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REVIEW ARTICLE

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Modern Markers of Renal Damage in Diabetes Mellitus

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Abstract

Diabetes mellitus (DM) is a public health problem worldwide. Despite the presence of various sugar-lowering drugs, correcting hyperglycemia and preventing late DM complications remain a difficult task. Unfortunately, DM complications are often detected at late stages, when there are irreversible changes and a global decrease in the function of damaged organs and systems. One of the most dangerous complications leading to early death of patients is diabetic nephropathy. However, the problem of finding early markers of renal damage that can detect the earliest renal damage in global clinical practice remains unresolved. The following are the modern markers that are likely to be able to timely reflect the preclinical manifestations of diabetic nephropathy (DN): Type-IV collagen, NGAL, b2MG, cystatin C, E-cadherin, podocalyxin, and nephrin. Heparan sulfate, mindin, TGF-β, ICAM-1, KIM-1, uromodulin, and LFABP are also being studied. The task of modern medicine is to find the most sensitive of these markers to diagnose DN in a timely manner. (**International Journal of Biomedicine. 2020;10(1):9-15.**)

Key Words: diabetic nephropathy • markers of renal damage • glomerular filtration rate • albuminuria

Abbreviations

β2MG, β2-microglobulin; **AngII**, Angiotensin II; **CysC**, cystatin C; **CKD**, chronic kidney disease; **DM**, diabetes mellitus; **DN**, diabetic nephropathy; **GFR**, glomerular filtration rate; **ICAM-1**, inter-cellular adhesion molecule-1; **KIM-1**, kidney injury molecule-1; **LFABP**, liver-type fatty-acid-binding proteins; **MAU**, microalbuminuria; **NAU**, normoalbuminuria; **NGAL**, neutrophil gelatinase-associated lipocalin; **TGF-β**, transforming growth factor-β; **T1DM**, type 1 diabetes mellitus; **T2DM**, type 2 diabetes mellitus

Worldwide, the prevalence of diabetes mellitus (DM) is growing every year, acquiring the scale of an epidemic.[1,2] Diabetic nephropathy (DN) is one of the most dangerous complications of DM that lead to patients` early death. Despite the available screening algorithms for this complication, timely detection of DN remains an urgent problem among DM patients.[2]

Hyperglycemia, intraglomerular hypertension, dyslipidemia and chronic inflammation play a role in the formation of this complication,[3,4] as does oxidative stress.[5-7] According to the current classification, there are 3 stages of DN: albuminuria, proteinuria, and renal failure.[3] The stages of proteinuria and renal failure are irreversible, since by the time proteinuria occurs, 50%-70% of the renal mass has already been sclerosed.[3,8] During the progression of nephropathy, the number of actively filtering nephrons continues to decrease, which leads to a drop in the GFR and to uremia formation, so diagnosing DN at an early preclinical stage is a major problem, the solution of which will protect patients from early disability and death. According to current data, 29.5% of patients with T1DM and 36.9% of patients with T2DM have DN in the albuminuria stage.[9] It is known that in the conditions of timely appointed nephron-protective therapy, this stage of DN is capable of regression (remission). These data are confirmed in several studies. In the Araki S. study, 216 patients with T2DM took part, among which the regression rate was 51%

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over the 6-year follow-up period.[10] In STENO-2, 151 patients with T2DM and MAU were studied for 7 years, and 46(30.4%) patients showed regression to NAU stage.[11] Two hundred and seventy-three patients with T2DM were involved in the Kashiwa study for 8 years. Among the studied patients, 94 exhibited DN at the MAU stage, and regression against the background of carbohydrate metabolism correction and blood pressure control was recorded in 44% of patients.[12] Three hundred and fifty-two T1DM patients participated in the EURODIAB study for 7 years, and MAU regression to NAU on the background of carbohydrate metabolism correction reached 50.6%.[13] In this study, patients were not compromised by an additional risk factor in the form of hypertension. In a study of 1513 DM patients, de Zeeuw et al.[14] found a relationship between the level of albuminuria and the prospective kidney condition—the higher the albumin excretion with the urine, the less likely that kidney function will return to NAU. However, the concept of "normoalbuminuric" nephropathy, which requires active study of early markers of renal damage in DM, is increasingly encountered in modern literature data.[15]

To date, determination of serum creatinine and albuminuria levels, GFR calculation, and determination of the albumin to creatinine ratio[3,4] are actively used to diagnose kidney damage in DM in clinical practice. However, these indicators are affected by various factors: The level of serum creatinine and albuminuria may increase transiently with ketoacidosis, a high-protein diet, and increased physical activity; and a decrease in GFR may be temporary in urinary infection and DM decompensation.[8,9] A persistent decrease in GFR occurs if a large mass of working nephrons is lost and renal dysfunction is irreversible. Currently, the well-known physiological reasons make the serum creatinine an imperfect GFR biomarker. Because the relationship between serum creatinine and glomerular filtration rate is hyperbolic, all known analytical limitations will impact not only the precision of serum creatinine but still more the precision of different creatinine-based equations, especially in low or normal-low creatinine levels (or high or normal-high glomerular filtration rate range).[8,16] The task of modern diabetology is to find more sensitive and specific markers of early preclinical kidney damage in order to be able to timely prescribe nephron-protective therapy in order to influence the formation of renal failure. In addition to glomerulosclerosis, tubulointerstitial kidney damage occurs in DN.[17] Tubular changes occur as a result of hyperglycemia and the subsequent cascade of inflammatory reactions with the activation of protein kinase C, which is a catalyst for stimulating collagen synthesis in the kidneys. In addition, when hyperglycemia and intraglomerular arterial hypertension are combined, when AngII levels increase, TFR-β is synthesized, which also leads to increased collagen synthesis, and AngII itself contributes to the death of tubulointerstitial cells.[18] Tubular dysfunction is formed, including in diabetic nephropathy, probably ahead of the glomerular apparatus damage.[19] An additional damaging factor of the tubules is enhanced filtration of plasma proteins, resulting in a "vicious circle," where tubulointerstitial damage is a process that provokes glomerular dysfunction, and against this background, the dysfunction of the tubular apparatus is aggravated.

Type IV collagen. Among the currently actively studied markers of renal damage that are proposed for consideration is type IV collagen (ColIV), which is one of the components of the glomerular mesangial matrix and provides mechanical stability of the membrane, forming a support network. The tendency to the increased excretion of ColIV is observed in DM patients who are already at the NAU stage and increases with further progression of DN. Thus, in the study by Bondar et al.,[19] the urinary ColIV level increased as albuminuria progressed. Among NAU patients, an increased level of ColIV was recorded in 39.1% of cases, which is comparable to the indicators in the control group of healthy individuals. It was noted that in NAU patients, ColIV increased among those who had hypertension. As albuminuria progresses,[20] the amount of ColIV in the urine increases (during the MAU formation): 53.6% of patients had an increased ColIV level, and at the stage of proteinuria – 100%. Similar data were obtained by Cawood et al.[21]: an increased ColIV level was found in 26% and 58% of patients with NAU and MAU, respectively, and at the stage of proteinuria – in 65% of patients. In T2DM 254 patients (185 with NAU and 69 with MAU), Araki et al.[22] revealed an increase in ColIV level in both groups. In a prospective follow-up of 8 years, 16 out of 185(8.6%) patients in the NAU group had elevated levels of ColIV, and 9 out of 69(13%) patients in the MAU group had DN progression. In the study, a clear inverse correlation was found in the relationship between the ColIV level and the GFR level. When observing patients in the NAU group, the annual decrease in GFR was more pronounced among the patients with elevated ColIV levels than among normal ColIV level patients. The same pattern was found in the group of patients with MAU. Morita et al.[23] also recorded data confirming the results in a study of 231 T1DM patients over a period of 7.4±1.3 years: the GFR reduction rate was higher in patients with elevated levels of ColIV in both the NAU and MAU groups. Thus, it is likely that the determination of ColIV excretion can serve for early DN diagnosis and have a prognostic value regarding changes in GFR.

Neutrophil Gelatinase-Associated Lipocalin. NGAL, which is expressed in small concentrations in many tissues, including the kidneys, being an indicator of the tubular apparatus condition, is also considered as one of the markers of kidney damage that may help in early DN diagnosis. In the study of the NGAL level as an early marker of renal damage, Lacquaniti et al.[15] found an increase of it in blood serum of T1DM patients up to 193.7 ng/ml at NAU, while in healthy individuals the median was 46.4ng/ml, in urine – 25.5 ng/ml and 6.5 ng/ml, respectively. Mahfouz et al.,[24] who studied NGAL levels in 150 T2DM patients, obtained similar data. The patients were divided into groups according to the albuminuria level. An increase in the NGAL level was recorded at the NAU stage, in comparison with the group of healthy individuals. The NGAL level increased progressively with the increase in albuminuria level: 46.46±8.56 ng/ml in healthy individuals, 55.6±16.95 ng/ml in DM patients with NAU, 97.8±10.97 ng/ml in DM patients with MAU, and 131.0±27.29 ng/ml in DM patients with proteinuria.

In several studies, NGAL has shown a relationship with creatinine and GFR levels: An increase in the level of

this marker in the urine has a positive correlation with an increase in blood creatinine levels, while in DM patients, the NGAL level increased earlier than did creatinine.[25] A negative correlation with GFR level was found in a study by Woo et al.:[26] In patients with GFR<60 ml/min, the NGAL level was 96.0 [2.7–975.2] ng/ml, and in the control group of healthy individuals with normal GFR - 18.8 [1.3–81.9] ng/ml. An increase in NGAL levels at normal serum creatinine concentrations is a sign of subclinical acute renal damage associated with the risk of rapid progression of this condition to the clinical stage. Nielsen et al.,[27] studying the relationship between NGAL and GFR in 177 T2DM patients, found that a higher NGAL level was associated with a faster decrease in GFR. Similar data were obtained in a study by Zylka et al.:[28] the increased NGAL level was inversely correlated with GFR in 80 T2DM patients at the NAU and MAU stages. Currently, NGAL can probably be considered as a marker of acute renal damage. Its timely detection should make it possible to start treatment earlier in order to prevent the progression and chronization of the renal failure process.[29]

Cystatin C. CysC, a protein with a molecular weight of 13 kDa, is offered as a marker that can participate in a more reliable GFR calculation than can creatinine. Studying the CysC level in 58 T2DM patients, Klimontov et al. compared the GFR levels calculated using CysC and creatinine levels. It was found that in 14(24.1%) patients the difference between these indicators was 11-19ml/min/1.73m^2, and in 12(20.7%) patients the difference was more than 20 ml/min/1.73m^2.[30] Waheed et al.[31] described an earlier decrease in GFR calculated using CysC levels compared with GFR calculated by creatinine levels in patients at the NAU stage. The high sensitivity of CysC in comparison with creatinine was also confirmed by Inker et al.,[32] who analyzed 13 studies involving 5352 DM patients. When recalculating GFR by CusC level, CKD was further reclassified in 19.4% of patients with GFR>60 ml/min/1.73m^2 and 16.9% of patients with GFR in the range of 45-59 ml/min/1.73m^2. This fact is extremely important for establishing the stage of CKD, especially in patients with NAU. It is also interesting to determine the excretion of this marker with the urine.[33] Given that CysC is normally metabolized in the renal tubules, an increase in its level in the urine is an indicator of damage to the tubular apparatus, which precedes MAU. Early detection of an increase in the CysC level in the urine makes it possible to identify patients with the maximum risk of renal complications.[34] The relationship between the patients' body weight and the blood level of this protein is that the higher the proportion of the fat component in the body weight, the lower the GFR calculated by the CysC level.[30] CysC is more sensitive than creatinine for determining GFR, but in some patients (morbid obesity, elderly patients), additional research is required.[30]

β2-microglobulin (β2MG) is a protein with a molecular weight of 11.8 kDa, which is synthesized in all cells of the body that have nuclei; its amount in the blood reflects both the process of cell synthesis and the level of cell decay. β2MG is filtered by glomerular capsules, followed by reabsorption and metabolism in the proximal tubules, so the amount of β2MG in the urine is minimal in normal kidney function. Therefore, an increase in the plasma β2MG level may be evidence of a dysfunction in renal glomerulus capsules, and in the urine the concentration of this marker increases when the renal tubules are disturbed, indicating a pathology of renal filtration.[34] One of the factors that allows β2MG to penetrate the filtration barrier earlier than albumin is the small molecular weight compared to the mass of albumin (69 kDa). Available studies have shown that the level of β2MG excretion in DM patients is higher than in people without DM.(34) This was confirmed by Monteiro et al.[35] in 51 patients with T1DM, compared to healthy individuals. In addition, β2MG has a positive correlation with the levels of creatinine and CysC,[36] and a negative correlation with GFR. In a study by Kim et al.,[37] an increased serum β2MG level was found in T2DM patients with higher MAU. Zeng et al.[38] confirmed the relationship of β2MG level with tubal lesions during subsequent renal biopsy in 46 patients: 30 patients had increased β2MG level, and tubal changes were found in 100% of cases during biopsy. Given the presence of tubular damage in DN formation, it is likely that β2MG can be identified as a marker of renal damage.

E-cadherin. E-cadherin, previously known as an oncomarker for lesions of the colon, breast, etc., is being studied as another suspected indicator of kidney damage in DM. According to available data, the ratio of the E-cadherin fragment to creatinine (80 kDa) in the urine increases in patients with CD at MAU (2751.5±164 mcg/g) and macroalbuminuria (5839.6±428 mcg/g), compared with the control group of patients(652.7±87 mcg/g), and also increases in patients with NAU (721.9±93 mcg/kg).[39] Taking into account the change in the expression of this protein with excessive caloric intake, it is suggested that E-cadherin is involved in the formation of cancer in DN with T2DM,[40] but still, the question of its role in the formation of fibrosis in DN remains controversial to date. Further research is needed to answer this question.

Podocalyxin. Another process that occurs when the glomerulus is damaged is a decrease in the adhesion of podocytes to the basal membrane, which increases the number of podocytes in the urine, which includes both cells that have already undergone apoptosis and viable ones. Podocalyxin, a protein whose amount correlates with the level of glycated hemoglobin and albumin in the urine, is expressed on the surface of podocytes.[41] When studying this marker, it was found that in DM patients at the NAU stage, its number increases in 53.8% of patients, in the MAU stage – in 64.7% of patients, and in the proteinuria stage – in 66.7% of patients, but there was no correlation between the podocalixin level and GFR.[42] A deeper study of this marker is required to accurately answer the question whether its increase can be a predictor of renal damage in DM.

Nephrin. Diagnosis of podocyte damage is an interesting direction in diagnosis of nephropathy. The nephrin protein can also act as a DN marker.[43,44] Normally, nephrin is part of the podocytes involved in the formation of the filtration barrier. An increase in the amount of nephrin in the urine is a consequence of the destruction of podocytes when podocytopathies of various geneses occur. When studying the nephrin level in 381 DM patients, it was found that nephrinuria is closely associated with a decrease in GFR and the ratio of albumin to

creatinine;[43] similar indicators were found by Tai et al.[44] in a study of 70 DM patients. Jim et al.[45] identified the presence of this marker in the urine of 54% of patients with NAU and 100% of patients with micro- and macroalbuminuria. Similar results were recorded by do Nascimento et al.[46]: nephrin was detected in the urine at the NAU stage in 53% of DM patients with DM, at the MAU stage – in 71% of DM patients, and in 90% of DM patients with proteinuria. Another important point is the increase of nephrin in the urine before the level of GFR decreases. In the above study, the average GFR was 85 ml/min/1.73m^2, meaning that the level of this protein increases even before the glomerular function decreases, and nephrin can act as an early marker of tubular damage in DN.

Mindin. Another protein specific for podocyte damage is mindin. Murakoshi et al.,[47] studying the mindin level in mice with DM, found an increase in this marker, compared to a group of healthy animals. In addition, an increased excretion of mindin was found in T2DM patients, in comparison with the control healthy group, and a correlation was established between the mindin level and the ratio of albumin to creatinine.[48] Determining markers of podocyte damage, such as nephrin and mindin, is probably one of the promising areas, but the question of specificity of changes in these indicators remains open.

Heparan sulfate. The glomerular basal membrane contains various types of glycosaminoglycans, one of them is heparan sulfate, the amount of which increases in the urine when the glomerular apparatus of the kidneys is damaged, including in patients with T1DM.[49] However, the question of the specificity of this indicator remains unresolved.

In several studies, the above-described TGF-β has been shown as the marker whose excretion is increased during the formation of a fibroporous process in renal glomeruli[50] and in the formation of micro- and macroalbuminuria in DM patients, compared to healthy individuals.[51]

ICAM-1. A number of studies have shown the relationship between changes in the blood levels of ICAM-1 and the levels of ICAM-1 expression in the kidneys.[52] A study of 63 patients with T1DM revealed a tendency to an increase in ICAM-1 in MAU formation, and a significant increase in this marker was recorded in patients with proteinuria, but there were no significant differences between patients with NAU and healthy individuals.[52]

KIM-1. As an early marker of nephropathy, KIM-1 was studied in several papers. EL-Attar et al. found an increase in the ratio of KIM-1 to creatinine (KIM-1/Cr) in T2DM patients with MAU and proteinuria, and the KIM-1/Cr ratio was a more sensitive method for determining renal damage than determining only the KIM-1 level. The KIM-1/Cr ratio was positively correlated with the AU/Cr ratio.[53]

Uromodulin. Uromodulin is the most abundant protein secreted in urine. Chang et al. recorded a progressive decrease in uromodulin excretion in 62.5% of DM patients with renal insufficiency, compared to 20% of individuals with non-diabetic nephropathy.[54]

L-FABP. *L-FABP* is a protein with a molecular weight of 15 kDa. According to modern concepts, L-FABP can be used as a promising marker for assessing tubule damage. Kamijo-Ikemori et al. examined 142 patients with T2DM, compared to a control group of healthy individuals. During the study, the progression of nephropathy was considered as an increase in albuminuria, the formation of end-stage renal failure, and the initiation of renal replacement therapy (program hemodialysis). According to the study results, a progressive increase in L-FABP was found in DM patients: the level of urinary L-FABP increased according to the stage of nephropathy. The concentration of urinary l-FABP was higher in patients with DM and NAU compared to the control group (P<0.05).[55]

The question whether the indicators of the lipid peroxidation system-antioxidant protection can autonomously act as additional markers of DN remains open. Their role in the formation of vascular complications is known;[5,6,56-60] there are also data on the increase in the level of diene conjugates, malondialdehyde, and ketodienes directly in DN,[61] but whether it is possible to predict the formation or outcome of DN based on changes in their level is a task that modern medicine has yet to solve.

Thus, currently, the main early marker of kidney damage in diabetic patients due to hyperglycemia is albuminuria; at the same time, there is a need to expand the range of markers that allow high accuracy in diagnosing DN in DM at an early preclinical stage with preserved kidney function and laboratory NAU. Probably, a more promising direction in the search for early diagnosis is the study of tubular damage markers, the level of which increases earlier than laboratory indicators of glomerular dysfunction. The direction of studying proteins with a small molecular weight is also seen as promising, so that they penetrate the filtration barrier earlier than albumin. The question about reduction in the level of markers that are likely to participate in the earlier diagnosis of DN remains insufficiently studied— whether the existing renal lesions in DM undergo complete regression or are still irreversible. If there is a decrease in them, then under what circumstances: whether the existing classical nephron-protective therapy and correction of carbohydrate metabolism and dyslipidemia are sufficient or additional methods of influence are required, and whether markers behave the same in patients with T1DM and T2DM, or genetic factors in T1DM, age and body weight in T2DM and additional aspects of pathogenesis will require modern medicine to develop different corrective approaches. The presence of a high level of tubular and glomerular damage markers in DM patients in the absence of a high level of albuminuria increasingly leads to talk about the presence of normoalbuminuric nephropathy in some patients, which may require adjustments to the modern classification of DN.

Competing Interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Methods for Treatment of Malignant Pleural Effusion

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Abstract

This brief review provides up-to-date information on the management of malignant pleural effusions (MPE). In general, selection of the most appropriate treatment approach should be individualized. Management of MPE relies on tumor type, pulmonary re-expansion, performance status, symptoms, and life expectancy. Pleurodesis and IPC placement are two effective treatments recommended for recurrent MPE, both of which can effectively improve dyspnea and quality of life of patients. Other options such as intrapleural therapies, radiation therapy, and pleuroperitoneal shunting are alternative treatments. However, most of these treatments are temporary, and MPE would recur soon. Hence, further palliative treatments to effectively control pleural effusions and relieve symptoms are necessary. (**International Journal of Biomedicine. 2020;10(1)16-19.**)

Key Words: malignant pleural effusions • thoracentesis • pleurodesis • indwelling pleural catheters

More than 300,000 patients in the Russian Federation die every year from malignant neoplasms, which thus occupy the third place in the mortality structure of the country's population and remain the most important medical and social problem.

One of the most common complications of tumor diseases is malignant pleural effusions (MPE). In the structure of the general incidence, the proportion of pleurisy reaches 4%, while the oncological etiology accounts for 63% of all exudative pleurisy.

The majority of MPE is caused by metastatic disease: most commonly lung cancer in men and breast cancer in women.[1] These two cancers combined account for 50%–65% of all MPE.[2-5] Mesothelioma is the most common type of primary pleural tumor and is associated with MPE in more than 90% of cases.[3] In 12% of patients with MPE, it is not possible to establish the nature of the primary tumor. The presence of MPE indicates an advanced stage of the disease with a median life expectancy of 3 to 12 months, depending on the stage and type of underlying malignancy.[6] There are more than 100,000 new cases of MPE yearly in Russia.

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Although the first randomized trial for MPE treatment methods was performed in 1977,[7] the optimum management of the disease remains under debate and research. In MPE patients, dyspnea is the most common presenting symptom followed by chest discomfort and cough.[3,8,9] The quality of life is improved by local treatment methods, which not only help reduce the symptoms of pleurisy, but also extend the life of patients from several months to 1-3 years. Prior to considering any definitive treatment intervention, all patients with MPE should undergo a therapeutic aspiration to assess symptomatic improvement and rate of fluid reaccumulation.

During the past two decades, there has been a change in direction in MPE research and management.[1] Historically, studies were focused on halting pleural fluid accumulation and often employed aggressive surgical methods (pleurectomy);[10-12] and most clinical trials[13,14] aimed at identifying the best agent that would achieve obliteration of the pleural space (pleurodesis). The most common end-point of these early studies was radiological improvement at 1-3 months post-pleurodesis, without consideration of the patients' symptoms.[15] Currently, the treatment approach for patients with MPE is mainly aimed at alleviating their symptoms and improving quality of life indicators, which is a key goal of treatment.[16]

In general, selection of the most appropriate treatment approach should be individualized. Management of MPE relies on tumor type, pulmonary re-expansion, performance

status, symptoms, and life expectancy. Asymptomatic patients with a known tumor type who are responding well to systemic therapies should be under observation.[3] Some cancers, such as small cell lung cancer, lymphoma, breast cancer, prostate, and ovarian cancer, may respond well to chemotherapy.[3,17-19]

Patients who have a life expectancy of more than 3 months or are resistant to chemotherapy should be given palliative treatments,(20) such as observation, thoracentesis, indwelling pleural catheters (IPCs), pleurodesis, intrapleural therapies, radiation therapy, and pleuroperitoneal shunting (PPS).[3,20-22]

Thoracentesis is generally safe, especially if it is performed with ultrasound guidance.[23] Thoracentesis is a good choice for patients with advanced disease and a short life expectancy (1-3 months), slow pleural fluid reaccumulation, or poor performance status that precludes the patient from other interventional therapies.[21,24] The amount of fluid evacuated by pleural aspiration will be guided by patient symptoms and should be limited to 1.5L on a single occasion. [3,25] Pneumothorax is one of the most common complications associated with thoracentesis, with an incidence rate as high as 20%-39%.[26] Re-expansion pulmonary edema occurs rapidly if the removed fluid is more than 1.5L.[27,28] As known, almost all patients experience recurrence of symptoms and effusions within 1 month.[3,29] Although thoracentesis does not improve survival, it can significantly improve the patient's condition and avoid hospitalization.

Pleurodesis refers to the process of chemically or mechanically inducing pleural inflammation to the visceral and parietal pleura to obliterate the area and prevent the accumulation of air or liquid in the pleural space. Instillation of the sclerosing agent is thereafter followed by a profound inflammatory response between the layers, which, in turn, result in fibrin accumulation and pleural fibrosis. Pleurodesis is a better option for recurrent MPE than thoracocentesis unless the patient has a very poor performance status, a short life expectancy, or a trapped lung.[27] A variety of different chemicals (e.g. talc, bleomycin, tetracycline, iodopovide) and bacterial products (Corynobactum parvum, Streptococcus pyogenes, Staphylococcus aureus, and others) have been used in clinical studies to achieve pleurodesis.[30-33] The profound inflammatory response they may result in adverse events, such as pain and fever, but it is believed that the level of inflammation correlates with the likelihood of successful pleurodesis.[34] Chest pain and fever are the most common complications of chemical pleurodesis. Other complications include a cough, empyema, local site infection, trapped lung, and acute respiratory distress syndrome.[29] The type of sclerosant, the method of administration and the method of selecting patients to be treated with pleurodesis are still unclear. Talc is the most effective sclerosant available for pleurodesis, especially graded talc, which can be delivered as slurry via an intercostal catheter or by dry-powder poudrage during a thoracoscopy.[3] Talc was first used for pleurodesis in 1935.[35] It has been proven that graded preparations (as opposed to small particle talc) should be used to minimize systemic dissemination of talc particles and the risk of acute respiratory distress syndrome.[36,37]

Results from the largest randomized trial in MPE revealed that the success rates of talc slurry and talc poudrage are not significantly different.[38,39-41] A subgroup analysis of patients with lung and breast cancer suggested a better success rate for talc poudrage.[38] On the contrary, a metanalysis performed by Xia H et al.[42] demonstrated that talc poudrage was superior to talc slurry in pleurodesis success. According to Zhestkov& Iaduta,[43] in a series of 132 patients, the effectiveness of the method was 97.7%, pleurisy recurrence was diagnosed in only 3 patients, and no serious postoperative complications were recorded. Domestic authors recommend talc pleurodesis with daily exudation of pleural effusion up to 300 ml, bleomycin pleurodesis with daily exudation up to 700 ml and combined pleurodesis at more than 700 ml; in case of failure, pleurodesis by videothoracoscopy is recommended.

IPCs have gained popularity during the last decade as they offer ambulatory management, thereby minimizing hospital stay and healthcare costs.[44] An IPC is a silicone tube placed in the pleural cavity and tunneled subcutaneously. The proximal end of the exposed tube has a one-way valve, which connects to drainage bottles. Drainage is guided by symptoms and is patient-driven, offering a sense of control to most patients. The optimal schedule of MPE drainage through an IPC is still not clear. This system provides the patients, or people who care for them, complete control over the removal of fluid from the pleural cavity. Such systems are rarely used in Russia; domestic scientific literature on this issue could not be found. The British Thoracic Society Pleural Disease Guideline recommends the use of IPCs in those patients with MPE that have failed pleurodesis or in those with trapped lung (unsuitable for pleurodesis).[3] A meta-analysis of 1348 patients with MPE treated with IPCs revealed that 95.6% had symptomatic improvement and 45.6% achieved spontaneous pleurodesis after a median of 52 days.[45] The TIME2 randomised controlled trial showed that IPCs achieved control of breathlessness and quality of life comparable to talc pleurodesis, median length of hospitalization was significantly shorter in the IPC group than talc group (0 vs. 4 days; $P<0.001$). Fewer patients with IPCs required further pleural procedures than talc group (6% vs. 22%, $P=0.03$).[16] Another prospective multicenter study achieved the same results.[46] There is ongoing research on possible combinations of IPC with sclerosant agents in order to enhance pleurodesis success.[47] Recent data also provide reassurance on the safety of IPC use, with a risk of death from pleural infection below 0.3%.[48] As IPCs offer long-term access to the pleural cavity, they represent ideal potential portals for local drug delivery.

There is another method proposed by Plaksin and Farshatova[49] for effective control of pleural effusions and obliteration of the pleural cavity: Through a drainage tube installed during thoracoscopy or during drainage of the pleural cavity, 50 ml of a 1% lidocaine solution is injected into the pleural cavity for pain relief, after which the tube is closed for 20 minutes. After that, 40-80 ml of a previously prepared mixture consisting of 1% iodopyron solution and 40% glucose solution in a 1:4 ratio is injected into the drainage tube using a syringe. The drainage is closed for 2 hours. At this time, the patient repeatedly changes the position of the body

so that the drugs get into all parts of the hemithorax. Then the drainage is opened and active aspiration is continued. Drainage is removed from the pleural cavity with a decrease in the volume of exudation to ≤100 ml of effusion per day. The disadvantages of this method of treatment are (1) insufficient analgesia; since there is no premedication (anesthesia before surgical procedures), anesthetic (lidocaine) is administered once; and (2) the use of a standard drainage tube of the same diameter without through holes, which does not allow effective irrigation of the pleural cavity with drugs, thereby reducing their effectiveness.

In conclusion, pleurodesis and IPC placement are two effective treatments recommended for recurrent MPE, both of which can effectively improve dyspnea and quality of life of patients. Other options such as intrapleural therapies, radiation therapy, and PPS are alternative treatments. However, most of these treatments are temporary, and MPE would recur soon. Hence, further palliative treatments to effectively control pleural effusions and relieve symptoms are necessary.

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ORIGINAL ARTICLE

Cardiology

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Predictive Markers of Atrial Fibrillation Progression in Heart Failure

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Abstract

Atrial fibrillation (AF) is one of the most common arrhythmias encountered in clinical practice. It has been found that the presence of chronic heart failure increases the risk of AF by 5 times. Furthermore, with an increase in the severity of symptoms of heart failure, the incidence and severity of AF increases. To date, markers of AF progression are not clearly defined. In this regard, it is relevant and reasonable to identify such markers in patients with heart failure.

The aim of this research was to study the prognostic value of the 6-minute walk test (6MWT), NT-proBNP level, and left ventricular diastolic dysfunction for AF progression in patients with chronic heart failure (CHF).

The study involved 96 participants with stable Class II and III CHF (the NYHA Functional Classification), who were included in the regional registry of CHF patients in September-November 2014. The data obtained showed that in patients with CHF, an increase in the level of Nt-proBNP, impaired myocardial relaxation, and a decrease in 6MWT distance may serve as predictors of AF progression with the transition of arrhythmias to stable forms. (**International Journal of Biomedicine. 2020;10(1):20-23.**)

Key Words: atrial fibrillation • NT-proBNP • 6-minute walking test • diastolic dysfunction

Abbreviations

6MWT, the six-minute walk test; **AF**, atrial fibrillation; **CHF**, chronic heart failure; **IVST**, interventricular septal thickness; **LA**, *l*eft atrium; **LVEF**, left ventricular ejection fraction; **LVDD**, left ventricular diastolic dysfunction; **LVESD**, left ventricular end-systolic dimension; **LVEDD**, left ventricular end-diastolic dimension; **LVPWT**, left ventricular posterior wall thickness.

Introduction

Atrial fibrillation (AF) is one of the most common arrhythmias encountered in clinical practice.[1] In developed countries, there has been a steady increase in the number of patients with AF. This increase is associated with an increase in life expectancy, an increase in the occurrence of pathology of the cardiovascular system, and an improvement in the detection of latent forms of arrhythmia. At the same time, despite significant progress in diagnosis and treatment, this pathology remains one of the main causes of stroke, heart failure (HF), sudden cardiac death, and premature dementia.

There are three main mechanisms for the appearance and progression of AF: genetic predisposition, arrhythmogenic cardiomyopathy and structural myocardial remission in various heart pathologies.[2,3] The course of AF and its form depend on the prevalence of one of these mechanisms over others. In most patients with a paroxysmal or persistent form of AF, the role of genetic factors in the development of arrhythmia is

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small. The progression of arrhythmias is associated with age and the presence of cardiovascular disease. In patients with persistent AF, the transformation of arrhythmias into more stable forms is determined by the presence of cardiovascular predictors of progression.[2]

It has been found that the presence of chronic heart failure increases the risk of AF by 5 times.[4] The occurrence of AF in patients with heart failure is associated with atrial fibrosis, which is a hallmark of arrhythmogenic remodeling. Atrial fibrosis triggers are the activation of the renin-angiotensin-aldosterone system, inflammation, and oxidative stress.[5-8] Furthermore, with an increase in the severity of symptoms of heart failure, the incidence and severity of AF increases.

To date, markers of AF progression are not clearly defined. In this regard, it is relevant and reasonable to identify such markers in patients with HF.

The aim of this research was to study the prognostic value of the 6-minute walk test (6MWT), NT-proBNP level, and left ventricular diastolic dysfunction (LVDD) for AF progression in patients with CHF.

Materials and Methods

The study was open-label, prospective and observational. It involved 96 participants with stable Class II and III CHF (the NYHA Functional Classification), who were included in the regional registry of CHF patients in September-November 2014. The diagnosis of CHF was made on the basis of the "ESC Recommendation for the Diagnosis and Treatment of Acute and Chronic Heart Failure," developed by the Working Group of European Society of Cardiology (ESC) for the Diagnosis and Treatment of Acute and Chronic HF with the participation of the Heart Failure Association as part of the ESC.

The 6MWT was used to measure the functional status of patients. The average follow-up was 35±2 months and included a routine examination by the researcher every 3 months, telephone contacts with patients in case of a heartbeat/interruption with ECG recording, and annual general clinical and laboratory instrumental examination: ECG, 24-hour ECG monitoring, echocardiography. AF progression was assessed by the results of 24-hour ECG monitoring and patient diary data. The appearance of a long-term, persistent (up to 1 year) or persistent form of AF was considered as a progression of arrhythmia.

All patients underwent a standard examination, which included clinical, laboratory and instrumental methods. Exercise tolerance (ET) was determined by the 6MWT. In order to assess ET, a complex of cardiorespiratory analysis was used to carry out functional medical tests, recording the distance in 6MWT, pulse oximetry, and heart rate.[9-11] Laboratory methods included a general blood test, general urine analysis, and biochemical and enzyme-linked immunosorbent assay for determining the level of NT-pro-BNP using the "sandwich" version of the solid-phase ELISA and reagent kits "NT-proBNP-IFA-Best" (Vector Best, Russia) on the IMMULITE 2000 automatic analyzer (Siemens Diagnostics, USA). Measurements were performed at the time of inclusion in the study and annually during the observation time.

LVDD was evaluated using Doppler flow studies. Measurements of transmitral inflow included the peak early filling (E-wave) and late diastolic filling (A-wave) velocities (cm/s), the E/A ratio, deceleration time of the E-wave (ms), and isovolumetric relaxation time (IVRT) (ms).

The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. The study was approved by the Ethics Committee of Voronezh State Medical University. Written informed consent was obtained from each patient.

All data was evaluated with STATGRAPHICS Plus 5.1 For descriptive analysis, results are presented as mean±standard deviation (SD), median (Me), and 95% confidence interval (95%CI). For data with normal distribution, inter-group comparisons were performed using Student's t-test. Mann-Whitney U test and Wilcoxon criterion were used to compare means of variables not normally distributed. Group comparisons with respect to categorical variables were performed using chi-square tests 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

During the entire observation period, none of the patients were dropped out due to death. Cardiovascular complications were detected in 5(5.2%) patients: myocardial infarction was diagnosed and confirmed by laboratory and instrumental methods in 4(4.1%) patients, and acute cerebrovascular accident in 1(1%) of the patients. Arrhythmia progression was observed in 31(32.3%) patients. Two groups of patients were formed depending on the clinical course of AF. Group 1 included AF patients who did not show any progression of arrhythmia during the observation period (n=65); Group 2 included patients with AF progression (n=31). Groups 1 and 2 were comparable in gender and age composition, the presence of arterial hypertension, coronary heart disease, diabetes mellitus, and chronic obstructive pulmonary disease. The proportions of patients who previously had myocardial infarction were also comparable in both groups.

The initial 6MWT distance reflects a statistically significantly lower ET in patients of Group 2 compared with patients of Group 1 (by 33 m, $P=0.0159$) (Fig.1). At the time, in Groups 1 and 2 there was no significant difference in LVEF (43.03±1.23% and 44.18±0.65%, $P=0.08$), LVESD (4.11±0.07 cm and 4.06±0.06 cm, $P=0.46$), LVEDD (5.63±0.09 cm and 5.25±0.05 cm, $P=0,77$), the size of LA(4.51±0.2 cm and 4.46±0.14 cm, $P=0.32$), LVPWT (1.24±0.05 cm and 1.27±0.02 cm, $P=0.71$), and IVST (1.19±0.03 cm and 1.24±0.02 cm, $P=0.09$).

The analysis of the initial parameters of LVDD in Groups 1 and 2 showed that LVDD type I was found in 80.6% and 23%, respectively ($P=0.013$), LVDD type II in 19.4% and 70.7%, respectively ($P=0.008$), and LVDD type III in 0 and 6%, respectively ($P=0.024$).

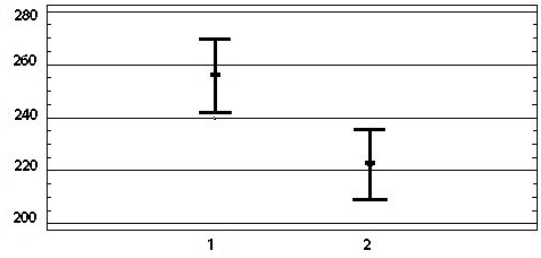

Fig. 1. *The initial 6MWT distance (m) in Groups 1 and 2 (P=0.0159).*

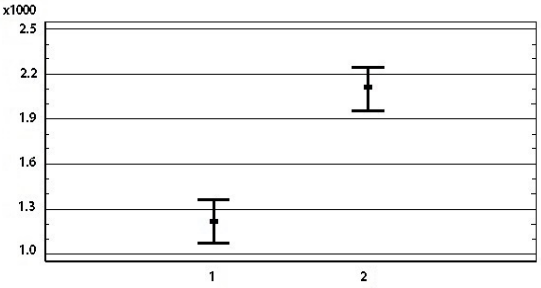

Fig. 2. *The initial Nt-proBNP level (pg/ml) in Groups 1 and 2 (P<0.001).*

The initial Nt-proBNP level in Group 1 was 1.7 times lower than in Group 2 (P<0.001) (Fig. 2).

Discussion

Thus, the data obtained showed a higher Nt-proBNP level in the group of patients with progressive AF compared with the group of patients with a stable course of arrhythmia. The higher initial Nt-proBNP level in these patients can be explained by the following structural and functional changes in the myocardium: a higher level of myocardial fibrosis and impaired relaxation of cardiomyocytes, which caused arrhythmogenic remodeling of the myocardium of the LA and AF progression (i.e. the transition of paroxysmal AF into more stable forms of arrhythmia—persistent and permanent).

In CHF patients, a statistically significant effect on the progression of arrhythmias, in addition to an increase in the Nt-proBNP level, was exerted by the following factors: impaired myocardial relaxation and decreased 6MWT distance (reduced ET). Impairment of the diastolic function of the left ventricular myocardium in patients with arrhythmia progression, revealed upon inclusion of patients in the study, could be the result of hypertrophy and/or decreased elasticity of the cardiomyocytes/myocardium. Since the frequency of arterial hypertension was comparable in the study groups, and the initial echocardiography data did not show a statistically significant difference in the values of LVPWT and IVST, it can be assumed that it was the decrease in myocardial elasticity (fibrosis) that made the most important contribution to the progression of AF into more stable forms. Impairment of LV myocardial relaxation leads to a redistribution of the transmitral blood flow: Most of the blood enters the LV during the LA systole. This causes an increase in the load on LA cardiomyocytes, which is accompanied by a compensatory increase in the filling pressure and subsequent structural reorganization of the LA, which, in our opinion, also contributed to the progression of AF and lower initial results of 6MWT.

The results of our study are consistent with data from other authors, in particular, a retrospective analysis of patients who were included in the BOREAS-CAG registry. In that study, it was found that an increased Nt-proBNP level in patients with coronary artery disease was the main factor predisposing to the development of new cases of AF and the development of heart failure.[12]

Thus, in patients with CHF, an increase in the level of Nt-proBNP, impaired myocardial relaxation, and a decrease in 6MWT distance may serve as predictors of AF progression with the transition of arrhythmias to stable forms.

Competing Interests

The authors declare that they have no competing interests.

Disclaimers

The opinions expressed in this article are the authors' own and do not reflect the view of the institutions or funder.

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ORIGINAL ARTICLE

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Spatial Synchronization of Hemodynamics and Metabolism in Norm

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Abstract

Synchronization of the curves of central and peripheral hemodynamics, according to the data obtained during catheterization, showed the intersection points of the graphical pressure curves, which were shown in previous works. The three-chamber block of the ventricles (with leading role of left ventricle) in the isometric contraction phase (D(Q)) generates three wave impulses: 1) retrograde impulse of coronary sinus directed to the zone of the integral combination (hemodynamics, metabolites of metabolic zones, hormones) and partial interference of venous flows coming to right atrium (end section of systemic circulation); 2) anterograde impulse of right ventricle, which heads to the exchange zone of the lung (initial section of pulmonary circulation) and outrunning of the "venous bolus"; 3) anterograde impulse of left ventricle, ahead of the "arterial bolus" (initial section of systemic circulation), leaving along Ao into the vascular bed to the exchange zones of peripheral organs.

We have interpreted intersections of hemodynamic curves as "zone of temporal equalization of pressure" (ZTEP), both in the heart and in remote topographic zones. Based on the results of previous works, we built separate ZTEP plots for the high- and low-energy phases of the cardiac cycle (CC), relative to cardiac mean integral pressure (CMIP). The genesis and development of key points of the low-energy phase are considered. We believe that the distributed ZTEP matrix created in the vascular bed during each CC, consisting of short-term synchronous equal-sized pressor structures interfering with the aorta wave impulse, has a regulatory effect on the CC phase sequence, the hemodynamics of organs (including peripheral resistance), and metabolism. The systemic distributed ZTEP association—a formed CMIP—is a synchronized (between ZTEPs, with the phases of CC, and electrocardiography), high-speed, information-regulatory structure of the interaction of hemodynamics and metabolism of both central and peripheral organs, which is involved in the control and regulation of homeostasis as a whole. (**International Journal of Biomedicine. 2020;10(1):24-28.**)

Key Words: cardiosynchronization • pulse wave • cardiac hemodynamics • hemostasis

Abbreviations

Ao, aorta; **CC**, cardiac cycle; **CBF**, cerebral blood flow; **CS**, coronary sinus; **Cat**, catalase; **CMIP**, cardiac mean integral pressure; **EF**, ejection fraction; **ECG**, electrocardiography; **Er**, erythrocyte; **F-n**, fibrinogen; **IC**, integral curve; **IVC**, inferior vena cava; **LA**, left atrium; **LV**, left ventricle; **MV**, mitral valve; **PC**, pulmonary circulation; **PEP**, pressure equalization point; **P-n**, total protein; **PV**, pulmonary valve; **PT**, pulmonary trunk; **RA**, right atrium; **RHV**, right hepatic vein; **PW**, pulse wave; **Plas**, plasma; **Prot**, protein; **RV**, right ventricle; **SC**, systemic circulation; **SVC**, superior vena cava; **SS**, sigmoid sinus; **SAH**, stabile arterial hypertension; **TV**, tricuspid valve; **TP**, trigger point; **ZTEP**, zone of temporal equalization of pressure.

Materials and Methods

The aim of this study was to determine the mechanisms of synchronization of zones of temporal equalization of pressure (ZTEPs) obtained during the construction of graphs of central and peripheral hemodynamics.

ZTEPs in the points of intersection of pressure curves (calculated by average values) on the graphs that we obtained during catheterization of vessels in the normal state[1-5] denote short periods of equality of pressure indicators in blood sampling points. The intersection of the graphical pressure curves for a particular subject is a PEP (pressure equalization

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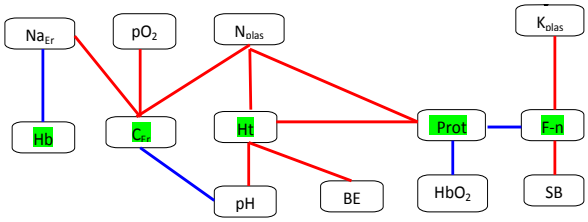
point), which does not have a range of confidence interval for coefficient value (as opposed to average values), which means a short-term alignment of pressure indicators at two or more exchange entry/exit exchange zones of two or more organs. In the graphs at the intersections of the curves, we registered point coincidence and pressure equalization for hemodynamic curves synchronized in time with the CC phase and ECG, which together constitute a distributed system.

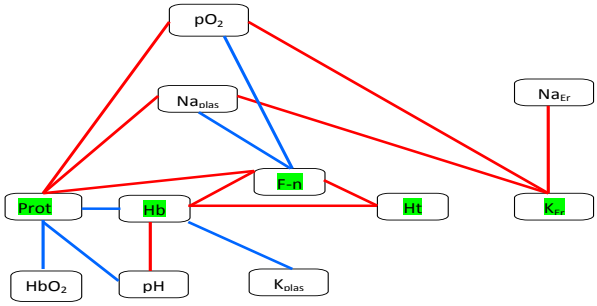
Results and Discussion

The tables we gave earlier[6,7] contain the data of a correlation analysis between the parameters of metabolism and hemodynamics obtained at the points of blood sampling during catheterization in all organs we examined. We believe that each topographically stable point of catheterization, a complete set of which is presented in the form of "pressure curves" at all stages of pressor dynamics, is synchronous to the phase of metabolism (metabolic activity) in the corresponding organ at the time the initial data were obtained (hemodynamics, biochemistry, hormones). As an example, we present the data of correlations obtained at the input (Ao) and output (SS) from the cerebral blood flow of metabolites (i.e. a dynamic summary structure of brain metabolic connections, without impurities of extracranial blood) (Tables 1 and 2).



Scheme 1. The input in the CBF system (control group) - Ao

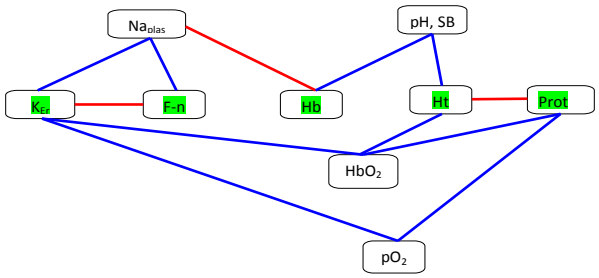
- negative correlations
- direct correlations



Scheme 2. The output from the CBF system (control group) - SS

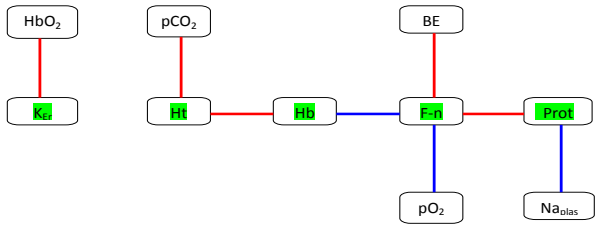
- negative correlations
- direct correlations

As the "structural" parameters, we chose a number of indicators whose concentration does not change throughout one CC (F-n, P-n, K_{Er}, and other). For a comparative analysis, we present similar indicators obtained by catheterization in patients with SAH[7] (Tables 3 and 4).



Scheme 3. The input in the CBF system (SAH) - Ao

- negative correlations
- direct correlations



Scheme 4. The output from the CBF system (SAH) – SS

- negative correlations
- direct correlations

We note radical differences from the norm in the entire set of correlation relationships (i.e. the structures of cerebral metabolism, both at the entrance and at the exit from the system of cerebral blood flow in SAH).

The differences we found in the mechanisms (including metabolic) of maintaining stable cerebral blood flow in normal conditions and hypertension were significant. We presented our analysis[7] of the differences between the norm and SAH earlier. Here, we emphasize the synchronism of hemodynamic and metabolic organ processes at the informational point when receiving data by catheterization.

In other words, the data obtained during catheterization made it possible to obtain not only certain indicators, but also (through a set of correlation connections) the ability to build synchronous graphical portraits of hemodynamics and intraorgan metabolism (similar to the above for cerebral blood flow) for each organ examined.

Our data [1-5] made it possible to construct separate ZTEP plots of the high- and low-energy phases of CC relative to each other and the average integral pressure of CC as a universal rhythmic cycle that sets the main parameters of the frequency and intensity of all hemodynamic wave processes in the body (Fig.1).

Damping and dissociation of the stroke energy of the aortic flow wave structures and targeted selection of segments

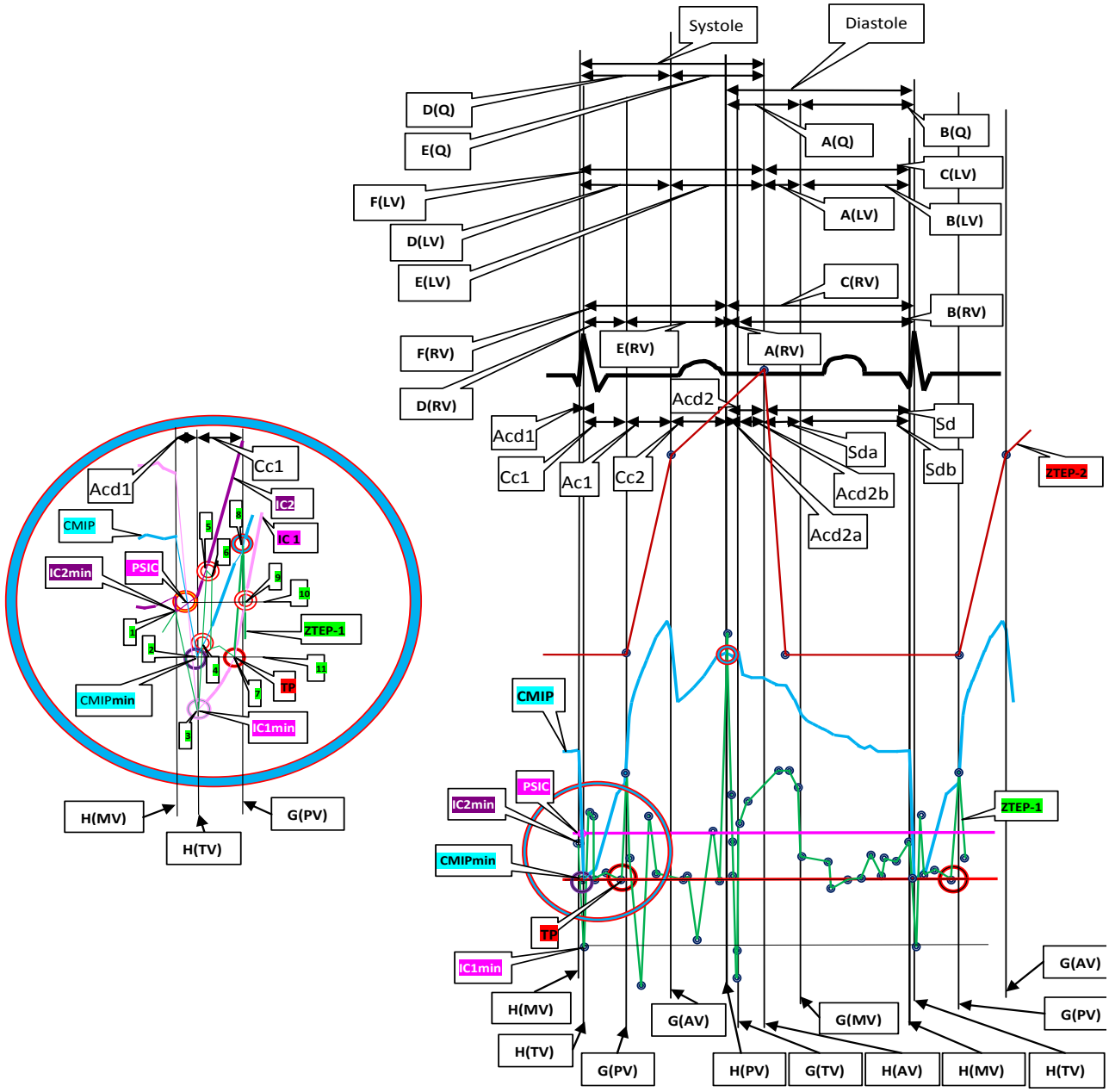


Fig.1. ZTEP of the high- and low-energy phases of CC

A(Q) – isometric ventricular relaxation
B(Q) – actual ventricular diastole
C(LV) – LV diastole
A(LV) – isometric LV relaxation
B(LV) – actual LV diastole
C(RV) – RV diastole
A(RV) – isometric RV relaxation
B(RV) – actual RV diastole
D(Q) – isometric ventricular contraction
E(Q) – actual ventricular systole
F(LV) – LV systole
D(LV) – isometric LV contraction
E(LV) – actual LV systole
F(RV) – RV systole
D(RV) – isometric RV contraction
E(RV) – actual RV systole
G(AV) – opening of AV
H(PV) – closing of PV
G(TV) – opening of TV
H(AV) – closing of AV
G(MV) – opening of MV
H(TV) – closing of TV
H(MV) – closing of MV
G(PV) – opening of PV **H(PV)** – closing of PV

Acd1 – asynchronous period of ventricular systole-diastole -1
Cc1 – synchronization period of isometric ventricular contraction -1
Ac1 – asynchronous period of ventricular systole -1
Cc2 – synchronization of the actual ventricular systole -2
Acd2 – asynchronous period of ventricular systole-diastole -2
Acd2a – from closing of PV to opening of TV
Acd2b – from opening of TV to closing of AV
Sd – period of synchronization of ventricular relaxation
Sda – isometric relaxation of LV
Sdb – actual LV diastole

– TP including ZTEP: SS-VH-SVC-CS-RV-LA
– intersection point for ZTEP
– point of "stabilization" of ICs of the right and left parts of the heart (PSIC)
– CMIP; – ZTEP-1; – ZTEP-2
– intersection of ZTEP-1 with CMIP
– IC1; – IC2
– point of "stabilization" of ZTEP-1 with IC1 and IC2

ZTEP : 1 – H(MV)-IC 2_{min}; 2 – CMIPmin-(SS_{med}-VH_{med}); 3 – H(TV)-$IC1_{min}$; 4 – $CMIP_{min\uparrow}$-($La_{max\downarrow}$-$SS_{min\uparrow}$);

5 – IC $2_{min\uparrow}$-($LK_{max1\downarrow}$ -$PT_{min\uparrow}$); 6 – ($LV_{cdp\uparrow}$- $PT_{min\uparrow}$); 7 – TP(SS-VH-SVC-CS-RV-LA); 8 – G(PV- CMIP-LK_{max1};

9 – IC $1_{min\uparrow}$- $RV_{cdp\uparrow}$- CS_{max}. Equality of spaced points: 10 – PSIC = 9; 11 – CMIPmin = TP

(spheroid sectors) of the arterial bolus directed to the exchange zones are ensured by evolutionarily determined angles of departure from Ao and further branching of the great vessels conducting the impulse and then the blood substrate from Ao to each organ of the body.

For example, Truncus coeliacus, departing from the aorta at a right angle, is divided into A. lienalis and A. hepatica, which depart from it at sharp angles, transforming the propagation and influence of the impulse wave to the exchange zones, adapting (specializing) PW for each organ. Previously, we described the damping effect of SS for the exchange fields of brain tissue.[7] Also, we presented data [6,8] on the mechanism of the influence of PW propagating along the vascular wall and outrunning of the movement of the blood substrate along the vascular bed. In other words, the angular characteristics of the "Ao-main artery" relationship determine the segment of the aortic spheroid, speed, and volume of the arterial bolus fragment, targeted from the aortic bed to the exchange zones of organs. That is, each organ receives an individual wave impulse specific to it ("information packet") at the entrance to the organ's exchange zone, with the same biochemical composition as the arterial blood that is coming.

In the venous sectors of SC and PC, hemodynamic gradients ("organ-heart") form boost pressure at the exit from organs (exchange zones). In the arterial sector, hemodynamic gradients ("heart-organ") are involved in adaptive changes in blood vessels (address distribution of aortic spheroid segments) at the entrance to the exchange zone to optimize the intake of blood substrate brought by the arterial bolus.

The ZTEPs on the graphs indicate PEP characterizing topographic stability, equality of pressor indices, and short-term synchronism in time and CC phase, making it possible to construct an objective picture of metabolism in PEP coincidence organs.

In other words, ZTEPs (PEPs) make it possible to obtain simultaneous, synchronous information about organ hemodynamics and the structure of intraorgan metabolism in a certain phase of CC for organs with coincident ZTEPs (PEPs). The sequential distribution of ZTEPs among the SC and PC hemodynamic vectors, synchronized with ECG and CC phases, makes it possible to construct a design for monitoring and regulating hemodynamic and metabolic homeostasis as a whole.

We consider it appropriate to divide the ZTEP routing into two routes, high- and low-energy, in accordance with the CC phases (on the graph: above and below CMIP). High-energy ZTEPs are points of pressure-mediated effects (through ductile walls at points of direct contacts with LV and Ao; ligamentous apparatus, for example, lig. arteriosum, connecting Ao and PT, etc.) of the dynamics of isometric transformation of the ventricles (RV/LV systole) and arterial wave impulse on RA, PT, LA and peripheral vessels (shown in the part of the graph above CMIP).

In this paper, we deem it appropriate to consider some ZTEPs related to the low-energy phase of CC. Each CC begins with IC-2min, attributable to the end of the previous CC with the closure of MV and the beginning of the asynchronous period of isometric contraction of the ventricles (Acd1) of the current CC. During this period, against the background of the completion phase of the RV diastole and contraction of the RA appendage of the previous CC, a period of isometric contraction of LV of the subsequent CC begins, forming the CS impulse. This impulse, interfering with the RA appendage impulse, reaches RV (i.e. the final stages of the dynamics of the exchange zones of the brain (SVC) and liver (IVC) and other organs, participating in the creation of boost pressure in these zones).

In the middle of Acd1, a point of stabilization of the integral pressure curves (PSIC) of the right and left heart (IC-1, IC-2) is formed. PSIC is a point of temporary pressure equalization at the SC inlet and outlet, described in a previous work.[5] We believe that PSIC: 1) determines the sequence of triggering phases of the isometric contractions of the ventricles (RV after LV); and 2) determines the level of perfusion pressure in the brain, liver and lung after the opening of PV. The mechanism of synchronization of the "ventricular block" in the period of the formation of the spheroids of RVEF (current cycle) and LVEF (the end of the previous cycle, which influences the RV by the CS ejection impulse) is initiated in PSIC.

The next ZTEP point in the Acd1 period is CMIPmin, which coincides with ZTEP at the pressor level of exit from the brain and liver exchange zones (SSmed-VHmed). It creates equal hemodynamic conditions in the exchange zones (including equal pressure levels), forming hemodynamic parameters of the next stage (TP).

The Acd1 period ends with IC-1min, attributable to the closure of TV and the beginning of the synchronization period of the isometric ventricular contraction (Cc-1). IC-1min is characterized by the creation of a base pressure in RV at the time TV closes (VDcdd).

The following is the period of synchronization of isometric ventricular contraction (Cc1), characterized by: 1) involvement of RV to CS impulse formation in LV, and 2) complete isolation of the three-chamber block of the ventricles (except CS) from the SC/PC collectors of variable capacitance. Thus, the CS impulse is twice corrected during the phase of the isometric contraction of the ventricles (D(Q), interfering with the RA appendage systole and the isometric contraction of RV, which during the period Cc1 is "superimposed" on the isometric contraction of LV. During this period, due to the systolic suction function of the ventricles and the dynamics of pressure gradients in the variable capacities of the venous SC segment and arterial PC segment, TP is formed, uniting SS-VH-SVC-CS-RV-LA in ZTEP. This TP is synchronized (vertical) with Aomin and PTmin.[2-4] TP determines equal hemodynamic conditions (including boost pressure at the exit from the organ), at all the indicated ZTEP points, ensuring equal peripheral resistance for the incoming Ao wave impulse and the spheroid of the arterial bolus in the corresponding exchange zones: brain, heart, lung, and liver. The main role of TP is to create the initial hemodynamic matrix for the upcoming CC, for which purpose the pressor indices at the exit from the SC and PC organ exchange zones are optimized, synchronizing and creating equal conditions in the microcirculatory bed of all exchange zones, adequate for the current homeostasis.

We believe that it is TP, completing the diastolic evolution of CC, which is the beginning pressure level for

starting high-energy processes of the right heart, initiating the following phases of CC: the opening of PV, the beginning of LA systole (from the TP pressure level - boost pressure for pulmonary veins) and the subsequent CC sequence. In other words, the isometric contraction pressure of LV increasing during the Acd1 period forms CMIPmin, which at the end of the next period (Cc1), before the opening of PV, creates TP in the corresponding zones of the vascular bed. Thus, the diastole phase of the previous CC is completed, forming the base pressure in the venous section of the next CC.

We believe that during the period of isometric contraction of the ventricles ((D (Q)), along with others, the hemodynamic components of the next CC are formed: 1) the CS impulse, corrected by the RA systole and isometric RV contraction (Cc1), is involved in the formation of the boost pressure at the final exit stage from SC exchange zones (in RA); 2) PSIC, which determines the perfusion pressure levels of the SC (brain, liver, heart, and kidneys) and PC (lung) exchange zones after the opening of PV and initiating synchronization of the ventricular block during the formation of the spheroids of RVEF (current cycle) and LVEF (the end of the previous CC, affecting the RV by the CS impulse); 3) CMIPmin, creating equal hemodynamic conditions (including equal pressure levels from the exchange zones), defining the TP parameters, which structures the hemodynamic matrix of the next CC; and 4) IC-1min, which forms the RV base pressure at the time of TV closing, while isolating the "ventricular block" from the SC/PC collectors of variable capacitance.

The distributed ZTEP system is formed by the universal, multidimensional structure of CMIP,[5] changing the architectonics of the relationship between the venous capacitive vessels, arterial vessels, chambers of the heart and the vascular bed as a whole, creating targeted pressor impulses in the structure of systemic hemodynamics.

We believe that ZTEPs are short-term synchronized isometric pressor structures that exert a regulatory effect on the metabolism and hemodynamics of various organs, affecting peripheral resistance and interfering with the Ao impulse. The combination of several ZTEPs (for example, TP) can have a significant range of effects on hemodynamics, metabolism (see above) and the formation of subsequent hemodynamic structures

Conclusion

CMIP, forming the hemodynamics of the vascular bed as a whole, creates a distributed high-speed structure of short-term equilibrium hemodynamic formations (ZTEPs), affecting the phase sequence of CC, peripheral resistance, and organ metabolism, as well as interacting with the Ao hemodynamic impulse. We believe that the systemic distributed ZTEP association—a formed CMIP—is a synchronized (between ZTEPs, with the phases of CC, and ECG), high-speed, information-regulatory structure of the interaction of hemodynamics and metabolism of both central and peripheral organs, which is involved in the control and regulation of homeostasis as a whole.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Use of the FINDRISC Questionnaire in the Uzbek Population as a First Stage of Screening for Type 2 Diabetes Mellitus

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Abstract

Background: Active diagnosis of rapidly growing type 2 diabetes (T2D) is very important. Whether the FINDRISC questionnaire can be used as a first-stage screening tool in the Uzbek population is unclear, as anthropometric parameters and the impact of each risk factor may differ in different populations.

Materials and Results: In 2015–2016, regardless of nationality 2521 people (711 men and 1464 women) living in urban and rural areas of Uzbekistan were examined. The study included filling out a FINDRISC questionnaire with an assessment of the risk for T2D. All subjects underwent OGTT and HbA1c testing. The average score on the FINDRISC questionnaire among people with newly diagnosed diabetes was 12.8±0.4 (P<0.001), for IGT – 11.4±0.4 (P<0.001), for IFG – 11.0±0.8 (P<0.001) compared with individuals without carbohydrate metabolism disorders (8.0±0.09). Among people with diabetes diagnosed during the screening, only 33.9% had a high (30%) and very high (3.9%) risk of T2D, while 27.6% had a moderate risk, 30% - an increased risk, and 8.7% - a low risk of T2D. For the FINDRISC score, the sensitivity with a threshold value of 9 points was 87.9% and specificity was 45.3%; with a value of 12 points, sensitivity was 71.6% and specificity was 54.8%.

Conclusion: These results were the basis for a multivariate analysis of the risk for T2D among people of Uzbek nationality, and for the development of our own risk assessment program. (**International Journal of Biomedicine. 2020;10(1):29-35.**)

Key Words: type 2 diabetes • screening • ethnicity • risk assessment

Abbreviations

ADA, American Diabetes Association; **AH**, arterial hypertension; **BMI**, body mass index; **CMD**, carbohydrate metabolism disorders; **CDA**, Canadian Diabetes Association; **CVD**, cardiovascular disease; **DM**, diabetes mellitus; **HC**, hip circumference; **IFG**, impaired fasting glucose; **IGT**, impaired glucose tolerance; **OGTT**, oral glucose tolerance test; **PhA**, physical activity; **T2D**, type 2 diabetes; **WC**, waist circumference; **WHR**, waist-to-hip ratio

Introduction

Given the dangerously rapid growth of type 2 diabetes (T2D) prevalence worldwide,[1] active diagnosis is very important, since it has been shown that patients at the stage of prediabetes already have diabetes-specific complications (IFG and IGT).[2-4] The diabetes screening strategy proposed by WHO in 2003 has a two-step approach.[5] At the first stage, the risk groups for developing diabetes are identified:

1. All persons aged 45 or older
2. Persons younger than 45 having at least one of the following factors:
   - Obesity
   - Family history of diabetes

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• Ethnicity
• History of gestational diabetes
• Birth of a child weighing more than 4.5 kg
• Arterial hypertension
• Hyperlipidemia
• Previously diagnosed IFG or IGT

At the second stage of screening, OGTT is performed in these risk groups. In cases of normal results of OGTT, diabetes prevention strategies are introduced in risk groups, and regular repeated screening for diabetes is carried out at least every 3 years.[5]

The diabetes screening strategy proposed by the International Federation of Diabetes (2017) differs in its list of risk factors:[3,6] age over 40; obesity, increased WC; family history of diabetes; and arterial hypertension.

Local questionnaires (for example, FINDRISC)[7,8] are recommended to identify risk groups; if there are no such a questionnaires, testing for fasting glycemia is recommended.[3]

ADA[9] considers all people age 45 or older to be at risk for diabetes, and persons of any age with obesity plus:

• Family history of diabetes (first-degree relatives)
• Racial and ethnic groups at increased risk for diabetes
• History of cerebro-vascular disease (CVD)
• History of gestational diabetes
• Arterial hypertension
• Dyslipidemia (HDL<0.9 mmol/L and / or TG>2.82 mmol/L)
• Women with polycystic ovary syndrome (PCOS);
• Insulin resistance (severe obesity, acantosis nigricans)
• Previously diagnosed IFG or IGT

In risk groups, it is recommended to perform OGTT and/or HbA1c testing.

The strategy proposed by the CDA[10] differs from the ADA strategy in that it has an even more extended list of risk factors, and the threshold age for the beginning of mandatory testing is 40 years.

Risk groups for diabetes, according to CDA[10] are:

1. All persons aged 40 or older
2. Persons of any age with obesity plus:

• Family history of diabetes (first-degree relatives)
• Racial and ethnic groups at increased risk for diabetes
• Micro and macrovascular complications
• History of gestational diabetes
• History of fetal macrosomia
• Arterial hypertension
• Dyslipidemia (HDL <0.9 mmol/L and / or TG>2.82 mmol/L)
• Women with PCOS
• Insulin resistance (severe obesity, acantosis nigricans), obstructive sleep apnea, mental disorders, HIV infection, taking glucocorticoids, atypical antipsychotics, or antiretroviral therapy
• Previously diagnosed IFG or IGT

Australian Diabetes Risk Questionnaire[11] includes:

• Age 35 or older
• Gender (male)
• Racial and ethnic groups at increased risk for diabetes
• Family history of diabetes (first-degree relatives)
• History of hyperglycemia, history of gestational diabetes
• Arterial hypertension
• Smoking
• Insufficient vegetables and fruits consumption
• Low physical activity
• Abdominal obesity (own standards of WC for each race)

Chinese authors[12] consider the following as risk factors for T2D:

• Age
• Gender (male)
• BMI (24 and 28 kg/m^2);
• Family history of diabetes
• Level of education
• Arterial hypertension
• Heart rate at rest (70–80–90 per min)
• Fasting glycemia (5.6–6.1–6.9 mmol/L)
• TG≥1.7 mmol/L

In Japan,[13,14] 2 strategies for determining the risk of T2D are proposed. The first takes into account age, gender, BMI, WC, arterial hypertension, and smoking. The second one includes age (40 years or older), gender (male), family history of diabetes (first-degree relatives), smoking, low PhA, BMI>23 kg/m^2, and arterial hypertension.

In Peru,[15] the Ministry of Health recommends screening by testing for fasting glycemia in people aged 40 to 70 who have risk factors. However, fasting glucose testing is not available in rural settings. The risk questionnaire includes age 55 or older, family history of diabetes, and WC above 90 cm.

The Finnish Diabetes Risk Score (FINDRISC) is one of the most frequently used instruments for assessing the risk of DM.[7] The main points of the questionnaire include age, BMI, WC (separately for men and women), PhA, the consumption of vegetables, fruits and berries, regular use of antihypertensive drugs, a history of hyperglycemia and a family history of diabetes. The threshold for WC in this questionnaire is 94cm for men and 80cm for women; the threshold for BMI is 25 kg/m^2. FINDRISC assesses whether an individual has undiagnosed T2D or dysglycaemia or the probability of developing T2DM during the following 10 years. The applicability of the FINDRISC questionnaire has been evaluated in many countries.[16-19]

The recommendations on diabetes, prediabetes and cardiovascular diseases by EASD/ESC[19] indicate the need to develop risk assessment scales for T2D and cardiovascular diseases for each specific population.

The scales and questionnaires described above are non-specific for the Uzbek population. At the same time, there are no data on the standards of WC, WHR and BMI for those of Uzbek nationality. In this regard, conducting epidemiological screening studies to identify diabetes and prediabetes, with the study of anthropometric indicators, is very important.

Materials and Methods

General characteristics of the examined persons

In 2015–2016, regardless of nationality 2521 people (711 men and 1464 women) living in urban and rural areas of the Tashkent, Kashkadarya and Khorezm regions were examined. The target group of the study was the adult population aged 35

or older with stratification by gender in each age group: from 35 to 44, from 45 to 64 and over 65, without known CMD. The average age of those examined was 48.1±11.6 years; 68% of the examined were women.

Epidemiological method

The sample was formed by the method of random numbers, and the design of sample formation was weighed cluster. Examination was done by the same group of endocrinologists in all areas in order to eliminate errors in data collection and filling out questionnaires. A group of researchers was pre-trained and instructed on the rules of data collection, anthropometric measurements, and blood sampling for biochemical analysis.

The study included filling out a questionnaire with an assessment of the risk for T2D. The basis of the questionnaire was the WHO recommendations for screening for T2D, as well as the FINDRISC questionnaire [7] for identifying the risk of developing T2D over the next 10 years.

Anthropometry

Previously, we proposed reference values for WC, HC, WHR and BMI for men and women of the Uzbek population.[20,21]

General clinical research

Blood pressure was measured using Korotkov's method on two hands twice. Assessment of PhA was carried out on the basis of a survey of subjects, taking into account the duration, intensity and frequency of PhA. PhA was assessed as high if a person had moderate-intensity physical exercises (walking at a moderate pace, aerobic exercise) lasting more than 300 minutes a week; high-intensity exercises (running, training on simulators, anaerobic exercises) lasting more than 150 minutes a week, or when combining medium and high-intensity exercises for more than 150 minutes a week. PhA was regarded as moderate when performing physical exercises of medium intensity for 150–300 minutes per week or high intensity from 60 to 150 minutes per week. Low PhA was recorded if PhA of average intensity was less than 150 minutes per week or of high intensity less than an hour per week.[22]

Biochemical tests

All subjects underwent OGTT; glucose in the capillary blood plasma was measured using an iXell glucometer (Poland) with test strips calibrated in accordance with the reference values for venous blood plasma. The level of glycated hemoglobin was determined by a direct photometric immunochemical method using monoclonal antibodies to HbA1c (Human, HbA1c liquidirect, Germany) by means of a Human biochemical analyzer in the laboratory of the Republican Specialized Scientific-Practical Medical Center of Endocrinology named after Academician Ya. Kh. Turakulov. This method for HbA1c testing is standardized in accordance with the requirements of NGSP/DCCT and IFCC.

Prediabetes and diabetes were diagnosed according to the international recommendations.[23] All persons with identified CMD were referred to an endocrinologist at the place of residence for registration and further monitoring.

Statistical analysis was performed using the Microsoft Office Excel-2016 software package. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±standard deviation (SD) for continuous variables. For data with normal distribution, inter-group comparisons were performed using Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

Results

A total of 2521 people aged 35 or older living in urban and rural areas of the Tashkent, Kashkadarya and Khorezm regions were examined. T2D was diagnosed in 7.9% of the examined individuals, and in 74% of them the disease was diagnosed during the screening. Prevalence of T2D was 8.7% among men aged 35 years or older, and 7.5% among women. IFG was diagnosed in 4.4% of the examined individuals (in 2.6% of men and 5.3% of women); IGT was diagnosed in 1.4% of the examined individuals (in 0.5% of men and 1.8% of women). The average age of men and women with identified CMD was 53.3±1.27, and for newly diagnosed T2D it was 55.7±1.07; for IFG it was 58.5±4.63 (men) and 54.1±2.15 (women); for IGT it was 53.8±2.1 (men) and 55.6±1.41 (women); the average age of people without CMD was 48.3±0.44 for men and 46.4±0.29 for women.

All persons with newly diagnosed DM and prediabetes were referred to an endocrinologist at the place of residence for treatment and monitoring.

The overwhelming majority of people with newly diagnosed CMD admitted that they do not spend enough time on PhA: 59% of women and 70% of men with newly diagnosed DM rated their PhA as moderate, and 34% and 23%, respectively, rated it as low. Patients with IFG (66% of women and 52% of men) rated their PhA as moderate, and 25% and 38%, respectively, as low. As for patients with IGT, 74% of women and 50% of men rated their PhA as moderate, 19% of women as low. Since IFG was detected in only 4 men, it is premature to draw conclusions on the analysis of IFG in men.

A family history of diabetes was found in 40% of people with newly diagnosed diabetes, 36% of people with IGT and 26% of people with IFG. A family history of obesity was found in 39% of people with newly diagnosed diabetes, 34% of people with IGT, and 51% of people with IFG. An extremely high percentage of families had a history of cardiovascular diseases, in particular arterial hypertension, which are significant risk factors for T2D (Table 1).

Table 1.

Family history of individuals with identified CMD

Family history	Individuals with newly diagnosed diabetes		Individuals with IGT		Individuals with IFG		Individuals without CMD	
	men	women	men	women	men	women	men	women
n	43	104	21	91	4	31	711	1464
Obesity, %	35	41	29	35	25	55	26	39
Arterial hypertension, %	53	60	52	62	25	65	44	56
Ischemic heart disease, %	28	38	24	41	-	45	23	37
Diabetes, %	37	41	62	30	25	26	15	25

We analyzed the prevalence of overweight and obesity among the subjects. In general, in the entire population of the examined, only 28.7% of people had normal weight (BMI of 18–25 kg/m^2); 70.1% were overweight or obese. Even among individuals without identified CMD, only 31% had normal weight.

Figure 1 shows the average BMI of individuals with impaired carbohydrate metabolism compared with individuals without identified carbohydrate disorders. As can be seen from the figure, the average BMI among individuals with newly diagnosed T2D was 33.0±0.7kg/m^2 for women and 30.1±0.8 kg/m^2 for men. The average BMI among individuals with IGT was 31.6±0.7 kg/m^2 for women and 30.2±1.2 kg/m^2 for men. The average BMI among individuals with IFG was 32.1±1.3 kg/m^2 for women and 28.8±2.8 kg/m^2 for men. Among people without CMD, the average BMI was 27.3±0.18 kg/m^2 for men and 28.4±0.15 kg/m^2 for women.

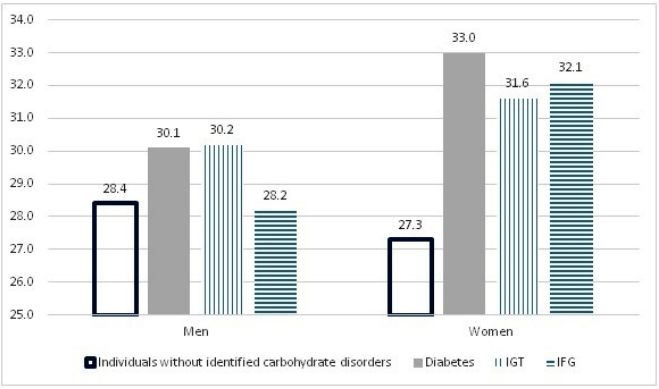

Fig.1. *The average BMI (kg/m^2) in individuals with impaired carbohydrate metabolism compared with individuals without identified carbohydrate disorders.*

WC is one of the key indicators in diagnosing obesity and determining the risk of diabetes, according to many questionnaires. Among the individuals we examined, WC exceeded the WHO recommended values for a healthy population, even in the absence of CMD (Table 2).

Table 2.

WC and WHR in individuals without CMD and in patients with diabetes and prediabetes

		Individuals without CMD	Diabetes	IGT	IFG
WC, cm	Men	96.3±0.48	106.9±2.1**	103.7±2.8*	103.5±5.2
	Women	88.7±0.37	101.0±1.38**	95.6±1.48**	96.3±2.7**
WHR	Men	0.95±0.003	1.0±0.009**	0.98±0.016	0.94±0.016
	Women	0.84±0.002	0.89±0.007**	0.87±0.007**	0.85±0.015

** - P <0.05 and ** - P <0.001 – compared with individuals without CMD*

Despite the high percentage of obesity among people without impaired carbohydrate metabolism, the overall average score for the FINDRISC questionnaire in these individuals was significantly lower than in groups in which diabetes, IGT, and IFG were detected (Table 3). Thus, the average score on the FINDRISC questionnaire among people with newly diagnosed diabetes was 12.8±0.4 (P<0.001), for IGT – 11.4±0.4 (P<0.001), for IFG – 11.0±0.8 (P<0.001), and for people without CMD – 8.0±0.09.

Table 3.

Biochemical and anthropometric risk factors depending on the type of CMD

		T2D	IGT	IFG	Healthy subjects
Age		55.0±0.8"	55.3±1.2"	58.5±4.6*	47.0±0.24
Fasting glucose, mmol/L		9.6±0.3"	5.5±0.1"	6.3±0.04"	4.8±0.014
OGTT, mmol/L		13.7±0.5"	9.6±0.1"	6.8±0.31"	5.6±0.02
HbA1c, %		9.4±0.24"	6.2±0.1"	5.7±0.2"	4.9±0.015
Body mass, kg		85.2±1.5"	80.1±1.6^	83.7±3.4*	75.2±0.3
BMI, kg/m^2		32.2±0.5"	31.3±0.6"	31.7±1.2^	28.0±0.1
% of obesity		63	55	50	32
WC, cm	men	106.9±2.1"	103.7±2.8*	103.5±5.2	96.3±0.48
	women	101.0±1.38"	95.6±1.48"	96.3±2.7^	88.7±0.37
HC, cm	men	106.7±1.7^	105.9±2.8	110.3±4.7	101.2±0.3
	women	113.8±1.3"	110.2±1.4^	113.0±2.5^	105.8±0.3
WHR	men	1.0±0.009"	0.98±0.016	0.94±0.016	0.95±0.003
	women	0.89±0.007"	0.87±0.007"	0.85±0.015	0.84±0.002
Average BP, mmHg		135.2±2.0"	125.6±1.7"	129.6±3.4^	119.7±0.4
		86.8±1.0"	82.4±1.0"	83.3±2.0*	78.3±0.3
AH, %		60	50	47	28
CVD, %		66%	58	50	31
IVC, %		12.2	14.3	42	15
Low physical activity, %		31	28	17	16
History of hyperglycemia, %		28	5.4	11	2.2
Average FINDRISC score		12.8±0.4"	11.4±0.4"	11.0±0.8"	8.0±0.09

IVC - Inadequate vegetables consumption; *- P <0.05, ^- P<0.01, and "- P <0.001 – *compared with individuals without CMD*

J.Lindstrom and J.Tuomilehto [7] wrote that PhA and consumption of fruits and vegetables did not have a big predictive value, but they were still included in the questionnaire to emphasize the importance of PhA and diet in diabetes prevention. In their study, DM was revealed in 3.5% in 1987 and in 5.7% in 1992 (people who did not know about their disease before the study, and underwent a FINDRISC questionnaire scoring and OGTT). Interestingly, in the 1987 cohort, among

people with a moderate risk of diabetes (score 4–8), diabetes developed in 2.4% within 10 years after the study, and only in 0.4% in the 1992 cohort. Perhaps this fact indicates the need for regular review and reassessment of the risk scale for the development of T2D, even in the same region.

The FINDRISC questionnaire was tested in adapted versions in 32 countries (at the time of publication of the seventh IDF Atlas in 2015[1]) with different efficiency. The difference in the effectiveness of the application of the questionnaire in different populations is determined not only by the characteristics of the surveyed population itself, but also by different values of the total score of the questionnaire, taken as a threshold for further examination—OTTG and/or HbA1c. Thus, in Botswana,[16] the FINDRISC questionnaire showed moderate efficacy with a threshold value of 13 points: out of 14.4% of people with newly diagnosed diabetes, only 55% had a high and very high risk, according to the questionnaire. Authors from Nigeria[24] and Colombia[25] showed the effective use of the questionnaire with a threshold value of 14 points. In Spain[26] and New Zealand,[18] the threshold value was 12 points. In addition, Spanish authors have shown the effectiveness of a simplified version of the MADRISK questionnaire, which includes only BMI, a history of hyperglycemia, and antihypertensive therapy. In Greece,[27] a second stage of screening has been proposed for individuals who score 15 points or more on the FINDRISC scale. In Germany,[28] a simplified version has also been adopted, including age, BMI, and a history of hyperglycemia.

B. Omech et al.[16] evaluated the applicability of the FINDRISC scale to the Botswana outpatient population. Sensitivity and specificity was 48% and 73%, respectively. The authors concluded that the main limitation of the application of this questionnaire was the use of a non-standardized method for determining HbA1c in the country, as well as the lack of consideration given to cardiovascular risk factors and other conditions accompanied by insulin resistance, which need additional laboratory tests for diagnosis.

M.P. Silvestre et al.[18] evaluated the applicability of the FINDRISC questionnaire to the New Zealand overweight population. The sensitivity of the questionnaire was 0.6026 and specificity - 0.5536. However, OTTG was performed only on individuals who scored more than 12 points on the questionnaire. The authors concluded that, in the New Zealand population, FINDRISC is an effective method for T2D and prediabetes screening for overweight residents.

A. Bernabe-Ortiz et al.[15] attempted to develop and evaluate the use of a simple questionnaire, not including biochemical measurements, to screen for diabetes among the population of Peru. The predictors were age, diabetes in first-line relatives, and WC. The sensitivity of this evaluation method was 69% and specificity - 59%.

In addition to the widespread FINDRISC questionnaire, questionnaires are being developed for individual countries, some of which include diabetes screening in the state program, and questionnaires are publicly available on government and health sites (for example, Canadian and Australian questionnaires for assessing the risk of T2D).[10,11]

In addition, the ADA has a test to determine the degree of risk for T2D, which is intended to be self-administered.[9,29] It is understood that a person taking this test has information about the permissible weight and clearly represents the criteria for a sedentary lifestyle.

In a study among people of Uzbek nationality without impaired carbohydrate metabolism, 94.6% of the examined had a low, increased or moderate risk, according to the FINDRISC questionnaire (below 15 points). Among people with IFG, only 25% had a high risk of developing T2D (from 15 to 20 points), 21% - a moderate risk, 33% - an increased risk (from 7 to 11 points), and 21% - a low risk (lower 7 points). Among people with IGT, only 28.4% had a high risk of T2D, 25.9% - a moderate risk, 28.4% - an increased risk, and 17.3% - a low risk. Among people with diabetes diagnosed during the screening, only 33.9% had a high (30%) and very high (3.9%) risk of T2D, while 27.6% had a moderate risk, 30% - an increased risk, and 8.7% - a low risk of T2D. Therefore, if, with the active screening for T2D among people of Uzbek nationality, we had only performed OGTT for people with a high and very high risk of T2D, according to the FINDRISC questionnaire, we would not have identified 66.3% of people with diabetes.

In our study among people of Uzbek nationality, in 61.4% of people with newly diagnosed diabetes and in 54.3% of people with IFG diagnosed by OGTT, the total score for the FINDRISC questionnaire was 12 or higher. At the same time, only 19.9% of people with normal OGTT results had a score above 12 (Table 4).

Table 4.

Percentage of people with and without CMD depending on the FINDRISC score in Uzbekistan

FINDRISC score	Without CMD	IFG	IGT	IFG+IGT	Newly diagnosed T2D
≥4	80.8	91.7	93.8	100	97.6
≥9	44.2	70.8	72.8	75	86.6
≥12	19.9	45.8	54.3	62.5	61.4

Thus, the sensitivity with a threshold value of 9 points was 87.9% and specificity was 45.3%; with a value of 12 points, sensitivity was 71.6% and specificity was 54.8%.

These results were the basis for a multivariate analysis of the risk for T2D among people of Uzbek nationality, and for the development of our own risk assessment program, taking into account new reference values of such anthropometric indicators as WC, HC and BMI.[20,21]

Thus, active screening is necessary for the early detection of T2D. The use of questionnaires to identify the risk for T2D has obvious advantages in order to increase the effectiveness of screening. However, the characteristics of the population (age, major risk factors) to be actively screened vary depending on nationality and place of residence. The use of the FINDRISC questionnaire in the Uzbek population

is not effective enough to identify the maximum number of people in the high-risk group. In this regard, we recommend using our program for assessing the risk of T2D in the Uzbek population.[30]

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Disclosures

None.

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ORIGINAL ARTICLE

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Potential of Routine X-ray Examinations in Detecting Signs of Asymptomatic Carotid Disease

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Abstract

***Background*:** The aim of our study was to determine potential opportunities for routine radiological examinations (dental panoramic radiography (DPR), cervical spine radiography (CSR), cone beam computed tomography (CBCT) and multislice computed tomography (MSCT)) in the identification of carotid artery calcifications (CAC) as radiological signs of asymptomatic carotid artery disease (ACAD).

Methods and Results: The retrospectively evaluated results of the digital DPR were used for 4367 patients, CSR - 857 patients, CBCT - 582 patients, and MSCT - 377 patients. Mean age of patients was more than 55 years. The overall detectability of CAC during DPR, CSR, CBCT, and MSCT was 8.3%, 15.9%, 13.1%, and 40.6%, respectively. The gender difference in favor of women was observed during DPR, CBCT and MSCT and in favor of men - only during CSR. CAC should be sought at the level of C3-C4 intravertebral discs in the cervical soft tissues, more often on the one side, in the form of solitary/multiple, friable, homogenous/heterogeneous radiopaque shadows smaller than 0.5 cm.

Conclusion: CAC indicates the presence of a high risk of developing ischemic stroke, which means that the above modalities have to be used as a tool to identify the predictor of this pathological condition of the cardiovascular system. (**International Journal of Biomedicine. 2020;10(1):36-40.**)

Key Words: carotid artery calcifications • screening • radiological examinations • ischemic stroke

Abbreviations

ACAD, asymptomatic carotid artery disease; **BCA**, brachiocephalic artery; **CBCT**, cone beam computed tomography; **CAC**, carotid artery calcifications; **CSR**, cervical spine radiography; **DPR**, dental panoramic radiography; **MSCT**, multislice computed tomography; **US**, ultrasound

Introduction

Asymptomatic carotid artery disease (ACAD) is a disorder identified in people who did not have either a previous history of ischemic stroke or transient ischemic attack in the ipsilateral carotid artery area with neurological signs, such as transient blindness, fatigue, numbness in the extremities or contralateral side of the face, dysarthria or aphasia over the past six months.[1-2] The underlying cause of ACAD is an atherosclerotic lesion of the vascular wall. At certain stages of atherosclerotic plaque formation, the lesion can be accompanied by vascular calcinosis due to the deposition of calcium salts; the plaque becomes radiopaque and it results in the appearance of carotid artery calcifications (CAC).[3]

Routine examinations, including radiological ones, are widely available, inexpensive, standardized and diagnostic; they are also frequently applied and performed due to numerous

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indications.[4] According to the WHO, more than 60% of radiological examinations are dental.[5] The data obtained in 2017 showed that tens of thousands of cervical spine radiographs and dental panoramic radiographs are performed annually in Russia.[6] Moreover, computed tomography (both CBCT and MSCT) is no longer an exotic radiological method available only in extreme cases and for specific indications. Nowadays, this is a routine radiological procedure in our country, which is normally applied for diagnosing different types of problems.[7]

All the above-mentioned statistical data and regulations allow us to consider dental panoramic radiography (DPR), cervical spine radiography (CSR), cone beam computed tomography (CBCT), and multislice computed tomography (MSCT) as routine radiological examinations.

The first mention of using of X-ray methods of examinations for the detection of CCA dates back to 1912, when Schuller, in his published monograph, indicated the possibility of detecting calcifications of "vascular" origin during X-ray of the skull.[8]

Later in 1951, Fisher M., in an article about the occlusion of carotid arteries, mentioned the possibility of detecting linear shadows of calcifications in the projection of the bulb of the internal carotid artery during radiography of the cervical spine.[9]

In 1963, Ring and Eddy published an article in which they evaluated 1000 chest radiographs and identified 216 cases of CAC. The detection rate was noted to be higher in elderly people, and in a group of patients over 80 years of age, CAC was revealed in more than 70% of cases.[10] In addition, in 1963, Hayler and Fischer were the first to use CSR for detecting CAC.[11]

In Russia, the pioneer and certainly the best expert on the problem in those years, was L.K. Bragina (1962), who carefully compared various invasive and non-invasive methods of X-ray diagnosis.

Since the 1970s, numerous studies of Stulin et al., dedicated to screening clinical and instrumental examinations, showed the possibility and perspective of identifying extra- and intracerebral atherosclerosis, often combined with coronary atherosclerosis.[12,13]

In 1981, Friedlander and Lande identified CAC for the first time in dental panoramic radiograms, which were performed in general dental practice. The authors paid attention to the fact that DPR should be performed for detecting dental abnormalities, pathologies of the temporomandibular joint and adjacent structures. Special focus should be given to the peripheral areas in the projection of the common carotid artery, which may provide the vital information.[14]

Since that time, there has been an increasing interest in the issue, and groups of scientists all over the world have been actively involved in its research.

The aim of our study was to determine potential opportunities for routine radiological examinations (DPR, CSR, CBCT and MSCT) in the identification of CAC as radiological signs of ACAD.

In order to achieve this aim, the following tasks were defined:

1.To analyze the current status of diagnosing ACAD.

2.To carry out a retrospective analysis of the results of routine radiological examinations (DPR, CSR, CBCT, and MSCT).

3.To evaluate the opportunities for routine radiological techniques (DPR, CSR, CBCT, MSCT) in the visualization of CAC.

4.To study and to specify radiological changes in CAC as radiological signs of ACAD.

5.To specify radiological signs of anatomic and pathological structures that are necessary for differentiation of CAC.

6.To improve and to complete the algorithm for analysis of the results of routine radiological examinations in diagnosis of carotid artery atherosclerosis as ACAD manifestation.

Materials and Methods

During the first stage of the study, we performed a retrospective evaluation of the results of digital DPR (Group 1) in 4637 patients (mean age of 66.1±8.6 years), digital CSR (Group 2) in 857 patients (mean age of 61.7±6.4 years), CBCT (Group 3) in 582 patients (mean age of 63.2±6.9 years), and MSCT (Group 4) of lower facial zone and neck in 377 patients (mean age 65.1±7.5 years).

A total of 6453 results were retrospectively evaluated. All patients in each group were divided into the following age subgroups: 55-59 years, 60-64 years, 65-69 years, 70-74 years, 75-79 years, and over 80 years.

Inclusion criteria were patient's age over 55 years, the absence of neurological signs of ACAD for the past 6 months minimum, according to the patient's previous history, and the possibility of visualizing cervical soft tissues.

Exclusion criteria were patient's age under 55 years, the presence of neurological signs of ACAD, according to the patient's previous history, the absence of visualization of cervical soft tissues due to the insufficient area of investigation and/or wrong physical and technical parameters of the examination.

During the second stage of the study, we evaluated the previous histories of all patients in the first and second groups in order to identify those patients who underwent a US examination of BCA within a year after the radiological examination. The US of BCA is considered to be a gold standard in radiological diagnosis of atherosclerotic lesions of the carotid arteries in patients without neurological symptoms. If the patients underwent the US of BCA within a year, its protocol and conclusion were assessed to determine the presence or absence of evidence of calcified atherosclerotic plaques located in the carotid arteries. The obtained data were compared with the findings received after the first stage of the study in order to evaluate diagnostic effectiveness of radiological examinations.

Among 4637 patients included in Group 1, 1523(32.8%) patients had US of BCA in their histories. Patients who underwent US of BCA later than DPR were selected first from this group; their number was 289 people, including 183 patients who underwent US of BCA within a year after DPR. The protocols of US of BCA of these 183 patients were chosen for the final analysis, which allowed us to evaluate the diagnostic effectiveness of DPR in revealing CAC.

Among 857 patients composing Group 2, 271(31.6%) people had US of BCA in their histories. All these patients had US of BCA within a year after CSR, and due to this fact, they were all included in the final analysis for evaluation of indicators of CSR diagnostic effectiveness in detecting CAC.

Results

Normality of distribution by age was determined for each group. As the number of investigations exceeded 50 in each case, a one-sample Kolmogorov-Smirnov test was applied for this purpose, and additionally, skewness and kurtosis parameters were evaluated.

For all four techniques the obtained findings of significance level were <0.05, skewness and kurtosis parameters were not equal to 0, which allowed us to consider the distribution for each of the groups as different from normal and to apply nonparametric statistical methods in statistical processing (odds ratio (OR), Fischer's exact test).

Total detection rate of the patients with suspected CAC using DPR was 8.3% (mean age of 66.7±8.7 years, ratio of men to women was 2.8:1), using CSR it was 15.9% (mean age of 64.9±7.3, ratio of men to women was 0.8:1), using CBCT it was 13.1% (mean age of 66.4±7.7, ratio of men to women was 1.8:1), and for MSCT it was 40.6% (mean age of 67.9±7.3, ratio of men to women was 1.3:1).

Moreover, the odds ratio parameters and Fischer's exact test were estimated for each radiological technique: The parameters for DPR, CSR, CBCT and MSCT were 1.848 (95% CI: 1.462-2.335; P<0.001), 0.358 (95% CI: 0.247-0.520; P<0.001), 0.768 (95% CI:0.462-1.277; P=0.346), and 0.518 (95% CI: 0.336-0.800; P=0.04), respectively.

During the second stage of the study, the data of routine radiological examinations and US of BCA were compared and the following groups of findings were identified:

1. True-positive findings. The data of retrospective analysis of DPR or CSR on CAC presence were consistent with the protocol of US of BCA, proving the evidence of calcified atherosclerotic plaques in the carotid arteries.

The indicator of true-positive results was 12 for DPR, and 37 for CSR.

2. False-positive findings. The data of retrospective analysis of DPR or CSR showed the presence of CAC, but according to the protocols of the US of BCA, there were not any atherosclerotic plaques in the carotid arteries.

The indicator of false-positive results was 8 for DPR, and 6 for CSR.

3. False-negative findings. The retrospective analysis of DPR or CSR did not reveal any pathological changes suggesting the presence of CAC; however, calcified atherosclerotic plaques in the carotid arteries were detected using the US of BCA, according to the protocols.

The indicator of false-negative results was 6 for DPR, and 13 for CSR.

4. True-negative findings. The data of retrospective analysis of DPR or CSR on the absence of CAC were consistent with the protocol of US of BCA, proving the absence of calcified atherosclerotic plaques in the carotid arteries.

The indicator of true-negative results was 157 for DPR, and 213 for CSR.

Based on the above-mentioned findings, we estimated sensitivity, specificity and accuracy parameters for DPR and CSR, which were 66.7%, 95.1%, and 92.3% and 71.2%, 97.3%, and 92.2%, respectively.

The next stage of our research was the evaluation of radiological characteristics of CAC signs on the basis of DPR, CSR, CBCT and MSCT findings, in order to reveal the most common characteristics of the following parameters (Table 1, Figure 1):

- Level of CAC location toward the cervical vertebrae
- Side of location (left-sided, right-sided, located on each side from the cervical spine)
- Intensity (hardly visible, moderately dense, pronounced calcifications)
- Number (solitary, multiple)
- Shape (linear, annual, friable)
- Structure (homogeneous, heterogenous)
- Size (<0.5 cm, from 0.5 to 1 cm, >1 cm)

Table 1

Radiological characteristics of CAC

	DPR	CSR	CBCT	MSCT
Level of location	C3-C4 (34%) C4 (34%)	C3-C4 (37.5%) C4 (25.8%) C4-C5 (25.0%)	C3-C4 (39.5%) C4 (31.6%) C3 (19.7%)	C3-C4(32.7%) C4 (24.8%) C3 (24.2%)
Side of location	Unilateral (76.1%)	Unilateral (76.5%)	Unilateral (81.6%)	Unilateral (50.3%)
Intensity	Moderately dense (89.1%)	Moderately dense (76.5%)	Moderately dense (78.9%)	Moderately dense (85.6%)
Number	Solitary (57.9%)	Solitary (66.2%)	Solitary (57.9%)	Multiple (64.1%)
Shape	Friable (87.2%)	Friable (83.1%)	Friable (73.7%)	Friable (78.5%)
Structure	Homo-geneous (51.4%)	Hetero-geneous (50.1%)	Homo-geneous (61.8%)	Hetero-geneous (62.1%)
Size	Smaller than 0.5 cm (74%)	Smaller than 0.5 cm (66.9%)	Smaller than 0.5 cm (84.2%)	Smaller than 0.5 cm (70.6%)

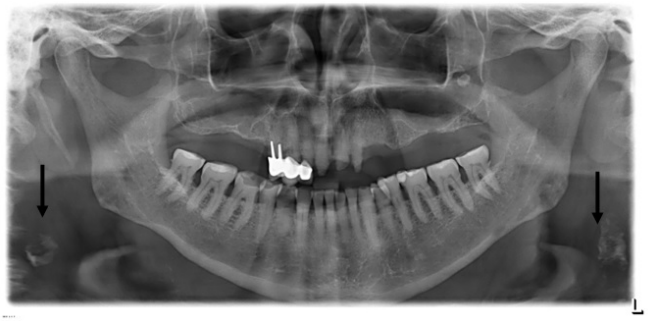

Fig. 1. *DPR. Heterogeneous moderately dense radiopaque shadows, semicircular on the right and linear on the left, with the diameter of 15.0 mm and length of 16.0 mm, respectively (arrows). They can be visualized on both sides closer to the cervical spine in the cervical soft tissues.*

It is worth noting that while performing two-dimensional investigations (DPR and CSR) it is necessary to differentiate CAC from various calcified anatomical and pathological structures. In our research, we used a dental panoramic radiogram for this purpose.

Among 4637 patients, 282(6.1%) had solitary/multiple calcifications in the soft tissues of the head and neck; they were revealed by dental panoramic radiograms. Table 2 shows the distribution of these calcifications.

Table 2.

Revealed calcifications in the soft tissues of the head and neck using DPR

Anatomical/pathological structure	Absolute number of patients	% of total number of patients with calcifications	% of total number of patients in the study (DPR)
Calcified triticeal cartilage	84	29.8	1.8
Calcified stylohyoid ligament unilateral/bilateral	9	3.2	0.2
Calcified stylomandibular ligament unilateral/bilateral	9	3.2	0.2
Parotid gland calculi unilateral/bilateral	66	23.4	1.4
Submandibular gland calculi unilateral/bilateral	44	15.6	0.9
Calcified lymph node	7	2.5	0.1
Vascular stent	5	1.8	0.1
Calculi in the parotid and submandibular salivary glands	1	0.4	0.02
Idiopathic calculi (solitary/multiple)	57	20.1	1.2

Discussion

CAC can be revealed during routine radiological investigations (DPR, CSR, CBCT and MSCT) that allow us to use them as examinations for the primary detection of ACAD. According to different authors, the total detection rate of CAC using DPR ranges from 0.43%[15] to 38.8%,[16] and using CSR it is 13.0%.[17] In our research, the total detection rate of CAC for DPR, CSR, CBCT and MSCT was 8.3%, 15.9%, 13.1% and 40.6%, respectively.

Many researchers emphasize that most of the patients with CAC are women.[18-21] In our study, CAC was more frequently detected in women than in men while using three out of four radiological techniques (DPR – 73.8%, CBCT – 64.5%, MSCT – 57.5%). However, this association turned out to be statistically significant only for DPR (OR=1.848, 95% CI: 1.462-2.235, $P<0.001$). For CBCT this association was not statistically significant (OR=0.768, 95% CI: 0.462-1.277, $P=0.346$). CSR, as the primary diagnostic technique in ACAD, was used only in one scientific work, but there was not any indication of the gender ratio among the patients with CAC.[17] In our study, using CSR investigation, we revealed more male patients with CAC, and this association was statistically significant (OR=0.358, 95% CI: 0.247-0.520; $P<0.001$).

Diagnostic effectiveness indicators of two-dimensional routine radiological techniques were evaluated by many researchers. Yoon et al.[22] estimated the sensitivity, specificity and accuracy of DPR as 22.2%, 62.3% and 90.0%, respectively; Alman et al.[23] assessed sensitivity and specificity as 77.8% and 84.0%; Khambete et al.[24] – as 76.0% and 98.66%; Constantine et al.[25] – as 76.9% and 46.9%. In our research, sensitivity, specificity and accuracy of DPR were determined as 66.7%, 95.1% and 92.3%, respectively; CSR – 71.2%, 97.3% and 92.2%, respectively.

Based on the obtained data of DPR, CSR, CBCT and MSCT, we determined that the most frequent significant radiological signs of CAC were presented by unilateral, moderately dense, solitary/multiple, friable, homogenous/heterogeneous radiopaque shadows smaller than 0.5 cm in the cervical soft tissues at the level of the C3-C4 intravertebral discs (Figure 1).

It is necessary to differentiate CAC from other anatomical and pathological structures; the most frequent anatomical one is calcified triticeal cartilage (n=84, 29.8%), and the most common pathological structure is parotid gland calculus (n=66, 23.4%) (Figure 2). The obtained findings are consistent with the data of foreign researchers.[26-30]

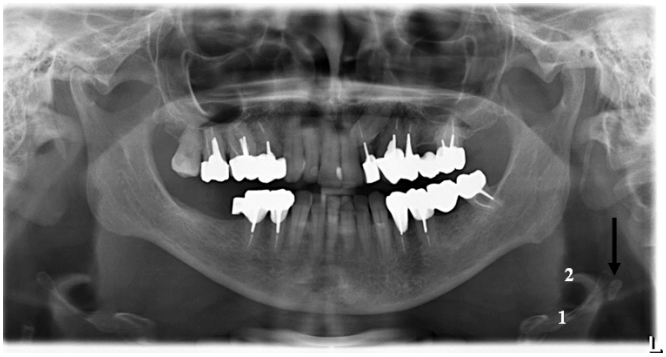

Fig. 2. *DPR. Solitary radiopaque oval-shaped shadow with well-defined smooth edges can be visualized in the cervical soft tissues on the left under the great horn of hyoid bone (1) and epiglottis (2). The shadow may refer to calcified triticeal cartilage (arrow).*

Thus, carotid artery calcifications suggest the presence of atherosclerosis of the particular localization and consequently the presence of high risk for developing ischemic stroke; hence, the above-mentioned techniques (DPR, CSR, CBCT, MSCT) have to be used as an instrument for detecting a predictor of this socially and economically significant pathological condition of the cardiovascular system.

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Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

General Surgery

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Conceptual and Methodological Approaches to Choosing a Method for Marking a Surgical Site for Reconstructive Surgery on the Anterior Abdominal Wall

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Abstract

This article discusses the effectiveness of the author's method of marking a surgical site (Ukraine patent UA 99427) for surgical treatment of patients with postoperative ventral hernias (POVH). Based on the study of quality of life (QOL) indicators obtained using the SF-36 questionnaire, it was found that the proposed therapeutic tactics for surgical correction of POVH can increase medical, social and aesthetic efficacy to a statistically significant improvement in QOL. (**International Journal of Biomedicine. 2020;10(1):41-44.**)

Key Words: postoperative ventral hernias • surgical site • quality of life

Introduction

Current trends in the development of medicine put in the range of health problems not only the safety, functionality and social orientation of surgical techniques, but also the aesthetics of the consequences of surgical intervention.[1] Surgeons began paying attention to this problem in the second half of the 20th century. This was facilitated by the development of plastic surgery, as well as an increasing understanding in civilized countries of the importance of the role of human appearance.[2-4]

Elimination of defects of the anterior abdominal wall (AAW) takes first place among all planned surgical interventions. A significant percentage of these interventions consists of surgery for postoperative ventral hernias (POVH).[5,6] This category of patients is a constant contingent of surgical hospitals. Thus, over the past 25 years, due to the increase in the number of surgical interventions on the abdominal organs, the frequency of POVH has increased by 9 times or more.[7]

Marking a surgical site precedes the main stage of any surgical intervention, including during reconstructive operations on AAW.[8] Each surgical operation ends with suturing the skin. The skin scar that the patient sees is evaluated precisely from an aesthetic point of view. The quality of the seam and the further formation of the scar, from the standpoint of aesthetics, determine the psycho-emotional state of the patient, especially women, for many years.

In this regard, the correct determination and marking of the alleged borders of the excision of the AAW tissues, with the subsequent determination of the symmetry of the applied lines, is of extreme importance from the standpoint of aesthetics and cosmetics.[9,10] Most surgeons mark surgical sites "by eye," which does not allow for achieving the ideal symmetry of the drawn lines,[11,12] thus significantly worsening the cosmetic characteristics of the postoperative scar, the aesthetic consequences of surgery, and as a result, QOL of the patients. Since the perception of one's appearance is one of the key components that forms a patient's satisfaction with a surgery, we consider it worthwhile to study QOL in this category of patients.[13]

The purpose of this study was to develop a unified approach to choosing a method for marking a surgical site (access) during reconstructive surgical interventions on AAW, aimed at improving the aesthetic effectiveness of surgical correction and QOL.

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Materials and Methods

The study was based on a clinical and laboratory examination of 128 patients with POVH who underwent surgical treatment in the Simferopol Central District Clinical Hospital in the period from 2009 to 2017. The study was carried out in compliance with Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 59th WMA General Assembly, Seoul, Republic of Korea, October 2008. Written informed consent was obtained from all patients before inclusion in the study.

All surgical interventions were planned. The examined patients were divided into two groups. Group 1 (comparison group) included 64 patients with POVH who underwent AAW plastic surgery with an allograft according to the "classical" sublay technique and arbitrary marking of surgical access by eye. Group 2 (main group) included 64 patients with similar POVH and cosmetic defects, as well as AAW deformities, who underwent surgical treatment using the author's proposed cutting techniques[14] with allograft fixation [15] when performing retro-muscle hernioplasty.[16] Among the operated patients there were 86 women (67.2%) and 42 men (32.8%).

When performing operative access in all cases, wide bordering incisions were considered rational with the complete removal of postoperative scars and the excess skin and subcutaneous fat. The correctly selected shape and direction of the incision create convenient access to a hernial defect and provide a good cosmetic effect. In cases of localization of a hernia or a pathological postoperative scar in the epigastrium, longitudinal and oblique transverse incisions were preferred; in the mesogastrium, longitudinal and transverse incisions; and in the hypogastrium, a T-shaped incision with complete removal of the cutaneous-subcutaneous apron and removal or relocation of the navel.

When determining the width of the carved skin flap, we were guided by the rule: with longitudinal access, the maximum width of the flap should be such that the skin fold on the right side meets without tension the skin fold on the left side when the skin is drawn together by the index fingers along the line of the proposed incision; with horizontal access, respectively, the upper skin fold should meet the lower without tension.

Considering the above-mentioned, a method for marking a surgical site for reconstructive surgery on AAW was proposed, which is carried out as follows:[16] Preoperative marking of the surgical site is carried out with the patient in the vertical position, when the soft tissue of AAW go down under the influence of gravity. We mark the midline from the xiphoid process through the navel to the pubic symphysis, and the transverse line connecting the anterior superior iliac spine of the iliac wing on both sides (Fig.1).

Given the individual mobility of the skin-fat layer, the surgeon, with an assistant, pulls the ligature through the midline between the opposite symmetrical parts of the abdomen above the skin-fat flap and marks the upper access line along a ligature (Fig.2). Similarly, the lower access line is marked on the skin-fat flap (Fig.3). At the end of the marking, the surgeon once again determines the symmetry of the drawn lines (Fig.4) and, creating with the fingers a skin-fat fold on AAW, determines the coincidence of the upper and lower surgical access lines.

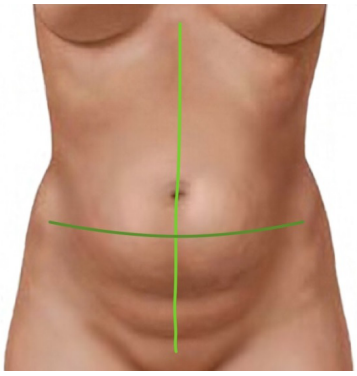

Fig.1. *Marking the midline from the xiphoid process through the navel to the pubic symphysis, and the transverse line connecting the anterior superior iliac spine of the iliac wing on both sides*

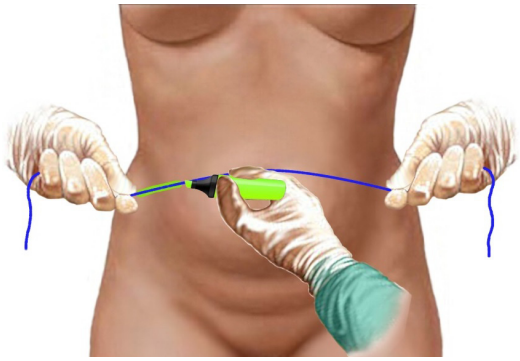

Fig.2. *Marking the upper access line along a ligature*

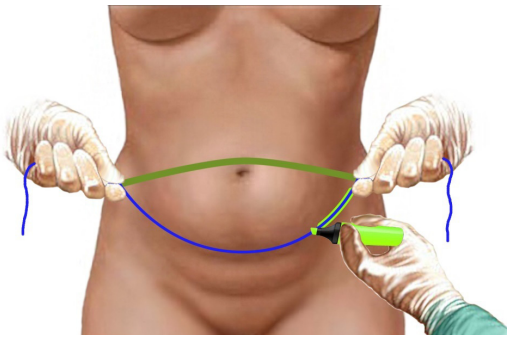

Fig.3. *Marking the lower access line along a ligature*

It is necessary to make sure that the edges of the future wound will be "without tension," including both in the "direct" (Fig.4) and in the "lateral" projections (Fig.5).

The features of the course of the remote postoperative period were studied in the period from 1 year to 3 years. In order to evaluate QOL after surgical treatment in patients of both clinical groups, we analyzed 128 questionnaires. The QOL of patients was assessed using the MOS SF-36 questionnaire (translation into Russian, validation and testing - Institute of

Clinical and Pharmacological Research, St. Petersburg), which is a universal standardized questionnaire intended for use in clinical practice and for scientific research.[17] The SF-36 measures eight scales: physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH).

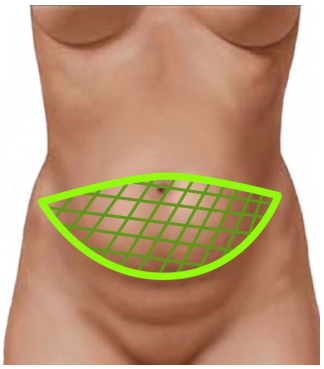

Fig.4. *The final view of the marking of surgical site (in the "direct" projection) during reconstructive surgical interventions on AAW*

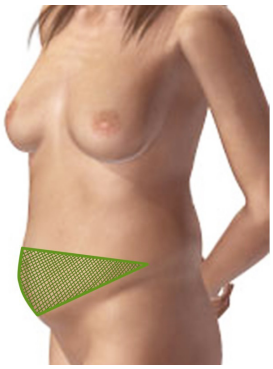

Fig.5. *The final view of the marking of surgical site (in the "lateral" projection) during reconstructive surgical interventions on AAW*

The statistical analysis was performed using the statistical software Microsoft Excel 2010. The mean (M) and standard error of the mean (SEM) were calculated. Student's unpaired and paired t-tests were used to compare average values for data with normal distribution. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

When analyzing these questionnaires, it was noted that in the remote postoperative period, the QOL of patients in both clinical groups was high. However, in the comparison group, QOL indicators were significantly lower than in patients of the main group. After 1 year (Table 1), there was an increase in all indicators, reflecting the restoration of working capacity, adaptation to role-based functioning (work, everyday activities), and improvement of emotional well-being. During this period, VT in patients of the main group approached the values of healthy people. SF, RE, and MH were significantly lower than normal values; therefore, patients experienced certain limitations of social activity, negative emotions and depression due to concomitant neuropsychiatric syndrome. However, in the patients of the main group in the first year, all indicators of QOL were significantly higher than in patients of the comparison group and were practically in the lower values of the norm of healthy indicators.

Table 1.

QOL indicators in study groups 1 year after surgical treatment

Scales	PF	RP	BP	GH	VT	SF	RE	MH
Group 1	65.41 ±0.38	62.16 ±0.34	67.84 ±0.31	61.01 ±0.33	48.84 ±0.27	72.94 ±0.18	72.01 ±0.24	66.37 ±0.29
Group 2	67.76 ±0.44	64.17 ±0.34	69.23 ±0.28	63.84 ±0.28	51.84 ±0.33	75.66 ±0.17	74.67 ±0.25	69.16 ±0.36
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

After 3 years (Table 2), there was an increase in all indicators, including SF. The VT indicator increased more significantly in the main group than in the comparison group. During this period, none of the patients of both clinical groups changed to easier work, nor did they reduce the level of physical activity. The high rate of increase in the GH indicator, among other indicators, reflected a certain reassessment of physical strengths, the denial of the disease and the necessary restrictions.

Table 2.

QOL indicators in study groups 3 years after surgical treatment

Scales	PF	RP	BP	GH	VT	SF	RE	MH
Group 1	69.30 ±0.24	61.08 ±0.13	68.53 ±0.12	63.80 ±0.18	52.83 ±0.15	74.37 ±0.30	72.64 ±0.29	73.33 ±0.21
Group 2	73.06 ±0.29	63.09 ±0.11	71.44 ±0.16	66.78 ±0.19	56.22 ±0.32	76.05 ±0.23	75.01 ±0.25	74.31 ±0.28
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

A positive fact is the absence of differences between patients in the study and healthy individuals in the way they characterized their fullness of strength and the absence of depression (VT and MN), because such differences, as a rule, become the background for the development of dissatisfaction with the appearance of the abdomen. Thus, the proposed method for marking a surgical site for surgical treatment of patients with POVH had a positive effect on the QOL of the main group of patients, which made it possible to improve the immediate and long-term results of treatment, increasing both the aesthetic and functional state of the operated patients.

The proposed procedures for the surgical correction of POVH made it possible to increase the medical, social and aesthetic effectiveness to statistically significant improvement in QOL, which makes it possible to recommend the proposed method for marking a surgical site for surgical treatment of patients with POVH, cosmetic defects, and AAW deformities for widespread use in surgical practice.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Experimental Surgery

INTERNATIONAL JOURNAL OF BIOMEDICINE

Morphological Substantiation for the Effectiveness of the Proposed Method of Gastrostomy using a Polypropylene Endoprosthesis

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Abstract

The article discusses the morphological changes in the area of gastrostomy performed by the proposed original method of gastrostomy using an implant in laboratory animals (rabbits). Morphological changes in the tissues at gastrostomy sites were studied on Days 10 and 20 after the proposed method of gastrostomy, in comparison with the classical Witzel gastrostomy and Depage-Janeway gastrostomy using the GIA stapler. Negative morphological changes in the tissues around gastrostomy sites were revealed in the form of necrosis of the microenvironment and disturbance in microcirculation according to the stagnant type, caused by the damaging effect of the surgical suture material at the microscopic level, the least pronounced when using a polypropylene implant. (**International Journal of Biomedicine. 2020;10(1):45-49.**)

Key Words: gastrostomy • original method • polypropylene mesh • histology

Introduction

Currently, there are about 100 different modifications of gastrostomy. However, the expansion of indications for gastrostomy increases the frequency of its use, which leads to the need for an improvement in technique.(1) Thus, the development of new gastrostomy methods is still relevant, on the one hand contributing to improving the quality of life of patients, and on the other, reducing the risk of complications. Despite the fact that the complication rate for surgical gastrostomy is from 2% to 8%, and the complication rate for endoscopic gastrostomy is only 1% to 3%, surgical gastrostomy continues to be used, since the absolute contraindications for performing endoscopic gastrostomy are decompensated stenosis of the stomach, complete obstruction of the pharynx and esophagus, obesity grade 3, severe coagulopathy, and other morbidities.(2) At the same time, the expansion of indications for gastrostomy requires the development of new methods for its implementation and assessment of the effectiveness of interventions.

The purpose of the work was to characterize the morphological changes in the tissues around gastrostomy sites in the original method of operation using a polypropylene mesh, in comparison with the classical Witzel gastrostomy and Depage-Janeway gastrostomy using the GIA stapler. The experiment was performed on rabbits.

Materials and Methods

For the experiment, we selected 18 sexually mature male rabbits (3 months old) of the Chinchilla breed, weighing 2500–3400g. The animals were divided by the method of pair analogues into 3 groups (6 animals each).(3) Group 1 included rabbits that underwent the Witzel's gastrostomy;

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Group 2 included rabbits that underwent the Depage-Janeway gastrostomy using the GIA apparatus; Group 3 included rabbits that underwent the original method of gastrostomy using a polypropylene endoprosthesis (Patent RU No. 2691924 dated 06/18/2019 Bulletin No. 17).[4]

For the original method of gastrostomy, we used the synthetic polymer Esfil - Standard of the Lintex company. Esfil is a classic mesh endoprosthesis made from monofilament polypropylene for soft tissue repair, mainly used in herniology. This endoprosthesis combines high rates of biological inertness, resistance to infection and mechanical strength. One of the main disadvantages of the polypropylene implant, which excludes its use in the intraperitoneal plastic of hernias of the anterior abdominal wall, is the development of a massive adhesive process, due to the expressed adhesive properties of the implant, with the possible formation of intestinal fistulas.[3] In our model, this "negative" property of polypropylene material becomes a key advantage, providing a tight attachment of the stomach with a gastrostomy to the anterior abdominal wall (AAW).

The rabbit was chosen for the experiment because it is a standard laboratory animal in the development of new surgical methods.[5] In addition, the rabbit is phylogenetically closer to primates than rodents[6] and is a large enough laboratory animal to monitor physiological changes without euthanasia. The animals were kept in a vivarium in isolated cages with a 12-hour light cycle at a temperature of 18-21ºC.

The method of gastrostomy using a polypropylene mesh was implemented as follows: A median laparotomy was performed; the stomach wall was pulled up to AAW and was taken on two Babcock clamps. A GIA type stapler was placed perpendicular to the greater curvature of the stomach, and a gastric tube (GT) was formed from the stomach wall. From a polypropylene mesh, 2 polypropylene mesh implants were modeled. The first was formed as an oval plate with a central hole. The second implant was formed as a single-layer mesh clutch covering GT. GT was passed through the hole of the first implant, then a second implant was put on it in the form of a clutch. The first implant was fixed to the gastric wall and the second implant to GT, then the two implants were sutured together. Through a hole of 1.5–2 cm in size, GT was pulled out onto AAW to the left of the midline incision in the projection of the left rectus abdominis muscle.

GT was fixed with sutures to the parietal peritoneum and the muscular aponeurotic layer. The GT end was dissected, 3 fixing sutures were placed, stitching the AAW through, as well as the wall of the stomach together with the first implant attached to it in three places with polypropylene 1/0 thread, ensuring gastropexy. The threads were tied with a knot on the skin, hemostasis was carried out, and then layer-by-layer the laparotomy wound was sutured. Further, in the postoperative period, after 3 weeks, as the implants germinated with connective tissue, which provided an increasingly tight attachment of the stomach wall to AAW, the gastric-fixing sutures were gradually removed.

The animals were withdrawn from the study on Days 10 and 20 after surgery. Tissue fragments were taken from the gastrostomy zone and fixed for at least 2 hours in a 10% solution of neutral formalin. Further sample processing was carried out by intermediate Blick mixtures. Subsequently, paraffin sections 5–7 μm thick were made, which were stained with H&E. To identify acidic glycosaminoglycans, which are of particular importance in formation of connective tissue, staining with Alcian blue was used.

Morphometry was performed using the ImageJ-1.45s. We performed quantitative and qualitative analysis of the composition of cell infiltrates in the newly formed connective tissue of the gastrostoma. The severity of the inflammatory reaction was assessed by determining the area of infiltrate in mm^2. To determine the prevalence of inflammatory or reparative tendencies, the percentage of cell composition per 100 cells was calculated. Quantitative assessment of reparative changes was determined by counting the resident cells (fibrocytes, fibroblasts, and macrophages), and the inflammatory process was determined by counting the number of the non-resident cells (lymphocytes, neutrophils, and monocytes). The ratio of these cell groups was subsequently presented as a percentage. The relative area of the dermal vessels was calculated as a percentage of the area of the dermal connective tissue in one field of view.

The work with animals was carried out in accordance with the principles of humanism laid down in the directives of the European Community (86/609/EEC) and the Declaration of Helsinki, in accordance with the "Animal experimentation legislations".

Statistical analysis was performed using the statistical software «Statistica». (v10.0, StatSoft, USA) and Microsoft Excel 2007. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. 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.

Results

Day 10 after surgery

In Group 1, we found healing of the tissue defect with the formation and maturation of granulation tissue, and its reorganization, closing of the defect with the epidermis and scar formation in the zone of the imposed gastrostoma from the side of AAW. In the epidermis, all layers characteristic of intact skin were differentiated. An increase in the thickness of the epidermis was caused by a thickening of the prickly and granular layers, while the epithelial papillae were virtually absent, due to which the relief of the epithelium was smoothed. Skin appendages were not numerous (Fig.1.1). In the deep layers of the newly formed connective tissue, we found randomly located, newly formed, thickened collagen fibers surrounded by fibroblasts. On a number of micropreparations, a lymphocytic infiltrate separating the fibers was visualized. In the area adjacent to the suture material, cellular detritus was formed as a result of tissue necrosis (Fig.1.2).

In Group 2, we found the formation of a thicker epidermal layer, due to an increase in the thickness of the granular and horny layers. The appendages of the skin were more numerous and represented not only by hair follicles,

but also by the terminal sections of the sebaceous glands. Subepidermal vessels were significantly dilated. The red blood cells in them were in a state of sludge. Venous lumen was characterized by the dissociation of blood plasma, and signs of perivascular edema were noted (Fig.1.3). The same signs of impaired microcirculation by stagnant type were noted in the deep sections of the connective tissue of the gastrostoma. The dilated venules, with dissociated plasma, were surrounded with numerous clusters of non-resident cells. In the area adjacent to the suture material, less pronounced cellular detritus, due to tissue necrosis, was formed.

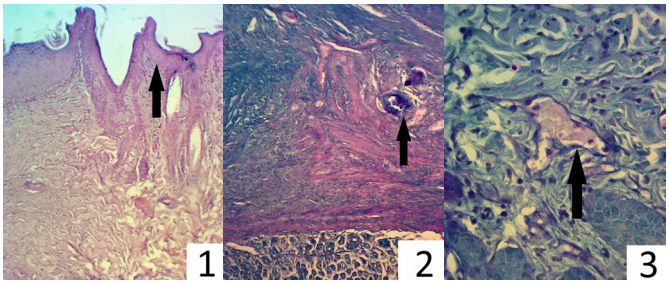

Fig.1. *The area of the anastomosis at the transition zone between the stratified squamous keratinized epithelium of AAW and simple columnar epithelium of the stomach; Day 10 after surgery.*

1.1.The Witzel's gastrostomy. Skin epidermis with few hair follicles.
1.2.The Witzel's gastrostomy. Cellular detritus in the newly formed connective tissue, located in the deep sections of the anastomosis, closer to the gastric mucosa.
1.3.The Depage-Janeway gastrostomy. The dissociation of blood plasma in the expanded venule of newly formed connective tissue with a large number of non-resident cells.

In Group 3, a complicated running of the epidermal basement membrane was noted. It acquired a convoluted character with the formation of numerous papillary papillae of complex shape that layered on top of each other, due to which the entire surface of the epithelium was corrugated. As in Group 2, the granular and horny layers were more pronounced, in comparison with Group 1, and skin appendages were more numerous (Fig.2.1).The subepidermal connective tissue was filled with dense inflammatory-cellular infiltrates; however, unlike in Group 2, we did not reveal paretically dilated subepidermal vessels. In the deep sections of the anastomosis, around artifacts associated with the threads of the polypropylene mesh, already at this time, the ordered dense intercellular connective tissue structures were determined. In the area adjacent to the suture material, we did not observe accumulations of cellular detritus characteristic of Groups 1 and 2.

Day 20 after surgery

In Group 1, in the zone of superimposed gastrostoma from the side of AAW, we found the completion of re-epithelialization of the contact site of the stomach epithelium and skin epidermis, with complete differentiation of the epidermal layers. The uneven thickness of the epidermis persisted. A local increase in the thickness of the epidermis was caused by a thickening of the prickly and granular layers. Epithelial papillae were more numerous, and in their form were close to those of intact rabbits. An increase in the number of skin appendages, represented by numerous hair follicles collected in groups, was noted. Collagen fibers filled the entire volume of the zone of the formed scar and had a convoluted course. The underlying connective tissue scaffold was more mature, compared to the morphological picture of the previous period in this group. In the deep layers of the newly formed connective tissue, chaotically located collagen fibers were preserved, alternating with the connective tissue sites depleted in acidic glycosaminoglycans. Cellular detritus, previously formed at the site of surgical sutures, was surrounded by a connective tissue capsule with sites of lymphocytic infiltrate. Foci were preserved in this group and Group 2, and fresh cell detritus was contained.

In Group 2, as in Group 1, the thicker epidermal layer, as it approaches the zone of artificial transition into a simple columnar epithelium of the stomach, was smoothed and lost the horny layer, as well as the appendages of the skin. Numerous subepidermal vessels, characteristic for Day 10, became empty after Day 20. Similar changes on the part of the microvasculature were noted in the deep sections of the connective tissue of the gastrostoma (Fig.2.2).

In Group 3, a complicated running of the epidermal basement membrane persisted over the entire surface of the epidermis adjacent to the anastomosis zone. Moreover, it spread to the transition zone of direct contact with the epithelium of the stomach (Fig.2.3). In this place, the epithelium was reduced to a single or double layer, in some places acquiring a uni-layer, multirow, prismatic character. However, keratinization processes persisted. In the deep sections of the anastomosis, numerous ordered, dense, intercellular connective tissue structures were observed. Between the fibers and in the immediate vicinity of artifacts associated with the elements of the polypropylene network, clusters of cells of inflammatory-cell infiltrate, mainly represented by resident cells, were determined, which characterized the high activity of reparative processes. No sites of necrosis or accumulations of cellular detritus were detected.

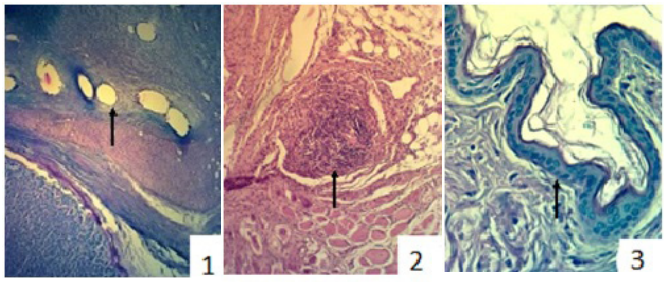

Fig.2. *The area of the anastomosis at the transition zone between the stratified squamous keratinized epithelium of AAW and simple columnar epithelium of the stomach.*

2.1. Gastrostomy using a polypropylene mesh. Artifacts in the threads of the polypropylene mesh. There are no sites of necrosis.
2.2. The Depage-Janeway gastrostomy. The encapsulated area of necrosis in the deep layers of the anastomosis adjoining to abdominal striated muscles.
2.3. Gastrostomy using a polypropylene mesh. Epithelial metaplasia in the transition region between the skin epidermis and simple columnar epithelium of the stomach.

According to a morphometric study, 10 days after surgery the largest number of inflammatory infiltrate cells in

the field of view was characteristic of Group 1 and Group 2 micropreparations, due to the accumulation of non-resident cells (Table 1). The identified features characterized the high activity of the acute phase of inflammation. Later, 20 days after the operation, the situation was changed: the maximum cell area was observed in animals of Group 3, where the original method of gastrostomy with a polypropylene mesh was used. The increase in cell area was associated with a high percentage of resident cells, which amounted to 23.8%, while in Groups 1 and 2 these indicators were 15.7% and 13.4%, respectively.

Table 1.

Morphometric indicators of connective tissue in the anastomotic zone

Variable	Day	Group 1	Group 2	Group 3
The number of inflammatory infiltrate cells	10	509±34	456±35	401±35*”
	20	248±15	282±25	356±30*^”
Resident cells.%	10	64.5±4.1	62.8±4.1	50.1±5.2*^
	20	84.3±6.1	86.6±6.9	76.2±5.9*^”
Non-resident cells.%	10	35.5±4.1	37.2±4.1	49.9±5.2*^
	20	15.7±6.1	13.4±6.9	23.8±5.9*^”
Area of inflammatory cell infiltrate	10	0.68±0.07	0.63±0.05	1.08±0.09*^
	20	0.32±0.02	0.37±0.03	0.89±0.07*^”
The relative area of the blood vessels of the dermis	10	10.15±0.08	12.43±1.1	8.72±0.71*^
	20	6.98±0.06	9.5±0.78	5.44±0.36*^”

** - P<0.05 compared to Group 1; ^ - P<0.05 compared to Group 2; “ - P<0.05 between Days 10 and 20 in Group 3*

On Day 20 after surgery, the largest specific area of the microvasculature of the newly formed connective tissue was noted in Group 2; it was 75% higher than in Group 3 and 36% higher than in Group 1. This indicator was inversely proportional to the number of resident cells in the cell infiltrates of the newly formed connective tissue.

Discussion

This study confirmed the development of negative necrotic changes around the suture during the formation of anastomoses. In the early stages, these changes are accompanied by the formation of cellular detritus, and in the later stages, the appearance of thick connective tissue capsules with gross fibro-degenerative changes and signs of inflammatory reactions associated with the “sawing effect” of the thread during tension.[7]

In addition, the alterative processes are caused not only by mechanical damage to tissues or the penetration of infectious agents, but also by a violation of microcirculation, which is of great importance for the formation of a full anastomosis between the hollow organs.[8] As known, there are significant differences in the nature of the microvasculature of the skin and the stomach wall, which provokes a violation of hemodynamics in the gastrostomy zone, which, against the background of inflammatory reactions, is accompanied by a violation of tissue metabolism and an increase in lipid peroxidation markers.[9] Activation of apoptosis mechanisms in endotheliocytes under these conditions may inhibit the expansion of newly formed vessels and inhibit neoangiogenesis. Thus, the circulatory disturbance observed in our study in the group of laboratory animals with the Depage-Janeway gastrostomy can play a significant role in the development of a complex of wound complications that can be minimized by performing the original method of gastrostomy with a polypropylene implant.

The revealed microcirculatory disorders and necrosis in the area of suture material (in the Witzel and Depage–Janeway models of gastrostomy) are leveled using a polypropylene mesh. In the literature, there are indications of the features of reparative processes under the influence of polypropylene implants, namely the activation of fibroblasts on the fifth day after the operation, the formation of granulation tissue with many thin-walled vessels, and collagenization of the interstitial matrix on the 10th day. By the 14th day, according to published data, the interstitial inflammatory infiltration by lymphocytes and macrophages is significantly reduced,[10] which is consistent with the data obtained in our study.

Thus, the following conclusions can be drawn:

1. The morphological changes in the tissue ensemble in the gastrostoma zone, which determine the development of complications in the postoperative period, are usually based on the surgical technique for performing a gastrostomy.

2. Marked negative morphological changes in the gastrostoma zone in the form of necrosis of the microenvironment and disturbances of microcirculation according to the stagnant type are caused by the damaging effect of the surgical suture material and occur in groups of animals that underwent gastrostomy without the use of a polypropylene implant.

3. The use of a polypropylene implant significantly reduces the severity of necrotic changes in tissues in the area of suture material, primarily due to tissue remodeling caused by the specificity of fibrillogenesis under the influence of polypropylene, which contributes to more reliable attachment of the gastrostoma and lower mechanical load on the ligatures due to an increase in the strength of the newly formed connective tissue.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Population Genetics

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Genetic Predictors for the Development of Congenital Orofacial Clefts

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Abstract

Background: The aim of this study was to search for associations between polymorphisms of the *IRF6, MDR1*, and *MTHFR* genes and the risk of congenital orofacial cleft (OFCs) among the population of the Republic of Sakha (Yakutia).

Methods and Results: The sample of the studied individuals consisted of 94 children (46 girls and 48 boys) with OFCs and their parents (75 mothers and 18 fathers). The children with OFCs were divided into 3 groups. Group 1 included 48 children with cleft lip and palate (CLP); Group 2 included 22 children with cleft lip (CL); Group 3 included 24 children with cleft palate (CP). The comparison group included 156 healthy volunteers (118 women and 38 men) who did not have a history of relatives with OFCs. Analysis of the distribution of alleles and genotypes of studied SNPs in children with all OFCs and healthy children showed a significant (P=0.000) difference only in *MDR1* genetic variant rs1045642 SNP. The carriage of the TT genotype of the *MDR1* rs1045642 SNP was associated with increased risk of OFCs (OR=2.711, 95% CI=1.459-5.037; P=0.000). Analysis of the frequency distribution of alleles and genotypes depending on the severity of clefts showed that the carriage of the TT genotype of the *MDR1* rs1045642 SNP was associated with significant risk for development of CL (OR=3.114; 95% CI=1.123-8.634) and CLP (OR=2.804; 95% CI=1.333-5.895). In children with CP, we found significant risk with carriage of the TT genotype of the *IRF6* rs2235371 SNP (OR=5.429, 95% CI=1.135-25.962; P=0.035).

Conclusion: A study of four SNPs in the *IRF6*, *MDR1*, and *MTHFR* genes revealed statistically significant increased risks for OFCs in carriers of the TT genotype of the *MDR1* rs1045642 SNP; in addition, the carriage of the TT genotype of the *IRF6* rs2235371 SNP significantly increased the risk of CP development. (**International Journal of Biomedicine. 2020;10(1):50-53.**)

Key Words: orofacial cleft • cleft lip and palate • *IRF6* • *MTHFR* • *MDR1*

Abbreviations

OFC, orofacial cleft; **CLP**, cleft lip and palate; **CL**, cleft lip; **CP**, cleft palate; **IRF6**, Interferon regulatory factor 6; **MTHFR**, Methylenetetra-hydrofolate reductase; **MDR1**, Multidrug resistance1; **SNP**, single nucleotide polymorphism

Introduction

Annually, about 30,000 children with congenital malformations of the face, neck and skull are born in the Russian Federation; 70% of them are anomalies of the maxillofacial system. The total frequency of morphological malformations in children under 1 year is approximately 27.2 per 1000 population. About 60% of them are detected in the first 7 days of life in obstetric institutions. One of the leading places among malformations is occupied by orofacial clefts (OFCs). Congenital clefts of the lip and/or palate (CLP, CL, CP) are some of the most common birth defects in children; they can occur as isolated conditions or can be a symptom of hereditary syndromes. The average frequency of nonsyndromic

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OFCs is from 0.41 to 1.2 cases per 1000 newborns in the RF and does not significantly differ from the EUROCAT register data (0.39–1.39).[1]

In most cases, congenital clefts of the lip and/or palate are multifactorial congenital malformations, where both genetic and exogenous factors are essential. Among the exogenous factors, the effects of physical and chemical factors, poor and unbalanced nutrition, hormonal disorders, poisons, a number of drugs and biological agents (viruses, bacteria and their toxins, protozoa) and other substances during pregnancy have been widely studied. Reliable information has been obtained on the effect of genetic polymorphisms of a number of genes on the risk of OFC development. Modern diagnostic methods allow us to study the factors of genetic predisposition involved in the morphogenesis of the lips and palate. This serves for the development of methods to prevent OFCs in families with a burdened history. In recent years, much attention has been paid to the study of gene polymorphism as a factor in the genetic predisposition to the development of this pathology. Reliable associations of gene variations (*IRF6*, *MDR1,* and *MTHFR)* with an increased risk for CLP, CL, and CP have been obtained in different populations.[1-5]

IRF6 is a protein encoded by the *IRF6* gene in humans. This gene encodes a member of the interferon regulatory transcription factor family. Family members are divided into a highly conserved N-terminal helix-turn-helix DNA binding domain and a less conservative C-terminal protein-binding domain. The function of *IRF6* is associated with the formation of connective tissue, such as in the palate. A mutation in the *IRF6* gene can lead to autosomal dominant Van der Wood syndrome or its associated popliteal pterygium syndrome. In addition, variants of the *IRF6* gene have a demonstrated association with congenital clefts of the oral cavity.[1,2]

Functional SNPs in the *MDR1* gene can affect the expression and activity of transport proteins located on the apical and basolateral surfaces of syncytiotrophoblast and endothelial cells of fetal placental fetal capillaries. These proteins are able to remove toxins or drugs from the environment that enter the mother's body, into the mother's bloodstream, and can lead to an altered response of the fetus on xenobiotics and a subsequent increase in the risk of complex genetic disorders or birth defects.[3]

The *MTHFR* gene, encoding the synthesis of the MTHFR enzyme, is located on chromosome 1p36.3. MTHFR plays a key role in folic acid metabolism. The rs1801133 SNP (also known as 677C>T) is localized in exon 4 of the *MTHFR* gene and is formed by the transition from cytosine (C) to thymine (T). The 222nd genetic code of the *MTHFR* gene changes from GCC to GTC, which leads to the replacement of alanine (Ala) with valine (Val) in the MTHFR polypeptide. Animal studies have shown that reducing the formation of methionine from homocysteine plays a key role in the development of neural tube defects. A number of studies investigated the relationship between the polymorphisms of the *MTHFR* gene and OFCs, but with mixed results.[4,5]

The aim of this study was to search for associations between polymorphisms of the *IRF6, MDR1*, and *MTHFR* genes and the risk of congenital OFCs among the population of the Republic of Sakha (Yakutia).

Materials and Methods

The experimental part of the work on the genotyping of the *IRF6* SNPs (rs2235371, rs861019), the *MDR1* rs1045642 SNP, and the *MTHFR* rs1801133 SNP was performed in the Department of Molecular Genetics at YSC CMP. For the study, we used DNA samples from the collection of biomaterials of the YSC CMP (Project "The Genome of Yakutia"; No. USE_507512). The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems (YSC CMP). Written informed consent was obtained from each research participant (or the participant's parent/guardian).

The sample of the studied individuals consisted of 94 children (46 girls and 48 boys) with OFCs and their parents (75 mothers and 18 fathers). The children with OFCs were divided into 3 groups. Group 1 included 48 children with CLP; Group 2 included 22 children with CL; Group 3 included 24 children with CP. About one third of the OFC children (32 patients) had a family history of the malformation. According to indications, a cytogenetic examination was performed to exclude chromosomal pathology in this group of children. The comparison group included 156 healthy volunteers who did not have a history of relatives with OFCs.

Genomic DNA was isolated from the peripheral blood leukocytes using a commercial DNA-isolation kit (Excell Biotech Corporation; Yakutsk, Russia). The study of the *IRF6* SNPs (rs2235371, rs861019), the *MDR1* rs1045642 SNP, and the *MTHFR* rs1801133 SNP was performed by PCR and RFLP analysis.

Primer sequences, conditions for amplification, restriction pattern, and restriction enzymes for study SNPs are presented in Table 1. Genotypes were determined by analyzing the sizes of the resulting fragments by gel electrophoresis on 4% agarose gel with ethidium bromide in standard Tris-acetate buffer at 120V for 1 hour. Restriction products were visualized using a gel documentation system in a Vilber Lourmat Compact UV Transilluminator (France). Electrophoretograms of the studied SNPs are presented in Figures 1,2,3 and 4.

Statistical analysis was performed using Microsoft Excel 2010. Differences in the allele distribution between the two groups were assessed by $\chi 2$- test with Yates correction. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A probability value of $P<0.05$ was considered statistically significant.

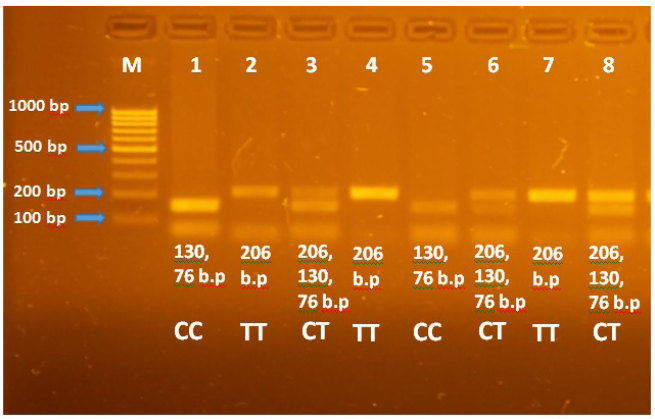

Fig. 1. *A 4% agarose gel electrophoresis of PCR-RFLP products for the MDR1 rs1045642 SNP*

M - marker Step100

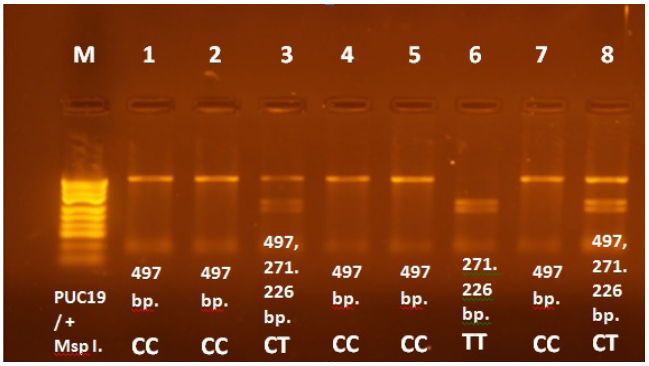

Fig. 2. *A 4% agarose gel electrophoresis of PCR-RFLP products for the MTHFR rs1801133 SNP*
M – marker pUC19 / Msp I

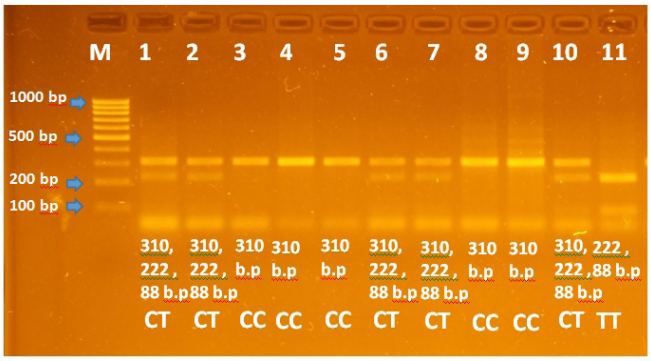

Fig. 3. *A 4% agarose gel electrophoresis of PCR-RFLP products for the IRF6 rs2235371 SNP*
M - marker Step100

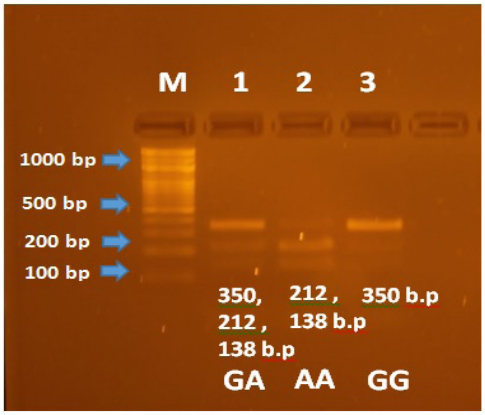

Fig. 4. *A 4% agarose gel electrophoresis of PCR-RFLP products for the IRF6 rs861019 SNP*
M - marker Step100

Results and Discussion

Analysis of the distribution of alleles and genotypes of studied SNPs in children with all OFCs and healthy children showed a significant (*P*=0.000) difference only in *MDR1* genetic variant rs1045642 SNP. The carriage of the TT genotype of the *MDR1* rs1045642 SNP was associated with increased *risk* of OFCs (OR=2.711, 95% CI=1.459-5.037; *P*=0.000) (Table 2).

Table 1.
Conditions for PCR-RFLP analysis

SNP	Primer	AT, °C	AL, bp	RE	Restriction fragment length, bp
IRF6 (rs2235371)	F:5'- ATC AGT CCT CTG TCC ATG ACG -3' R:5'- GCA TGA GTC ACA GGG ATG AAC -3'	61	310	MboI	CC: 310 CT: 310,222,88 TT: 222, 88
IRF6 (rs861019)	F:5′-ATG ACA CCA CCA TGA TGAGGGA-3′ R:5′-CTA GCC ATG CAA AGCTTGTCTC-3′	61	350	TfiI	GG: 350 GA: 350,212,138 AA: 212,138
MDR1 (rs1045642)	F:5′-TTG ATG GCA AAG AAA TAA AGC-3′ R:5′-CTT ACA TTA GGC AGT GAC TCG-3′	54	207	DpnI	CC: 130,76 CT: 206,130,76 TT: 206
MTHFR (rs1801133)	F:5'- TGG GGT CAG AAG CAT ATC AGT CA -3' R:5'- CTG GGA AGA ACT CAG CGA AC-3'	62	497	TaqI	CC: 497 TC: 497,271,226 TT: 271,226

Note: bp - base pairs; AT- Annealing temperature; AL - Amplicon length; RE- Restriction enzyme

Table 2.
Distribution of alleles and genotypes of studied SNPs in children with all OFCs and healthy children

Genotype/ allele	Genotype/allele frequency,%		χ2	*P*	OR (95% CI)
	OFCs	Control			
IRF6 (rs2235371)					
CC	52.1	47.4	2.188	0.335	
CT	42.6	50.0			
TT	5.3	2.6			
C	73.4	72.4	0.056	0.813	
T	26.6	27.6			
IRF6 (rs861019)					
AA	18.1	30.8	5.29	0.071	
GA	44.7	34.6			
GG	37.2	34.6			
A	40.4	48.1	2.773	0.096	
G	59.6	51.9			
MDR1 (rs1045642)					
CC	18.1	10.9	15.724	0.000	2.711 (1.459-5.037)
CT	50	74.4			
TT	31.9	14.7			
C	43.1	48.1	1.176	0.278	1.223 (0.850-1.761)
T	56.9	51.9			
MTHFR (rs1801133)					
CC	75.5	70.5	0.474	0.789	
CT	24.5	28.8			
TT	0.0	0.64			
C	87.8	84.9	0.78	0.377	
T	12.2	15.1			

Analysis of the frequency distribution of alleles and genotypes depending on the severity of clefts showed that the carriage of the TT genotype of the *MDR1* rs1045642 SNP was associated with significant risk for development of CL (OR=3.114; 95% CI=1.123-8.634) and CLP (OR=2.804; 95% CI=1.333-5.895). In children with CP, we found significant risk with carriage of the TT genotype of the *IRF6* rs2235371 SNP (OR=5.429; 95% CI=1.135-25.962; *P*=0.035).

In conclusion, a study of four SNPs in the *IRF6*, *MDR1*, and *MTHFR* genes revealed statistically significant increased risks for OFCs in carriers of the TT genotype of the *MDR1* rs1045642 SNP; in addition, the carriage of the TT genotype of the *IRF6* rs2235371 SNP significantly increased the risk of CP development.

Acknowledgments

The study was carried out within the framework of the project "The study of the genetic structure and burden of hereditary pathology of populations of the Republic of Sakha (Yakutia), using the Unique scientific equipment (USE) "The Genome of Yakutia" (Reg. No. USU_507512).

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Population Genetics

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Genetic Profile of Patients with Classical Ph-negative Chronic Myeloproliferative Diseases in the Republic of Sakha (Yakutia)

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Abstract

Background: Mutations in the *JAK2*, *CALR*, and *MPL* genes are key factors of the classical Ph-negative CMPD pathogenesis with demonstrated diagnostic and prognostic value. The aim of this research was to study the prevalence of *JAK2*, *CALR,* and *MPL* mutations in patients with CMPD and healthy individuals in the Republic of Sakha (Yakutia) (RS(Y)).

Methods and Results: The study included patients with previously confirmed diagnoses of PV (n=15), ET (n=16), and PMF (n=11) and 68 people with peripheral blood changes, suspected to have CMPD. The control group included 184 healthy volunteers. All patients and participants in the control group were genotyped according to the following SNPs: the *JAK2* rs77375493 SNP, the *CALR* rs765476509 SNP, the *CALR* rs1450785140 SNP, the *MPL* rs121913616 SNP, and the *MPL* rs121913615. The prevalence of the *JAK2*V617F mutation among PV patients in the RS(Y) was 90.9%. Patients with ET in 61.3% of cases were carriers of the *JAK2*V617F mutation, in 6.4% of *CALR* mutations, and in 3.2% of the *MPL*W515L mutations. In PMF patients, the *JAK2*V617F mutation was detected in 64.7% of cases, and the Type 1 *CALR* mutation was detected in 17.6% of cases. Carriage of the *JAK2*V617F mutation was revealed in 1.1% of healthy individuals and in 4.4% of individuals with initial signs of a myeloproliferative process.

Conclusion: Early molecular genetic testing will improve the timely diagnosis of CMPD and possibly reduce the number of complications. (**International Journal of Biomedicine. 2020;10(1):54-57.**)

Key Words: chronic myeloproliferative diseases • gene • mutations • single nucleotide polymorphism

Abbreviations

AS-PCR, allele-specific polymerase chain reaction; **CMPD**, chronic myeloproliferative diseases; **CALR**, Calreticulin; **ET**, essential thrombocythemia; **PV**, polycythemia vera; **PMF**, primary myelofibrosis; **SNPs**, single nucleotide polymorphisms

Introduction

Recent decades have been marked by a major breakthrough in understanding of the classical Ph-negative CMPD pathogenesis. In 2005, a sense mutation V617F (also known as *JAK2*Val617Phe) at exon 14 of the Janus Kinase *(JAK)2* gene (a valine-to-phenylalanine substitution in position 617) was described. This mutation can also be described using the SNP ID rs77375493 (the wild-type (normal) allele is rs77375493(G), and the (very rare) variant allele is rs77375493(T)). The mutation results in loss of autoinhibition of JAK2 tyrosine kinase, its hyperactivation and cytokine-independent differentiation of myeloid cells.[1] A little later, mutations in the MPL, CALR genes were described,

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which play a key role in the pathogenesis of megakaryocyte proliferation. Among mutations in the MPL gene encoding the thrombopoietin receptor, mutations W515L (a tryptophan-to-leucine substitution in position 515) and W515K (a tryptophan-to-lysine substitution in position 515) have a major clinical importance.[2] They lead to spontaneous activation of the MPL receptor and increase its sensitivity to thrombopoietin. The role of *CALR* mutations, in particular, Type 1 (52-bp deletion; p.L367fs*46) mutation and Type 2 (5-bp TTGTC insertion; p.K385fs*47) mutation, include the loss of KDEL signal sequence due to a shift of the reading frame by 1 nucleotide.[3]

The diagnostic value of the *JAK2*, *CALR*, and *MPL* mutations has been determined; they are included in the WHO criteria for the diagnosis of classical Ph-negative CMPD.[4] In addition, many studies have been published that describe the impact of mutational status on the clinical course, the risk of complications, and disease outcome.[5] Despite the progress, the problem of early diagnosis of diseases and the prevention of thrombotic complications remains unresolved. According to findings in the literature, at the time of diagnosis thrombotic complications are recorded in 12%-39% of PV patients, 7.14%-26.3% of ET patients and 4%-7% of PMF patients.[6] In recent years, we have been getting more information about the prevalence of the *JAK2*V617F mutation among individuals with thrombosis of different localization. In addition, the observed frequency of the *JAK2* (0.3%-3.1 %) and *CALR* (0.16 %) gene mutations among healthy populations in different countries exceeds the officially recorded incidence of chronic myeloid neoplasm (0.002%-0.02%).[7,8] In this regard, the *JAK2*V617F mutation may be an early diagnostic criterion for identifying individuals with latent clonal hematopoiesis of the myeloid germline, and the role of the *CALR* and *MPL* mutations remains unclear.

The aim of this research was to study the prevalence of *JAK2*, *CALR,* and *MPL* mutations in patients with CMPD and healthy individuals in the RS(Y).

Materials and Methods

The study included patients with previously confirmed diagnoses of PV (n=15), ET (n=16), and PMF (n=11) and 68 people with peripheral blood changes, suspected to have CMPD. All patients underwent outpatient consultation in the Republican Hospital №1 "National Center of Medicine"; the diagnosis was verified based on WHO diagnostic criteria valid at the time of diagnosis.[4] The control group included 184 healthy volunteers.

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems (YSC CMP). Written informed consent was obtained from each research participant.

The average age of patients ranged from 50 to 60 years: 52±16.63 for ET, 60±12.84 for PV, and 50±21.17 for PMF. In all investigated subgroups of patients, women predominated (80.6% in ET, 54.5% in PV and 52.9% in PMF). Further analysis of ethnic groups demonstrated a predominance of Yakuts among ET patients (67.7%) and Russians among patients with PV and PMF (54.5% and 52.9%, respectively). The median follow-up time was 48 months (from 0 to 252 months).

The experimental part of the work was carried out in the Department of Molecular Genetics at YSC CMP. All patients and participants in the control group were genotyped according to the following SNPs: the *JAK2* rs77375493 SNP, the *CALR* rs765476509 SNP, the *CALR* rs1450785140 SNP, the *MPL* rs121913616 SNP, and the *MPL* rs121913615.

DNA was isolated from peripheral blood lymphocytes with a commercial DNA-isolation kit (Excell Biotech Corporation; Yakutsk, Russia). SNP was determined using AS-PCR. Amplification of the gene region containing the polymorphic variant was carried out using standard pairs of primers produced by SybEnzime (Novosibirsk, Russia).[9-11] Primer sequences and conditions for amplification are presented in Table 1.

Table 1.
Primers and conditions for PCR

SNP	Primers	Amplicon length	Annealing temperature
JAK2 rs77375493	Forward mutant-specific 5'-AGCATTTGGTTTTAAATTATGGAGTATATT-3'	Allele T – 364 bp and 203 bp Allele G – 364 bp	56°C
	Forward 5'-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3'		
	Reverse 5'-CTGACACCTAGCTGTGATCCTG-3'		
MPL rs121913616 rs121913615	Forward 5'-GCCGAAGTCTGACCCTTTTT-3'	Wild-type – 209 bp Mutation W515L– 124 bp Mutation W515K – 125 bp	55°C
	Reverse 5'-ACAGAGCGAACCAAGAATGCCTGTTTACA-3'		
	Forward mutant-specific for W515L 5'-GGCCTGCTGCTGCTGAAGTT-3'		
	Reverse mutant-specific for W515K 5'-TGTAGTGTGCAGGAAACTGCTT-3'		
CALR rs765476509 rs1450785140	Forward 1 5'-GCAGCAGAGAAACAAATGAAGG-3' Forward 2 5'-GCAGAGGACAATTGTCGG-3' Reverse 5'-AGAGTGGAGGAGGGGAACAA-3'	Wild-type – 357 bp Type 1 mutation – 302 bp Type 2 mutation – 272 bp	56°C

Detection of PCR products was carried out on a 3% agarose gel stained with ethidium bromide using a standard Tris-acetate buffer at 120V for 45 minutes.

Statistical analysis was performed using Microsoft Excel 2010. For descriptive analysis, results are presented as mean±standard deviation (SD). The Mann-Whitney U Test was used to compare the differences between the two independent groups. Differences in the allele distribution between the two groups were assessed by $\chi 2$- test with Yates correction. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

According to the results of genotyping studied groups, the T allele of the *JAK2*V617F mutation was detected in 48.2% of patients and 1.1% of healthy individuals. Mutations in the *CALR* gene were detected in 4.5% of patients, of which 3.6% had a mutation of Type 1, and 0.9% had a mutation of Type 2. Mutations in the *MPL* gene were detected in 1.8% of patients. Among the healthy group, mutations in the *CALR* and *MPL* genes were not detected (Table 2).

Table 2.

Prevalence of JAK2, CALR, MPL mutations in patients and control group

Alleles/ Type of mutation	Patients (n=110) %	Control (n =184) %	$\chi 2$	OR (95% CI) *P*
JAK2				
T	48.2)	1.1	197.7	84.614 (30.504-234.708) *P*=0.000
G	51.8	98.9		
CALR				
Wild-type	95.4	100	6.007	*P*=0.015
1 type mutation	3.6	0		
2 type mutation	0.9	0		
MPL				
Wild-type	98.2	100	1.215	*P*=0.271
W515L mutation	1,8	0		
W515K mutation	0	0		

The genetic profiles of patients with verified diagnoses (n=70) demonstrated that PV patients in 90.9% of cases carry the *JAK2*V617F mutation. Among ET patients the *JAK2*V617F mutation was detected in 61.3% of cases, *CALR* mutations in 6.4%, and *MPL* mutation in 3.2%. PMF patients were carriers of the *JAK2*V617F mutation in 64.7% of cases, and *CALR* mutations in 17.6% of cases (Table 3). Mutations in the *CALR* and *MPL* genes were found in patients with PV. In 3 *JAK2*V617F-positive young patients (24, 29, and 33 years old) with borderline changes in the peripheral blood count, clinical and laboratory parameters did not meet the criteria for the diagnosis of CMPD; therefore, they were recommended for dynamic follow-up.

Table 3.

Prevalence of JAK2, MPL, CALR mutations in patients with CMPD

Mutation	PV, n=22 %	ET, n=31 %	PMF, n=17 %
JAK2V617F	90.9	61.3	64.7
CALR, total	0	6.4	17.6
CALR Type 1	0	3.2	0
CALR Type 2	0	3.2	17.6
MPL, total	0	3.2	0

Next, we evaluated the impact of the *JAK2*V617F mutation in clinical presentation of patients with ET and PMF. It was found that the average age of the *JAK2*V617F(T allele)-positive ET patients was significantly higher than that of carriers of the wild-type G allele (58.9±13.25 and 44.3±16.33, $P<0.05$) (Table 4). In patients with PMF, the *JAK2*V617F(T allele)-positive individuals demonstrated a higher level of leukocytosis ($20.2\times10^9\pm11.01$ vs $10.2\times10^9\pm6.24$, $P<0.05$).

Table 4.

The JAK2V617F mutation and clinical presentation of patients with ET and PMF

Variable	ET (n=31)			PMF (n=17)		
	JAK2V617F positive	JAK2V617F negative	*P*	JAK2V617F positive	JAK2V617F negative	*P*
Age, yrs	58.9±13.25	44.3±16.33	<0.05	54±16.95	54±15.39	>0.05
RBC, $\times10^{12}$/L	4.8±1.03	4.6±0.89	>0.05	5.1±1.47	4.0±0.96	>0.05
Hb, g/L	130±20.94	140±22.75	>0.05	125±26.59	125±30.98	>0.05
Hct, %	39.9±6.65	42.5±6.13	>0.05	40.2±9.26	38.9±8.06	>0.05
WBC, $\times10^9$/L	9.2±4.26	11.2±9.40	>0.05	20.2±11.01	10.2±6.24	<0.05
Platelets, $\times10^9$/L	934.4±278.3	940.9±172.9	>0.05	868±576.21	671.8±577.94	>0.05
Spleen, cm^2	42.1±21.2	40.4±17.60	>0.05	60±31.23	103.2±64.83	>0.05

RBC - Red Blood Cells; Hb - Hemoglobin; Hct - Hematocrit; WBC- -White Blood Cells

Our results are comparable with well-known data and confirm the diagnostic value of genetic testing. According to the results of numerous studies, the prevalence of the *JAK2*V617F mutation among patients with PV is more than 95%, with ET and PMF – 60%. Among patients with ET and PMF, *CALR* mutations were detected in 20%–25% of cases, *MPL* in 5%, and triple-negative status in 5%–10%.[12]

In clinical practice, mutations in the *JAK2*, *CALR*, and *MPL* genes play a role in predicting the clinical course of diseases and stratifying the risk of thrombotic complications. The *JAK2*V617F mutation is known to significantly increase the risk of thrombotic complications among patients with ET and PMF. The *JAK2*V617F-positive patients with ET are primarily among the elderly. They are characterized

by a high level of hemoglobin, leukocytosis and moderate thrombocytosis. Extreme trombocytosis, a lower risk of thrombotic complications and a high risk of transformation into secondary myelofibrosis are characteristic of *CALR* mutation-positive patients. In cases of PMF, patients with the *JAK2*V617F mutation have a worse prognosis than those with the *CALR* mutation. Leukocytosis and a lower incidence of severe anemia is common for *JAK2*V617F-positive PMF patients.[13]

The results of numerous epidemiological studies that include patients with thrombosis at different sites led to the hypothesis that the *JAK2*V617F mutation can be used as a universal marker of thrombogenic risk in various clinical conditions.[14] A high prevalence of mutation was observed among individuals with cerebral vascular thrombosis (3.8%–6.6%), splanchnic vein thrombosis (16%), and Budd–Chiari syndrome (up to 40%).[7] A screening study of Russian donors revealed carriage of the *JAK2*V617F mutation in 0.65% of cases, and the highest frequency was recorded in the Danish population – 3.1%.[7,8] Long-term monitoring of mutation carriers has demonstrated that the *JAK2*V617F-positive individuals have a higher risk of developing not only myeloid neoplasms, but also solid tumors.[15]

In conclusion: The prevalence of the *JAK2*V617F mutation among PV patients in the RS(Y) was 90.9%. Patients with ET in 61.3% of cases were carriers of the *JAK2*V617F mutation, in 6.4% of *CALR* mutations, and in 3.2% of the *MPL*W515L mutations. In PMF patients, the *JAK2*V617F mutation was detected in 64.7% of cases, and the Type 1 *CALR* mutation was detected in 17.6% of cases. Carriage of the *JAK2*V617F mutation was revealed in 1.1% of healthy individuals and in 4.4% of individuals with initial signs of a myeloproliferative process. The results of clinical evaluation demonstrated that the *JAK2*V617F mutation affects disease phenotype. For the *JAK2*V617F-positive ET patients, disease manifestation in older age is characteristic, and in the case of PMF, the *JAK2*V617F mutation was associated with higher leukocytosis. Early molecular genetic testing will improve the timely diagnosis of CMPD and possibly reduce the number of complications.

Acknowledgments

The study was carried out within the framework of the project “The study of the genetic structure and burden of hereditary pathology of populations of the Republic of Sakha (Yakutia), using the Unique scientific equipment (USE) “The Genome of Yakutia” (Reg. No. USU_507512).

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Epidemiology/Population Health

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Ethnic-Related Characteristics of Lipid and Carbohydrate Metabolism in the Indigenous Population of Yakutia

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Abstract

Background: The objective of our research was to study the ethnic-related characteristics of lipid and carbohydrate metabolism in the indigenous population of Yakutia.

Methods and Results: The study was conducted under expeditionary conditions in the North of Yakutia (Nizhnekolymsky, Anabarsky, Verkhnekolymsky, Tomponsky, and Srednekolymsky districts). In total, 529 people aged between 20 and 70 years were examined in the primary health care units. For a comparative analysis, we formed 6 ethnic groups (Yakuts, Evenks, Evens, Dolgans, Chukchi, Yukagirs). In all ethnic groups, there was a high prevalence of atherogenic dyslipidemia, with the highest frequency in Evenks and Yakuts. Women had a higher frequency of lipid metabolism disorders than men did. The frequency of hyperglycemia was significantly higher among the Dolgans, Evenks and Yakuts than in other ethnic groups.

Conclusion: This study showed a high frequency of metabolic syndrome in the examined ethnic groups, which is caused by a change in the traditional lifestyle and the nature of nutrition. (**International Journal of Biomedicine. 2020;10(1):58-60.**)

Key Words: hypercholesterolemia • hyperglycemia • indigenous population • Yakutia

Abbreviations

BMI, body mass index; **CE**, cholesterolemia; **FPG**, fasting plasma glucose; **HTG**, hypertriglyceridemia; **HCE**, hypercholesterolemia; **HG**, hyperglycemia; **HDL-C**, high-density lipoprotein cholesterol; **IGT**, impaired glucose tolerance; **LDL-C**, low-density lipoprotein cholesterol; **PG**, postload glucose; **TC**, total cholesterol; **TG**, triglycerides; **MetS**, metabolic syndrome

Introduction

Cardiovascular disease (CVD) is one of the leading causes of mortality in Yakutia, as it is across Russia. According to the Federal State Statistics Service, the CVD morbidity rate of the population remained on the same level from 2013 to 2015, and the mortality decreased slightly by 0.9%, making the mortality rate 45.4%.[1]

The association between metabolic syndrome (MetS) and the total mortality from cardiovascular diseases is becoming more and more evident.[2-6] An important point in the study of MetS is the identification of its characteristics in different ethnic groups with the peculiarities of culture and lifestyle.[7]

The objective of our research was to study the ethnic-related characteristics of lipid and carbohydrate metabolism in the indigenous population of Yakutia.

Materials and Methods

The study was conducted under expeditionary conditions in the North of Yakutia (Nizhnekolymsky, Anabarsky, Verkhnekolymsky, Tomponsky, and Srednekolymsky districts). In total, 893 people aged between 20 and 70 years were examined in the primary health care units. The sample was formed according to the lists of employees located in the administrations of the villages. The response was 76%.

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The present study included 529 indigenous people of Yakutia. For a comparative analysis, we formed 6 ethnic groups (Yakuts, Evenks, Evens, Dolgans, Chukchi, Yukagirs) (Table 1). The average age of respondents was 45.59±0.55 years.

Table 1.

Ethnic characteristics of the indigenous population of Yakutia

	Yakuts (n=119)	Dolgans (n=85)	Evens (n=141)	Evenks (n=67)	Chukchi (n=40)	Yukagirs (n=77)
Men, n/%	30/25.2	26/30.6	51/36.2	13/19.4	20/50	34/44.2
Women, n/%	89/74.8	59/69.4	90/63.8	54/80.6	20/50	43/55.8
Average age, yrs	48.94± 1.0	44.93± 1.56	43.02± 0.98	48.37± 1.64	39.73± 1.93	46.49± 1.54

The research program included the following sections: 1) The collection of anamnestic data, physical examination, and anthropometric data analysis [BMI (kg/cm^2)]; 2) Assessment of FPG, OGTT, and blood levels of TG, HDL-C, LDL-C.

Glucose and lipid metabolism disorders were diagnosed according to the Russian national recommendations (the All-Russian Scientific Society of Cardiologists [VNOK, 2012])[2] based on the European recommendations (2011)[3]: TC ≥5.0 mmol/l; LDL-C >3.0 mmol/l; HDL-C <1.0 mmol/l in males and <1.2 mmol/l in females; TG ≥1.7 mmol/l; FPG >6.1 mmol/l; IGT 2Hr PG ≥7.8 mmol/l and ≤11 mmol/l.

Blood was taken from the ulnar vein in accordance with the existing requirements in the morning after an overnight fast. After blood centrifugation, the serum was stored at -70°C until analysis. The serum lipid spectrum was determined by enzymatic method on a Labio 200 automatic biochemical analyzer using Analyticon reagents (Germany).

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems. Written informed consent was obtained from each patient.

Statistical analysis was performed using SPSS (version 19.0). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Mann-Whitney U test was used to compare means of 2 groups of variables not normally distributed. The frequencies of categorical variables were compared using the Chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

An ethnicity-dependent comparative characteristic of the average values of the lipid profile and blood glucose is presented in Table 2. Data obtained showed that the average values of TC exceeded the normal range in Yakuts and Evenks, compared with other ethnic groups. Women had higher values of TC than men did. The level of LDL-C was higher than the reference values in all the studied representatives of the indigenous population, both in men and women. For other indicators, the average concentration of blood parameters did not exceed the normal range.

The average frequency of HCE, HTG, hyper-LDL-CE, hypo-HDL-CE, and carbohydrate spectrum disorders depending on ethnicity is presented in Figure 1. The frequency of HCE was high in all ethnic representatives. The HCE frequency was significantly higher in the Yakuts (58.8%) than in the Yukagirs ($P<0.001$), Evens ($P<0.004$), and Dolgans ($P=0.01$). The lowest frequency of HCE was observed in the Yukagirs (37.7%). These data are consistent with literature data. The prevalence of HCE in Russia, according to ESSE epidemiological studies, was 62%.[8,9]

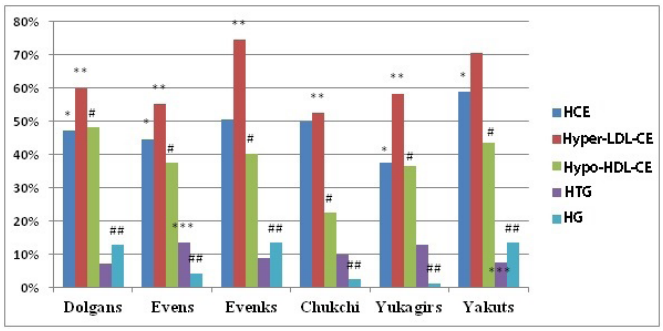

Fig.1. *The frequency of dyslipidemia and hyperglycemia depending on ethnicity*

** - P<0.05 for HCE; ** - P<0.05 for Hyper-LDL-CE; # - P<0.05 for Hypo-HDL-CE; *** - P<0.05 for HTG; ## - P <0.05 for HG*

Table 2.

The average values of the lipid profile and blood glucose in the indigenous population of Yakutia

Variable	Yakuts			Evenks			Evens			Dolgans			Chukchi			Yukagirs		
	total	men	women	total	men	women	total	men	women	total	men	women	total	men	women	total	men	women
FPG, mmol/l	5.12± 0.16	4.93± 0.25	5.19± 0.19	5.41± 0.24	5.66± 0.61	5.35± 0.27	4.76± 0.07	4.69± 0.08	4.79± 0.11	5.17± 0.09	5.38± 0.12	5.07± 0.12	4.27± 0.17	4.44± 0.31	4.09± 0.10	3.82± 0.12	3.81± 0.22	3.83± 0.14
TG, mmol/l	1.03± 0.04	1.01± 0.06	1.03± 0.05	1.02± 0.06	1.02± 0.14	1.03± 0.07	1.12± 0.04	1.18± 0.08	1.08± 0.05	0.84± 0.05	0.76± 0.08	0.88± 0.06	0.98± 0.08	1.04± 0.11	0.93± 0.09	1.09± 0.06	1.13± 0.09	1.05± 0.08
TC, mmol/l	5.18± 0.08	4.96± 0.16	5.26± 0.09	5.17± 0.10	5.15± 0.15	5.18± 0.12	4.93± 0.08	5.04± 0.14	4.86± 0.09	4.86± 0.10	4.77± 0.21	4.90± 0.11	4.96± 0.15	4.94± 0.26	4.98± 0.14	4.89± 0.10	4.78± 0.15	4.98± 0.15
HDL, mmol/l	1.23± 0.02	1.11± 0.05	1.27± 0.03	1.27± 0.06	1.10± 0.09	1.31± 0.07	1.25± 0.02	1.25± 0.05	1.25± 0.03	1.20± 0.04	1.16± 0.08	1.22± 0.04	1.42± 0.07	1.37± 0.11	1.48± 0.09	1.22± 0.03	1.20± 0.66	1.23± 0.03
LDL, mmol/l	3.47± 0.07	3.36± 0.15	3.51± 0.08	3.49± 0.08	3.57± 0.11	3.47± 0.10	3.16± 0.07	3.27± 0.13	3.11± 0.08	3.26± 0.08	3.27± 0.16	3.26± 0.10	3.08± 0.12	3.09± 0.20	3.06± 0.12	3.15± 0.08	3.07± 0.12	3.21± 0.12

The frequency of hyper-LDL-CE was high in all members of the indigenous nationality (more than half), with the highest rate (74.6%) among the Evenks and Yakuts (70.6%). Statistically significant differences were noted between the Evenks and the Chukchi (P<0.001), Evens (P<0.001), Yukagirs (P=0.003), and Dolgans (P=0.007). We found a high direct correlation (r=0.929, P<0.000) between the levels of TC and LDL-C.

The frequency of hypo-HDL-CE was significantly higher among the Dolgans (48.5%), Evens (37.6%), Evenks (40.3%), Yukagirs (36.4%), and Yakuts (43.7%) than it was among the Chukchi (22.5%). Frequency of HTG varied in the range from 7.1% in Dolgans to 13.5% in Evens. Statistically significant differences were noted between Yakuts and Evens (P=0.02).

The frequency of HG was significantly higher among Dolgans (12.9%), Evenks and Yakuts (13.4%) than in Evens (4.3%), Chukchi (2.5%), and Yukagirs (1.3%).

When comparing the frequency of lipid and carbohydrate metabolism disorders on a gender basis, the data obtained were comparable to the data on ethnic groups in general. It should be noted that Yakut and Dolgan women had a greater frequency of HCE than men did. Hypo-HDL-CE prevailed among women, compared to men, in all ethnic groups except for the Chukchi. The frequency of HG was highest among Dolgan men and Yakut women.

Thus, in all ethnic groups, there was a high prevalence of atherogenic dyslipidemia, with the highest frequency in Evenks and Yakuts. Women had a higher frequency of lipid metabolism disorders than men did. The frequency of hyperglycemia was significantly higher among the Dolgans, Evenks and Yakuts than in other ethnic groups. Our study showed a high frequency of MetS in the examined ethnic groups, which is caused by a change in the traditional lifestyle and the nature of nutrition. The high frequency of atherogenic dyslipidemia in representatives of various ethnic groups of the indigenous population of Yakutia is of scientific-clinical interest. In the public health arena, the results of this study are important for the development of regional policies to preserve the basic traditional principles of lifestyle and nutrition for the indigenous population in order to optimize preventive measures for cardiovascular diseases.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

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Applying a Translated Version of the Adolescent Sleep Habits Survey in Russian High School Children with Obesity

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Abstract

Background: Inadequate sleep duration and sleep patterns have been associated with metabolic and circadian changes that promote obesity. The aim of this study was to applicate a translated version of the Adolescent Sleep Habits Survey to assess sleep habits and schedules in Russian obese adolescents.

Methods and Results: We questioned 87 adolescents aged between 15 and 17 years: 57 with obesity and 30 with a normal weight (NW). In total, some sleep problems were observed in 60.8% of obese respondents and 28.6% of NW participants (P=0.048). School-night wake times in obese adolescents did not differ significantly from the same variables in NW adolescents. School-night bedtimes and TST only in an obese sample were later and shorter, respectively, than in NW subjects (P<0.001 for both variables). Surprisingly, NW adolescents had a greater bedtime shift than obese peers (P<0.001). Finally, about half of obese respondents reported that they usually eat (62.7%) and watch TV (44.4%) in bed (P<0.001 for both variables compared with controls).

Conclusions: Applying a translated version of ASHS helps assess sleep habits and schedules in Russian adolescents, including obese patients. (**International Journal of Biomedicine. 2020;10(1):61-65.**)

Key Words: adolescents • obesity • sleep-wake rtythm • sleep habits • self-reported survey

Abbreviations

ASHS, Adolescent Sleep Habits Survey; **BMI**, body mass index; **NW**, normal weight; **TST**, total sleep times

Introduction

The World Health Organization has identified obesity as a global epidemic with rates of obesity, poor diet, and lack of physical activity rapidly rising in children, adolescents, and adults.[1,2] On average, the prevalence of obesity in school-age children in Europe was 4.9%.[3] According to the results of a multicenter study conducted in Russia, 19.9% of children and adolescents are overweight, 5% are obese.[4] Primary or exogenous obesity is a multi-factorial disorder that results from the interaction between an unfavorable socio-cultural environment and polygenic predisposition in an individual.[5] Sleep is a risk factor linked to the development and maintenance of obesity, and it has emerged as a potential target for obesity prevention.[6] It is hypothesized that sleep impacts weight through a variety of biological and behavioral pathways. For example, sleep restriction has been shown to negatively impact energy and glucose metabolism, alter appetitive hormones, and allow for more time to engage in obesogenic behaviors (e.g., television watching, poor diet choices).[7]

It is known that during adolescence physiological sleep patterns and psychosocial influences on sleep change. Some studies show that adolescents, compared to adults, have shorter sleep durations, later bedtimes, and greater discrepancies between weekday and weekend sleep schedules.[8,9] Regular

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insufficient sleep and excessive daytime sleepiness are serious epidemics among adolescents. In several studies with large samples of adolescents, researchers reported that from 45% to 80% of adolescents experience insufficient sleep on school nights.[10-12] Thereby, adolescents may be particularly vulnerable to sleep-related changes in their weight. Most research on sleep and obesity in adolescents has focused on the associations between sleep duration and sleep quality. Recent studies suggest that patterns of sleep, in addition to sleep duration and quality, may be related to the weight status and may provide a more comprehensive picture of the relationship between BMI and sleep.[13] Specifically, later bedtimes show positive relationships with obesity and negative relationships with physical activity and fruit and vegetable consumption in children and adolescents.[14-16] Furthermore, discrepancies between weekday and weekend bedtimes(bedtime shift) may dysregulate circadian rhythms, as the circadian system is slow to adapt to rapid shifts in sleep, which can then influence metabolic processes.[17] So, a combination of factors, including circadian phase delay, reduced sleep pressure, early school start times, caffeine use, electronic media usage, modern lifestyles, and social obligations, has minimized the opportunities for adolescents to obtain adequate sleep and thus has increased the risk of obesity.

The subjective assessment of sleep patterns is widely performed in sleep studies. From the earliest times of epidemiology, questionnaires have been used as a basic instrument for data collecting and screening.[18] Self-reported sleeping habit questionnaires are relatively easy to utilize and continue to be the most requested and widely used method. Respondents usually estimate bedtimes, waking times, hours of sleep, sleep habits, sleepiness, and so on. Most of the questionnaires are valid only for pre-pubertal children or adults, but not adolescents. Wolfson and colleagues (1998) successfully attempted to study sleep and waking behaviors in American high school children using the Sleep Habits Survey and showed that most of the adolescents surveyed do not get enough sleep, and their sleep loss interferes with daytime functioning.[19] In 2003, the above-mentioned authors examined the validity of this questionnaire through a comparison of retrospective survey descriptions of usual school- and weekend-night sleep habits with diary-reported sleep patterns and actigraphically estimated sleep behaviors. Their results support the validity of the Sleep Habits Survey estimates in comparison with sleep diary and actigraphy in high school children.[20] Sung (2011) used the Youth Sleep Habits Survey to assess self-reported sleep duration in obese adolescents.[21] Shahid and colleagues described this questionnaire as ASHS for schoolchildren in grades 4 through 12, developed in 2009 as part of Pediatric Sleep Disorders Program.[22,23] However, the administration of this sleep questionnaire for Russian obese adolescents is not established.

Based on the description above, the problem of this research is that sleep is a risk factor linked to the development of obesity, and many adolescents report sleep-wake schedules that involve late bedtimes and short sleep in self-report survey questionnaires.

The purpose of this study was to translate and applicate the ASHS to assess sleep habits and schedules in Russian adolescents with obesity.

Materials and Methods

We conducted a cross-sectional study, which involved 57 obese patients (Group 1) aged 15 to 17 years, who were referred to the Clinic of the Scientific Center for Family Health and Human Reproduction in 2019. Thirty age- and sex-matched adolescents with NW were included in the control group (Group 2).

Study inclusion criteria were the 15-17 year age range; BMI Z-score >2 for age and sex for Group 1 and BMI Z-score from -1 to +1 for age and sex for Group 2; and a signed informed consent form. The study exclusion criterion was unwillingness to participate in this study.

The program of the study included a general medical examination with anthropometric measurements, questioning and statistical analysis.

Anthropometric parameters of adolescents were assessed once when they were included in the study. Body mass index (BMI, kg/m^2) was calculated. The growth and weight parameters of the adolescents were evaluated using the reference values of the WHO and the AnthroPlus calculator (2009). The nutritional status was determined by the values of the Z-score.[24]

To fulfill the purpose of this study, the sleeping habits were evaluated subjectively, using a questionnaire—the Russian version of the ASHS. This is a structured survey featuring both open-ended and multiple-choice questions, which allows for the collection of demographic details, familial and medical histories, and information regarding sleep habits, schedules, and behaviors. Two versions of the self-reported survey were used (differing only in their mention of either male- or female-related developmental milestones). Each version is a pencil-and-paper instrument, consisting of between 61 (for boys) and 62 (for girls) questions and should require between 20 and 30 min for completion. Research assistants met with the participants once upon admission to the hospital. The survey items queried adolescents about usual sleeping and waking behaviors (sleep problems, sleep habits, sleep history, daytime sleepiness and sleep/wake rhythms) over the last two weeks, which is a typical interval for point-assessment sleep habit surveys. This article examines the following the ASHS variables: (1) for sleep problems ("Do you have sleep problems(s)?" answered as "Yes" or "No"; (2) for usual sleep habits (school and weekend nights separately): (a) ASHS bedtime: usual bedtime ("What time do you usually go to bed on school days?" answered as one specific time, such as 10:30 p.m.); (b) ASHS wake time: usual wake time ("What time do you usually wake up on weekends?" answered as one time, such as 9:30 a.m.); (c) ASHS TST: usual total sleep time ("Figure out how long you usually sleep on a school night and fill it in here," answered as specific hours and minutes such as 7 hours, 30 minutes); (d) ASHS latency: usual sleep latency ("On weekends, after you go to bed at night, about how long does it usually take you to fall asleep?" answered as specific minutes, such as 20 minutes; if longer than one

hour, change to minutes); (3) for possible sleep habits: (a) ASHS activities: possible activities in bed ("How often have you done any of the following activities in bed?: read, watch TV, eat, do schoolwork, worry" answered as "Every night," "Several times," "Twice," "Once" or "Never"; (b) ASHS falling asleep: possible activities if difficulty falling asleep ("When you have difficulty falling asleep or getting back to sleep, what do you do?: try to get to sleep, do something in bed, get up and watch TV, get up and drink warm milk/water/tea/coffee, other" checked as "All that apply").

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Ethics Committee of Scientific Centre for Family Health and Human Reproduction Problems. Written informed consent was obtained from the patient/parent/guardian/ relative of each patient.

Statistical analysis was performed using the *Statistica* 6.1 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±standard deviation (SD) for continuous variables. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Mann-Whitney U test was used to compare means of variables not normally distributed. Group comparisons with respect to categorical variables were performed using chi-square tests or, alternatively, Fisher's exact test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

Characteristics and response to the ASHS for the participants were analyzed. Comparisons for age, sex, zBMI and survey variables (for usual sleep habits) between participant groups are shown in Table1. Adolescents of Group 1 had a mean age of 16.2 years with an average zBMI of 2.56, whereas Group 2 adolescents (n=30) had a mean age of 16.1 years ($P>0.05$) with an average zBMI of 0.7 ($P<0.05$). The proportion of girls in the study was 22.8% and 20.1% for Group 1 and Group 2, respectively ($P>0.05$).

School-night wake times in Group 1 did not differ significantly from the same variables in Group 2. On average, estimates were within 5 minutes, and all participants reported waking up at about 7:10 a.m. Conversely, survey-reported school-night bedtimes in Group 1 were significantly later (on average 60 minutes) and TST significantly less (on overage 65 minutes) than in Group 2 ($P<0.001$ for both variables). However, participants of Group 1 reported a trend toward longer times falling asleep versus adolescents of Group 2. Finally, survey-reported weekend bedtimes, TST and wake times in Group 1 did not differ significantly from the same variables in Group 2.

Next, we carried out a comparative analysis of the remaining questionnaire data and obtained the following results: About 60.8% of respondents in Group 1 answered that they have some sleep problems, compared with 28.6% in Group 2 ($P=0.048$). According to the self-assessment of possible sleep habits in the last two weeks, in the section "ASHS activities," 24.3% of respondents noted their activities in bed as "Read" in Group 1 and 25.1% in Group 2 ($P>0.05$); as "Watched TV" 37.4% and 31.1% ($P>0.05$), respectively; as "Ate" 54.8% in Group 1 and 27.1% in Group 2 ($P<0.05$); as "Did schoolwork" 16.9% and 17.1%, respectively ($P>0.05$); as "Worried" 24.6% in Group 1 and 19.8% in Group 2 ($P>0.05$). Furthermore, more than half of Group 1 adolescents (62.7%) answered "ate in the bed" as "Every day/night" compared with 28.3% of Group 2 respondents ($P=0.034$); and 44.4% of participants in Group 1 answered "watched TV in the bed" as "Every night" compared to 18.5% participants in Group 2 ($P=0.048$). In section "ASHS falling asleep" respondents noted when they had difficulty falling asleep: 42.8% of Group 1 respondents and 71.8% of Group 2 respondents "stayed in bed and tried to get to sleep" ($P=0.047$); 33% vs. 18.1% ($P=0.63$), respectively, "Did something in bed"(e.g., read, ate or watched TV); 9.1% of Group 1 adolescents and 10.1% of Group 2 participants "got up and watched TV" ($P=0.93$); also, 15.1% of Group 1 respondents answered that they "got up and drunk warm milk/water/tea/coffee."

Table 1.

Comparisons for age, sex, zBMI and ASHS assessment between participant groups

Variable	Group 1	Group 2
Age, y	16.3 ± 0.5	16.1 ± 0.3
Gender (M/F)	44 (77.2)/13(22.8)	24(80)/6(20)
BMI, kg/m2	34.9 ± 2.3*	19.8 ± 1.9
zBMI	2.4± 0.4*	0.7± 0.2
School-night TST, min	450.2(82.4)*	515.1(45.8)
Weekend-night TST, min	612.7(124.1)	618.5(83.2)
School-night bedtime^	23:07(1:22)*	22:05(45)
School-night wake time^	7:12(50)	7:07(42)
Weekend-night bedtime^	23:52(1:34)	23:47(53)
Weekend-night wake time^	10:05(1:31)	10:15(1:23)
Bedtime shift^	53(1:17)	1:47(52)*
School-night sleep latency, min	17(9.2)	13.6(8.4)
Weekend-night sleep latency, min	20.1(15.7)	15.7(10.1)

*^Bedtimes, wake times and bedtime shift are expressed in 24-h clock; zBMI, body mass index-for-age z-score; *P<0.05*

Our study found that adolescents with obesity have later school-night bedtimes, trend toward greater sleep latency and significantly shorter weekday sleep duration versus a NW peer. Of note is that as student sleep opportunities are curtailed most in the morning so as not to be late for school, the time that adolescents go to bed is a key determinant of how much sleep they obtain.[25] Surprisingly, NW adolescents had a greater bedtime shift than obese peers ($P<0.001$); that result differs from the results of Hayes et al. (2018), who found the same schedule pattern in adolescents with overweight and obesity.[26]

In that study, 186 respondents aged from 12 to 17 years reported typical sleep and wake times on weekdays and weekends using the Pittsburgh Sleep Quality Index questionnaire. In contrast to our results, that study found that zBMI was not related to weekday bedtimes, but significantly related to weekend bedtimes as well as bedtime shift in obese adolescents. However, shorter school-night TST in obese participants confirms the availability of relationships between sleep duration and overweight, which have been reported in the majority of the extant literature.[7,14,27,28] In the current study, we found that self-reported average sleep duration on the weekdays in an obese group was not within the recommended 8–10, hours, in contrast to the control group.[29,30] Specifically, only 14% of the obese adolescents reported more than 8 hours of sleep on school nights compared with 83% of NW adolescents.

It is argued that later bedtimes and sleep deficit occur due to the confluence of psychosocial and biological factors of adolescent development.[31] Much work has been done to determine how the types of activities adolescents engage in immediately before sleep impact subsequent sleep.[32,33] As the current study shows, about half of obese respondents reported that they "ate and watched TV in bed every night" in the last two weeks, which significantly differed from NW adolescents. The same behavioral patterns are also an important risk factor for obesity and are significantly associated with the severity of overweight. Many studies have found that screen time is adversely associated with both sleep outcomes [34] and obesity.[35] A previous study in children and adolescents with overweight and obesity showed that later bedtimes were related to increased daily high calorie food intake and screen time.[36]

The present study adds additional evidence and extends the current literature on the association of sleep habits with weight in adolescents with obesity. It showed that about two thirds of obese respondents had some sleep problems compared with one third of controls (P<0.05). Despite some limitations of the study, such as small sample size and self-reported survey estimates of sleep patterns only, we found certain differences between usual school- and weekend-night sleep habits in obese and NW participants, which allow justifying the possibility of using ASHS to assess sleep habits and schedules in Russian adolescents, including those with obesity. However, despite the results, future studies should use objective measures of sleep (polysomnography) and consider accurate measurement, particularly as self-reported sleep duration tends to be overestimated.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Dentistry

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Treatment of Class II Caries Lesions with Application of Packable and Conventional Resin Composites: Clinical and Experimental Study

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Abstract

Background: The aim of the present study was to compare the efficacy of packable and conventional composites in treatment of patients with class II caries lesions (CIICLs).

Methods and Results: The clinical part of the study was conducted on patients with indications for composite restoration of at least two adjacent CIICCLs. There were 32 patients in whom 72 (36 pairs) teeth with caries pathology were treated. One tooth in every pair was randomly assigned for restoration with Filtek Z250 (Cl-Group 1, n=36) and another one with preheated Filtek-P60 (Cl-Group 2, n=36). The mean observation time for composite restorations was 47.1±13.8 months. The modified USPHS criteria list was applied for clinical evaluation of the four following clinical parameters, which were used in the study: secondary caries, anatomy form, occlusal contact, and surface texture. The experimental study was conducted on 40 filling samples, which were made of conventional Filtek Z250 (Exp-Group 1, n=20) and packable Filtek P60 (Exp-Group 2, n=20) with the help of a transparent plastic mold. Every experimental group was randomly divided into two subgroups (A and B): polymerized filling samples of Exp-Subgroups 1A (n=10) and 2A (n=10) were put into the test right after polymerization; samples of Exp-Subgroups 1B (n=10) and 2B (n=10) were passed through a shear-strength test after exposure to four consecutive cycles of autoclaving. Shear-strength measurements were made in an Ultratest Machine (Ultradent, USA), which was adapted to perform a proper test.

The incidence of secondary caries in both clinical groups (two incidents in Gl-Group 1 and one incident in Cl-Croup 2) was very low, and comparative analysis of obtained results did not reveal any significant difference between them. In relation to criteria of anatomy form, the percentage of *alpha* level of tooth restorations that were done with packable Filtek P60, was 38.9%. *Bravo* estimates had 50% of restored teeth. Tooth restorations with conventional Filtek Z250 had *alpha* level in 58.3% and *bravo* in 41.7% of cases. Occlusal contact and surface texture, the efficacy of CIICL management was better in teeth that had been treated with packable composite. Analysis of experimental findings revealed that the mean value of shear strength for Filtek P60 filling samples, which were tested immediately after polymerization, was lower than the same parameter for Filtek Z250 on 20.5% ($P<0.01$). In addition, it was established that the studied parameter for filling samples of Filtek Z250 had decreased by 1.4 times (28.1%) after cycles of autoclaving ($P<0.05$). A similar tendency was observed for Filtek-P60 too, but only 10.2% showed a decrease in value ($P<0.05$).

Conclusions: Based on obtained clinical findings it can be concluded that treatment of CIICLs with application of packable Filtek P60 and conventional Filtek Z250 does not lead to a recurrence of caries in a period of 47.1±13.8 months. The issue of more frequent chipping of composite restorations that were made of packable Filtek P60, but not of conventional Filtek Z250, could be of clinical value in treatment planning of patients with excessive occlusal load and tooth wear. In addition, it was clinically noticed that tooth composite restorations made of packable Filtek P60 had values of surface texture that were close to *alpha* level. **(International Journal of Biomedicine. 2020;10(1):66-69.)**

Key Words: class II caries lesions • packable and conventional composites • shear strength

Introduction

Since the moment of their appearance on the market, dental composites have had narrow indications for application and are usually used for restoration of anterior teeth. However, during the last several decades, resin restoratives took one of leading places among the materials with indications for direct and indirect restoration of posterior teeth.[1-3]

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Present achievements in diversifying applications of resin composite filling material could mostly be attributed to the rapidly growing new technologies, directed toward the synthesis of durable, high molecular weight, polymeric matrices with low polymerization shrinkage and stress, which were loaded with nanoparticles in different organic and inorganic ratios.[4-7]

Nowadays, the application of dental resins can meet the needs of an enormous variety of clinical situations that require a low invasive approach to the treatment of caries lesions. However, management of patients with indications for direct restoration of class II cavities with composite materials still may be a challenge for dentists, and the invention of packable composites could be of great assistance to them.[8,9]

Silver amalgam was the basic material of choice for direct restoration of class I and II cavities for a prolonged period of time. The longevity of this type of restoration was about 20 years, which was due to the long-lasting marginal seal, appropriate wear resistance and cariostatic behavior.[10,11]

However, the presence of probable mercury toxicity, tarnish, stains and color of silver amalgam were the main reasons for the application of alternative packable composites that had manipulability and consistency close to silver amalgam, but without any of the abovementioned drawbacks.[12-14]

In accordance with their definition, packable composites are hybrid resin restoratives designed for use in posterior dentition, where a stiffer consistency facilitates condensation during cavity filling and restoration. It is noteworthy that the increased stiffness of packable resins is due to the high filler load with particle sizes measured from nanometers to micrometers.[15] Packable composite materials, which are often called condensable, have one substantial advantage over conventional hybrids—they are not sticky. Absence of the tendency to stick to dental instruments allows placing and shaping this type of resin in an unstressful manner during restoration of posterior teeth. However, there are a few other differences between conventional materials and packable composites, which may not be in favor of the latter.[16,17]

The results of several studies showed that highly filled composites have better wear resistance and flexural strength than resins with lower filler content.[18,19] On the contrary, the data obtained from other studies indicated that values of the wear resistance and strength of packable resins were the same as for conventional composites, and sometimes they were even worse.[20,21]

Previously, a group of researchers stated that in dental composites an inorganic filler part must not exceed 70% by volume because of technical difficulties and poor handling characteristics. Increased viscosity of resin composites may be of help with respect to low stickiness while inserting filling material, and may cause a problem during the time it is adapting to cavity walls.[22] Many of the abovementioned facts may explain why the prevalence of conventional composites with lower inorganic content and viscosity in posterior teeth is still high.

Therefore, because there is no solid opinion on whether to use a resin material with high filler content or a universal composite, the aim of the present study was to compare the efficacy of packable and conventional composites in treatment of patients with class II caries lesions (CIICLs).

Material and Methods

The clinical part of the study was conducted on patients with indications for composite restoration of at least two adjacent class II carious cavities. There were 32 patients in whom 72 (36 pairs) teeth with caries pathology were treated.

Patients were treated by one dentist. One tooth in every pair was randomly assigned for restoration with Filtek Z250 (Cl-Group 1, n=36) and another one with preheated Filtek-P60 (Cl-Group 2, n=36). The packable Filtek-P60 was preheated to facilitate easy material adaptation to prepared cavity walls.

Basic inclusion criteria into the study were: 1) similar periodontal status and mobility of adjacent teeth with CIICL; 2) similar periodontal status and mobility of antagonist teeth, which should be sound. Presence of satisfactory porcelain or metal restorations on antagonist teeth was not a contraindication for inclusion into the study. The mean observation time for composite restorations was 47.1±13.8 months.

Considering that there are only three shades in a restorative system of Filtek-P60 and that in a posterior region of the mouth there is not a high demand for color matching or excessive esthetics, and a reasonable priority of function, the modified USPHS criteria list (Table 1) was applied for clinical evaluation of the four following clinical parameters, which were used in the study: secondary caries, anatomy form, occlusal contact, and surface texture. In order to make an interpretation of final results more informative, every character rating was expressed in a proper conventional unit (CU).

Table 1.

Modified USPHS criteria used for clinical evaluation

Category	Rating, CU	Description
Secondary caries	*alpha* (2) *bravo* (1)	No caries present Caries present
Occlusal contact	*alpha* (3) *bravo* (2) *charlie* (1)	Normal Slight No contact
Anatomy form	*alpha* (3) *bravo* (2) *charlie* (1)	No presence of a material chipping Slight loss of a material, dentin or base are not exposed Sufficient loss of a material with dentin or base exposure
Surface texture	*alpha* (3) *bravo* (2) *charlie* (1)	Polished surface of a composite restoration Slightly pitted surface of a composite restoration, possible to refinish Deeply pitted surface of a composite restoration, not possible to refinish

The experimental study was conducted on 40 filling samples, which were made of conventional Filtek Z250 (Exp-Group 1, n=20) and packable Filtek P60 (Exp-Group 2, n=20) with the help of a transparent plastic mold. Every

sample was of a standardized cylindrical shape with a mean diameter of 2.46±0.03 mm and length of 8.14±0.12 mm. Light polymerization was initiated with application of Blue Phase iG20 (Ivoclar) in a "High" mode with 40 sec exposure time for every sample in a similar manner.

Every experimental group was randomly divided into two subgroups (A and B): polymerized filling samples of Exp-Subgroups 1A (n=10) and 2A (n=10) were put into the test right after polymerization; samples of Exp-Subgroups 1B (n=10) and 2B (n=10) were passed through a shear-strength test after exposure to four consecutive cycles of autoclaving.

Shear-strength measurements were made in an Ultratest Machine (Ultradent, USA), which was adapted to perform a proper test. Values were registered in pounds (lb).

Statistical analysis was performed using StatSoft Statistica v7.0. The mean (M) and standard deviation (SD) were calculated. The Mann-Whitney U Test was used to compare the differences between the two groups. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

In the present study, the mean observation time to assess the quality of treatment of CIICLs with application of Filtek Z250 and Filtek P60 was about four years (Table 2). Within this time period, the incidence of secondary caries in both clinical groups (two incidents in Gl-Group 1 and one incident in Cl-Croup 2) was very low, and comparative analysis of obtained results did not reveal any significant difference between them.

Table 2.

Treatment efficacy of CIICL and clinical appearance of composite restorations

	Criterion of anatomy form (CU)	Criterion of secondary caries (CU)	Observation time, (mos)	Criterion of occlusal contact (CU)	Criterion of surface texture (CU)
Cl-Group 1	2.58±0.5	1.89±0.32	47.1±13.8	2.36±0.72	2.39±0.6
Cl-Group 2	2.28±0.66	1.97±0.16	47.1±13.8	2.69±0.62	2.8±0.4
P	<0.05	>0.05	-	<0.05	<0.01

However, in relation to criteria of anatomy form, in occlusal contact and surface texture some differences were detected. Thus, the anatomy of composite restorations in Cl-Groups 1 and 2 had deteriorated on 14% and 24%, respectively. In addition, there was a statistically significant difference between them ($P<0.05$), which could be explained by a noticeable predisposition of Filtek-P60 material to chipping.

Therefore, in relation to parameters of anatomy form, the percentage of *alpha* level of tooth restorations that were done with packable material, was 38.9%. *Bravo* estimates had 50% of restored teeth. In the same time, tooth restorations, which were of conventional Filtek Z250, had *alpha* level in 58.3% and *bravo* in 41.7% of cases.

Taking into account parameters of occlusal contact and surface texture, the efficacy of CIICL management was better in teeth that had been treated with a packable composite.

Analysis of experimental findings revealed (Table 3) that the mean value of shear strength for Filtek P60 filling samples, which were tested immediately after polymerization, was lower than the same parameter for Filtek Z250 on 20.5% ($P<0.01$). In addition, it was established that the studied parameter for filling samples of Filtek Z250 had decreased by 1.4 times (28.1%) after cycles of autoclaving ($P<0.05$). A similar tendency was observed for Filtek P60 too, but only 10.2% showed a decrease in value ($P<0.05$).

Table 3.

Shear strength of cured resin composites before and after cycles of autoclaving

Subgroups	Exp-Group 1 (lb)	Exp-Group 2 (lb)	*P*
A (n=10)	85.9 ± 15.1	68.3 ± 11.2	<0.01
P	<0.01	<0.05	
B (n=10)	61.8 ± 14.5	61.3 ± 15.2	>0.05

It is noteworthy, that the difference between mean values of shear-strength findings for packable Filtek P60 and conventional Filtek Z250, which were obtained after an impact of four consecutive cycles of autoclaving, was not of any statistical significance.

The efficacy of CIICL management is strongly material-dependent, since a proper longevity and function of used restorations are the prerequisites of long-term tooth vitality and the healthy status of surrounding periodontal tissues.

Resin composites have been recognized by the dental community as materials of choice because of tooth color esthetics and ease of application. However, durability and wear resistance of this type of restoration remains under question.

Performance of particular laboratory tests and specially designed clinical studies might shed a light on probable clinical behavior of the restoratives that are used. Thus, in the present study it was revealed that lower shear-strength values for filling samples made of Filtek P60 packable composite might explain the chipping of proper restoration in a patient's mouth.

At the same time, it was found that more pronounced degradation of the organic matrix of Filtek Z250 samples *in vitro* was a reasonable issue of poor surface texture and occlusal contact on this type of tooth restoration *in vivo*.

Therefore, based on obtained clinical findings it can be concluded that treatment of CIICLs with application of packable Filtek P60 and conventional Filtek Z250 does not lead to a recurrence of caries in a period of 47.1±13.8 months.

The issue of more frequent chipping of composite restorations that were made of packable Filtek P60, but not of conventional Filtek Z250, could be of clinical value in

treatment planning of patients with excessive occlusal load and tooth wear. In addition, it was clinically noticed that tooth composite restorations made of packable Filtek P60 had values of surface texture that were close to *alpha* level.

Competing Interests

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Microscopic Techniques

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Using Scanning Electron Microscopy and Atomic Force Microscopy to Study the Formation of Nanoparticles on Red Blood Cell Surface in Cervical Cancer Patients

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Abstract

Background: In this study, we used scanning electron microscopy (SEM) and atomic force microscopy (AFM) to examine the changes in morphology of red blood cells (RBCs) and to investigate the nanoparticles (NPs) found on their surface in cervical cancer (CC) patients undergoing radiation therapy (RT).

Methods and Results: We obtained smears of venous blood from 12 CC patients at the start, midway and at the end of external beam RT and then midway and at the end of brachytherapy. It was found that in CC patients, the number of RBCs with abnormal morphology increased and NPs appeared on their surface. During RT, the total number of abnormally shaped RBCs and the number and size of NPs increased. The RBC diameter was 8.38±0.36 μm in the control group and 9.41±0.47 μm in CC patients. The average diameter of NPs on the RBC surface was 69.91±12.15 nm and their average height 23.75±3.70 nm. After RT, the morphology of RBCs was restored, and the formation of NPs decreased.

Conclusion: The changes observed could serve as the basis for developing efficacy indicators of cancer radiation therapy. **(International Journal of Biomedicine. 2020;10(1):70-75.)**

Key Words: extracellular vesicle • red blood cell • nanoscale • morphology • radiation therapy

Abbreviations

AFM, atomic force microscopy; **CC**, cervical cancer; **EV**, extracellular vesicle; **HPV**, human papillomavirus; **NP**, nanoparticle; **REM**, reflection electron microscopy; **RT**, radiation therapy; **RBC**, red blood cell; **SEM**, scanning electron microscopy

Introduction

Every year approximately 500,000 women are diagnosed with CC.[1] It is the second most common cancer among women and is very deadly, accounting for around 260,000 deaths worldwide,[2] making it the leading cause of death by cancer among the female population of developing countries.[3]

While there is overwhelming evidence to suggest that persistent HPV infection is the key reason for the development of cervical carcinoma, other factors also contribute to the establishment and progression of cancer. Several studies have shown elevated levels of EVs in the bodily fluids of cancer patients,[4] including CC patients specifically.[5] EVs can be broadly defined as membrane particles released into the extracellular space by a cell of any type.[6] EVs typically range from 30nm to 2,000 nm in diameter[7] and can be broadly classified into exosomes, microvesicles and apoptotic bodies, according to their biogenesis.[7,8] While microvesicles and apoptotic bodies arise through direct blebbing off the membrane of a normal or apoptotic cell, respectively, exosomes are generated through the endolysosomal pathway.[7] Existing research recognises that an increased abundance of exosomes in cancer patients' blood [9,10] may play a role in the so-called cancer "field effect," in which cancer cells induce malignant changes in the surrounding healthy cells.[11] In fact, there is a large body of research dedicated to the role that exosomes play in cell-to-cell communication by carrying molecular messages, often in the form of miRNA, from the parent cell to the target cell.[5,12,13]

RT, which is a common treatment type for cervical carcinoma, may exacerbate the cancer field effect as irradiation has been shown to produce non-targeted effects in the cells that have not themselves been irradiated.[14] This can, at least partially, be attributed to the stimulating effect radiation has been shown to have on the secretion of exosomes and their subsequent uptake by the surrounding cells.[14-17]

While there is a wealth of studies dedicated to tumor-derived exosomes in the bloodstream of oncology patients,[9,10] it appears that cancer cells may not be the only perpetrators of elevated EV levels in cancer sufferers. In fact, a study by Kim et al.(2003) has demonstrated that the quantity of platelet MVs in gastric cancer patients' blood was more than 3 times higher than in healthy controls, with platelet MV quantities of $>2.70\times10^9$/ml correlating with the presence of distant metastasis and poor prognosis.[4] This indicates a need for a more complex understanding of various blood parameters, including EV abundance, as biomarkers of cancer progression. Moreover, the current focus of scientific inquiry rests on freely circulating EVs or their uptake by the surrounding cells; however, a recent study has found that roughly two thirds of the total blood EV count in breast cancer patients exists in the blood cell-bound state.[18] A thorough investigation of blood cell-bound EVs as potential cancer biomarkers is therefore entirely warranted.

In the present study, we use SEM and AFM to examine the changes in morphology of RBCs and to investigate the nanoscale objects found on their surface in CC patients undergoing RT. While it is beyond the scope of the current study to identify the precise biogenesis of these objects, we propose that they could be cell-bound EVs of various origins.

Materials and Methods

Blood Samples

Smears of venous blood containing K3-EDTA from 12 patients with CC and from 3 patients of the control group were obtained. The age range was from 45 to 55 years. For 4 CC patients, analyses were taken at each stage of the full course of treatment, and for the remaining 8 CC patients, right after the first stage. A thin, even layer of blood was smeared onto a clean degreased glass slide and dried.

SEM and AFM imaging

SEM and AFM were used to investigate the morphology and surface of RBCs in CC patients at the start, midway and at the end of external beam radiation therapy and then midway and at the end of brachytherapy. A high-resolution SEM JSM-7800F (Japanese Electron Optics Laboratory, JEOL, Japan) equipped with a Schottky thermal field emission cathode and a super hybrid objective lens was used. The microscope is equipped with a Gentle Beam system, which reduces the speed of electron propagation of the emission beam and the acceleration of emitted electrons, which significantly increases the signal-to-noise ratio and image quality at low accelerating voltages. The following microscope parameters make it possible to study the morphology of the RBC surface in blood smears without spraying conductive coatings, to eliminate damage to the object and to identify NPs (resolution from 0.08 nm to 1.2 nm, magnification range 25–1000000x, at voltage 15 kV and 1 kV, respectively).

To investigate the morphology of RBCs, glass and graphene substrates and various SEM amplification modes were used. The studies were carried out at accelerating voltages of 1 kV and 2 kV with a voltage of 8–10 V applied to the test object. The maximum size of the samples was 20 mm.

The size analysis of RBCs and NPs was carried out using the software JMicroVision v1.2.7 (Roduit, 2007). The data determined were entered in Excel and histograms of the sizes of RBCs and NPs were constructed using standard methods. Average values, dispersions, standard deviations, and size distributions of RBCs and NPs were determined using the Gauss distribution.

AFM was performed using a Solver Next microscope (NT-MDT company) with scanning area 50×50 μm (512 points) and 5×5 μm (512 points); scanning speed 0.5 Hz, 0.25 Hz; NSG10 cantilever with a radius of curvature of not more than 10nm. The AFM images were recorded using Nova Px and Image Analysis (NT-MDT company) software. The S1 ruler was used to assess the RBC surface unevenness and thickness.

Radiation Treatment

In the Yakut Republican Oncology Dispensary (Yakutsk, Russia), patients were treated with RT, which consists of two stages: first, distance RT using the Elekta Synergy accelerator (United Kingdom, external beam radiation therapy, 6-18 MeV) and then brachytherapy using the MultiSource HDR device (Germany, brachytherapy with Cobalt-60 source). At the first stage, the treatment was performed on a linear accelerator in the mode of working with electrons with energy of 6 MeV. The therapy was carried out as follows: 3 times for 5 days daily with a break of 2 days (i.e. at the first stage only 15 fractions of 2Gy were carried out). Then, without a break between the first and second stage, contact RT of 5 Gy was performed, interspersed with distanced RT of 2 Gy of 5 fractions of each type of RT.

Blood samples were collected at the beginning, middle and end of the remote therapy, then in the middle and at the end of the contact RT. Multiple methods (3D CRT, IMRT, VMAT) were employed in the course of therapy to ensure precision delivery of high doses to the tumor and low doses to healthy tissue.

Primary methods for processing experimental results

To determine the linear size of RBCs, REM images were used at magnification of 1000x, and for NP sizes, REM images were used at magnification from 30,000x to 50,000x. Primary methods of statistical processing of experimental data were used to determine the linear sizes of RBCs and NPs. The diameters of RBCs and NPs were measured using the computer program JMicroVision 1.2.7, which is freely available. Using Excel, we constructed histograms of linear sizes of RBCs and NPs on the RBC surface in normal and pathological conditions for each patient; based on these histograms, the distribution of linear sizes was determined as a normal Gauss distribution. Then, based on a sample of the average size of RBCs and NPs of all patients using the Shapiro-Wilk test, the hypothesis of a normal distribution of linear sizes was tested. This criterion was used, taking into account that it is reliable for a test volume of $8 \le n \le 50$.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Ethics Committee of the M.K. Ammosov North-Eastern Federal University (protocol No. 13 of April 4, 2018, decision No. 2). Written informed consent was obtained from each patient.

Results and Discussion

In the course of the present study, linear size distribution histograms for RBCs and NPs were obtained, and abnormally shaped RBCs, or poikilocytes were detected (Fig.1 a,b). An increased abundance of poikilocytes and NPs (Fig.1 c, d) was observed in CC patients. The RBC diameter was 8.38±0.36μm in the control group and 9.41±0.47 μm in CC patients.

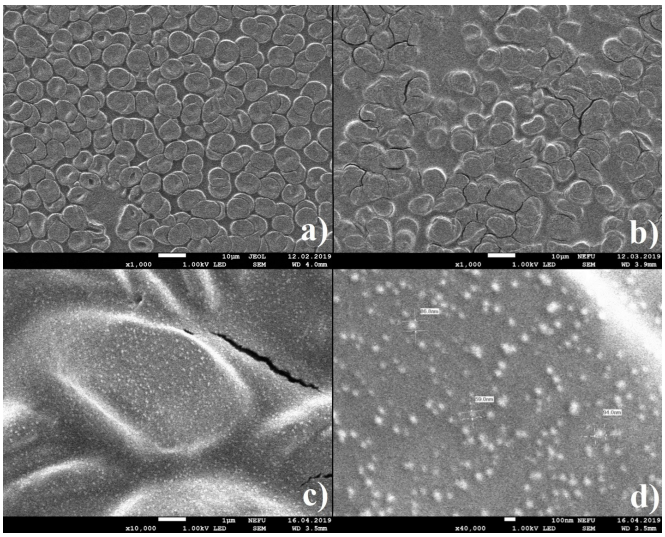

Fig. 1. *SEM image of RBCs in patients with CC a) before RT; b) after RT, ×1000; c) SEM images of a blood sample in case of CC, ×10000 , and d) ×40000*

In this paper, discussions of the results and the authors' conclusions were based on certain sizes of NPs on the RBC surface. Obviously, determining the size of these particles is a major and important part of the study. Accordingly, improving image quality is a prerequisite for the closest-to-reality identification of NPs. In the future, the authors plan to use the most accurate approaches to study the morphology of RBCs and NPs on their surface. One such approach is presented below by improving the contours of NPs on the RBC surface in the SEM image at a magnification of 100,000x. In some situations, these microscope images may suffer from noise. In our case, "pepper-and-salt" noise and Gaussian noise occur frequently under the high-magnification, high-resolution imaging situations. In general, the size of the nanoparticles is around 10–50 pixels in 100,000x, and that of noise can be 3–5 pixels. In this case, it is always difficult to measure the size of nanoparticles with high accuracy. In order to reduce the interference of the noise, we introduced two denoising methods: a median filter and an L0-norm smoothing filter. The median filter is proved to be helpful in removing the pepper-and-salt noise, while the L0-norm smoothing filter shows a strong ability to filter out the noise and to preserve the salient edges. The median filter is very easy. We considered a small local path centroid in pixel k, and vector $\boldsymbol{I}_k = (\boldsymbol{I}_{k,1}, \boldsymbol{I}_{k,2}, \boldsymbol{I}_{k,n})$ to represent the values of all pixels in this patch. Then we sorted the $\boldsymbol{I}_{k,1}, \boldsymbol{I}_{k,2}, \boldsymbol{I}_{k,n}$ in ascending order, and the filter output $\bar{\boldsymbol{I}}_k = \boldsymbol{I}_{k\lfloor \mathbf{n/2} \rfloor}$, where $\lfloor \mathbf{n/2} \rfloor$ is the largest natural number less than $\mathbf{n/2}$. We set the patch size w=5, n=5×5. As shown in Figure 2 (in the middle of first row), the pepper-and-salt noise is significantly suppressed in comparison with the original image. Moreover, in order to compute the size of nanoparticles more accurately, we introduced the L0-norm smoothing filter to boost the edge. Mathematically, this method is based on an optimization framework and can preserve the main edges of the nanoparticles while smoothing the local small gradients. The reader is referred to the algorithm[19] for more details. As shown in Figure 2 (top right), the outline of NPs is very clear now compared to the source image. It is easy to compute the size by counting the number of pixels. In Figure 2 (bottom), we also compared the results in 1D case. Obviously, the original data (blue) has strong pepper-and-salt noise, and the median filter (red) can reduce some noise but the outline of NPs is still not clear, while these residual noises can be further reduced with L0-norm filter (yellow).

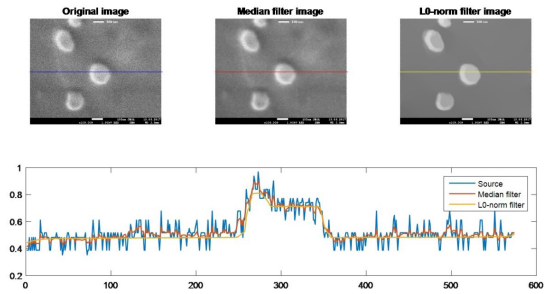

Fig. 2. *Microscope Image Filtering Results*

Ionising radiation was found to influence the size and morphology of RBCs. The overall quantity of poikilocytes

increased from 19% to 23% in the course of RT with approximately a 1% increase at each stage of therapy. The ratio of poikilocytes also varied according to the RT stage. The number of ruptured RBCs increased from 11% to 24% while the number of discocytes decreased from 77% to 71%. Drepanocytes-like cells (7% midway through and 5% at the end of RT) and echinocytes (12% at the start of RT) were also detected.

Comparative analysis of the RBC size in the control group and the group with CC by mathematical statistics shows that the distribution of linear sizes of RBCs has the form of single peaks with the same width at half-height of the peaks shifted relative to each other. The average value of the linear size of the RBC smears of the control group was 6.91 microns. The average value of the RBC diameter of CC patients was 7.64 microns.

In the present study, the number of NPs (from 20 nm to 110 nm in diameter) in the blood of CC patients was increased (Fig.3a). The average diameter of the discovered objects was 69.91±12.15nm and their average height 23.75±3.70nm. Changes in the size and quantity of NPs during RT were observed in the course of this study. The size range of NPs was 20 nm to 70 nm at the start of the treatment and 20 nm to 110 nm midway through the treatment. The quantity of NPs increased with each stage of RT from 136 at the start to 192 in the middle of the second stage of the treatment. Once RT was finished the number of NPs dropped (Fig. 3a).

SEM images before and after RT revealed RBC agglutination (Fig.1b), or "clumping" in all samples. Characteristic peaks, which are presumed to be NPs, were observed on the RBC surface on the AFM images (Fig. 4). The figure shows the linear dimensions of NPs on the RBC surface (Fig. 3 b,c).

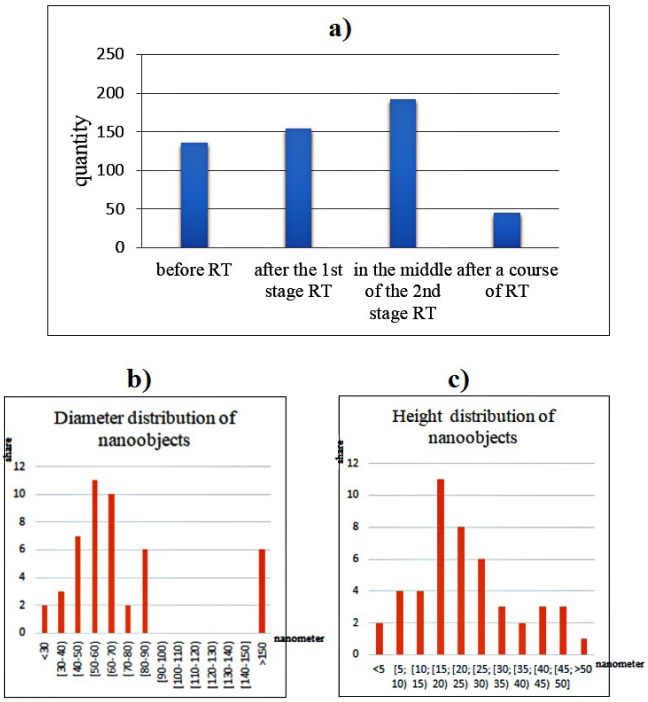

Fig. 3. *a) Changes in the number of NPs on the surface of RBCs during RT; b) distribution of the diameter and height (c) of NPs on the RBC surface of patients.*

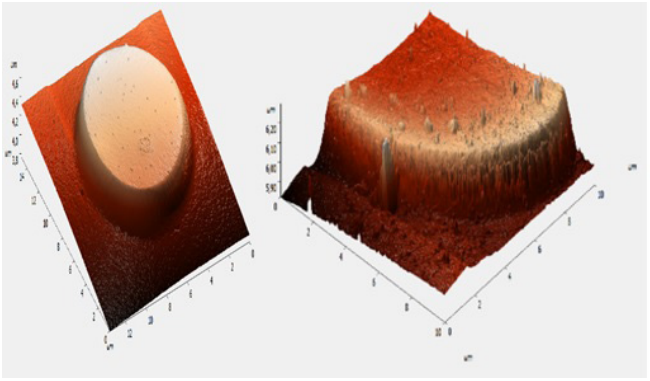

Fig. 4. *AFM image of RBCs of a healthy (a) and sick (b) person.*

The present study shows that RT results in morphological abnormalities and agglutination in RBCs. This is in line with previous findings that gamma radiation increases the relative number of echinocytes, sphero-echinocytes and other types of poikilocytes in a dose-dependent manner.[20] The authors suggested that such changes in RBC morphology could stem from protein structure modifications, changes in deformability and membrane permeability as well as membrane rupture induced by radiation.[20] In a study of rat blood following gamma irradiation treatment, an increase in RBC size and distribution width was observed, which could point to the possible radiation-induced morphological changes.[21]

Our study has also found elevated levels of NPs in CC patients' blood. The observed diameter values for NPs (69.91±12.15nm) bound to RBC surface fall within the typical range for exosomes of 30 nm to 100 nm.[22] Here we propose that these RBC-bound NPs could be EVs of various biogenesis as multiple reports have shown that elevated levels of EVs are typical for oncological conditions[4,10] and that a significant proportion of them exist in a blood cell-bound state.[18] One possible source of these EVs could be the CC cells, which are known to secrete abundant exosomes.[5] These exosomes contain abnormally high levels of microRNA-21 and microRNA-146a associated with CC tumorigenesis, as compared to cancer-free cells.[5] It appears that HPVs, which are the main reported cause of CC, affect the miRNA composition of cancer exosomes. The study by Honegger et al.[13] confirmed that the silencing of viral E6/E7 oncogenes approximately doubled the relative percentage of miRNAs inside exosomes in relation to other small RNA fractions. Interestingly, however, another study found no small RNA sequences corresponding to HPV in HPV-infected cervical carcinoma cell lines.[23] This data points to the fact that while the link between HPV and exosomal content of CC cells cannot be denied, further research is required to determine the precise mechanisms of exosomal sorting for HPV-infected cancer cells.

It should be noted that a study by Mata-Rocha et al. found that not only exosomes derived from HeLa cells contained viral HPV DNA but also non-cancerous HPV-positive cervical samples with and without low-grade squamous intraepithelial lesions had HPV DNA (including the E6 and E7 oncogenes).[24] These findings point to another potential source of RBC-bound EVs in CC patients: cancer-free, HPV-infected cells. While it is

likely that RBC-bound EVs in cervical oncology patients could be attributed to cancer cells or HPV-infected cells the possibility of them being of RBC origin cannot be ruled out, especially since the average diameter of discovered EVs (69.91±12.15 nm) closely matches that of RBC exosomes reported by Huang et al. to be 64.08 nm.[25] RBCs release vesicles into the extracellular space in the course of their normal lifespan, increasing their vesiculation levels in pathology.[26] Their role in pathological conditions, including CC, is under-documented; however, they have been shown to modulate immune response by raising the proliferation of T cells in an antigen-presenting, cell-dependent manner.[27] In fact, depending on storage day the numbers of T cells were augmented by more than 50% in comparison to phytohemagglutinin stimulation alone.[27] In addition, RBC-derived EVs could be involved in inflammation processes as in a study by Danesh et al. [27] they caused significant upregulation of 14 proinflammatory cytokines in peripheral blood mononuclear cells.

In the present study, an increase in the size of RBC-bound NPs was observed in the course of RT; however, reports on the effect of irradiation on EV size present conflicting evidence. A recent study found that RT had a significant impact on exosome size, increasing it by approximately 37% at 10Gy.[28] However, other research groups have reported no change in exosome size post-irradiation [16,29] or an insignificant increase of around 5%.[14] It has been hypothesised that the alterations in exosome size may stem from the impact of radiation on multivesicular body cargo sorting pathways;[28] however, the evidence to support this hypothesis remains limited.

The elevated abundance of NPs detected in the patients during radiotherapy is consistent with the findings that irradiated cells tend to increase their EV secretion.[14-17] Enhanced exosome release after irradiation could be the result of upregulation of certain genes. One study found significantly elevated levels of Rab11, Rab27a, Rab27b, TSAP6, CD63, and Alix transcripts in irradiated cells, compared to controls.[28] These genes are thought to be responsible for exosome secretion, and the effect of radiation on their abundance was dose-dependent, reaching its peak at 10Gy.[16] While the study by Jabbari et al.[28] investigated breast cancer, it has also been observed that exosomes specifically increased secretion of EVs post-irradiation in normal astrocytes,[15] indicating that similar genetic changes may take place in healthy cells. These findings are consistent with our assumption that RBC-bound EVs could originate not only from CC cells but also from non-malignant HPV-infected cells or RBCs.

In conclusion, using SEM and AFM, our results reveal that CC patients exhibit changes in morphology of RBCs and in the number and size of NPs found on their surface during different stages of RT. Ionising radiation was found to influence the size and morphology of RBCs. The average value of the diameter of red blood cells in CC patients was higher than in the control group. The overall quantity of poikilocytes increased approximately 1% at each stage of therapy. The ratio of poikilocytes also varied according to the RT stage. In addition, elevated levels of NPs were found in CC patients' blood, and the quantity of NPs increased with each stage of RT. Once RT was finished the number of NPs dropped. SEM images before and after RT revealed RBC agglutination The changes observed could serve as the basis for developing efficacy indicators of cancer radiation therapy.

In addition, further research is needed to develop a precise explanation of the origin of NPs of various sizes observed on the surface of RBCs, the number of which varies during RT.

Acknowledgments

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Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

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Treatment of Pleural Effusion after Lobectomy and Lymphadenectomy for Primary Lung Cancer: A Case Report

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Abstract

The majority of malignant pleural effusion (MPE) is caused by metastatic disease: most commonly lung cancer in men and breast cancer in women. MPE worsens the quality of life in patients due to the occurrence of respiratory failure, compression of internal organs and violation of homeostasis. Existing methods for MPE treatment have a number of disadvantages, including insufficient analgesia and the use of standard drainage tubes that do not adequately irrigate the pleural space with drugs, thereby reducing the drugs' effectiveness. The proposed method for the treatment of MPE improves the treatment results by improving the drainage and introduction of drugs into the pleural cavity. (**International Journal of Biomedicine. 2020;10(1):76-78.**)

Key Words: malignant pleural effusions • thoracentesis • pleurodesis

Introduction

Worldwide, the incidence of cancer and malignant pleural effusion (MPE) is increasing annually. There are more than 100,000 new cases of MPE yearly in Russia.[1-3]

MPE worsens the quality of life in patients due to the occurrence of respiratory failure, compression of internal organs and violation of homeostasis. Repeated thoracentesis has the potential risks of inducing hypoproteinemia, empyema and pneumothorax. Without adequate therapy, all these circumstances lead to decompensation of the main body systems and death in a short period of several months.[3,4] More than 800 ml of pleural effusion leads to respiratory failure, lung atelectasis that causes hypercapnia and hypoxemia.[3-5]

Existing methods for MPE treatment have a number of disadvantages, including insufficient analgesia and the use of standard drainage tubes that do not adequately irrigate the pleural space with drugs, thereby reducing the drugs' effectiveness.[4-6] As a result, palliative treatments are needed to effectively control pleural effusions and relieve symptoms.

The aim of our study was to improve the results of treatment of patients with MPE by improving the drainage and introduction of drugs into the pleural cavity.

"A method for the treatment of exudative pleurisy" was developed in the Department of Faculty Surgery at Ulyanovsk State University (Application for invention No. 2019103176; Priority of 02/02/2019) (Authors: Charyshkin AL, Toneev EA, Martynov AA, Khusnutdinov BI).

The proposed method is performed as follows: Thoracentesis is performed in the posterior axillary line (6 cm - 10 cm lateral to spine) at the level of the eighth intercostal space using a silicone tube with a diameter of 5 mm. A chest X-ray is performed one day after the pleural cavity drainage. If the pleural cavity is dry and the lung is fully inflated, the silicone tube is removed and replaced by a polyurethane catheter (a diameter of 2 mm). The inner part of the catheter, located in the pleural cavity, has 8 through holes with a diameter of 1 mm. As premedication, Tramal is intramuscularly administered, and 50 ml of Naropin (100 mg) and physiological saline 50 ml are injected through the catheter. The external end of the catheter is pinched and the patient lies in different positions for 30 minutes. Next, the clamp is removed and 30 ml of 10% Betadine solution and 60

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ml of Naropine (120 mg) are introduced through the catheter. The external end of the catheter is squeezed for 8 hours. During this time the patient is lying down, and every 2 hours changes the position of the body; then the clamp is removed and active aspiration is performed within 12 hours. Finally, the catheter is removed.

We believe that applying the developed method prevents purulent-inflammatory complications, which often develop with prolonged drainage of the pleural cavity.[3,7-9] The described technique is used in the Ulyanovsk Regional Oncology Clinical Dispensary and has been performed on 23 patients with a positive result.

Case presentation

A 54-year-old white male was admitted to the surgical thoracic department of the Regional Oncology Clinical Center of Ulyanovsk for surgical treatment with a diagnosis of a lung cancer of the right upper lobe. The planned operation: Right thoracotomy, upper lobectomy, and systemic lymph node dissection.

On Day 6 after surgery, the postoperative period was complicated by exudative pleurisy. We drained the pleural cavity according to the developed methods, and 400 ml of exudates was removed. A day after the pleural cavity was drained, an X-ray control was performed, which showed the dry pleural cavity and fully inflated lung. Subsequent stages of the intervention were performed in accordance with the method described above. After the active aspiration was completed, repeated X-ray examination showed the presence of a dry pleural cavity and fully inflated lung; the catheter was removed. Pain intensity according to the Visual Analogue Scale scored 2 points. The postoperative period was uneventful; wound healing passed by primary intention. The patient was discharged from the hospital in satisfactory condition under the supervision of an oncologist at his place of residence. The patient was examined after one year; no recurrence of the disease was detected.

The majority of MPE is caused by metastatic disease: most commonly lung cancer in men and breast cancer in women.[3,10-13] The presence of MPE indicates an advanced stage of the disease with a median life expectancy of 3 to 12 months, depending on the stage and type of underlying malignancy.[14] During the past two decades, there has been a change in direction in MPE research and management. Advanced, minimally invasive methods are becoming increasingly important.[3,15,16] Instead of aggressive surgical methods, the current treatment approach for patients with MPE is mainly aimed at alleviating symptoms and improving quality of life indicators, which is a key goal of treatment.[17]

Among treatment methods, two effective treatments recommended for recurrent MPE are pleurodesis and IPC placement, both of which can effectively improve dyspnea and quality of life of patients.[11,17-21] However, these treatments are also temporary, and MPE would recur soon. The proposed method for the treatment of MPE contributes to a pronounced analgesic effect, and reduces treatment time and recurrence of the disease. The described clinical case confirms the method's effectiveness.

Competing Interests

The authors declare that they have no competing interests.

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CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Iliocaval Fistula - A rare Option for Permanent Vascular Access

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Abstract

The number of patients receiving maintenance dialysis treatment has been growing in recent years. Unfortunately, the lifetime of permanent vascular access is not unlimited, and some patients initially have problems with its formation. Because of these problems, there is a need to develop new methods of forming constant vascular access in this cohort of patients. This case shows the successful surgical approach to forming permanent vascular access in patients who are intravenous drug users and who have post-thrombotic syndrome of most veins, making them unsuitable for creating an arteriovenous fistula. (**International Journal of Biomedicine. 2020;10(1):79-81.**)

Key Words: permanent vascular access • haemodialysis • continuous ambulatory peritoneal dialysis

Introduction

The number of patients receiving maintenance dialysis treatment has been growing in recent years.[1,2] The quality of this procedure is constantly improving; this fact has a positive effect on the life expectancy of patients receiving the procedure.[3,4] Different regimens of antithrombotic therapy are used widely in different algorithms of thrombosis and embolism prophylaxis and in various options for perioperative therapy in surgical treatment of vascular pathology, such as reconstructive surgery for atherosclerosis, phlebological manipulations, and others. In spite of this fact, anticoagulants are not widely used for haemodialysis (HD) patients with permanent vascular access (PVA).[5-7] Unfortunately, the lifetime of PVA is not unlimited, and some patients initially have problems with its formation.[8-10] Because of these problems, there is a need to develop new methods of forming constant vascular access in this cohort of patients. We present our approach to surgical treatment of a patient with severe renal failure.[11-15]

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Case Presentation

A 54-year-old white male, intravenous drug user, has received continuous ambulatory peritoneal dialysis (CAPD) for two years. This type of renal replacement therapy was chosen because the patient's veins (deep and superficial, both on the upper and lower extremities) were unsuitable for the formation of permanent vascular access due to persistent obliteration. During the last 12 months, the patient has undergone four episodes of St. Aureus and E. Coli peritonitis. As a result, use of CAPD became inadequate due to a dramatic decrease in peritoneum resorption capabilities.

In such circumstances, patients are usually transferred to HD. When making a decision about the formation of a PVA, we encountered the following issues: ultrasound signs of postthrombotic syndrome of jugular, subclavian and iliac veins, and of deep and superficial veins of the upper and lower extremities from both sides. An extensive network of collaterals with a maximum diameter of 3mm was present across all segments, but they were absolutely unsuitable for forming a

PVA. The left common iliac vein (CIV) and the inferior vena cava (IVC) were found to be intact, suitable both for catheterization and surgery. We decided to create a PVA using a VENAFLO®2 ePTFE straight vascular graft 6mm×50cm. Before surgery, the dialysis solution was drained from the abdominal cavity. Epidural anaesthesia was used. An extraperitoneal approach to the left CIV was used. Its length was 3 cm, which was not enough for clamping and implanting a vascular prosthesis. The iliac arteries, the lower part of the abdominal aorta and the IVC were mobilized. An end-to-side anastomosis was created between the lower part of the IVC involving the beginning of the left CIV and the vascular graft. Another part of the vascular graft was moved down under the skin of the left thigh, and the second anastomosis was performed between the end of the vascular graft and the side of the left external iliac artery.

The stages of the intervention are presented in Figures 1, 2, and 3.

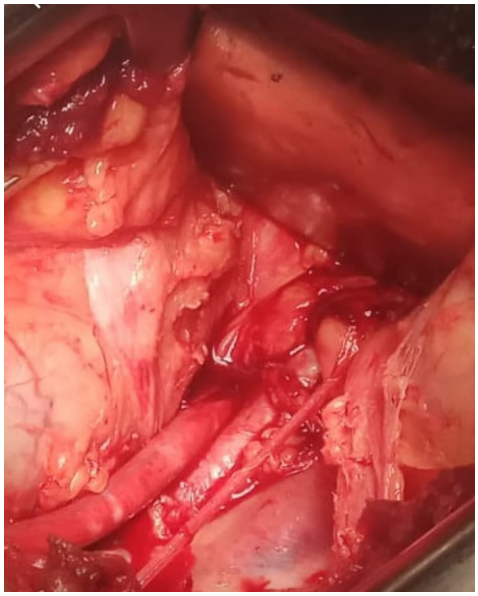

Fig. 1. *The inferior vena cava.*

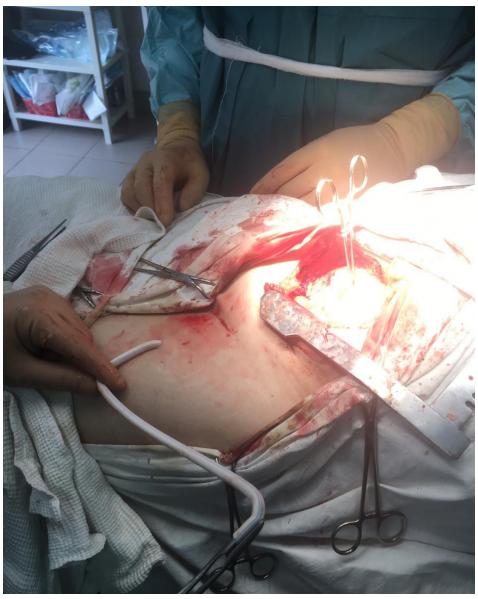

Fig. 2. *The synthetic vascular prosthesis on the front surface of left thigh.*

Fig 3. *The view of postoperative wounds.*

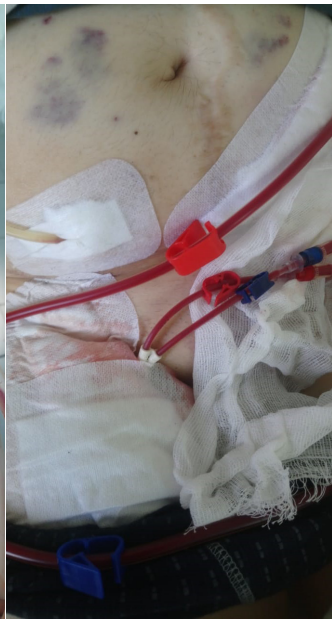

Fig. 4. *Femoral artery catheter.*

Two hours after the operation, the patient was moved to the cath lab, and a double-lumen catheter was implanted into the right superficial femoral artery.

Three hours after this procedure, the patient underwent the first HD session using this catheter. Figure 4 shows HD using a femoral artery catheter.

The HD session lasted 3 hours; ultrafiltration of 2,000 ml was completed. The next day, the duration of dialysis was the same, and the volume of ultrafiltration was 2,500 ml. A transfusion of 400 ml of red blood cells was added. The patient received antibiotic and anti-inflammatory therapy. HD was performed every second day for 2 weeks. That was followed by three sessions of HD using the access with the punction of vascular graft, then the catheters were surgically removed from the femoral artery and abdominal cavity. On the 17th day, the patient was discharged for outpatient treatment with programmed HD.

The search for new solutions to the problem of PVA formation in patients for whom standard methods cannot be used is extremely relevant and is carried out by many surgical scientists. Our proposed method allows HD in a patient with persistent vascular obliteration. This case shows the successful surgical approach to forming permanent vascular access in patients who are intravenous drug users and who have post-thrombotic syndrome of most veins, making them unsuitable for creating an arteriovenous fistula.

Competing Interests

The authors declare that they have no competing interests.

Disclaimers

The opinions expressed in this article are the authors' own and do not reflect the view of the institutions or funder.

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CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Computational Aerodynamics in Nasal Septal Perforation

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Abstract

Nasal septal perforation (NSP) remains a problem, and treatment of it is still controversial. NSP leads not only to disturbances in the NC aerodynamics, but also to disturbances in warming and moistening of the inhaled air. By using the computational fluid dynamics (CFD) method, we evaluate disturbances in warming and moistening of the inhaled air in cases of NSP. We emphasize that an adverse effect of NSP begins with a disorder of air heating and moisturizing, and believe that a NSP must and can be closed as early as it is possible. (**International Journal of Biomedicine. 2020;10(1):82-85.**)

Key Words: nasal septum • nasal septal perforation • computational fluid dynamics • surgical reconstruction

Abbreviations

CFD, computational fluid dynamics; **NS**, nasal septum; **NSP**, nasal septal perforation; **NC**, nasal cavity

Introduction

Nasal septal perforation (NSP) is an anatomical defect of the cartilaginous and/or bone NS and it remains a severe problem to many patients. It is well recognized that NSP has a negative impact on the NC aerodynamics, leading to whistling during inspiration, crusting, recurrent nasal bleeding, nasal obstruction and other symptoms.[1-3] CFD simulation is a modern method to evaluate the influence of nasal passage geometry on the NC aerodynamics.[4-6] Today, by using CFD simulation, we can not only quantify the flow variables, such as velocity, pressure, and streamline, but also simulate the warming and moisturizing function of the inhaled air.[7] According to the general practice, only symptomatic perforations require surgical treatment to relieve symptoms; however, nobody takes into account heating and moisturizing disorders of the inhaled air.[8,9]

The aim of this study was to evaluate how NSP affects the NC physiology *in vivo* and consider the significance of early surgical closure of the NS defect.

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Case Report

A 24-year-old white male was referred to the department of otorhinolaryngology with complaints of nasal bleeding, crusting and noisy nasal breathing. These symptoms appeared at the age of 18, and NSP was diagnosed. Medical treatment had been performed for 6 years, but therapy proved to be ineffective. Endoscopic examination of the patient's NC revealed NSP with a width of 15 mm and height of 10.5 mm in the anterior part of NS, crusting and signs of blood. CT examination revealed NSP; paranasal sinuses were intact (Figures 1 and 2).

Surgical reconstruction of the NSP was performed. The quadrangular cartilage was removed, turned in a front-to-back direction and returned to its former place. The mucosa from inferior turbinates was fitted to the place of the removed NS mucosa. Two years after the surgery, a CT examination was performed, which revealed that the anatomy of the NC was normal (Figures 3 and 4).

With the purpose of studying the NSP aerodynamics, we used high-resolution CT images, which were taken before and after surgical closure. Based on CT data, appropriate geometric models were generated, using a combination of several commercially available, pre-processing software

programs. Our patient had an NSP of elliptical shape (width of 15 mm and height of 10.5 mm). The cross-sectional area of the NSP was 12.2 cm^2. The perforation started at a distance of 9mm from the nostril and ended at 24mm from the nostril.

Meshing is an important part of CFD simulation. The grid independency test was performed, and an unstructured three-dimensional mesh was generated, consisting of 2,500,000 cells (we used the program tool Ansys Meshing).

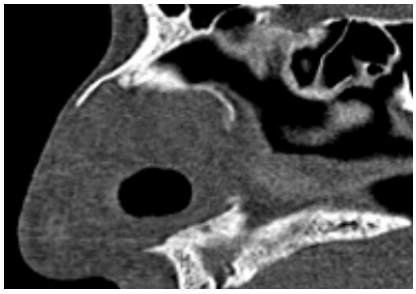

Fig. 1. *Sagittal CT scan. Septal perforation of the cartilaginous part of NS.*

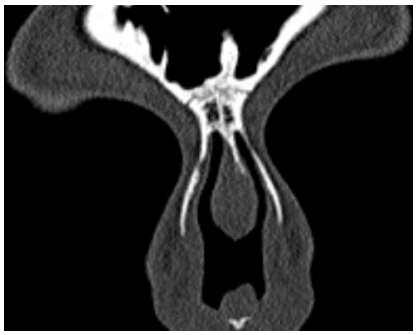

Fig. 2. *Coronal CT scan. Septal perforation of the cartilaginous part of the NS.*

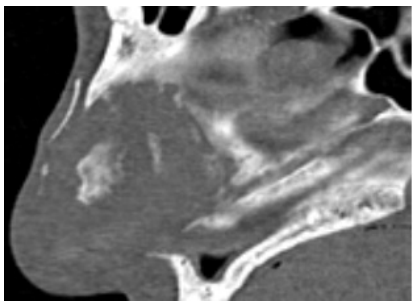

Fig. 3. *Sagittal CT scan. Two years after the surgery: the normal anatomy of the NS.*

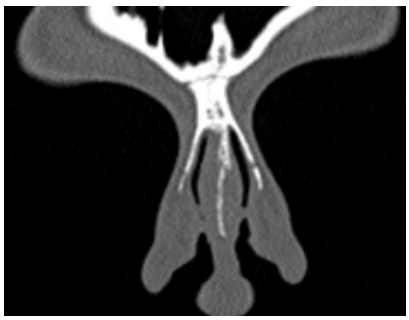

Fig. 4. *Coronal CT scan. Two years after the surgery: the normal anatomy of the NS.*

Boundary conditions

CFD simulation is based on an approximate solving of the Navier-Stokes equations on unstructured meshes. The inhaled air was assumed to be a turbulent flow in boundary conditions of a large pressure drop (a range of pressure drops from 0 Pa in the inlet of the nostril to 50 Pa in the nasopharynx) and a flow rate of 300ml/s. That required using the so-called Reynolds averaged Navier-Stokes equation to account for the turbulence effects. A no-slip velocity boundary condition was assumed on the airway walls and the gravitational effects on the airflow were neglected. Numerical fluid simulation with heat and humidity transport was performed according to the method of Kumahata.[10] The temperature of the inhaled air was 25°C and the relative humidity was 35%.

The NS is an important part of the nasal cavity. In cases of NSP, the NS function is not executed, causing airflow exchange between two nostril sides of the NC through the NSP. According to the study performed by Cannon et al., the larger size of the NSP in the anterior location of the NS causes a larger cross-flow volume through the NSP.[3]

The detrimental effect of airflow leakage through the NSP is based not only on airflow exchange through the NSP, but also on an increase of total velocity of the inhaled air. In our study, total airflow velocity in a case of NSP was 17m/s (normal value is <12 m/s) (Figure 5). After the NSP reconstruction, total airflow velocity decreased and reached normal values (Figure 6).

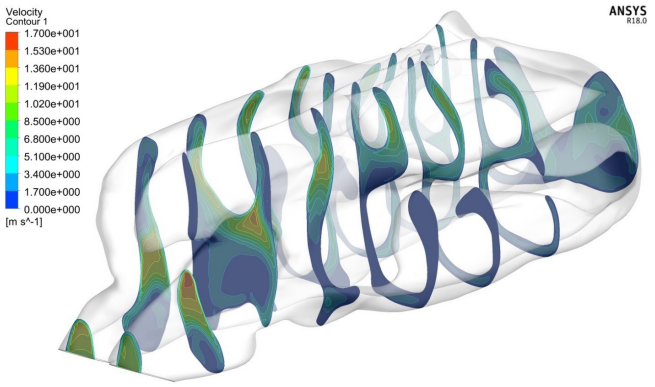

Fig. 5. *Sagittal CT scan of the nasal cavity. Airflow velocity streamlines and cross-sectional air exchange through the NSP.*

The NC anatomy is created so that anterior parts of the inferior turbinates keep the inhaled air, giving time to contact the inhaled air with mucosa for heating and moisturizing. In the case of NSP, this process is disturbed as the total airflow velocity increases in the cartilaginous part of the NS. We found that the temperature and relative humidity of the airflow in the nasopharynx was lower than normal. The first diapason of temperature was from 32°C to 34°C and relative humidity was from 93% to 96%; the second diapason of temperature was from 35°C to 37°C and relative humidity 100% (Figure 7).

As seen from Figure 8, the coolest area in the NC was located in the NSP area. Thus, inhaled air was heated not only

because of high total air velocity, but also because of large air leakage through the perforation. After the NSP reconstruction, the process of heating and moisturizing of the inhaled air was recovered (Figure 8).

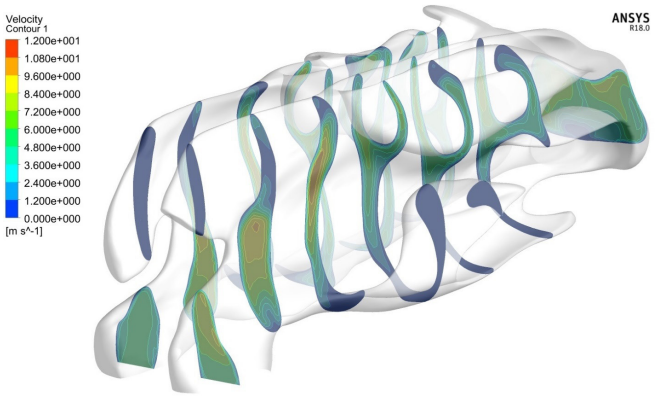

Fig. 6. *Sagittal view of the nasal cavity after the NSP reconstruction. Airflow streamline passes through each side of the nasal cavity. Total airflow velocity decreased and reached normal values*

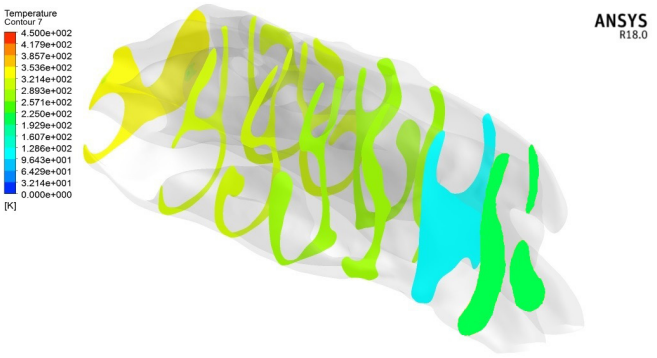

Fig. 7. *Sagittal view of the nasal cavity. The temperature and relative humidity of the airflow in the nasopharynx is lower than normal. The coolest area in the nasal cavity is corresponded to the area of the NSP.*

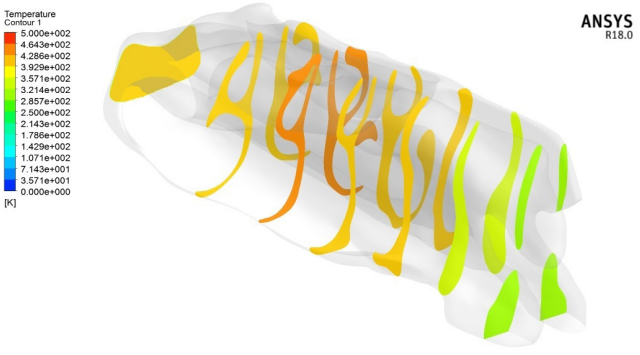

Fig. 8. *Sagittal view of the nasal cavity after the NSP reconstruction. The process of heating and moisturizing of the inhaled air is normal.*

Discussion

Nasal septal perforation remains a problem, and treatment of it is still controversial. As is common, only clinically significant nasal septal perforations are recommended to surgery, while small-sized nasal septal perforations are ignored. This happens because only obvious symptoms of nasal septal perforation such as whistling during breathing, frequent nasal bleeding, and crusting, are taken into account as a reason for surgery, but disturbances of the temperature and humidity in the nasal cavity are ignored. Our study shows that decreased temperature of the inhaled air in the nasopharynx provides an adverse effect on nasal physiology, initiating an atrophic rhinitis progression. We emphasize that an adverse effect of nasal septal perforation begins with a disorder in air heating and moisturizing. Cold and dry air leads to crusting, increasing the size of the perforation and contributing to the mucosa's atrophy. Therefore, over time, nasal septal perforation becomes symptomatic and, according to modern concepts, is indicated to surgical reconstruction.

In conclusion, we would like to quote the famous Meyer's statement: "Nasal septal perforations must and can be closed," the title of his article published in 1994.[11] We emphasize that an adverse effect of nasal septal perforation begins with a disorder of air heating and moisturizing, and believe that a nasal septal perforation must and can be closed as early as it is possible.

Competing Interests

The authors declare that they have no competing interests.

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SHORT
COMMUNICATION

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Prevalence of Otitis Media with Effusion in Children

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Abstract

The article presents the prevalence of otitis media with effusion (OME) in children in the Tyumen region. Based on the 12-year experience of treating children in a children's regional clinical hospital, it has been established that the children most prone to having OME are pre-school boys (under age 7), and that OME makes up one third of all ear pathologies and accounts for 10.7% of all treated ENT diseases in children. (**International Journal of Biomedicine. 2020;10(1):86-88.**)

Key Words: otitis media with effusion • radiofrequency myringotomy • tympanostomy tube procedure

Abbreviations

CT, computed tomography; **ENT**, Ear, Nose and Throat; **OME**, otitis media with effusion

Introduction

Otitis media with effusion (OME) is one of the most widespread and symptomless forms of the clinical course of otitis among children under age 7. OME presents with an accumulation of fluid in the middle ear, which in turn results in conductive and mixed hearing loss. On average about 90% of children suffer from at least one episode of OME before the age of 7.[1] More than a third of all cases of hearing loss and deafness are associated with the dysfunction of auditory tubes.[2] According to many authors, the main cause for this condition is its obstruction (obturation), which may be caused by the inflammation of auditory tubes, secondary edema, and hypertrophy of nasopharynx and pharynx lymph tissues.

Comprehensive diagnostics of auditory tube dysfunction with 3D computer tomography allows discovering pathologies in the nasal cavity, paranasal sinus and pharynx in 96.3% of patients.[3,4] The allergy factor and its role in OME development is not conclusive. Allergy processes in the nasal cavity and pharynx may lead to the worsening of auditory tube dysfunction, and, as a result, lead to OME development.[5] Medical sources contain contradictory data on the prevalence of OME in allergic rhinitis among children.[6,7]

Gushchin et al., performing medical examinations of children with allergic rhinitis, found OME in 17.9% of cases.[8] The highest rate (67.5%) of OME occurrence was documented among children between the ages of 3 and 6; the majority of children with OME (90.7%) were diagnosed with second- and third-degree of adenoid hypertrophy with upper-airway obstruction.[9]

The aim of this study was to define the prevalence of OME among children according to the data of the ENT-department at Tyumen Region Clinical Hospital №2.

Materials and Methods

Based on these data, 20,000 medical histories were retrospectively analyzed for the period from January 1, 2007 to November 31, 2018. All the children were examined either as scheduled patients or emergency cases with acute or chronic OME who underwent surgical interventions followed by a course of conservative treatment in the postoperative period. A routine check-up of ear, nose and throat was undertaken for all children, as well as otomicroscopy with the LEICA-14 diagnostic microscope, endoscopy of the pharynx, audiometry and tympanometry with an Interacoustics AA222 audiometer

and an AT235 tympanometer, along with clinical and lab diagnostics, CT of the temporal bone and, if necessary, CT of the paranasal sinus.

Children with acute otitis media who were admitted to a hospital as emergencies underwent a myringotomy under local or general anaesthesia with the aid of a Heine HR binocular loupe. Children with chronic OME (for scheduled operations) received (under an endotracheal anaesthesia and with the help of a LEICA-F40 surgical microscope) one of the following surgical treatments: radiofrequency myringotomy with the Ellman-Russ Surgitron TEE-240 electrode, tympanostomy tube placement with insertion of metal grommets, fluid drainage and transtympanic injection of Fluimucil and Otofa solutions during intra- and postoperative periods for 7 days. After being checked out, all children were referred to regular ENT-specialists, paediatricians, and audiology specialists with a further obligatory audio tympanometry to be done in 3, 6 and 12 months. Results were statistically processed using Microsoft Excel 2007. The study was approved by the Tyumen State Medical University Ethics Committee. Written informed consent was obtained from the child's parents.

Results and Discussion

A total of 20,000 children with various ENT pathologies were treated over the control period. The estimated percentage of children with otitis was 33%, the rest of the children (67%) had other ENT disorders (Figure 1). Among all ear pathologies, one third of the total number of patients was diagnosed with OME (Figure 2).

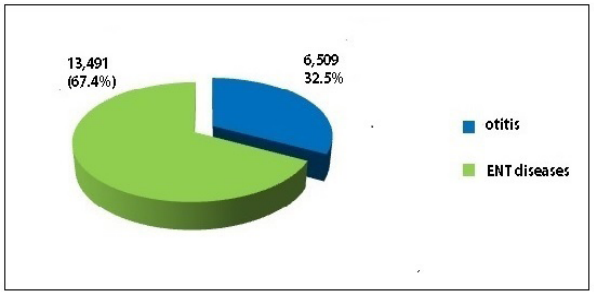

Fig. 1. *The prevalence of otitis among ENT diseases.*

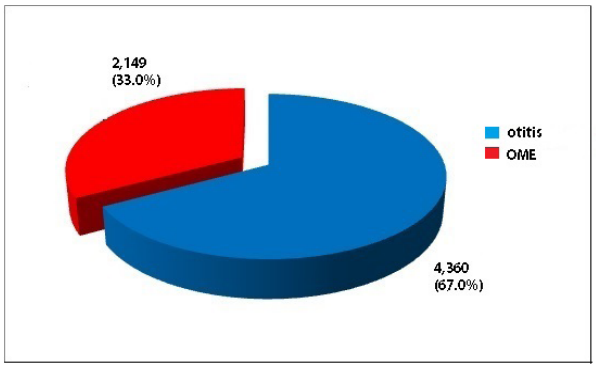

Fig. 2. *The prevalence of OME among all ear pathologies.*

According to our study, among all patients with ENT diseases treated over the 12-year period from 2007 to 2018, the percentage of children with OME accounted for 10.7% (Figure 3).

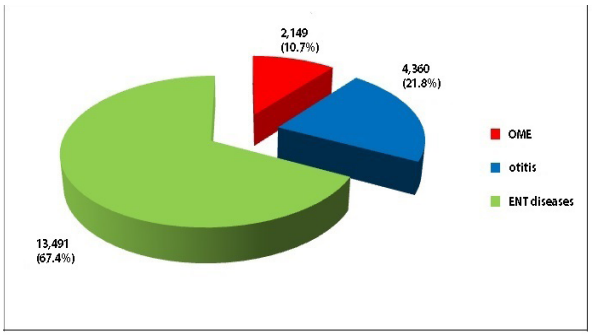

Fig. 3. *The prevalence of OME among all ENT diseases.*

Among all children with OME, there were 1354(63.0%) boys and 795(37.0%) girls. Looking closely at the prevalence of this disorder throughout the year, we can see the following picture: 537(25.0%) cases in the winter, 507(23.6%) in the spring, 546(25.4%) in the summer, and 559(26.0%) cases in the autumn. Thus, OME is evenly spread over the four seasons with a slight predominance in the autumn. Evaluating the dynamics of the number of OME patients over the years, we found a high prevalence of this pathology with no tendency to decrease. The growth of this ear disorder is more or less stable and is consistent with the rise of other types of otitis among children who undergo treatments at the above-mentioned ENT-department. The prevalence of OME among other types of otitis for the studied years is presented in Figure 4.

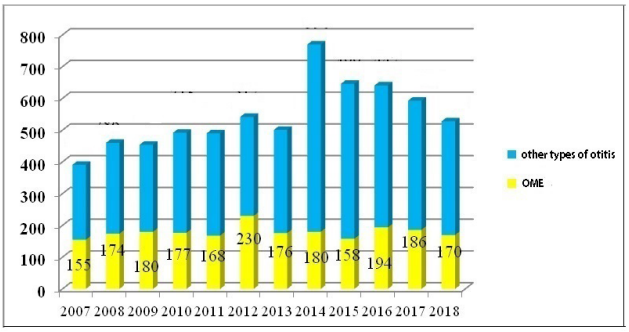

Fig. 4. *The prevalence of OME among other types of otitis for the studied years.*

OME has been diagnosed in 1133(52.7%) children in the age group of 3-4 years (mean age of 3.1±0.03 years), in 690(32.1%) children in the age group of 5-7 years (mean age of 5.88±0.03 years), in 249(11.6%) children in the age group of 8-12 years (mean age of 9.55±0.09 years), and in 77(3.6%) patients over 12 years of age (mean age of 14±0.14 years). Thus, the prevalence of OME among children of pre-kindergarten age (52.7%) and pre-school age (32.1%) was predominant. These age groups are at risk for developing

stable forms of hearing loss and speech disabilities that later may result in difficulties with social adaptation.

In conclusion, the prevalence of OME among children has been quite high (33% of all ear diseases) for the past 10 years without a downward trend. OME is mainly diagnosed in boys of pre-school age. Equal prevalence of OME in all seasons indicates the relevance of the problem throughout the year.

Competing Interests

The authors declare that they have no competing interests.

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