage of the polymorphic variants of the genes *GSTT1* and *GSTM1*, as well as determination of the enzymes of the thiol-disulfide system, can be recommended for additional estimation of the risk of developing reproductive disorders in men.

Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Male Reproductive Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Role of Serum Follicle-Stimulating Hormone Level as Predictor of Sperm Retrieval in Patients with Non-Obstructive Azoospermia

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Abstract

The aim of this study was to determine the predictive value and use of noninvasive, pre-operative estimation of serum follicle-stimulating hormone (FSH) on sperm retrieval rate (SRR) during microdissection testicular sperm extraction (mTESE) in patients with non-obstructive azoospermia (NOA).

Materials and Methods*:* A retrospective review of the charts of 74 patients (mean age of 36.7±6.7 years) with NOA who underwent mTESE was performed. Pre-operative serum levels of FSH, total testosterone, luteinizing hormone (LH), estradiol, prolactin and mTESE results were analyzed. Patients were divided into two groups according to the mTESE results: Group A (positive sperm retrieval) and Group B (negative sperm retrieval).

Results: Sperm was retrieved from 29 patients (positive SRR 39%). The mean serum FSH levels were 24.83±10.09 mIU/mL in Group A and 31±4.24 mIU/mL in Group B, without statistically significant differences between the two groups (P>0.05). In addition, there were no observed statistically significant differences between the two groups regarding serum LH, total testosterone, estradiol and prolactin levels.

Conclusion*:* The study found that pre-operative measurement of serum FSH level has no significant predictive value on SRR at mTESE in men with NOA.(**International Journal of Biomedicine. 2019;9(1):35-38.**)

Key Words: non-obstructive azoospermia • microdissection testicular sperm extraction • follicle-stimulating hormone • male infertility

Abbreviations

ART, assisted reproduction technologies; **FSH**, follicle-stimulating hormone; **ICSI**, intra-cytoplasmic sperm injection; **LH**, luteinizing hormone; **mTESE**, microdissection testicular sperm extraction; **NOA**, non-obstructive azoospermia; **SRR**, sperm retrieval rate.

Introduction

Infertility is defined as failure to achieve a clinical pregnancy following 12 months of regular, unprotected sexual intercourse.[1] It has been reported to affect 15% of couples, with a male factor contribution to the fertility problem in almost 50%.[2,3] The complete absence of sperm from the ejaculate is called *azoospermia* and occurs in 1% of all men and in 10%–12% of the infertile male population.[4] Azoospermia can be classified as obstructive and non-obstructive depending on the cause of it. NOA, which is caused by testicular failure, accounts for 60% of all cases of azoospermia and represents the most challenging case scenario.[5]

With advances in ART and the success of ICSI of surgically retrieved sperm, couples with NOA can have their own children.[6] There are different surgical procedures to retrieve sperm from patients with NOA, including percutaneous testicular biopsies, open conventional testicular biopsies and mTESE. In NOA patients, sperm can be found in small islands following extensive dissection of seminiferous tubules with SRR approaching 40%-50% with mTESE.[7]

A thorough medical history and examination are essential for proper evaluation and management of NOA

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patients. Before attempting sperm retrieval, use of pre-operative, noninvasive parameters to predict the success of finding sperms is helpful. There have been several studies in the literature with controversial predictive values about the use of non-invasive parameters and SRR during mTESE. FSH has been used as a marker for sperm production and considered one of the important pre-operative serum parameters in evaluating NOA patients. In the literature, elevated levels of serum FSH are postulated to have an inverse effect on finding sperm following sperm retrieval procedures.[8,9] In this study, the role of pre-operative, noninvasive measures of serum FSH in prediction of a successful sperm retrieval in patients with NOA who underwent mTESE procedures is evaluated.

Materials and Methods

We performed a retrospective review of the charts of 74 patients with NOA who underwent mTESE between August 2015 and December 2018 and met the inclusion criteria for this study. Enrolled subjects were required to have at least two separate azoospermia semen analyses of normal volume, palpable vas deferens on both sides and no history of sexually transmitted diseases, vasectomy or prior inguinal hernia repair. Data on reproductive history, a physical examination, semen analysis, and hormonal profile were analyzed. Serum FSH levels were determined by ELISA with a normal reference range of FSH between 1.5 mIU/mL and 15 mIU/mL. Based on serum FSH levels, patients were divided into Group 1 (FSH≤15) and Group 2 (FSH>15). Semen analysis was performed concurring with WHO criteria (WHO laboratory manual for the examination and processing of human semen; 2010).

mTESE was performed under general anesthesia on all enrolled subjects. A small midline scrotal incision was made to allow easy access to both testes. Patients were examined under anesthesia for testicular size and consistency, and the most promising of the two testicles was brought out first after dissecting the dartos muscle and opening the tunica vaginalis. The tunica albuginea was then opened in a horizontal plane under the operating microscope using a No.15 blade. Initial inspection of the exposed seminiferous tubules was performed, followed by bivalving the testicle and exposing the deep compartment. The seminiferous tubules were then thoroughly and systematically examined for opaque and dilated tubules. Identified tubules were then harvested with forceps and put in a small tube filled with HEPES-buffered sperm medium and handed over to the IVF lab. An embryologist in the IVF lab examined the harvested testicular biopsies for presence of sperm. If sperms were identified and enough for freezing and subsequent ICSI, hemostasis was achieved with bipolar diathermy and closure of the tunica albuginea with interrupted absorbable monofilament suture was performed. Closure of tunical vaginalis was performed, followed by placing the testicle back into the hemiscrotum and closure of dartos muscle, fascia and scrotal skin. However, if no sperm was identified, the same technique was carried out on the contralateral testicle.

Statistical analysis was performed using statistical software package SPSS version 17.0 (SPSS Inc, Chicago, IL). Data are expressed as mean±SD. Inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

The study was approved by the Qassim University Ethics Committee. Written informed consent was obtained from all the participants.

Results

A total of 74 patients with NOA (mean age of 36.7±6.7 years) were enrolled in this study and underwent mTESE as described in the methodology section. The baseline hormonal profiles showed in Table 1.

Table 1.

The baseline hormonal profiles of study patients

Variable	Mean±SD
Age, yrs	36.7 ± 6.7
Estradiol, pg/mL	101.56±42.37
FSH, mIU/mL	31±4.24
LH, mIU/mL	13.5±3.54
Total Testosterone, ng/mL	12.87±3.55
Prolactin, mIU/mL	9.83±5.91

Sperm was retrieved from 29 patients (positive SRR 39%) out of 74 in this cohort. There were 29 patients in Group A (positive sperm retrieval) and 45 in Group B (negative sperm retrieval). There were no statistically significant differences between the two groups with regard to age. The mean ages of Groups A and B were 37.86±6.83 and 35.89±5.90, respectively ($P>0.05$).

The mean serum FSH levels were 24.83±10.09 mIU/mL in Group A and 31±4.24 mIU/mL in Group B, without statistically significant differences between the two groups ($P>0.05$). In addition, there were no observed statistically significant differences between the two groups regarding serum LH, total testosterone, estradiol and prolactin levels (all *Ps*>0.05) (Table 2).

Table 2.

Age and serum hormones in patients of Group A and Group B

Variable	Group A	Group B	*P*-value
Age, yrs	37.86±6.83	35.89±5.90	>0.05
Estradiol, pg/mL	102.25±25.50	107.05±49.72	>0.05
FSH, mIU/mL	24.83±10.09	31±4.24	>0.05
LH, mIU/mL	14.73±6.26	13.5±3.54	>0.05
Total testosterone, ng/mL	12.43±2.84	12.85±4.12	>0.05
Prolactin, mIU/mL	10.27±5.16	11.21±6.53	>0.05

Discussion

From the first successful ICSI in 1994 by use of surgically retrieved sperm, finding testicular sperms when combined with ICSI offers men with NOA a chance to have

their own children.[10,11] For couples pursuing surgical sperm retrieval and the IVF/ICSI option, a discussion regarding the appropriate expectations and outcomes of their specific clinical scenario is crucial.[12] Although there are no specific clinical findings or investigations that accurately predict the finding of sperm in NOA patients, some clinical factors have been found that affect the sperm retrieval in these patients. These factors include genetic testing (Y chromosome microdeletions and chromosomal abnormalities), testicular biopsy and testicular histology. Testicular histology in NOA patients may reveal hypospermatogensis, maturation arrest, both early and late, and Sertoli Cell Only syndrome (SCOS).[13,14] Patients with SCOS carry the lowest SRR during mTESE.

One of the important pre-operative parameters obtained during evaluation of men with azoospermia is serum FSH. Overall, the higher the serum FSH level, the lower the rate of surgically retrieved sperms. Since the successful finding of sperms within some islands of seminiferous tubules in NOA patients, along with high FSH levels, some authors have not found any adverse relationships between SRR and serum FSH level. However, some other researchers have observed a small relationship between FSH levels and SRRs, with a decrease in SRRs as FSH levels increase. Ramasamy and colleagues reported SRR of 51% with serum level of FSH of less than 15I U/mL, 60% SRR with serum FSH value between 15 and 30 IU/mL, 67% SRR with FSH levels of 31–45 IU/mL and 60% SRR with FSH levels more than 45 IU/mL.[15] Lower SRRs have been reported in men with higher FSH levels, and different studies [16,17] have reported different SRRs, resulting in conflicting associations between serum FSH levels and SRRs. In this study, FSH levels have not predicted the success of sperm retrieval during mTESE in NOA patients. There was no significant association between serum LH, total testosterone, prolactin or estradiol levels and sperm retrieval rates.

Serum inhibin-B has been suggested as a direct measure of sertoli cell function and an indirect marker of active spermatogenesis.[18] In patients with SCOS, inhibin-B has been found undetectable despite normal testosterone levels.[19] Studies comparing inhibin-B to FSH as a predictor of sperm retrieval have demonstrated that inhibin-B has a sensitivity of 75.86% and a specificity of 80.85%, compared to 64.78% and 84.69% for FSH.[20] In the literature, some studies have shown a superior predictive value of inhibin-B over serum FSH while others have demonstrated similar predictive values. The combination of the two tests will ultimately give a better predictive value than doing only one of them.[21,22]

Q Yang et al. performed a systematic review and meta-analysis for FSH as a predictor of sperm retrieval in NOA. They did notice variations in the diagnostic value of the serum FSH level with patient age and region of residence. FSH was shown to have more diagnostic value if the patients are younger and of East Asian descent and concluded that FSH had moderate diagnostic value as an independent predictor for SRR in patients with NOA.[23]

The limitations of this study include retrospective design, small sample size and no information on the final histologic results of the testicular biopsies. Thus, a well-designed prospective study is needed and may provide more insights into the role of serum FSH and other pre-operative, noninvasive parameters in prediction of sperm retrieval during mTESE in patients with NOA. Combining serum FSH level with other pre-operative, noninvasive parameters in future research may add more diagnostic and therapeutic options to infertile men diagnosed with NOA.

Conclusion

Couples with NOA can have their own biological children when sperms are successfully retrieved from testicles. In the literature, there are conflicting results on the role of pre-operative, noninvasive parameters in prediction of sperm retrieval during mTESE. In this study, there was no statistically significant difference in serum FSH levels between NOA patients who have sperm retrieved and those who have no sperm retrieved.

Disclosures

None

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ORIGINAL ARTICLE

Women's Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Incidence of Cervical Disease in Women of Different Age Groups in the Republic of Sakha (Yakutia)

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Abstract

The purpose of this study was to investigate the incidence of cervical disease in women of different age groups in the Republic of Sakha (Yakutia).

Materials and Methods: The cytological material of the cervix of 7,600 women aged between 18 and 88 years was analyzed in the laboratory of pathomorphology, histology and cytology. The material of the cytological study consisted of smears of cervical mucosa and the cervical canal, stained according to the method of Romanovsky-Giemsa. The study was conducted with subjects grouped according to age: Group 1 (18-29), Group 2 (30-44), Group 3 (45-59), and Group 4 (60-74).

Results: According to the results of cytological analysis, inflammatory diseases of the cervix uteri were diagnosed in 4,629/61% cases. Among age groups, the highest rate of inflammatory diseases of the cervix uteri was registered in Group 1 and Group 2. Benign cervical lesions were found in 563/7.4% cases with the highest incidence in Groups 1 and 2. The most frequently diagnosed pathology was squamous cell metaplasia with maximum frequency in Group 2 and Group 1. Cervical intraepithelial neoplasia (CIN) was detected in 359/4.7% cases. CIN I, CIN II and CIN III were registered in 220/61.3%, 84/24.5%, and 38/10.6% women, respectively. At the same time, the maximum frequency of dysplasia was noted in Group 1 and Group 2. Thus, results obtained indicate a high incidence of cervical disease in women of reproductive age.(**International Journal of Biomedicine. 2019;9(1):39-42.**)

Key Words: cervical disease • inflammatory diseases • benign cervical lesions • cervical intraepithelial neoplasia

Introduction

The problem of cervical diseases (CD) is one of the topical issues in modern gynecology, and it has great importance for preventing the emergence and development of malignant tumors. The frequency of CD is very high and has no tendency to decrease: cervical pathology occupies a significant place in the structure of reproductive system diseases, as it is found in 15%-20% of women of reproductive age and 5%-9% of women of menopausal age.[1] Worldwide, cervical cancer (CC) is the fourth most frequent cancer in women, with an estimated 530,000 new cases in 2012 representing 7.5% of all female cancer deaths. CC is by far the most common HPV-related disease.[2] Most cervical carcinomas are etiologically related to the human papillomavirus (HPV) and about 70% of all cervical carcinomas are caused by 2 types of high-risk HPV, 16 and 18.[3] Once infected by HPV, cervical neoplasia undergoes a stepwise progression starting from preinvasive lesions that can be detected by screening and cured with complete excision.[4] In developed countries, programs are in place that enable women to get screened, making most pre-cancerous lesions identifiable at stages when they can easily be treated. Early treatment prevents up to 80% of CC in these countries.[2]

The purpose of this study was to investigate the incidence of cervical disease (CD) in women of different age groups in the Republic of Sakha (Yakutia).

Materials and Methods

The purpose of this study was to investigate the incidence of cervical disease (CD) in women of different age groups in the Republic of Sakha (Yakutia). The cytological

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material of the cervix of 7,600 women aged between 18 and 88 years, who applied to various medical institutions of the Republic of Sakha (Yakutia) (RS(Y)) for preventive and diagnostic purposes during 2017, was analyzed in the laboratory of pathomorphology, histology and cytology of the NEFU Medical Institute Clinic.

The material of the cytological study consisted of smears of cervical mucosa and the cervical canal, stained according to the method of Romanovsky-Giemsa. The study was conducted with subjects grouped according to age: Group 1 (18-29), Group 2 (30-44), Group 3 (45-59), and Group 4 (60-74). Bokhman's classification (1976) was used to assign a diagnosis.[5] According to Bokhman's classification, pathological processes in the cervix are divided into background processes (benign cervical lesions [BCL]), precancerous conditions (CIN II-III) and cancer.

Results and Discussion

Cytological material from 7,600 women was distributed as follows: Group 1 – 2,645/34.8%, Group 2 – 2,315/30.4%, Group 3 – 1,840/24.2%, and Group 4 – 800/10.5%. According to the results of cytological analysis, inflammatory diseases of the cervix uteri (IDCU) were diagnosed in 4,629/61% cases (Fig.1).

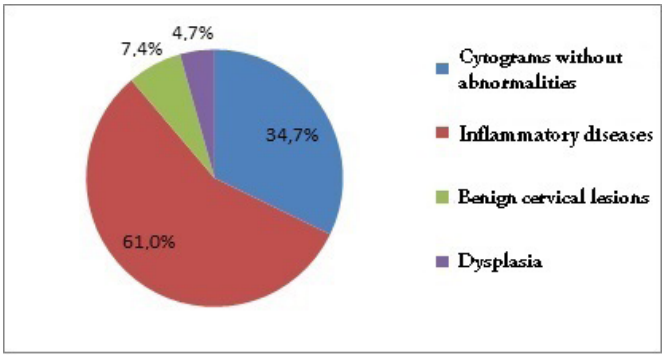


Fig.1. *Results of cytological analysis.*

Table 1.

The incidence of inflammatory diseases of the cervix in different age groups (in n/%)

	Group 1	Group 2	Group 3	Group 4	Total
Inflammatory process	540/29.8	348/19.2	542/29.9	379/20.9	1809/39
Flora dysbiosis	740/40.7	693/38.2	337/18.5	45/2.5	1815/39.2
Candidal colpitis	57/49.5	38/33	17/14.8	3/2.6	115/2.5
Trichomonas colpitis	29/49.1	16/27.1	13/22	1/1.7	59/1.3
Follicular cervicitis	47/49.5	25/26.3	13/13.7	10/10.5	95/2
Chlamydial infection	14/29.7	18/38.3	12/25.5	3/6.4	47/1
Viral infection (indirect signs)	269/39	269/39	131/19	20/2.9	689/14.9
Total	1696/36.6	1407/30.4	1065/23	461/9.9	4629/61

Among age groups, the highest rate of IDCU was registered in Group 1 (1696/36.6% cases) and Group 2 (1407/30.4% cases) (Table 1). In 1815/39.2% women, flora dysbiosis (bacterial vaginosis (BV) and *Lactobacillus* bacteremia (LB)) were found. The maximum frequency of flora dysbiosis was detected in Group 1 (740/40.7%) and Group 2 (693/38.2%). In BV, the cytogram presents an abundance of coccobacilli, "clue cell" and a small number of leukocytes (Fig. 2). Inflammatory processes have been identified in 1809/39% cases. The highest incidence was found in Group 1 (540/29.8%) and Group 3 (542/29.9%).

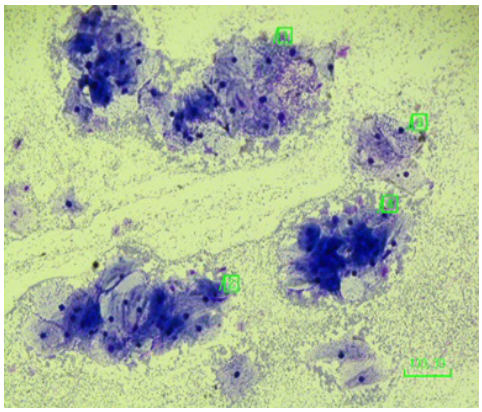
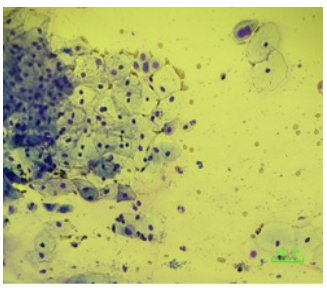
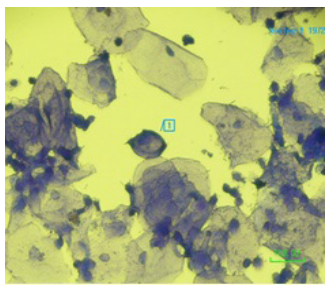


Fig. 2. *Bacterial vaginosis: an abundance of coccobacilli, "clue cell" and a small number of leukocytes; magnification x200.*

Currently, among sexually transmitted infections, the human papillomavirus (HPV) represents the greatest interest. HPV preferentially infects cells in the cervical transformation zone, an area of active cell turnover. Basal cells, which feature the HPV receptor, are a natural target for infection. The virus remains in the cell in a latent state until activation causes viral replication and squamous dysplasia. Low-grade squamous intraepithelial lesion/cervical intraepithelial neoplasia 1 (LSIL/CIN 1) is characterized by koilocytotic atypia, nuclear enlargement, hyperchromasia, and perinuclear cytoplasmic clearing (Figures 3.4). These features are the result of viral proteins that affect DNA synthesis and the structure of intermediate filaments in the host cell cytoplasm.[4] Indirect signs of viral infection were detected in 689/14.9% women, with a maximum frequency in Groups 1 and 2 (Table 1, Fig.3). These women were recommended to have HPV tests.



(3)



(4)

Figures 3, 4. *Binuclear cells and "kissing" nuclei in cervical smear for HPV infection (conventional smear) and koilocyte in liquid-based cytology (stained by Romanovsky-Giemsa); magnification x400.*

Candidal colpitis was registered in 115/2.5% women. In smears, budding yeast cells, spores, and pseudomycelium were found (Fig.5). Follicular cervicitis was detected in 2% of cases, with the highest frequency in Group 1 (47/49.5%) and Group 2 (25/26.3%). Cytological results of this pathology were characterized by the presence of lymphoid elements (Fig.6).

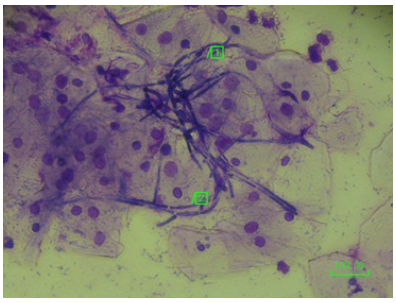


Fig. 5. Candidiasis. *Pseudomycelium and fungal spores in the cervical smear; magnification x400.*

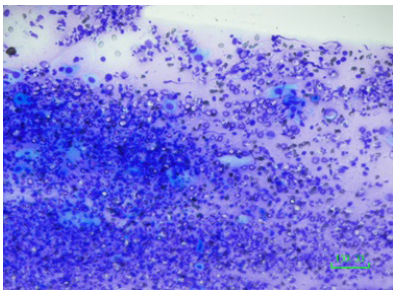


Fig. 6. *Follicular cervicitis; magnification x200.*

Trichomonas colpitis was detected in 1.3% of women, with the highest frequency in Group 1 (49.1%) (Table 1, Fig.7). Urogenital chlamydia was detected in 1% of women with the highest frequency in Group 2 (38.3%) (Figures 8,9). It should be noted that the data of cytological studies for the detection of chlamydial infection are only indicative and should be supplemented by other research methods (immunofluorescence analysis, PCR, etc.).

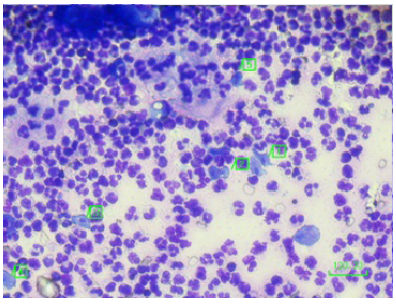
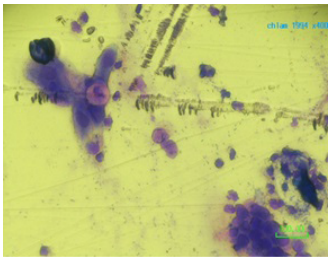
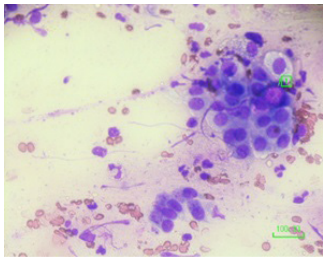


Fig. 7. *Trichomonas colpitis: Trichomonas and leukocytes in a ectocervical smear; magnification x400.*

BCLs include hyperplastic processes associated with hormonal disorders, as well as inflammatory and post-traumatic processes. The frequency of BCLs in different age groups is presented in Table 2. The highest incidence of BCLs was found in women of Group 2 (212/37.6%) and Group 1 (187/33.2 %). The most frequently diagnosed pathology was squamous cell metaplasia (74%) with maximum frequency in Group 2 (35.7%) and Group 1 (30.4%), obviously due to the intense influence of sex hormones (estrogens) in women of active reproductive age. Proliferation of glandular epithelial cells and cervical hyperkeratosis were registered much less frequently: 12% and 7.1%, respectively.



(8)



(9)

Figures 8, 9. *Chlamydial infection: the layers of metaplastic epithelial cells with vacuoles containing inclusions of various sizes; magnification x400.*

Table 2.

Benign cervical lesions in different age groups (in n/%)

	Group 1	Group 2	Group 3	Group 4	Total
Cervical hyperkeratosis	12/30	16/40	10/25	2/5	40/7.1
Squamous cell metaplasia. Microglandular hyperplasia	127/30.4	149/35.7	102/24.5	39/9.3	417/74
Proliferation of glandular epithelial cells	35/51.5	30/44.1	2/2.9	1/1.5	68/12
Dysplasia (atypical hyperplasia)	13/34.2	17/44.7	7/18.4	1/2.6	38/6.7
Total	187/33.2	212/37.6	121/21.5	43/7.6	563/7.4

Cervical intraepithelial neoplasia (or dysplasia) (CIN) was detected in 359/4.7% cases. Among them, CIN I, CIN II and CIN III were registered in 220/61.3%, 84/24.5%, and 38/10.6% women, respectively (Fig.10). At the same time, the maximum frequency of dysplasia was noted in Group 1 (32%) and Group 2 (33%). In Groups 3 and 4, dysplasia was found in 87(24.2%) and 39 (11%) cases, respectively.

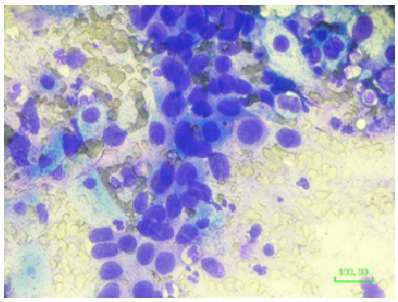


Fig. 10. *Parabasal cells: CIN II; magnification x400.*

Low-grade squamous lesions (CIN I), recognized as primarily the histologic manifestation of HPV infection,

usually resolve, but must be followed within 12 months to make sure that they do not progress to CIN 2 (moderate changes) and CIN 3 (high-grade changes). Depending on age groups (Fig.11), we found that the frequency of CIN I and CIN II was the same for women of Group 1 (19.7% and 7.2%) and Group 2 (19.2% and 7.5%). CIN III was found in 3.3%, 3.9%, and 3.1% for Groups 1, 2, and 3, respectively. Severe dysplasia with a transition to cancer was diagnosed in 4/1.1% women: two cases in Group 2, one case in Group 3, and one case in Group 4. Thus, our results indicate a high incidence of dysplasia in women of reproductive age.

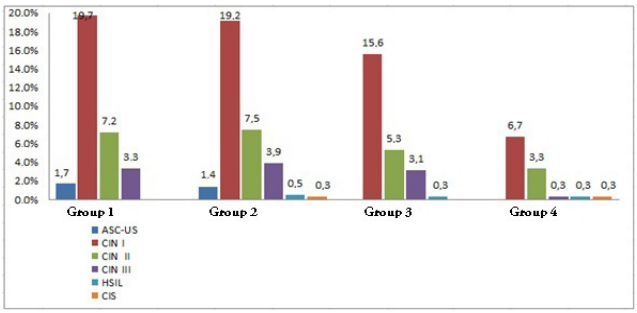


Fig. 11. *The incidence of cervical dysplasia in different age groups.*

As is well known, HPV infection increases the risk of dysplasia by 10 times. In the examined group of women, a combination of dysplasia with indirect symptoms of a viral infection was found in 207/57.6% cases. Taking into account that only a primary cytological examination was carried out in our Medical Institute Clinic, it is impossible to give a direct conclusion about the relationship between cervical dysplasia and a virus carrier. If a suspicious cervical lesion is identified in the first stage of the survey, the second stage of examination (colposcopy with target cervical biopsy, PCR methods for morphological verification of the diagnosis, histological examination) must be performed.

There have been a number of technological advances in CC screening since the introduction of Pap testing more than 60 years ago.[6] Among them, the implementation of routine screening programs is recognized as a public health success, whereas liquid-based cytology (LBC) represents a significant shift in testing methodology. This shift from conventional cytology to LBC has occurred due to improvements in sample quality, reproducibility, sensitivity, and specificity, as well as the ability to perform reflex molecular testing.[7,8] Pre-cancerous lesions detected during CC screening can easily be treated and cancer avoided. The new USPSTF [9] recommendations emphasize that the choice of screening strategy should consider the balance of benefit (disease detection) and potential harms (more frequent follow-up testing, invasive diagnostic procedures, and unnecessary treatment in women with false-positive results) and involve shared decision making between patients and their health care providers.

Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Polar Medicine

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The Relationship between Blood Thyroid Hormone and Dopamine Levels in Residents of the Arctic Regions of Russia

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Abstract

Due to the presence of contradictory results about the effect of dopamine on the synthesis and secretion of thyroid hormones, as well as the absence of this information in clinically healthy individuals, we were interested in studying this problem in permanent residents of the Arctic territories, which are characterized by high thyroid activity and dopamine content. ***The aim*** of this work was to study the hormone levels of hypothalamic–pituitary–thyroid axis (HPTA) under the different levels of dopamine in the blood of permanent residents of the Arctic territories.

***Materials and Methods*:** We examined 316 healthy individuals (the indigenous population, mestizos, and the local Russian population) born and permanently residing in the territories of the Russian Arctic zone. The examined participants were divided into 3 groups: Group 1 with undetectable levels of dopamine in the blood (0 nmol/l); Group 2 with reference levels of dopamine (<0.653 nmol/l); and Group 3 with increased levels of dopamine (>0.653 nmol/l). The serum levels of TSH, T4, free T4, T3, and free T3, and the plasma dopamine level were determined by enzyme immunoassay. The plasma cAMP level was determined by radioimmunoassay.

***Results*:** In individuals of Group 1, there was a decrease in the activity of hypothalamic–pituitary–thyroid axis (HTPA) and the peripheral conversion of iodothyronines compared with persons with reference or increased dopamine levels. In individuals of Groups 2 and 3, we found an increase in the activity of HTPA with an increase in the blood level of dopamine. The absence of the inhibitory effect of high levels of dopamine on HPTA hormones in the examined individuals may be a compensatory-adaptive response of the body under the conditions of permanently acting extreme factors of the North. (**International Journal of Biomedicine. 2019;9(1):43-47.**)

Key Words: dopamine • iodothyronines • cyclic adenosine monophosphate • Arctic

Abbreviations

cAMP, cyclic adenosine monophosphate; **HPTA**, hypothalamic–pituitary–thyroid axis; **TSH**, thyroid stimulating hormone; **T4**, thyroxine; **T3**, triiodothyronine; **fT3**, free T3; **fT4**, free T4.

Introduction

Numerous studies have shown that the activity of the sympathoadrenal system increases under adaptation of the body to the cold, which is especially important for people living in extreme conditions of high latitudes.[1,2] Thus, K. Harinath and colleagues[2] studied the role of the autonomic nervous system and adrenal system in acclimatization to cold in tropical men during short or prolonged sojourns at Antarctica. The study was carried out on volunteers of the winter over team (WOT) and summer team (ST) of an Indian Antarctic Expedition. This study suggested that Antarctic residency during austral summer

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resulted in gradual attenuation of sympathetic tone and a shift of autonomic balance toward the parasympathetic side. However, WOT members showed a predominance of sympathetic and adrenal activity compared with initial responses of ST members, suggesting deconditioning or possible resetting of the autonomic nervous system.

Most studies indicate the presence of a dopamine inhibitory effect on the secretion of thyroid hormones by changing the secretion of thyroliberin through the D2 receptors in the striatum, or on the secretion of TSH through the D2 receptors of thyrotropic cells, or by activating the dopamine receptors of thyroid cells.[3-7] Information about the stimulating effects of catecholamines, especially dopamine, on the synthesis of iodothyronines in modern literature is not enough,[8] although early studies have shown the direct stimulating effect of both sympathetic stimuli and exogenous catecholamines, including dopamine, on the synthesis and secretion of iodothyronines.[9]

Due to the presence of contradictory results about the effect of dopamine on the synthesis and secretion of thyroid hormones, as well as the absence of this information in clinically healthy individuals, we were interested in studying this problem in permanent residents of the Arctic territories, which are characterized by high thyroid activity and dopamine content.[10] Elucidation of the relationship between dopamine levels and the HPTA activity in the inhabitants of the Arctic territories will contribute to the development of human ecological physiology, as well as preventive medicine, to preserve the health of the population of the North.

The aim of this work was to study the hormone levels of HPTA under the different levels of dopamine in the blood of permanent residents of the Arctic territories.

Materials and Methods

An analytical cross-sectional uncontrolled study was conducted in the territories of the Russian Arctic zone in the period of increasing daylight hours (March). We examined permanent residents (110 men and 206 women between 22 and 65 years): the indigenous population (the Nenets and the Komi), mestizos, and the local Russian population that had been living in the North for at least 3 generations. The examined participants were divided into 3 groups: Group 1 included 11 men and 45 women with undetectable levels of dopamine in the blood (0 nmol/l); Group 2 included 70 men and 128 women with reference levels of dopamine (<0.653 nmol/l); and Group 3 included 29 men and 33 women with increased levels of dopamine (>0.653 nmol/l). The mean age of subjects in groups was 45.4±1.5, 45.5±0.9 and 45.1±1.5 years, respectively. At the time of the survey, subjects did not have a registered endocrine pathology and exacerbations of chronic diseases.

The study was conducted in accordance with the ethical principles stated in Declaration of Helsinki of 1964 (revised in Seoul in 2008). The study was approved by the Ethics Committee of N. Laverov Federal Center for Integrated Arctic Research. Written informed consent was obtained from all participants.

Blood samples (5 ml) were obtained from the ulnar vein on an empty stomach between 8:00 and 10.00 a.m. in IMPROVACUTER' Evacuated Blood Collection Tubes. On 1-plate fully automated ELISA analyzer (Elisys Uno, Germany), the serum levels of TSH, T4, fT4, T3, and fT3 (using test kits from Alkor-Bio, Russia), and the plasma dopamine level (using test kits from LDN, Germany), were determined by enzyme immunoassay. The plasma cAMP level was determined by radioimmunoassay on the ARIAN device (Vitaco, Russia) using RIA kits "Immunotech" (Czech Republic).

Statistical analysis was performed using the statistical software «STATISTICA 10». The normality of distribution of continuous variables was tested by Shapiro-Wilk's W test. Median (Me), the 95% confidence interval (95% CI), and percentage deviations from reference value were calculated. Spearman's rank correlation coefficient (r_s) was calculated to measure the strength and direction of the relationship between two variables. A probability value of $P<0.05$ was considered statistically significant.

Results

Most of the examined individuals, both men and women, had dopamine levels within the reference values (64% and 62%, respectively). Undetectable levels of dopamine were recorded twice as often in women than in men (22% versus 10%, respectively, $P=0.01$).

The increased levels of dopamine were detected in 26% of men and 16% of women. Analyzing the content of the HPTA hormones in the examined individuals with different concentrations of blood dopamine (Table 1), we found that both men and women of Group 1, compared to other groups, were characterized by minimum levels of TSH, T4, fT3, cAMP, and the fT3/fT4 ratio against the background of the maximum levels of fT4. A decrease in cAMP levels was especially pronounced. Thus, cAMP levels were lower than the normative levels in 57% of men and 61% of women of Group 1.

In persons of Group 2, the levels of TSH, T4, fT3, cAMP, and the fT3/fT4 ratio were higher, and the fT4 level was lower compared to Group 1. Interesting features were noted regarding the cAMP content in Group 2, taking into account the gender aspect. It turned out that the percentage of people with low cAMP levels was higher among women than among men (27% and 12%, $P=0.042$). At the same time, the levels of thyroid hormones, TSH and cAMP did not significantly differ in Groups 2 and 3, with the exception of the fT3/fT4 ratio. The analysis showed a statistically significant increase in the fT3/fT4 ratio, with increasing dopamine values in both men and women.

In Group 3, regardless of gender, we found an increase in the proportion of individuals with elevated values of fT3 and cAMP, compared with Group 2, (25% versus 13% and 18% versus 3%, respectively, $P<0.05$ in both cases). Correlation analysis revealed the presence of a significant negative relationship between increased levels of dopamine and fT4 levels in women ($r_s=-0.427$; $P=0.019$).

Table 1.

The content of the HPTA hormones and cAMP in the examined individuals with different concentrations of blood dopamine

Variable	Men			Women			*P*-value
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	
	Me 95%CI	Me 95%CI	Me 95%CI	Me 95%CI	Me 95%CI	Me 95%CI	
	1	2	3	4	5	6	
Dopamine, nmol/l	0 (0; 0)	0.43 (0.37; 0.46)	0.77 (0.74; 0.88)	0 (0; 0)	0.36 (0.33; 0.41)	0.78 (0.75; 0.89)	$P_{1\text{-}2;1\text{-}3;2\text{-}3}<0.001$ $P_{4\text{-}5;4\text{-}6;5\text{-}6}<0.001$
TSH, μIU/l	1.2 (0.9; 2.4)	2.0 (1.7; 2.4)	2.2 (1.6; 2.7)	1.6 (1.3; 2.1)	2.5 (2.1; 2.9)	2.3 (1.5; 2.5)	$P_{1\text{-}3}=0.041$ $P_{4\text{-}5}=0.006$
T4, nmol/l	79.7 (74.6; 105.7)	104.2 (96.7; 113.2)	105.3 (93.1; 112.3)	105.9 (101.6; 110.4)	107.5 (105.2; 110.6)	113.3 (108.5; 122.7)	$P_{1\text{-}2}=0.039$; $P_{1\text{-}3}=0.022$ $P_{4\text{-}6}=0.045$; $P_{1\text{-}4}=0.034$ $P_{2\text{-}5}=0.044$; $P_{3\text{-}6}=0.040$
fT4, pmol/l	15.5 (12.5; 18.7)	14.5 (13.6; 15.4)	13.7 (12.8; 15.2)	15.2 (14.5; 16.5)	14.3 (13.8; 14.7)	13.5 (12.8; 14.3)	$P_{4\text{-}5}=0.002$ $P_{4\text{-}6}=0.003$
T3, nmol/l	1.5 (1.3; 1.9)	1.7 (1.5; 1.8)	1.6 (1.3; 1.8)	1.7 (1.4; 1.9)	1.7 (1.5; 1.9)	1.6 (1.4; 2.2)	$P>0.05$
fT3, pmol/l	4.8 (3.9; 5.6)	5.6 (5.4;5.8)	5.9 (5.4; 6.3)	4.3 (3.9; 4.7)	5.4 (5.2; 5.6)	5.5 (5.1; 5.9)	$P_{1\text{-}2}=0.002$; $P_{1\text{-}3}=0.002$ $P_{4\text{-}5}<0.001$; $P_{4\text{-}6}<0.001$
cAMP, nmol/l	14.5 (11.8; 43.1)	22.1 (20.3; 23.9)	24.1 (19.0; 28.8)	15.3 (12.5; 19.4)	21.5 (18.4; 23.9)	22.6 (12.1; 30.4)	$P_{1\text{-}2}=0.049$; $P_{1\text{-}3}=0.048$ $P_{4\text{-}5}<0.001$; $P_{4\text{-}6}=0.026$
fT3/fT4	16.7 (8.3; 19.6)	10.8 (7.5; 10.8)	12.5 (6.8; 13.4)	12.4 (8.0; 15.9)	7.9 (6.3; 8.4)	8.2 (7.4; 14.9)	$P_{4\text{-}5}=0.020$
$\frac{(fT3+fT4)}{TSH}$	0.27 (0.25; 0.37)	0.39 (0.36; 0.39)	0.51 (0.35; 0.51)	0.27 (0.24; 0.29)	0.39 (0.37; 0.41)	0.41 (0.36; 0.44)	$P_{1\text{-}2;4\text{-}5;4\text{-}6}<0.001$ $P_{1\text{-}3}=0.004$; $P_{2\text{-}3}=0.04$

Discussion

Previous research has shown substantial seasonal changes in thyroid hormone activity among residents and sojourners to high latitudes.[11] In a series of studies among sojourners to Antarctica, Reed et al.[12-14] identified a constellation of physiological changes in response to severe cold exposure and reduced photoperiod. This cluster of responses, known as the "polar T3 syndrome," is associated with increased rates of production and clearance of T3, the active form of thyroid hormone, and T4, generally leading to reduced circulating levels of fT3 and fT4.[13-15] N.V. Do et al.[16] demonstrated that the adaptive changes in thyroid hormone economy with Antarctic residence reflect TSH dependent changes in thyroid synthetic activity, which may help explain a portion of the increases in T3 production found with Antarctic residence.

Research conducted in northern Europe, however, documents greater variability in seasonal changes in thyroid function, demonstrating both winter increases and decreases in fT3 and fT4.[17-18] Levy et al.[11] showed that the indigenous Yakut of northeastern Siberia experience marked seasonal changes in thyroid function. The marked declines in both fT3 and fT4 in Yakut men and women suggest an enhanced capacity to increase metabolic heat production during the severe winter cold.

Dopamine, a potent regulator of TSH secretion, can suppress TSH secretion in vivo[19] and in vitro.[20,21] The numerous studies show that the levels of thyroid hormones follow dopamine levels.[22,23]

The results of our study indicate parallel changes in the blood levels of dopamine and HPTA hormones in healthy people living in the Arctic territories. The absence of the inhibitory effect of high levels of dopamine on HPTA hormones in the examined individuals may be a compensatory-adaptive response of the body under the conditions of permanently acting extreme factors of the North.

Analysis of iodothyronines, their ratios and TSH in individuals with different blood levels of dopamine showed that in both men and women the most critical factor for thyroid activity and peripheral conversion of iodothyronines is undetectable levels of dopamine, which is confirmed by low cAMP levels, indicating a slowing down of the intracellular processes of synthesis of biologically active substances. It can be concluded that undetectable levels of dopamine in peripheral blood correlates with a lower activity of HPTA and peripheral conversion of iodothyronines, which can be an indicator of metabolic disturbances and a decline in overall well-being in conditions of iodine deficiency in the diet. Thus, the high frequency of undetectable blood dopamine levels among the female population may be a criterion for the risk of developing various somatic and psychological disorders.

Higher values of iodothyronines, cAMP and fT3/fT4 ratio in individuals with reference blood dopamine levels, compared with those with undetectable levels, may indicate

the stimulating effects of dopamine on the HPTA activity[24] and peripheral conversion of iodothyronines[25] at the reference dopamine values. MJ Obregon and colleagues[25] studied the direct effects of catecholamines on the activity of the type II iodothyronine 5'-deiodinase in dispersed rat brown adipocytes. Incubation with norepinephrine or phenylephrine for 3-4 h causes up to a 5-fold increase in deiodinase activity in these cells. At the same time, higher TSH levels in people with reference dopamine levels may be due to the adverse climatic factors causing both the activation of the sympathoadrenal system and the increased HPTA activity.[1,26]

In addition, there may be an opposite effect associated with the stimulation of synthesis and secretion of catecholamines by thyroid gland hormones.[27] It should be noted that increased levels of dopamine in the identified ranges, as well as its reference values, are associated with activation of HTPA, namely, an increase in T4 production and its conversion to more biologically active fT3, compared with the undetectable values of dopamine. Increased cAMP levels may also be an indirect sign of the activation of intracellular mechanisms by dopamine or TSH.[28]

Thus, the example of healthy individuals born and permanently residing in the territories of the Russian Arctic zone, regardless of gender, shows an increase in the activity of HTPA with an increase in the blood level of dopamine. In individuals with undetectable dopamine values, there is a decrease in the activity of HTPA and the peripheral conversion of iodothyronines compared with persons with reference or increased dopamine levels. The absence of the inhibitory effect of high levels of dopamine on HPTA hormones in the examined individuals may be a compensatory-adaptive response of the body under the conditions of permanently acting extreme factors of the North.

Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Psychophysiology

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Associations Between Neuropsychophysiological and Dermatoglyphic Indicators in the Assessment of Human Health

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Abstract

The purpose of this study was to explore the relationship between psycho-physiological markers of human health and dermatoglyphic indicators in young people.

Materials and Methods: The study included 920 healthy volunteers aged between 18 and 21 years. All volunteers underwent the following examinations: EEG, an assessment of the anxiety level according to the BAI, and dermatoglyphic scanning.

Results: According to the data obtained, there was a statistically significant strong negative correlation between the stress load indicator and dermatoglyphic data, such as the summary delta index (DI) and summary ridge count. A strong positive correlation was found between the percentage of whorls and stress (r=0.88). The predominant increase in anxiety is characteristic of persons with total ridge count (TRC) on the thumb of the right hand in the range from 19 to 23.

Conclusion: Results demonstrate the interrelationships (association) between psycho-physiological (anxiety level, stress load indicator) and dermatoglyphic markers (DI, TRC and whorl pattern type) in young healthy people. (**International Journal of Biomedicine. 2019;9(1):48-51.**)

Key Words: anxiety • electroencephalography • young people • dermatoglyphic markers

Abbreviations

BAI, the Beck Anxiety Inventory; **DI**, delta index; **EEG**, electroencephalography; **SLI**, stress load indicator; **SRC**, summary ridge count; **TRC**, total ridge count.

Introduction

Dermatoglyphic features begin developing during early stages of fetal life and are fully formed by the end of the fourth month of intrauterine life, and very importantly, they do not change throughout postnatal life.[1,2] Finger ridge counts and patterns are a model human polygenic trait in quantitative genetic analyses because they are some of the few age-independent human traits.[3] Dermatoglyphic studies are widely used in predicting the diversity of human individuality.[4-7] The prerequisites for such predictions are determined by the manifestation of not only the physical and physiological, but also the mental properties of the individual.[8-11] Dermatoglyphics as a diagnostic aid and supportive evidence in the diagnosis of several diseases, such as diabetes, mongolism, Tel Hashomer camptodactyly syndrome, and schizophrenia, has been reported in recent literature.[12-15]

The purpose of this study was to explore the relationship between psycho-physiological markers of human health and dermatoglyphic indicators in young people.

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Materials and Methods

The study was conducted on the basis of the Scientific and Educational Center "Morphology and Physiology of a Healthy Person" at the Krasnoyarsk State Medical University named after Professor V.F. Voino-Yasenetsky. The present study was approved by the local Ethics Committee. The subject of the research was the population of the Krasnoyarsk city and Krasnoyarsk Krai.

A total of 4,919 people aged over 18 years were surveyed. For this study, we identified a group of healthy volunteers (409 young men and 511 young women) aged between 18 and 21 years. Volunteers did not receive any remuneration for participating in the study. All volunteers underwent the following examinations: EEG, an assessment of the anxiety level according to the BAI, and dermatoglyphic scanning.

The EEG was performed using the computer system Neurokartograf (MBN, Russia), including spectral, power, and coherent analysis methods. We evaluated the characteristics of the neurophysiological maturation, the bioelectrical activity of the brain, and the state of the hemispheric and inter-hemispheric connections.[16-19]

The BAI was used to assess the level of clinical anxiety of the participants. The BAI is a 21-item self-report inventory, with high reliability for measuring clinical anxiety. The total score is calculated by finding the sum of the 21 items. Score of 0-21 = low anxiety, Score of 22-35 = moderate anxiety, Score of 36 and above = potentially concerning levels of anxiety. [20]

The fingerprint patterns were assessed for both hands, starting from the thumb to little finger. We analyzed the patterns of fingers according to Henry's system of classification (1900), which classifies fingertip patterns into three main types, namely, whorls (W), ulnar and radial loops (UL, RL), and arches (A). The percent distribution of these patterns was calculated for both hands, and the prevalence of dermatoglyphic phenotypes was assessed. To assess the asymmetry of different types of fingerprint patterns, DI was calculated separately on each finger of the right and left hands. We also calculated the local ridge count on each finger, TRC for the right and left hands, and the sum of the ridges on the 10 fingers of both hands (SRC).[21-23]

Statistical analysis was performed using IBM SPSS Statistics V22.0 (SPSS Inc., Chicago, IL, USA), StatPlus 2009 Professional 5.8.0, and Microsoft Excel (2013). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean and standard deviation (SD). Categorical variables were analyzed using the Chi-square test with the Yates' correction. Spearman's rank correlation coefficient was calculated to measure the strength and direction of the relationship between two variables. We used two-stage cluster analysis and data visualization with regression variable charts. Data mining analysis based on decision trees was used to evaluate the associations between various dermatoglyphic and neurophysiological parameters.[24] The logistic regression method was also applied. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

According to the data presented in Figure 1, there was a statistically significant strong negative correlation between the stress load indicator and dermatoglyphic data, such as the summary DI and SRC. A strong positive correlation was found between the percentage of whorls and stress (r=0.88).

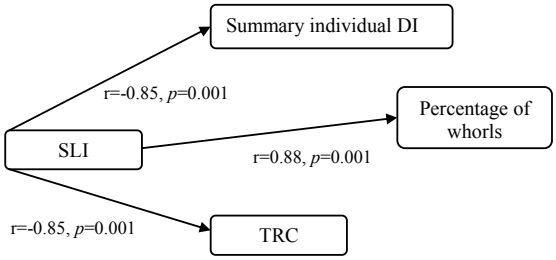

Fig. 1. *Correlations between psycho-physiological and dermatoglyphic markers.*

Two-stage cluster analysis was used to identify group similarities among the studied accounting features (Fig.2). A model with the quality of clusters on silhouette measure of connectivity and separation of clusters over 0.5 was obtained. Analysis of Figure 2 allows allocating three clusters. Cluster 2 has the average values of stress load index, delta indices, the percentage of whorls, TRC, and the fourth dermatoglyphic phenotype. Cluster 1 combined the lower values of dermatoglyphic indices, the first dermatoglyphic phenotype and the lower values of the stress load indicator. Cluster 3 is its opposite and includes the fifth dermatoglyphic phenotype.

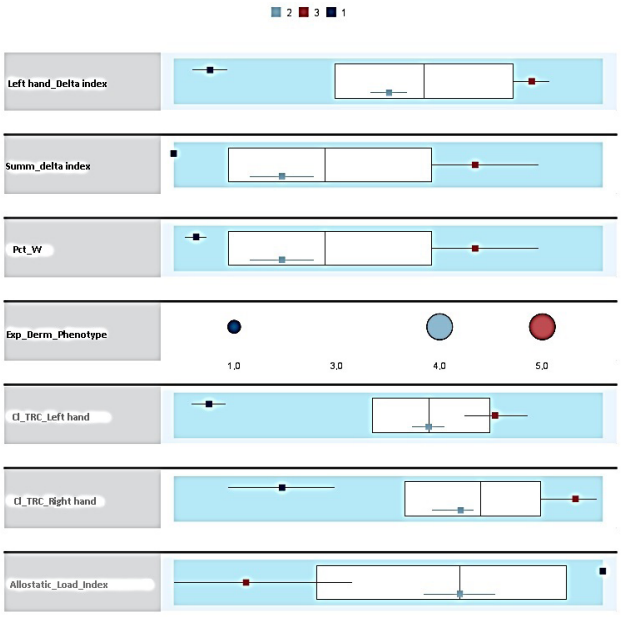

Fig. 2. *Comparison of clusters in psychophysiological and dermatoglyphic markers.*

The scatter plot (Fig.3) suggests the possibility of a non-linear relationship between the BAI score and the ridge

count on the thumb of right hand, which is confirmed by the decision tree data (Fig.4). The predominant increase in anxiety is characteristic of persons with TRC on the thumb of the right hand in the range from 19 to 23.

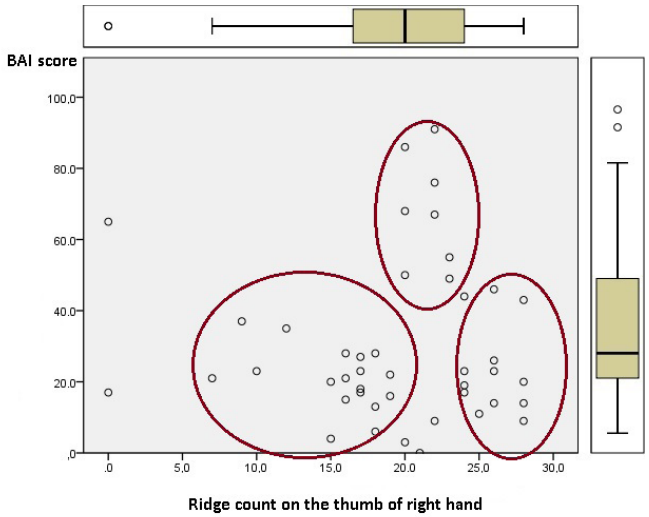

Fig. 3. *BAI score depending on the ridge count on the thumb of right hand.*

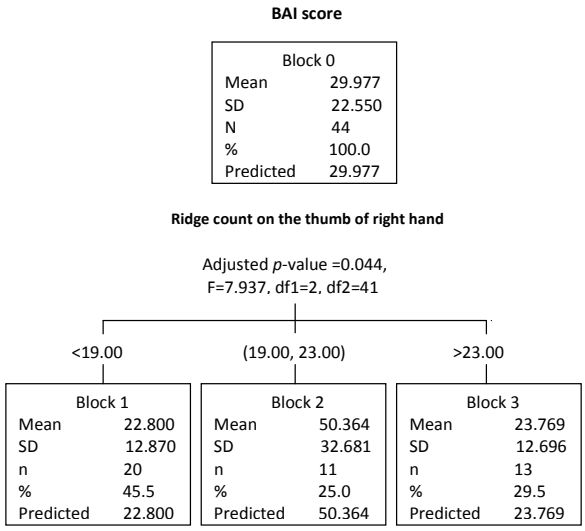

Fig.4. *The decision tree data.*

Table 1 presents the results of the construction of logistic regression, where the binary value of the stress factor up to 300 and below acts as a variable; dermatoglyphic indices (W,%) were chosen as influencing factors.

Table 1. Linear regression model

	Regression coeficient (b)	Standard Error	Wald test χ^2	DF	Sig. (p)	Exp (b)	95% CI for Exp(b)	
							Lower Bound	Upper Bound
Percentage of whorls	-.091	.046	3.981	1	.046	.913	.834	.998
Constant	1.952	1.060	3.391	1	.066	7.039		

After 6 iterations, a regression model was obtained with the Nagelkerke R^2 of 0.59 and a fraction of the predicted values of 77.8%.

Conclusion

This study demonstrated the i*nterrelationships* (association) between psycho-physiological (anxiety level, stress load indicator) and dermatoglyphic markers in young healthy people. Dermatoglyphic markers (DI, TRC and whorl pattern type) can be used as additional markers to assess the state of psycho-physiological health of the population.

Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Respiratory Infections

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Clinical Efficacy of Sodium Deoxyribonucleate in the Treatment of Acute Respiratory Infections

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Abstract

The aim of the study was to evaluate the effectiveness of sodium deoxyribonucleate (spray) in the treatment of patients with acute respiratory infections (ARI) in the outpatient setting.

Materials and Methods: We conducted a randomized controlled clinical trial. The study included 112 patients aged from 18 to 73 years with an established diagnosis of ARI and a duration of symptoms of the disease no more than 48 hours. The investigational drug – a 0.25% solution of sodium deoxyribonucleate (SD) for external and topical use (spray bottle, 10 ml). Group 1 included 54 patients who received SD: 2 intranasal doses into each nostril every 1-1.5 hours during the first days of the onset of the ARI symptoms; then - 2 doses into each nostril 3 times per day against the background of symptomatic therapy for 5 days. Symptomatic therapy included decongestants, antipyretics, mucolytics and antitussives (if necessary). Group 2 included 58 patients who received only symptomatic therapy. Medical examination was performed daily for 5 days.

Results: Including a 0.25% SD spray solution in the ARI treatment regimen effectively affects the dynamics of the ARI symptoms. SD in the treatment scheme from the first day allows eliminating the main ARI symptoms much faster and more efficiently than the standard treatment regimen; in particular, recovery is accelerated by 2-3 days. Complex therapy including SD causes a 3-fold increase in the level of sIgA in the nasal mucosa of patients on the fifth day of treatment, which indicates a pronounced immunomodulating effect of this intervention, a potential reduction in the risk of complications and recurrence of viral diseases.(**International Journal of Biomedicine. 2019;9(1):52-56.**)

Key Words: acute respiratory infections • secretory immunoglobulin A • squamous epithelium • symptomatic therapy

Abbreviations

ARI, acute respiratory infections; **ADR**, average degradation rate; **FO**, frequency of occurrence; **sIgA**, secretory immunoglobulin A; **SD**, sodium deoxyribonucleate; **SE**, squamous epithelium.

Introduction

Acute respiratory infections (ARI) are the leading cause of morbidity and mortality worldwide. ARI can occur in the upper and lower respiratory tracts and constitute a substantial disease burden in all age groups.[1,2] They are the most common reason patients seek ambulatory care.[3-6]

In recent decades, there has been a tendency toward an increase in the prevalence of diseases of the upper respiratory tract, which cover an increasing number of the working population. Untreated, neglected and inadequately treated respiratory infections cause an increase in the incidence of, and contribute to the chronicity of, the diseases of the bronchopulmonary system, covering up to 7% of the total adult population of Russia.[2,7,8]

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Diagnosis and treatment of ARI often cause certain difficulties for specialists. This circumstance is largely determined by the inability to establish the causative agent of the disease and the high cost of laboratory diagnosis, which, as a rule, is carried out in infectious hospitals and when an epidemically unfavorable situation occurs.[3] ARI are a group of diseases caused by different microorganisms. A viral etiological agent is estimated to be present in up to 80% of cases.[9-11] Influenza viruses are one of the main causative agents of ARI worldwide; however, many other respiratory viruses for which insufficient epidemiological information is available can also cause ARI. In the outpatient setting, it is extremely difficult to conduct a rapid diagnosis to identify the virus that caused the disease; therefore, the use of the term "acute respiratory viral infections" as a diagnosis is justified, since it indicates a non-bacterial etiology of the disease and makes it inappropriate to prescribe antibacterial medications.[2,7] On average, adults suffer from ARI 2-3 times a year.

The polyetiologic nature of respiratory diseases does not allow one to use only specific vaccine preparations[8,12] and dictates the need to use various nonspecific means[8,13,14] that stimulate the natural resistance of the human body. In addition, immunomodulators are widely represented in the pharmaceutical market; however, it is extremely difficult to assess the real declared effectiveness. Many studies [3,5,12,15,16] have shown that the maximum clinical effect of immunomodulating drugs in the complex treatment of ARI can be obtained with a combination of immunotropic action and reparative effects of drugs, which together form pronounced clinical efficacy.[13]

In light of the above, sodium deoxyribonucleate (SD) in the form of a "spray" is of certain interest for ARI therapy. The effects of this drug are due to the stimulation of the functional activity of T-helpers and T-killers, neutrophils and monocytes/macrophages,[13,17] and the enhancement of adaptive resources of mucosal immunity, which leads to the restoration of the epithelium lining the upper respiratory airways and potentiates local immunity.[18]

The aim of the study was to evaluate the effectiveness of SD (spray) in the treatment of ARI patients in the outpatient setting.

Materials and Methods

We conducted a randomized controlled clinical trial. The study included 112 patients aged from 18 to 73 years with an established diagnosis of ARI and a duration of symptoms of the disease no more than 48 hours.

Exclusion criteria: individual intolerance of the applied therapy; antiviral and immunomodulatory therapy; chronic diseases of the upper and lower respiratory tract; participation in any clinical trial during the previous month; violation of the procedure protocol. All participants provided the written informed consent.

The investigational drug – a 0.25% solution of SD for external and topical use (spray bottle, 10 ml).

The main group (Group 1) included 54 patients who received SD: 2 intranasal doses into each nostril every 1-1.5 hours during the first days of the onset of the ARI symptoms; then - 2 doses into each nostril 3 times per day against the background of symptomatic therapy for 5 days. Symptomatic therapy included decongestants, antipyretics (in cases of ≥38.5°C), mucolytics and antitussives (if necessary). The comparison group (Group 2) included 58 patients who received only symptomatic therapy.

All patients underwent treatment in the therapeutic department at the Voronezh City Clinical Polyclinic No. 4. Examination of patients included collecting anamnesis, complete physical examination, complete blood count using the automated analyzer ADAMS-A1C HA-8160 (Arkray, Kyoto, Japan), urine analysis, direct immunofluorescence study of the nasal scrapings for viral antigens with a luminescent microscope of MIKMED-2 option 11 (Russia), determination of sIgA level on the IMMULITE 2000 XPi immunoassay system (Germany) and the surface layer condition of the epithelium using the Nikon ECLIPSE Ni-U microscope (Germany).

To assess the state of cytological forms of the squamous epithelium of the nasal mucosa, the imprint smear technique was used. The manipulation was performed with a cotton swab dipped in sterile saline at the level of the inferior nasal concha. Rotational movements without pressure put smears on non-fat glass, dried, fixed and stained by Romanovsky-Giemsa. Microscopy was performed under immersion with magnification (7.0×9.0). Two hundred cells were counted on a slide. In nasal smears, the number and cytoforms of squamous epithelium were determined with an assessment of the nature of the cell location, vacuolation of the cytoplasm and nuclei, dyschromia of the cytoplasm and nuclei, the shape and size of cells and nuclei, and the presence of multicore cells. To assess destructive processes according to the methodology proposed by L. Matveeva (1993), 5 classes of destruction (0, 1, 2, 3, 4, 5), cell destruction index (CDI), mean destruction index (MDI) and cell cytolysis index (CCI) were determined in the nasal mucosa.

Medical examination was performed daily for 5 days. The severity of the symptoms was assessed in points (0 - complete absence, 1 – a mild severity, 2 – a medium severity, and 3 – a high severity). On the sixth day, at the end of the course of treatment, we registered complaints and conducted thorough physical and laboratory examinations. To determine the final score, the doctor counted the sum of points for each symptom separately. Thirty days after the start of treatment, the doctor interviewed patients by phone about well-being in the period since the end of the intake of the investigational drug. The safety and tolerability of the investigational drug was evaluated on the basis of an analysis of the frequency of occurrence (FO) of adverse events in all patients who received at least one dose of SD. The effectiveness of therapy was assessed according to the data obtained on the frequency of elimination of the pathogen, regression of clinical manifestations of ARI, the presence of complications, and repeated visits to the doctor caused by repeated episodes of respiratory infection. Symptom regression was considered to be 0 points, i.e. its complete absence.

Characteristics of the investigational drug

SD is an immunomodulatory agent, a regenerant, and a reparant. According to the literature, SD is an agonist for Toll-

like receptor 9 (TLR-9, CD289).[13,17] The immunomodulating effect of the drug is due to the interaction of the active substance (cytosine-guanine) with TLR9 on immunocompetent cells, which leads to the subsequent activation of a number of immune mechanisms. First, the stimulation of TLR in dendritic cells increases their ability to influence the differentiation of T helper cells in the direction of the formation of Type 2 T helper cell*s* (Th2). Under the influence of Th2, B-lymphocytes differentiate into plasma cells secreting IgG2, IgG4, and IgM. Stimulated by TLR9, epithelial cells enhance the secretion of sIgA, which performs both the barrier function and the function of opsonin for interaction with the cellular element of the local immune response: macrophages and NK. Thus, stimulation of macrophage TLR9 with an increase in IFNγ production leads to the activation of three levels of antiviral macrophage response.

Statistical analysis was performed using StatSoft Statistica v10.0 Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. Group comparisons with respect to categorical variables are performed using chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

During the initial examination, it was found that weakness and asthenia prevailed in patients of both groups. The febrile syndrome was most pronounced and manifested in 41 patients of Group 1 and in 46 patients of Group 2. FO for rhinorrhea, nasal congestion and oropharyngeal hyperemia (as components of the catarrhal syndrome) was 0.82, 0.91, and 0.65 in Group 1 and 0.78, 0.88, and 0.64 in Group 2, respectively. During the dynamic daily observation, it was found that FO for weakness and asthenia (symptoms of intoxication syndrome) was significantly lower ($P<0.05$) in Group 1, starting from the third day of treatment, compared to Group 2: 0.39 and 0.44 against 0.86 and 0.86, respectively. In Group 2, FO for these manifestations reached the values of Group 1 on day 5/6 of treatment. In the majority of patients of Group 1, the body temperature returned to a normal value on the third day of treatment; In Group 2, the value of 36.8°C was achieved only on the fifth day of treatment ($P<0.05$). In Group 1, catarrhal phenomena in the oropharynx significantly decreased on the third day of treatment with FO of 0.22 ($P<0.05$). A comparable FO of these phenomena in patients of Group 2 was achieved only by day 5/6 of therapy. In Group 1, in most cases, there was a clear positive trend in clinical symptoms: The nasal congestion almost completely disappeared by the end of the first day of treatment (FO was 0.12 on the second day of therapy); cough and symptoms of pharyngitis completely disappeared on the fourth day of treatment (FO was 0.03 on the third day of therapy). In Group 2, the vector of changes in clinical symptoms was similar to the dynamics of patients in Group 1; however, the minimum OF for the symptoms was shifted by 2-3 days. Thus, FO for nasal congestion was 0.11 only by the sixth day of treatment, and the cough did not stop with FO of 0.21 by the seventh day. It was possible to achieve a regression of swelling of the tonsils and signs of pharyngitis in 20% of cases in Group 2 only by the sixth day of treatment. Differences in the regression of pharyngitis symptoms were statistically significant ($P<0.05$). Compared to patients of Group 2, patients of Group 1 had a statistically higher incidence of regression for rhinorrhea symptoms by the third day of therapy—in 83% and 50% of cases, respectively ($P<0.05$). In patients of both groups, the same percentage (3%) of cases did not reveal the dynamics of rhinorrhea severity. After completion of treatment, the body temperature of all patients in Group 1 did not exceed normal values, while in Group 2, a subfebrile condition remained in one patient.

sIgA level assessment

Our analysis showed that the sIgA level in patients was lower the physiological norm by 65% ($P<0.05$) and amounted to 0.42±0.03 pg/ml. In Group 2, the initial level of sIgA was reduced more than 3.5 times compared with the physiological norm. In Group 1, after therapy, the level of sIgA significantly increased almost 4 times and reached 1.64±0.02 pg/ml ($P<0.05$). In Group 2, the sIgA level increased more than 2 times and reached 0.79±0.16 pg/ml, but this growth did not reach the level of the physiological norm, making up only 70% of the normal level.

Cytological characteristics of the nasal smears

An analysis of the morphological picture of imprints from the nasal mucosa in patients from both groups prior to the start of therapy showed a significantly similar picture (Table 1): The proportion of free flat epithelial cells exceeded the physiological norm by 3 times; CDI was 54% higher than the physiological norm. A large number of pycnomorphic cells and cells with reduced tinctorial characteristics were present. This condition of the nasal mucosa fully reflected the picture of ARI with dominant catarrhal and intoxication syndromes. After treatment, in Group 1 there was a significant decrease in the content of free squamous epithelium to the values of the physiological norm, cytolysis and destruction of epithelial cells also decreased, and multicore cells were absent.

Table 1.

Cytological characteristics of the nasal smears

Variable	Physiological norm	Group 1		Group 2	
		Before treatment	After treatment	Before treatment	After treatment
SE (%)	24.5±1.8	68.2±9.5*	26.4±2.1*	71.2±8.5*	51.3±4.5*
ADR	0.53±0.04	0.81±0.01	0.52±0.01*	0.81±0.01*	0.69±0.05*
CCI	0.04±0.02	0.08±0.01	0.04±0.01	0.08±0.01	0.06±0.02
CDI	0.48±0.08	0.83±0.04	0.41±0.03	0.79±0.04*	0.64±0.03
MDI	0.41±0.01	0.61±0.01	0.39±0.02*	0.68±0.01*	0.64±0.01*

**-P<0.05; ADR- average degradation rate; SE, squamous epithelium; CDI-cell destruction index; MDI- mean destruction index; CCI- cell cytolysis index.*

In Group 2, CDI and CCI decreased an insignificant amount as compared with the data before the start of therapy; there was a significant decrease (no more than 15%) in the free epithelium, but it did not reach the level of the physiological norm; pycnomorphic flat epithelial cells were freely detected in the fields of view. Thus, it can be concluded that in Group 1, by day 5 of therapy, the epithelium of the nasal mucosa had been morphologically restored and the possibility of local protection of the "entrance gate" of a viral infection was restored.

The timing of the regression of ARI symptoms

Analysis of the objective data from the physical examination led to the conclusion that the addition of SD to the symptomatic therapy in Group 1 contributed to a reliable regression of the pathological symptoms of ARI by the third to fourth day of therapy; a similar trend was observed in Group 2 by the sixth to seventh day. It should be noted that in Group 1, on the second day of therapy, only 2 patients remained with moderately marked rhinorrhea compared with Group 2, where the same symptom was observed in 38 patients. By the second to third day of treatment in Group 1, nasal congestion was stopped in 95% of patients and in 32% of patients in Group 2. In Group 1, the swelling of the tonsils was stopped on the second day of treatment in 87% of patients, and on the third day was absent in all patients. In Group 2, this symptom was stopped in 39% of patients by the fourth day of therapy. By the end of therapy, catarrhal syndrome was more often absent in Group 1 patients. The cough was completely stopped in Group 1 by the fourth day of therapy and persisted until the sixth to seventh day in Group 2. Intoxication syndrome was completely stopped by the fourth day of therapy in Group 1 and continued up to the sixth to seventh day of observation in Group 2. Weakness and asthenia persisted in patients of Group 2 until the sixth day of therapy with FO of 0.36 and 0.15, respectively. By the end of the observation, the need for symptomatic treatment had disappeared in 96% of patients in Group 1 and 86% of patients in Group 2; however, in Group 1, the need for symptomatic treatment by the third day of therapy was significantly lower, by 60%. Cases of undesirable reactions or side effects, which are not provided in the instructions for the medical use of SD (RU No. 002916 dated August 18, 2008), were not established during the study.

Remote assessment of the effectiveness of therapy

After the completion of therapy, the majority of patients in both groups (Group 1 - 46/85.2% and Group 2 - 50/86.2%) did not experience any symptoms of a past respiratory illness. Thirty days after the start of treatment, in order to detect repeated episodes of ARI and assess the safety of the treatment being performed, all patients were interviewed by phone. Repeated appeals regarding the symptoms of ARI within 30 days from the moment of discharge were recorded in 4/7.4% patients of Group 1 and in 16/27.6% patients of Group 2. It should be noted that 4 patients in Group 2 developed acute bronchitis; therefore, the treatment of ARI was regarded as ineffective. Acute bronchitis was stopped for several days while patients were taking antibacterial drugs. Additional data on the manifestation of complications in patients of both groups were not identified.

Discussion

The main aim of this study was to analyze the effectiveness of a complex treatment of ARI with SD spray solution at a dose of 0.25mg/ml with 2 sprays in each nostril 3 times a day for 5 days. The absence of any symptoms of ARI in patients of Group 1 against the inclusion of SD in the treatment regimen was already established by the third day of therapy, whereas in patients of Group 2, the disease symptoms (moderately pronounced rhinorrhea, cough, and nasal congestion) continued to be detected by day 6. In patients of Group 1, the viral agent was eliminated by the time therapy was completed, which was not observed in patients of Group 2. The data of the multiparameter table of a medical examination show that after the first day of therapy in Group 1, the severity of ARI symptoms (rhinorrhea, cough, and nasal congestion) significantly decreased. The use of SD spray solution was not accompanied by any side effects. Statistically significant differences in the levels of sIgA were recorded in patients of 2 groups, which clearly demonstrated the effectiveness of SD as an immunomodulator and reparant of the epithelium of the nasal mucosa. Dynamic observation allowed us to establish a pattern of the disappearance of individual symptoms. Thus, by the third day of treatment in patients of Group 1, with the regression of catarrhal phenomena, nasal congestion and rhinorrhea occurred significantly more often; in addition, the swelling of the tonsils had a pronounced tendency to disappear more rapidly. Starting from the third day of therapy, patients of Group 1 were less likely to need additional medications. Complications of ARI developed in an insignificant percentage of patients in both groups of the study, and the identified conditions proceeded in moderate form and were stopped by standard antibacterial therapy. Practical results of treatment of ARI obtained by district therapists indicate that SD is able to form a stable immunity and induce the growth of local immune protection, restore the morphofunctional state of the mucous membranes, to inhibit the growth of pathogenic microflora, and increase the functional reserves of immunological protection. It has been clinically proven that during the first 12 hours after the administration of SD spray solution, such symptoms as rhinorrhea and tickling and dryness in the throat are relieved; mucous hyperemia and follicular edema of the posterior pharyngeal wall disappear by the end of the first day; the area of inflammatory lesions of the nasal mucosa is also significantly reduced.

Conclusion

As a result of the study, it was found that including a 0.25% SD spray solution in the ARI treatment regimen effectively affects the dynamics of the ARI symptoms:

1. SD in the treatment scheme from the first day allows eliminating the main ARI symptoms much faster and more efficiently than the standard treatment regimen; in particular, recovery is accelerated by 2-3 days.

2. Complex therapy including SD causes a 3-fold increase in the level of sIgA in the nasal mucosa of patients on the fifth day of treatment, which indicates a pronounced immunomodulating effect of this intervention, a potential reduction in the risk of complications and recurrence of viral diseases.

Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Acarology

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Fractionating the Plant Extract in Mini Volumes to Purify the Potential Antivirals from *Terminalia Chebula*

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Abstract

Background: The preliminary experiments indicated that a 30% aqueous extract of seeds of the *Terminalia chebula* has a significant neutralizing activity against tick-borne encephalitis virus (TBEV). In this study, we developed the most gentle approach to purify the antiviral substance.

Methods and Results: The extract of *T. chebula* seeds was fractionated using gel filtration in Sephadex G-200 and G-50. The fractions were tested *in vitro* for the presence of a direct antiviral action against TBEV and profiled using liquid chromatography-mass spectrometry (LC-MS). Antiviral activity was detected in eight fractions from Sephadex G-200 and in three fractions from Sephadex G-50 gel filtration. The chromatogram of the most active Sephadex G-50 fraction exhibited three base peaks with a retention time of 3, 5 and 25.5 minutes. The MS of individual peaks revealed seven compounds with mass-to-charge ratios 114.03; 279.16; 290.91; 301.15; 579.3; 354.03, and 414.09 that were present at relatively high concentrations.

Conclusion: In spite of a small sample, it was possible to detect antiviral activity in the fractionated plant extract using convenient virological methods, as well as to separate the components of the single virus-neutralizing fraction using the LC-MS approach. The purity of resulting preparation needs to be improved. (**International Journal of Biomedicine. 2019;9(1):57-60.**)

Key Words: flavivirus • novel antiviral activity • chromatography • mass spectrometry

Introduction

Tick-borne encephalitis virus (TBEV) is one of the dangerous zoonotic pathogens transmitted to humans through the bite of hard ticks. The virus belongs to the *Flaviviridae* family. The disease causes damage to the nerve cells and their membranes, which leads to paralysis or even death of the patient.[1,2] Currently, the only specific drug with antiviral activity against TBEV is a human immunoglobulin. This drug has several major drawbacks, including potential risk of contamination with human pathogens (e.g., hepatitis C virus or HIV), the demanding conditions of storage and transportation ("cold chain"), and limited availability of competent donors—all of which contribute to the high price and limited amount of product. The efficacy of this drug is also disputable. Thus, the development of new antivirals is a very important scientific task.[3] Plants are believed to be very promising sources of new medical substances. Having a big diversity of plants, it can be assumed that they can serve as a source of medicines for the treatment of most currently known infections. One of the brightest examples of plant-derived drugs is aspirin, also known as acetylsalicylic acid, which was originally isolated from willow (*Salicaceae*). The effectiveness of aspirin has been repeatedly demonstrated, as a pain medication (analgesic), an antipyretic, and an anti-inflammatory, as well as an inhibitor of platelet aggregation.[4] In recent years, many other drugs possessing the antiviral,[5,6] anti-inflammatory,[7] antioxidant,[8] antimicrobial,[9] antifungal,[10] anti-cancer,[11] and other activities [12] have been derived from plants.

Previously,[13] we described a pronounced neutralizing activity against TBEV of aqueous extract of *T. chebula* seeds. To identify and characterize this neutralizing compound of the extract, it is necessary to purify it. However, the absence

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of any data on the physical and chemical properties of the substance (i.e. its molecular weight, chemical formula, or at least its concentration in the initial preparation) significantly complicates this task. In modern biochemical studies, one of the first steps in identifying biologically active molecules in multicomponent mixtures is molecular weight separation using column gel filtration.[14] In this case, various versions of the Sephadex polymer are successfully used as the solid phase, and distilled water as the mobile carrier.

In this study, we attempted to fractionate the extract of *T. chebula* under the gentlest conditions. Besides this, the starting amount of the sample was minimized to reduce the background as much as possible.

Thus, **the purpose** of this study was to develop a purification technique and the concentration of an unknown component of *T. chebula* extract, which has a neutralizing activity on TBEV, using minimal amounts of the original preparation.

Materials and Methods

The plant materials and preparation of the extract

Dried and crushed to a fine powder state seeds of *T. chebula* were kindly provided by Dr. N. Oyuntsetseg (Department of Traditional Medicine, Mongolian State Medical University, Ulaanbaatar, Mongolia). To prepare the extract, 30 g *T. chebula* seed powder were dissolved in 200 ml of sterile, double-distilled water and boiled at low heat for 15-30 minutes until the total volume of the mixture reached approximately 100 ml. The volume of the mixture was measured and brought to 100 ml sterile, bidistilled water. Then the mixture was sequentially filtered through a sterile cloth filter, a sterile paper filter with a pore diameter of 0.45 μm and sterilized by filtration through a vacuum 0.22 μm filter. The resulting solution was considered as a 30% aqueous extract of seeds of *T. chebula*.

Tick-borne encephalitis virus and cell culture

We used an isolate 92M of TBEV of the Siberian subtype.[15] Passaging of TBEV and determination of the concentration of infectious virus was carried out in the pig embryo kidney cell line (SPEV) purchased from the "Collection of human and animal cell lines for research in the field of virology" (FSI Influenza Research Institute, St. Petersburg, the Russian Federation). Cell culture was maintained on an RPMI1640 medium, supplemented with antibiotics and 5% fetal calf serum (ThermoScientific, UK). The concentration of the infectious virus in the stock suspension and in the experiments on the neutralization of TBEV were determined by titration of plaque-forming units (PFU) in the cell culture of SPEV and expressed as a decimal logarithm of PFU per milliliter of suspension (lg PFU/ml).[16]

Neutralization assay

The neutralization reaction was performed according to E. Gould.[16] In particular, 100 μl of the virus suspension containing 1×10^5 PFU of TBEV was mixed with 100 μl of the test or control sample. Human donor immunoglobulin against TBEV (FSUS «SIC Microgen», Tomsk) at a concentration of 1 mg/ml was used as a neutralization control. Sterile, bidistilled water was used as a reference control. The mixture of TBEV and the sample was incubated for 30 min at 37°C and then the concentration of the infectious virus was determined. The neutralization index was determined as the difference in titers (in logarithmic terms) of the test and reference samples. Each experiment was performed in three independent replications.

Column Gel Filtration

Gel filtration was performed under gravity force in a column of 1.2×10 cm with water as a mobile phase. The column was packed with a 5% aqueous suspension of Sephadex G-50 or G-200 (Sigma-Aldrich, Sweden). Before loading the sample, the column was additionally equilibrated with 10ml of sterile bidistilled water. Afterwards 0.05 ml of 30% *T. chebula* seed extract were loaded onto the gel, and after the extract entered the column, 10 ml of sterile bidistilled water was applied on top of the gel. Fractionation was performed at a flow rate of 0.5 ml/min. The void volume of the column was considered equal to 6ml, and eight fractions were collected with a volume of 4ml each. After that, the final fraction of 6 ml was collected.

Liquid chromatography and mass spectrometry

LC was performed using a Zorbax 300 SB-C18 reverse phase column with dimensions of 21×150 mm and 5 μm particle size and Agilent 1200 Series HPLC System. The separation was carried out in an acetonitrile gradient from 10% to 100% during 30 minutes, with the addition of a 0.1% heptafluorobutyric acid and a flow rate of 0.2 ml/min. The readings were taken in the ultraviolet spectrum at wavelengths of 200 nm, 230 nm, 270 nm, 300 nm, and 360 nm. Electrospray ionization was performed using the Agilent 6210 TOF MS instrument in a positive mode, the ratio of mass and charge was estimated in the range of 70-3200 m\z.

Presentation of results

The results were presented as the mean values of three independent replications. The standard deviation was used to evaluate the variability of data. The significance of differences was assessed using Student's t-test. Statistical analysis was performed using Microsoft EXCEL 2003. The LC-MS data were processed by the Agilent MassHunter Workstation software package (version B.01.03;Agilent Technologies Inc., USA).

Results and Discussion

Fractionation of the extract

The output of the colored part of the extract in Sephadex G-200 started with Fraction 4 and ended with the release of Fraction 8. In Sephadex G-50, the mobility of the colored part of the extract was one and a half times faster. The total volume of the eluate was 32 ml, which corresponded to a dilution of the initial sample by 640 times. Thus, the concentration of the extracted substances in each fraction approximately corresponded to a 0.05% *T. chebula* extract. We have previously described that a 2% *T. chebula* extract completely neutralizes infectious TBEV at a concentration of 1×10^5 PFU,[13] and the antiviral activity of the same extract against the influenza A virus is detectable up to a concentration of 0.01%.[17] Thus, the concentration of the active substance in any of the fractions, even if it is evenly distributed over

the entire volume of the column, should be sufficient for detectable neutralization of TBEV.

Neutralizing activity of fractions

After separation using the Sephadex G-200, the ability to neutralize TBEV was firstly detected in Fraction 2, just ahead of the colored fraction, reached a maximal values in Fractions 3 and 4, and then minor virus inhibition activity was detected in each fraction until the final one (Fig.1A). To increase the resolution ability of the column, the Sephadex G-50 was used as well. The release of the neutralizing agent in this sorbent started with Fraction 4, reached the maximal concentrations in Fractions 5 and 6, and some virus neutralizing activity was also detected up to the final fraction (Fig.1B).

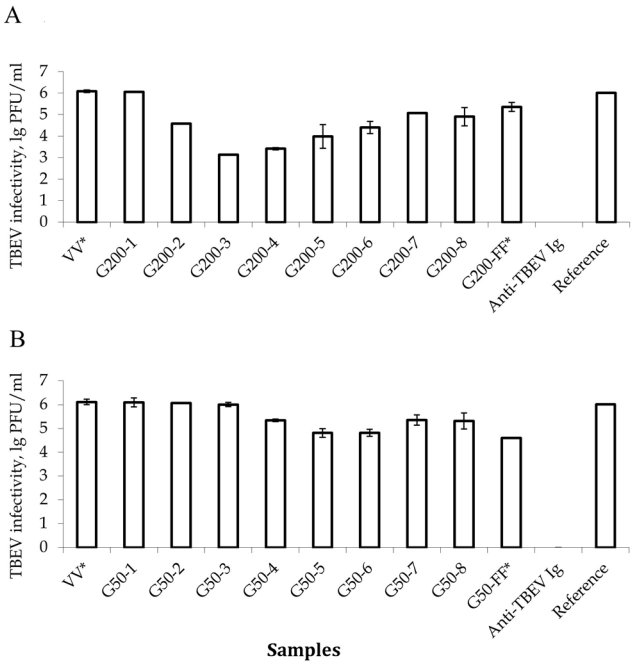

Fig. 1. *Virus neutralizing activity of eight fractions (4 ml each) of T. chebula extract purification on A) Sephadex G-200 and B) Sephadex G-50; * VV - void volume of the column, FF - the final fraction (6 ml); reference – sterile, double-distilled water.*

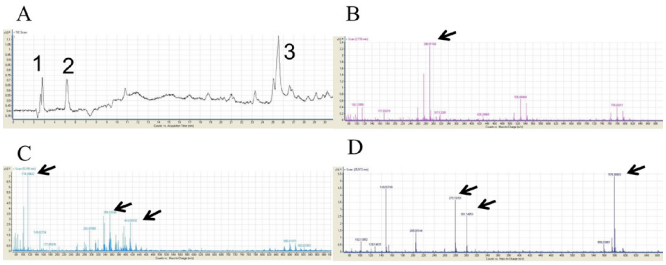

Fig. 2. *Chromato-mass-spectrometry profile of Fraction 5 of T. chebula extract after separation on a Sephadex G-50 column. A) Chromatogram of total ion current (TIC) of Fraction 5; the time of collection is 30 minutes, the total concentration of the substance is indicated on the abscissa axis, the elution time of the peak from the column is indicated on the ordinate axis; 1, 2, 3 – peaks of components of Fraction 5; B) The mass spectrum of peak 1; C) The mass spectrum of peak 2; D) The mass spectrum of peak 3. The arrows indicate the compounds with increased concentration.*

The method of column gel filtration used in this study is based on the ability of substances with a higher molecular weight to pass between the Sephadex particles easily and migrate faster, whereas the substances with a lower molecular weight are able to penetrate into the particles of Sephadex and, as a result, migrate more slowly. The substances with a molecular weight exceeding the separation limit of the corresponding Sephadex polymer migrate in the void volume of the column. Therefore, upon completion of the gel filtration, the components of the extract with a high molecular weight accumulate in the earlier fractions, while the concentration of substances with low molecular weight is increased in the later fractions. According to the manufacturer's data, the exclusion limit is approximately 600 kDa for Sephadex G-200 and 30 kDa for Sephadex G-50. Since the entire diapason of the neutralizing activity was detected both in G-200 and in G-50, it can be assumed that the molecular weight of the target substance is less than 30 kDa.

LC/MS spectrum of the fraction with a neutralizing effect

Sephadex G-50 Fraction 5 possessing the highest neutralizing activity was used for LC followed by MS separation of the components of the extract of *T. chebula*. On the chromatographic profile, three peaks are visually identified with a retention time of 3, 5 and 25.5 minutes (Fig. 2A). The mass spectra of each of these peaks indicated that neutralizing fraction includes about 30 individual components. Seven of these (values of m\z 114.03; 279.16; 290.91; 301.15; 579.3; 354.03, and 414.09) are present in elevated concentrations (Fig. 2B-D). It is likely that a substance with a neutralizing activity against TBEV is either one of these seven components, or some of these components are derivatives (for example, fragments) of the analyzed substance.

In this study, a relatively small sample was used (initially only 30 g dry samples were extracted with 100 ml of water, and only 50 µl were used in the experiment). Nevertheless, it was possible to detect virus-neutralizing activity in separate fractions using convenient virological methods, as well as to separate the components of the single fraction, possessing antiviral activity, using the LC-MS approach. However, the purity of the resulting preparation seems to be insufficient for further analysis. Probably, additional purification methods are necessary besides the Sephadex-based gel filtration.

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Conflict of Interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Endogenous Intoxication and the Role of Antioxidants in Motion Activity Correction with Traumatic Brain Injury in Rat Model

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Abstract

The aim of this study was to assess the effectiveness of cytoflavin and mexicor in the posttraumatic period of traumatic brain injury (TBI).

Materials and Methods: The experiments were carried out on 60 white non pedigree female rats weighing 180-200 g, with 15 rats in each series. TBI was modeled by a free-falling weight drop of 100 g from a height of 80 cm on the parietal-occipital area of the head. Blood samples were taken from the sublingual vein in an amount of 2.0 ml on Days 1, 3, 7, and 12 after the alteration. After TBI, in Group 1, 15 rats received an intraperitoneal injection of 2-ethyl-6-methyl-3-hydroxypyridine succinate (mexicor) for 10 days in a daily dose of 8.0 mg/kg. In Group 2, 15 rats received an intraperitoneal injection of cytoflavin for 10 days in a daily dose of 0.2 ml/kg. The activity of lipid peroxidation and antioxidant protection system in the blood plasma was determined by a biochemicoluminescence method. Analysis of animal motion activity included the determination of the ability to balance and to stay at the wooden bar, time spent for moving on the bar from the bright light source to the darkroom, and paw slip frequency.

Results: Mexicor and cytoflavin decreased the level of oxidative processes in rat model with TBI and the development of secondary brain injury. The positive dynamics in restoring pro- and antioxidant system balance was combined with positive changes in motor function. (**International Journal of Biomedicine. 2019;9(1):61-65.**)

Key Words: traumatic brain injury • rats • lipid peroxidation • antioxidant protection system

Abbreviations

AOS, antioxidant protection system; $\mathbf{I_{max}}$, the maximum chemiluminescence intensity; **DC**, diene conjugate; **LPO**, lipid peroxidation; **LMMWS**, low and medium molecular-weight substances; **SB**, Schiff bases**; PSF**, paw slip frequency; **SBI**, secondary brain injury; **TAS**, total antioxidant status; **TBI**, traumatic brain injury; **TC**, triene conjugate.

Introduction

Traumatic brain injury (TBI) has a high incidence worldwide and is associated with significant morbidity and mortality. According to the Centers for Disease Control, the total combined rates for TBI-related emergency department visits, hospitalizations, and deaths increased in the 2001–2010 decade.(1) The development of body hypoxic state is one of the main factors of acute TBI. In hypoxic state, the energy deficit, to say more exactly ATP deficit, provokes homotypic metabolic and structural changes in various organs and tissues,(2) as well as LPO activation. In turn, LPO products aggravate the disturbance of membrane structure and functions. They play a key role in the beginning and development of brain edema, which is manifested in TBI and may be realized in the disturbance of motion and cognitive function.(3-5)

One of the most effective ways to prevent and treat brain traumatic and ischemic lesions is the use of antihypoxic drugs. These are remedies that weaken or nullify hypoxic disturbances. Mexicor and cytoflavin are two such remedies.(6-9) These

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medicines are largely used to manage ischemic conditions.[10-12]

The aim of this study was to assess the effectiveness of cytoflavin and mexicor in the posttraumatic period of TBI.

Materials and Methods

The experiments were carried out on 60 white non-pedigree female rats weighing 180-200g, with 15 rats in each series. All animals were given access to food and water *ad libitum*. Experiment was performed in accordance with the Order of the Ministry of Health of the Russian Federation №267 (19.06.2003) "On approval of the rules of Good Laboratory Practice» (GLP).

Experimental model of TBI

The animals were fixed on a plate, and blood was collected from a sublingual vein in an amount of 2.0 ml. It was 8%–9% of the circulating blood volume. TBI was modeled by a free-falling weight drop of 100g from a height of 80 cm on the parietal-occipital area of the head.[13] Blood samples were taken from the sublingual vein in an amount of 2.0 ml on Days 1, 3, 7, and 12 after the alteration. Such blood sampling technology simulates the fractional blood loss, which, during the 10 days of the posttraumatic period, was 32%–36% of the volume of circulating blood in the rats.

Treatment

After TBI, in Group 1, 15 rats received an intraperitoneal injection of 2-ethyl-6-methyl-3-hydroxypyridine succinate (mexicor) for 10 days in a daily dose of 8.0mg/kg (a solution for intravenous and intramuscular administration, JSC EkoFarmInvest, Russia). In Group 2, 15 rats received an intraperitoneal injection of cytoflavin for 10 days in a daily dose of 0.2 ml/kg (a solution for intravenous administration, OOO NTFF Polisan», Russia). In Group 3 (control group-CG), 15 rats received an intraperitoneal injection of physiological saline solution in the same volume. The administration of the drugs was started 1 hour after TBI. The values of the physiological norm of the studied parameters were determined in intact rats (n=15).

The activity of LPO and ASO in the blood plasma was determined by a biochemicoluminescence method[14] using the biochemiluminometer Lum-5773. The following indices of chemiluminogram were analyzed: total activity of free-radical oxidation (I_{max}) and TAS (tgα). LPO intensity was defined by the concentration of DC/TC of polyunsaturated fatty acids as well as SB by a spectrophotometry method.[15] using the RF-5301 PC, Shimadzu, (Japan). Each phase was analyzed in comparison with the respective group at the following wavelengths: 220 nm (isolated double bonds absorption), 232 nm (DC absorption), 278 nm (TC absorption), and 400 nm (SB absorption). The level of DC, TC, and SB was defined by E232/E220, E278/E220, E400/E220 and it was expressed in terms of relative units (RU).

The distribution index in relation to the content of middle molecules at wavelengths of 238 nm, 254 nm, 266 nm, and 282 nm was defined for assessment of LMMWS.[16] The calculation of the final result was made using the formula:
LMMWS=1.013×(8×E238+16×E254+44×E266/3+64×E282/3.

Animal motion activity analysis

The motor disorders were defined by the method of K.Saatman et al.[17] The ability to balance and to stay at the wooden bar, time spent for moving on the bar from the bright light source to the darkroom, and PSF were defined in the posttraumatic period.

The statistical analysis was performed using the statistical software Microsoft Excel. The mean (M) and standard error of the mean (SEM) were calculated. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney *U*-test. A probability value of $P<0.05$ was considered statistically significant.

Results

The research results showed that the endointoxication and LPO activation in blood plasma took place in the TBI groups of animals (Table 1).

Table 1.

Parameters of the endointoxication and LPO activation in the TBI posttraumatic period

Variable	Intact rats	Group	Period after TBI (day)			
			1	3	7	12
LMMW, RU	5.65±0.43	CG	9.09±0.55*	8.70±0.17*	8.78±0.33*	7.15±0.4*
		Group 1	7.69±0.74*	6.89±0.50^	6.84±0.37^	5.70±0.58
		Group 2	6.48±0.28^	6.86±0.86	5.79±0.49^	5.24±0.29^
I max, mV	1.64±0.14	CG	3.05±0.14*	2.74±0.16*	1.97±0.17	1.93±0.07
		Group 1	2.32±0.11*^	1.98±0.08*^	1.51±0.1^	1.27±0.012^
		Group 2	2.42±0.24*^	2.03±0.1*^	1.33±0.21^	1.36±0.24^
tga, mV/sec	3.86±0.06	CG	3.58±0.11*	3.56±0.18	3.41±0.23	3.31±0.15
		Group 1	6.69±0.51*^	4.87±0.31*^	4.33±0.42	4.88±0.07*^
		Group 2	6.43±0.38*^	4.91±0.31*^	4.59±0.19*^	4.17±0.08*^

**- $P<0.05$ between Group 1/Group 2 and a group of intact rats*
^- $P<0.05$ between Group 1/Group 2 and CG

Mexicor and cytoflavin induced a decrease in the intensity of the intoxication processes. This decrease manifested in a decrease of the plasma LMMWS level in all the stages of the TBI posttraumatic period. The findings showed that mexicor and cytoflavin supressed considerably the endointoxication in blood plasma and induced a decrease in the number of decay products. Besides, after the treatment with these medicaments, the exit of decay products to blood from injured tissues was less intensive.

Activation of free-radical processes and AOS depression were pronounced in the control group. Mexicor and cytoflavin restrained the free-radical oxidative stress. We found a decrease in I_{max} on Day 3 of the experiment by 28% in Group 1 and 26%, in Group 2 compared to the value of control gtoup. On Day 7, I_{max} in Groups 1 and 2 did not differ from that in the intact rats.

Mexicor and cytoflavin provoked positive changes in free-radical processes in the blood plasma (Table 2). Mexicor induced a decrease in the level of DC and TC on Day 3 of the experiment by 29% and 44% relative to CG, respectively. The correction effect of cytoflavin was weaker.

The levels of DG, TC and SB in Group 2 were normalized by Day 7 of the experiment.

We observed tonic and clonic seizures just after TBI for 2-4 sec. Animals lost sensitivity and they had been in a lateral position for 10-20 sec. The study of motor response showed a worsening of motor function up to the end of Day 1 of the experiment (Table 3).

The positive dynamics in restoring pro- and antioxidant system balance was combined with changes in motor function. Mexicor and cytoflavin injections induced the normalization of standing balance and walking ability. It manifested in a decrease in PSF as well as in time spent for moving on the bar. At the same time the mexicor effect was more strongly pronounced. PSF decreased by 55.6% and 44.4% by the end of Day 1 in Group 1 and Group 2, respectively. The positive dynamics in the motion activity indices was evident up to the end of Day 1 after the mexicor and cytoflavin injections. A significant improvement in motion reactions was registered on Day 3 of the experiment. The level of these indices achieved the intact animal value by Day 7 after the mexicor injection and by Day 12 after the cytoflavin injection.

Table 2.

Changes in the intensity of LPO in the blood plasma of rats in the posttraumatic period

Variable	Intact rats	Group	Period after TBI (day)			
			1	3	7	12
DC, RU	0.12±0.01	CG	0.22±0.02*	0.21±0.02*	0.16±0.02	0.12±0.02
		Group 1	0.16±0.01*^	0.15±0.01*^	0.13±0.02	0.09±0.01*
		Group 2	0.17±0.02*	0.17±0.03*	0.08±0.03^	0.08±0.04
TC, RU	0.08±0.01	CG	0.15±0.03*	0.16±0.03*	0.16±0.02*	0.08±0.02
		Group 1	0.12±0.02	0.09±0.01^	0.08±0.03^	0.07±0.01
		Croup 2	0.10±0.02	0.12±0.01*	0.09±0.02	0.06±0.03
SB, RU	8.43±0.84	CG	9.35±0.96	12.99±0.98*	11.06±0.92*	9.29±0.75
		Group 1	10.25±1.03	8.00±0.44^	8.03±1.33	8.25±0.41
		Group 2	9.89±0.89	10.94±0.84*	8.66±0.64^	8.90±0.87

**- P<0.05 between Group 1/Group 2 and a group of intact rats*
^- P<0.05 between Group 1/Group 2 and CG

Table 3.

Animal motion activity analysis

Variable	Intact rats	Group	Period after TBI (day)			
			1	3	7	12
Moving on bar, score	2.5±0.1	CG	6.8±0.9*	5.7±0.9*	4.9±0.5*	4.5±0.7*
		Group 1	4.2±0.8*^	4.0±0.4*	2.9±0.3^	2.6±0.4^
		Group 2	4.5±0.6*^	3.6±0.3*^	3.3±0.3*^	2.3±0.1^
PSF, numbers	1.0±0.1	CG	3.6±0.7*	2.8±0.6*	2.0±0.5	2.2±0.5*
		Group 1	1.6±0.7	1.3±0.4	1.1±0.2	0.8±0.2^
		Group 2	2.0±0.4*	1.7±0.3*	1.5±0.3	1.0±0.1^
Time spent for moving on the bar, score	1.5±0.1	CG	3.4±0.4*	2.9±0.3*	2.2±0.2*	2.0±0.4
		Group 1	1.8±0.2^	1.6±0.2^	1.4±0.2^	1.4±0.2
		Group 2	1.9±0.2^	1.8±0.1*^	1.8±0.2	1.4±0.1

**- P<0.05 between Group 1/Group 2 and a group of intact rats*
^- P<0.05 between Group 1/Group 2 and CG

Discussion

Our results showed that mexicor and cytoflavin decreased the level of oxidative processes with TBI and the development of secondary brain injury. Secondary brain injury represents the cascade of biochemical inflammatory stress reactions, which provoke brain ischemia and worsen considerably the severity of the general condition. These reactions also hinder the restoration of psychical and moving activity.[18] In addition, the processes of neuroregeneration and neuroprotection start at the same time as the secondary brain injury cascade. Specific intracellular neuroregulatory proteins and pluripotent stem cells, being neurotrophic factors, play a key role in these processes. However, in TBI (especially, in severe cases) the regeneration function of nerve tissue gives way to the processes of secondary brain injury because the site of primary brain injury is too large and the total body reactivity decreases; thus, the speed of the development of secondary brain injury is higher than that of the reparative processes.

The mexicor and cytoflavin injections in the posttraumatic period of TBI induced a decrease in endointoxication and oxidative stress by increasing the antioxidative blood potential in all stages of the experiment. These medications interrupted the formation of Schiff bases at the level of diene- and triene conjugates. Mexicor was the most effective in the first stage of the posttraumatic period (1-3 days after TBI). Probably, differences between effect manifestations are associated with differences in the composition of these "succinate" medications. Mexicor is a heteroaromatic antioxidant, in which the succinate is connected by covalent link with antioxidant emoxypine.[6] Cytoflavin is a complex preparation, the composition of which comprises the following components: succinate acid, riboflavin, riboxinum, and nicotinamide.[20] Earlier it was shown that this group of preparations decreases the intensity of LPO both in blood plasma and in cell membranes.[21]

It is very important to note that mexicor and cytoflavin promoted a decrease in the LMMWS content. The catabolic component of LMMWS is represented by low molecular weight compounds—protein breakdown products. The catabolic anabolic component of LMMWS is represented by a complex of peptide compounds—protein proteolysis products. Peptide compounds are similar by their structure to regulatory peptides, and they may provoke abnormality in the metabolism and function of cells. They may also provoke the appearance of drug resistance by blocking T-cell receptors. The study results showed that cytoflavin and mexicor restored the functioning of cell membranes due to antioxidant properties and decreasing LMMWS on the surface of the membranes. A decrease in LMMWS content is evidence of reduced inflammation.

Given that mexicor and cytoflavin are succinic acid drugs, it is possible to suppose that the restoration of metabolism in the cells involves restoring homeostasis. The restoration of movement function of the experimental animals proves this thesis. Thus, it can be concluded that mexicor and cytoflavin are multimodal drugs with neuroprotective, antioxidant and anti-inflammatory properties.

Conflict of Interest

The authors declare that they have no competing interests.

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CASE REPORT

Radiology

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A Case Report of Perineural Cysts, Also Known as Tarlov Cysts

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Abstract

Tarlov cysts (TCs) are a rare but intensive cause of chronic low back pain or lumbosacral radiculopathy and should be considered in the differential diagnosis of radicular symptoms. Magnetic resonance imaging (MRI) is the radiologic study of choice to visualize these cysts.

In the current study, we describe a case of a 49-year-old patient with intermittent pain on his coccigeal region, right buttock, and intensive pain in the right hip and inner side of the thigh, in 1.5 years of duration. MRI of the lumbosacral spine and hip showed right-sided, sacral S2 and S3 perineural cysts, the largest located at the right S2 nerve root. Conservative treatment of sacral perineurial cysts should be the first choice. The surgical treatment is recomended in cases when MRI scans reveal a large (1.5 cm) cyst associated with neurological symptoms and no response on conservative treatment.(**International Journal of Biomedicine. 2019;9(1):66-68.**)

Key Words: meningeal cyst • Tarlov cyst • CT myelography • MRI • neurological symptoms

Introduction

Spinal meningeal cysts have been classified by Nabors et al.[(1)] into three different types:

• Type I: Extradural meningeal cysts without spinal nerve root fibers

• Type II: Extradural meningeal cysts with spinal nerve root fibers (TCs)

• Type III: Spinal intradural meningeal cysts

Perineural (or Tarlov) cysts (TCs) are defined as cerebrospinal fluid (CSF)-filled saccular lesions located in the extradural space of the spinal canal and formed within the nerve root sheath of the dorsal root ganglion (between the peri- and endoneural spaces of the spinal posterior nerve root sheath). This saccular dilatation of the spinal posterior nerve root sheath has microconnections to the subarachnoid space.

Thus, when pulsatile and hydrodynamic forces of CSF, through a ball-valve mechanism and gravitational pressure within the cyst, cause these perineural cysts to fill and expand in size, it can cause neural compression, resulting in neurological symptoms.[(2)]

Perineural cysts typically exhibit delayed filling with a contrast medium on myelography (Figures 1 and 2), which is one of the criteria used by Tarlov to distinguish PCs from meningeal diverticula.

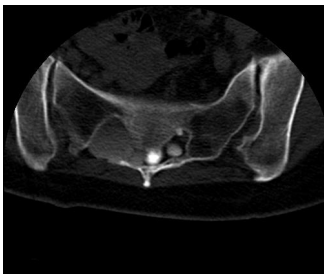

Fig. 1. *CT myelography: early postcontrast image.*

Fig. 2. *CT myelography: late post-contrast image.*

The incidence of TCs in the adult population is between 4.6% and 9%. The incidence does not significantly differ between sexes but is more prevalent in younger people: 4.0% in people less than 50 years of age versus 1.3% in people over 50 years.[(3)]

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On postmyelographic computed tomography immediately after contrast medium injection, the largest cyst at right S2 showed selective filling of the contrast medium, suggesting that inflow of cerebrospinal fluid to the cyst exceeded outflow. Six hours after the injection, the intensity of the cysts was similar to the intensity of the thecal sac.

Case presentation

A 49-year-old patient presented with intermittent pain on his coccigeal region, right buttock, and intensive pain in the right hip and inner side of the thigh, in 1.5 years of duration. Local pain is caused by cyst enlargement and by pressuring the periost or joint capsule. Radiculopathy results from a cyst compressing the nerve root, and in the case of ventral root compression, motor weakness occurs.

Patient reported exacerbation of symptoms when standing, coughing and lifting. Later the patient started having rest pain as well. On clinical examination, distribution of the pain corresponded to the S2-S3 dermatome. Straight leg raising was 40° on the right side and normal on the left side. There was significant blunting of sensations along the S1 and S2 dermatome on the right side, with a slight motor deficit in right lower limb. X-ray of the lumbosacral spine did not reveal any abnormality. Magnetic resonance imaging (MRI) of the lumbosacral spine and hip showed right-sided, sacral S2 and S3 perineural cysts, the largest located at the right S2 nerve root (Figures 3-6). Note the resulting denervation edema in the right gluteal muscle.

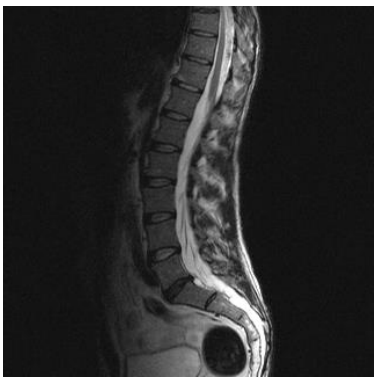

Fig. 3. *MRI of the lumbosacral spine: sagital plane; right-sided sacral S2 and S3 perineural cysts, the largest located at the right S2 nerve root.*

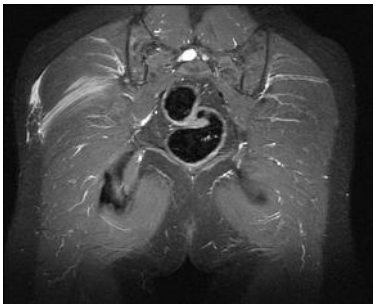

Fig. 4. *MRI of the lumbosacral spine: transversal plane.*

Fig. 5. *MRI of the lumbosacral spine: coronal plane.*

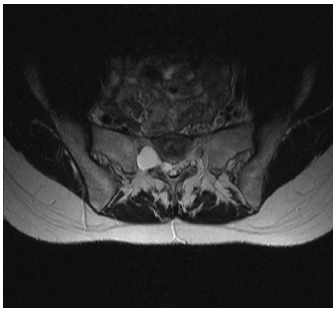

Fig. 6*. MRI axial T2WI: a CSF-filled cystic lesion, 1.5-1.8 cm in diameter, sitting opposite S2.*

The patient was, after 8 months of conservative treatment, taken for a sacral laminectomy, excision of the cyst and plication of the cyst wall, while retaining the nerve root. Histopathological examination of the cyst wall showed nerve cells, which confirmed the diagnosis of a Tarlov cyst. Patient experienced relief of pain immediately after the surgery. On follow-up, the patient had no pain in lower limbs and back.

Discussion

Tarlov cysts are rare causes of low back pain, but when they are present pain is very intense, leading to disability of normal working and living activities.

Plain X-rays are usually normal. They may reveal characteristic bone erosion of the spinal canal or anterior or posterior neural foramina.

MRI gives better soft tissue contrast and is currently the investigation of choice for perineural cysts. The cysts demonstrate a low signal on T-1 weighted images and a high signal on T-2 weighted images, similar to CSF.

Myelography showing the filling of the meningocele sac 1h after injection of the contrast medium is highly suggestive of a perineural Tarlov cyst.

A CT scan can demonstrate cystic masses isodense with CSF located at the foramina.

The pathogenesis of perineural cysts is uncertain. Tarlov felt that hemorrhage into the subarachnoid space caused accumulations of red cells, which impeded the drainage of the veins in the perineurium and epineurium, leading to rupture with subsequent cyst formation. There is no consensus on a single method of treatment. Various methods have been advocated. Tarlov advised that symptomatic, single perineural cysts should be completely excised together with the posterior root and ganglion from which they arise.[4]

According to Caspar, microsurgical excision of the cyst combined with duraplasty or plication of the cyst wall is an effective and safe treatment of symptomatic sacral cysts. The parent nerve root is always left intact.[5]

Conclusion

Tarlov cysts are rare, but they cause chronic low back pain or lumbosacral radiculopathy and should be considered in the differential diagnosis of radicular symptoms. MRI is the radiologic study of choice to visualize these cysts.

Conservative treatment of sacral perineurial cysts should be the first choice. The surgical treatment is recomended in cases when MRI scans reveal a large (1.5 cm) cyst associated with neurological symptoms and no response on conservative treatment.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgments

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CASE REPORT

Reumatology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Simultaneous Presentation of Ankylosing Spondylitis and Pancreatic Cancer: A Case Report

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Abstract

A clinical case of simultaneous presentation of ankylosing spondylitis and pancreatic cancer is described. Patients with rheumatic disorders must be closely followed to screen for malignancies. Most paraneoplastic rheumatic syndromes are difficult to distinguish from autoimmune rheumatic diseases; thus, cancer occurrence may constitute a major diagnostic challenge. (**International Journal of Biomedicine. 2019;9(1):69-71.**)

Key Words: ankylosing spondylitis • pancreatic cancer • paraneoplastic rheumatic syndromes

Abbreviations

AS, ankylosing spondylitis; **ARDs**, autoimmune rheumatic diseases; **NSAIDs,** nonsteroidal anti-inflammatory drugs; **PRS**, paraneoplastic rheumatic syndromes.

Introduction

Several studies have indicated connections between malignancy and autoimmune rheumatic diseases.[1-4] During the past 30 years there have been many attempts to determine more precisely the link between cancer and rheumatic disorders. The explanation of this association remains unclear. Rheumatic syndromes may be associated with malignancy as paraneoplastic conditions, which can antedate the neoplasm diagnosis. On the other hand, autoimmune rheumatic diseases (ARDs) have a higher risk of malignancy by themselves or because of the immunosuppressant treatments. Malignancies are associated with a wide variety of paraneoplastic rheumatic manifestations, which may arise in joints, fasciae, muscles, vessels or bones.[5,6] The pathogenesis of paraneoplastic rheumatic syndromes (PRS) is complex and not fully understood in the majority of instances. Rheumatic syndromes can be important clues to occult neoplasia.[3,7] Approximately 15% of patients with cancer develop paraneoplastic syndromes as a result of tumor-derived biologic mediators like hormones, peptides, antibodies, cytotoxic lymphocytes, autocrine and paracrine mediators.[5,8]

Until now, little has been understood regarding the pathogenesis of connective tissue diseases in association with neoplastic disease. Several factors, including autoimmune disease itself, common etiology between ARDs and malignancy, including genetic factors, viruses and

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smoking, have been implicated in the pathogenesis of tumor development.[9] Epidemiological studies have revealed that patients with chronic inflammation are at increased risk of developing cancer.[10,11] Pathologically, chemokines, cytokines and prostaglandins are able to shift the microenvironment of an organ from a healthy to a dysplastic state, which favors malignant changes in affected cells.[11] Elevated serum interleukin (IL)-1, IL-6, and TNF-α levels trigger inflammatory reactions in patients with spondyloarthritis.[12]

Chang et al.,[13] using the Taiwan National Health Insurance Research Database, have analyzed the data from patients diagnosed with AS between 2000 and 2008 who had no history of cancer prior to enrollment (n=5,452). Age- and sex-matched patients without AS served as controls (n=21,808). The results revealed that the overall incidence of cancer was elevated in AS patients (SIR 1.15; 95% CI 1.03-1.27). The cancer risk was increased during the first 3 years following the diagnosis of AS. In another study, Feltelius et al.[14] concluded that AS does not affect the overall cancer risk in Sweden. In studies reported by Hemminki et al., AS patients in Sweden showed an increased risk for multiple myeloma,[15] a decreased risk for digestive tract cancer,[16] and no altered risk for non-Hodgkin lymphoma.[17] In female cancer types of the breast, uterus, ovary and other genital organs, a low standardized incidence ratio (SIR) in female patients with AS was noted.[18] Thus, the potential association between AS and cancer is controversial.

Case presentation

A 34-year-old woman with severe pain in the upper abdomen that radiated to the back and was diagnosed with obstructive jaundice, was hospitalized in the surgical department (Clinical Emergency Hospital n.a. N.S. Karpovich). A biochemical blood test was performed and cytolysis syndrome was detected (increased ALT/AST up to three norms). Esophagogastroduodenoscopy showed a tumor-like formation with ulcerations in the area of the major duodenal papilla. The debut of articular syndrome with the development of pain and swelling, and severe stiffness in the knee joints, was registered in April 2018. Therapy for AS included NSAIDs (ketoprofen orally and topically) and parenteral glucocorticoids. It was possible to reduce the severity of articular syndrome only for a short time. In the same month, with suspicions that there were malignant tumors of the major duodenal papilla and pancreas, the patient was referred to the oncologic dispensary n.a. A.I. Kryzhanovsky.

The patient underwent gastropancreaticoduodenectomy with a histological examination of the removed material. The histological study detected a moderately differentiated pancreatic adenocarcinoma with invasion into the duodenal wall up to the mucosa. On the fifth day after the operation, the patient noted the return of pain and swelling in the knee joints. Nonsteroidal anti-inflammatory drugs and parenteral glucocorticoids were also included in the treatment regimen. Taking into account that her sister suffered from AS, the patient was examined by a rheumatologist at the place of residence. The examination confirmed the following: X-ray of the sacroiliac joints: bilateral grade 3 sacroiliitis. Multislice computed tomographyof the pelvic bones: bilateral erosive sacroiliitis. In addition, the patient was HLA-B27 positive. Sulfasalazine (2,000 mg), prednisolone (20 mg), and etoricoxib (90 mg) were additionally included in the treatment regimen. On the background of treatment, the pain and swelling of the knee joints and morning stiffness have decreased. The dose of prednisolone was reduced to 10 mg.

Conclusion

According to the clinical, instrumental and laboratory examinations, this patient simultaneously suffered from two diseases: pancreatic cancer and ankylosing spondylitis. The development of the latter can hardly be called paraneoplastic syndrome, since erosive sacroiliitis and HLA-B27 were detected. Recognizing that a relationship exists between malignancy and rheumatic diseases is important to our future understanding of the pathogenesis of the two entities.[5] Patients with rheumatic disorders must be closely followed to screen for malignancies. Most paraneoplastic rheumatic syndromes are difficult to distinguish from autoimmune rheumatic diseases; thus, cancer occurrence may constitute a major diagnostic challenge. Early detection and therapy may be of utmost clinical importance.

Conflict of Interest

The authors declare that they have no competing interests.

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SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Frequency and Neuroimaging Characteristics of Macro- and Giant Pituitary Adenomas

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Abstract

Data from 3 medical centers of Tashkent city, in which 156 patients (76 men and 80 women) with pituitary macroadenomas (PMA) and iant pituitary adenomas (GPA) were treated during 3 years (between 2015 and 2017), were retrospectively analyzed. The results of the study showed that the different variants of PMA/GPA suprasellar growth might serve as a neuroimaging marker of tumor aggressiveness, namely, supra-infra-latesellar and supra-infra-tellular growth observed in 68 (43.5%) patients, as well as invasive tumor growth in 108 (69.2%) patients. Transsphenoidal removal is a relatively safe and effective method, even in PMA and GPA with suprasellar extensions, in most of the cases.(**International Journal of Biomedicine. 2019;9(1):72-74.**)

Key Words: giant pituitary adenomas • pituitary macroadenomas • magnetic resonance imaging • transsphenoidal hypophysectomy

Abbreviations

GPA, giant pituitary adenomas; **NFPA**, non-functioning pituitary adenoma; **PA**, pituitary adenomas; **PMA**, pituitary macroadenomas; **PG**, the pituitary gland; **SIR**, Standardized Incidence Rates; **TSHS**, transsphenoidal hypophysectomy.

Introduction

Pituitary adenomas constitute 10%–15% of intracranial tumors, and occur in 17%–25% of the population.[1] Most pituitary adenomas are benign, but approximately one third of them are invasive, encroaching upon the bones, dura, and/or other adjacent structures.[2,3] Functioning PMA and GPA have large growth volumes and endocrinological abnormalities. Pituitary adenomas are classified as "giant" when their diameter exceeds 4 cm.[4] GPA has the potential for widespread, multi-directional extension. These tumors are very difficult to treat and can behave aggressively.[5,6]

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According to M. Gruppetta and J. Vassallo,[7] the prevalence for macroadenomas was 40–67/100 000 people and the SIR was 1–90/100 000/year. Giant pituitary adenomas (>40 mm) constituted 4–8% of the whole cohort of PAs and the SIR was 0–18/100 000/year. In 2004, WHO [4] classified pituitary adenomas into typical adenomas, atypical adenomas, or carcinomas.

Various classifications have been proposed but there is no evident terminology to describe large pituitary tumors.[8] JC Zakir et al. showed that parasellar invasion prevails as a strong predictive factor for tumor recurrence.[9] Severe suprasellar extension should be considered as invasion parameter and could impact prognosis.

An original study comparing the clinical spectrum of giant prolactinomas with ordinary macroprolactinomas was performed by Espinosa and colleagues.[10]

Despite the histological benign nature, the management

of PMA and GPA is very difficult.[6,7,11-17] Transsphenoidal surgery is recognized as the optimum technique for the management of pituitary adenomas, as it helps preserve normal pituitary function and allows recovery of visual function.[6,18,19] As a consequence, the transsphenoidal approach is the first choice in the surgical treatment of MPA or GPA with suprasellar extensions.[6,20-22]

The purpose of this study was to determine the frequency and neuroimaging characteristics of macro- and giant pituitary adenomas according to seeking medical care.

Materials and Methods

We retrospectively analyzed data from 3 medical centers of Tashkent city (RSSPMC of Endocrinology, the Republican Research Center of Neurosurgery, and the Republican Scientific Center for Emergency Medical Aid), in which 156 patients (76 men [mean age of 37.1 years] and 80 women [mean age of 38.2 years]) with PMA and GPA were treated during 3 years (between 2015 and 2017). The duration of the disease ranged from 2 months to 25 years. As the surgical management, TSHS was performed in 137 (87.8%) cases (surgeons: R.B. Fayzullaev, MD, PhD; Akbutaev MD; Prof. K. I. Makhkamov, MD, PhD; Prof. Michael Powell, MD, PhD) with repeated operations in 8 (5.1%) cases. Six (3.8%) patients received radiotherapy and 1 - chemotherapy.

Methods of investigation included: 1) general clinical examination, assessment of endocrine/neurological status; 2) instrumental methods of examination (visual field perimetry, colour vision, fundus oculi, visual acuity, ECG, CT/MRI of sella turcica and adrenal glands, ultrasound of reproductive organs); 3) determination of blood hormones (GH, IGF-1, LH, FSH, PRL, TTG, ACTH, testosterone, estradiol, prolactin, progesterone, cortisol) in RIA using "Gamma-12" and "Strantg 300," and 4) histological examination of postoperative specimens.

Statistical analysis was performed using statistical software package SPSS version 20.0 (SPSS Inc, Chicago, IL). Categorical variables were analyzed using the Chi-square test. A probability value of $P \leq 0.05$ was considered statistically significant.

Results

Depending on the size of PA detected on CT/MRI, the patients were divided into two groups: Group 1 included 70/44.9% patients with PMA (from 20 mm to 30 mm in maximum diameter), Group 2 - 86/55.1% patients with GPA (≥40 mm). Table 1 presents the distribution of patients by sex and age. In accordance with the topographic-anatomical classification by B. Kadashev,[23] endosellar adenomas were detected in 38/24.4% cases and endo-suprasellar in 5/3.2% cases, both of which were characterized by chiasmatic syndrome. Endo-supra-infra-ante-latero-retrosellar adenomas were detected in 48/30.7% cases, of which 3 were with extension into the anterior cranial fossa, 5 - into the ventricles, 1 - into the cavernous sinus, 2 - into both cavernous sinus and the middle cranial fossa. This group of adenomas contributed to the development of various neuro-endocrine disorders, as well as pyramidal symptoms due to affection of the motor path.

Table 1.

The age-gender distribution of patients

Age, years	Number of men		Number of women	
	Group 1	Group 2	Group 1	Group 2
13	-	-	1	1
16–29	8	14	10	10
30–44	10	15	13	10
45–59	8	13	12	11
60–74	3	5	4	5
≥75	-	-	1	2
Total: n = 156	29	47	41	39

Infra-supra-laterosellar and infra-suprasellar adenomas were detected with the same frequency - 28/17.9% cases. These cases had more vivid clinical symptoms due to the direction of tumor growth. Disorders of nasal breathing and swallowing (dysphagia) were characteristic of patients with these adenomas.

The distribution of tumors in the sellar region is presented in Table 2. Thus, NFPA was the most frequent type (96/61.5%) ($P<0.05$), whereas somatotropinoma and prolactinoma numbered 23/14.7% and 20/12.8%, respectively. Itsenko-Cushing's disease was detected in two cases (1.3%).

Table 2.

The distribution of patients according to the sellar region tumors and treatment

Nosology	Number of patients		TSHS		Radiation therapy	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
NFPA	43	53 (6*, 3^)	31	45 (5#)	1 (1#)	8
Craniopharyngioma	3	9 (2*,2^,1#)	3	6	-	1
Somatotropinoma	11	12 (2*)	8	10	-	2
Prolactinoma	12	8 (3*, 1^)	11	5	-	-
Hemangioblastoma	-	1	-	1 (1*,1#)	-	2
Itsenko-Cushing's disease	2	-	1	-	-	-
Astrocytoma	-	1 (1*)	-	1 (1CT)	-	1 (1ChT)
Meningioma	-	1	-	1	-	-

**- tumor regrowth, ^- hemorrhage in stroma, # - reoperation, ChT- chemotherapy, CT – combination therapy, TSHS - transsphenoidal hypophysectomy.*

According to MRI, necrosis of PG was diagnosed in one patient, hemorrhage in PG - in 6/3.8% cases. In 14 GPA patients, tumor growth continued after its removal. Malignant type of NFPA was detected in 2 patients.

Based on MRI of PG and X-ray classification by I.I. Dedov,[24] MPA with suprasellar growth were distributed as follows:

• Suprasellar growth within 10 mm above the pituitary fossa - 40 cases

• Suprasellar growth within 20 mm, with extension into the front pocket of the third ventricle (large PA) - 30 cases

• Suprasellar growth up to 30 mm, fills the anterior part of the third ventricle (very large PA) - 50 cases

• Suprasellar growth >30 mm (massive PA), rises above the level of the Monroe hole, or previous adenomas with asymmetrical lateral or multiple-sided growth (massive PA) - 36 cases

GPA showed invasive growth into surrounding anatomical structures in 69.2% of cases and pituitary apoplexy in 3.8% of cases, which was the main factor limiting the radical surgical capabilities and increasing the number of relapses.

In conclusion, the different variants of PMA/GPA suprasellar growth might serve as a neuroimaging marker of tumor aggressiveness, namely, supra-infra-latesellar and supra-infra-tellular growth observed in 68 (43.5%) patients, as well as invasive tumor growth in 108 (69.2%) patients. Transsphenoidal removal is a relatively safe and effective method, even in PMA and GPA with suprasellar extensions, in most of the cases.

Conflict of Interest

The authors declare that they have no competing interests.

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