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Clinical researches

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LONG-TERM RESULTS FOR THE TREATMENT OF PATIENTS WITH THE APPLICATION OF ORGAN-PRESERVING OPERATIONS IN PAPILARY THYROID CANCER

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In order to analyse the changes in the structural and functional state of the thyroid gland, a group of 185 patients (67.8 %) were examined after the surgical treatment of papillary thyroid cancer. Of these, 94 patients belonged to the first group – the comparison group where thyroidectomy was performed, and 91 to the second – the main group where, mainly, organ-preserving operations were performed. The average follow-up was 6.5 ± 5.1 years (varied from 2 to 11 years.

Hypoparathyroidism in patients receiving replacement therapy was observed in 20 (16.4 %) patients after thyroidectomy and only in 4 (6.3 %) patients after organ-preserving techniques (the differences are significant, $p < 0.05$). Side effects of replacement therapy were observed in 21 (17.2 %) patients after thyroidectomy and only in 4 (6.3 %) patients after organ-preserving techniques (the differences are significant, $p < 0.05$). Disease recurrence was detected in 4 (3.3 %) patients after thyroidectomy and in 1 (1.6 %) patient after organ-preserving techniques (the differences are not significant, $p > 0.05$).

The use of organ-preserving approaches in the main group allowed improving functional results by reducing the frequency of hypothyroidism and side effects of replacement therapy, due to the preservation of thyroid secretion and its regulation, without worsening the results of relapse-free survival.

KEY WORDS: organ-preserving interventions, papillary thyroid cancer, long-term results of treatment

ВІДДАЛЕНІ РЕЗУЛЬТАТИ ЛІКУВАННЯ ХВОРИХ З ВИКОРИСТАННЯМ ОРГАНОЗБЕРІГАЮЧИХ ОПЕРАЦІЙ ПРИ ПАПІЛЯРНОМУ РАКУ ЩИТОПОДІБНОЇ ЗАЛОЗИ

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З метою аналізу змін структурно-функціонального стану щитовидної залози у віддалений термін після оперативного лікування папілярного раку щитоподібної залози була обстежена група з 185 хворих (67,8 %). З них 94 хворих належали до першої групи – групі порівняння де виконували тиреоідектомію, а 91 до другої – основної групі, де, здебільшого, виконували органощадні операції. Середній термін спостереження склав 6,5 ± 5,1 років (варіював від 2 до 11 років).

Гіпопаратиреоз на тлі прийому замісної терапії було відмічено у 20 (16,4 %) хворих після тиреоїдектомії та тільки у 4 (6,3 %) хворих після органощадних методик (відмінності достовірні, $p < 0.05$). Побічні ефекти замісної терапії відзначалися у 21 (17,2 %) хворих після тиреоїдектомії та тільки у 4 (6,3 %) хворих після органощадних методик (відмінності достовірні, $p < 0.05$). Рецидив захворювання виявлено у 4 (3,3 %) хворих після тиреоїдектомії та у 1 (1,6 %) хворого після органощадних методик (відмінності недостовірні, $p > 0.05$).

Застосування органоощадних підходів в основній групі дозволило поліпшити функціональні результати, зменшивши частоту гіпотиреозу і побічних ефектів замісної терапії, за рахунок збереження тиреоїдної секреції і її регуляції, не погіршуючи результати безрецидивного виживання.

КЛЮЧОВІ СЛОВА: органощадні втручання, папілярний рак щитоподібної залози, віддалені результати лікування

ОТДАЛЕННЫЕ РЕЗУЛЬТАТЫ ЛЕЧЕНИЯ БОЛЬНЫХ С ПРИМЕНЕНИЕМ ОРГАНОСОХРАНЯЮЩИХ ОПЕРАЦИЙ ПРИ ПАПИЛЯРНОМ РАКЕ ЩИТОВИДНОЙ ЖЕЛЕЗЫ

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С целью анализа изменений структурно-функционального состояния щитовидной железы в отдаленные сроки после оперативного лечения папиллярного рака щитовидной железы была обследована группа из 185 больных (67,8 %). Из них 94 больных принадлежали к первой группе – группе сравнения, где выполняли тиреоидэктомию, а 91 ко второй – основной группе где, преимущественно, выполняли органощадящие операции. Средний срок наблюдения составил 6,5 ± 5,1 лет (варьировал от 2 до 11 лет).

Гипопаратиреоз на фоне приема заместительной терапии отмечался у 20 (16,4 %) больных после тиреоидэктомии и только у 4(6,3 %) больных после органощадящих методик (различия достоверны, $p < 0.05$). Побочные эффекты заместительной терапии отмечались у 21 (17,2 %) больных после тиреоидэктомии и только у 4 (6,3 %) больных после органощадящих методик (различия достоверны, $p < 0.05$). Рецидив заболевания выявлен у 4 (3,3 %) больных после тиреоидэктомии и у 1(1,6 %) больного после органощадящих методик (различия недостоверны, $p > 0.05$).

Применение органощадящих подходов в основной группе позволило улучшить функциональные результаты, уменьшив частоту гипотиреоза и побочных эффектов заместительной терапии за счет сохранения тиреоидной секреции, и ее регуляции, не ухудшая результаты безрецидивной выживаемости.

КЛЮЧЕВЫЕ СЛОВА: органощадящие вмешательства, папиллярный рак щитовидной железы, отдаленные результаты лечения

INTRODUCTION

In recent years, there has been a tendency to perform organ-preserving interventions for papillary thyroid cancer (PTC) such as hemithyroidectomy or subtotal resection [1–3]. This approach is considered in tumours with sizes less than 1 centimetre [2, 4]. However, for larger tumours, there is no clear agreement on the maximum size of the tumour in which organ-preserving operations can be performed [3].

There are several factors that influence the prognosis of disease recurrence and the tactics chosen by the surgeon during surgical treatment: multimodality, the presence of «aggressive» histological forms of PTC, tumour size, invasion into the capsule, and extrathyroid invasion [2, 5]. In addition, the biological properties of the tumour play an important role [4, 6]. This study presents an assessment of the long-term results of PTC treatment, where the choice of organ-preserving operations was carried out depending on the prediction of the aggressive behaviour of the tumour, which was determined in accordance with histological and immunohistochemical criteria.

OBJECTIVE

Evaluation of long-term results of organ-preserving operations in patients with papillary thyroid cancer.

MATERIALS AND METHODS

The work was performed in the State Institution «Institute of Problems of Endocrine Pathology named after V. Ya. Danilevsky of National Academy of Medical Sciences of Ukraine». There were 273 patients under observation with a final diagnosis of PTC. The patients were divided into 2 groups: Group I – the comparison group – 155 patients with PTC in whom only thyroidectomy was used. Group II – the main group – 118 patients, where the choice of surgical treatment method – thyroidectomy or organ-preserving surgery was carried out according to the developed algorithm. In order to analyse changes in the structural and functional state of the thyroid gland in a long-term period after the surgical treatment, a group of 185 patients (67.8 %) were examined. Of these, 94 patients belonged to the first group – the comparison group, and 91 to the second – the main group. The average follow-up was 6.5 ± 5.1 years (varied from 2 to 11 years).

The algorithm for choosing the method of operative treatment of patients with PTC: The surgical tactic in the comparison group was to perform thyroidectomy and lymph node dissection in the presence of metastatic lymph nodes in all patients. Surgical tactics in the main group were based on risk factors identified before the operation or after the operation. Hemithyroidectomy was performed on tumours up to 2 cm in size. If intraoperative histological examination revealed multifocal tumour growth, capsule invasion or extrathyroid invasion, as well as cancer from cylindrical cells, in such patients was performed thyroidectomy. In the postoperative period, resected material investigated immunohistochemicaly with quantitative determination of TTF1, NIS, Ki67. With overexpression of TTF1, the absence of expression of NIS, and the high expression level of Ki67, a repeated operation (total thyroidectomy) was considered to be shown.

RESULTS AND DISCUSSION

Analysis of the volume of surgical intervention in patients with PTC in the comparison group showed that most of them 64 (54.6 %) were operated on by organ-preserving techniques. Among 118 patients, only 55 (46.6 %) had thyroidectomy, the volume of other operations was a subtotal resection of the lobe of the gland in 21 (17.8 %) patients, a subtotal resection of the whole gland – 9 (7.6 %), hemithyroidectomy was performed in 17 (14.4 %) patients. In 16 (13.6 %) patients with a multinodular lesion, hemithyroidectomy on the affected side and subtotal resection on the opposite side were performed.

Of the 185 patients examined in the long-term period, 124 (67.02 %) received various types of radiation treatment. Of these, 50 patients (40.3 %) were treated with remote gamma-therapy on the thyroid bed and the lymphatic drainage path, 56 patients (45.2 %) were treated with radioactive iodine (from 1 to 4 courses), in 18 patients (14.5 %) radiation treatments were not used.

Of the 94 patients in the comparison group, a recurrence of thyroid cancer was detected in 3 (3.2 %) patients from 7 to 1 year after surgery.

From 91 patients of the main group (63 patients where organ-preserving techniques were performed and 28 who underwent total thyroidectomy), additional nodular formations in the residual thyroid tissue, according to ultrasound, were found in six patients (9.5 %) without an increase in the neck lymph nodes. When performing TAPB in these patients, in four cases, suspicious malignant changes were identified. In two cases, the recurrence of colloidal goiter.

Four patients with suspected malignancy were operated on. At the final histological examination after re-operation, PTC was diagnosed in one patient, in two patients a follicular thyroid adenoma and colloid goiter nodes were detected. In another patient, papillary microcarcinoma was found on the background of a multinodular goiter and a follicular adenoma – with a lesion of malignant growth of up to 4 mm in size.

Thus, in the late postoperative period, among 91 patients who operated on for PTC, a true recurrence of papillary thyroid cancer was detected only in 1 patient (1.1 %) 7 years after the first operation. Another lymphogenous relapse was detected in a patient undergoing thyroidectomy.

The analysis of follow-up observation of patients with PTC in the postoperative period showed very low patient compliance. Only the first two or three years the patients regularly visited the endocrinologist, in the future these visits became random. Patients either stopped taking thyroxin at all, or adjusted their dosing to their own sensations. As a result, out of 141 patients who received replacement therapy, only 38 (27 %) took thyroxin preparations in maintenance dosages.

An important problem was also the presence of side effects of substitution therapy – such as tachycardia, extrasystole, pressing pain behind the sternum, anxiety, hand tremor that occur after taking thyroxin or 30 minutes later, Considering that none of the patients taking thyroid hormone drugs in the replacement dose did not arise recurrence, then when prescribing hormonal therapy in a suppressive dosage it is necessary to carefully choose between the feasibility of achieving the recommended level of thyrotrophic hormone and the validity of such treatment, giving preference to the latter.

All of the above suggests that the treatment of patients with PTC (surgical treatment in organ-preserving volume, followed by radiation therapy and the use of suppressive drugs), has shown significant effectiveness. That is, removal of the tumour, leaving a small number of thyroid parenchyma, did not adversely affect the incidence of recurrent thyroid cancer.

At present, some specialists suggest that in case of surgical treatment of PTC, extrafascial thyroidectomy must be supplemented with prophylactic central lymphatic dissection. It is motivated by the fact that micrometastases in regional lymph nodes in patients with PTC occur very often (more than in 50 % of cases). Central lymphodissection is accompanied by an increase in the number of postoperative complications, such as laryngeal paresis and hypoparathyroidism. In the main group, we did not perform prophylactic lymphodissection. Performed only when we had clinically detected lymph node metastases, which were detected in 40 cases (14.65 %). Observation up to 11 years did not reveal a single case of regional or remote lymphogenous metastasis.

Hypothyroidism in patients receiving replacement therapy was observed in 20 (16.4 %) patients after thyroidectomy and only in 4 (6.3 %) patients after organ-preserving techniques (the differences are significant, $p < 0.05$). Side effects of replacement therapy were observed in 21 (17.2 %) patients after thyroidectomy and only in 4 (6.3 %) patients after organ preserving techniques (the differences are significant, $p < 0.05$). Disease recurrence was detected in 4 (3.3 %) patients after thyroidectomy and in 1 (1.6 %) patient after organ preserving techniques (the differences are not significant, $p > 0.05$).

These data indicate that the preservation of the parenchyma of the thyroid gland can reduce the frequency of hypothyroidism, as well as reduce the frequency of side effects of replacement therapy by maintaining thyroid secretion and its regulation. The use of organ preserving techniques, according to our data, does not significantly affect the risk of tumour recurrence.

CONCLUSIONS

The use of organ preserving approaches in the main group allowed improving functional results, reducing the frequency of hypothyroidism and side effects of replacement therapy, by maintaining thyroid secretion and its regulation, without worsening the results of relapse-free survival.

PROSPECTS FOR FUTURE STUDIES

Further study of the long-term results of surgical treatment of patients with papillary thyroid cancer will allow us to individualize the operational tactics and improve the results of treatment.

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DIAGNOSTICS OF INFLAMMATORY INSIDE THE PLEURAL COMPLICATIONS OF THORACIC INJURY

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The non-specificity of the clinical signs of posttraumatic purulent thoracic complications indicates the importance of instrumental diagnostic methods. There is no generally accepted algorithm for examining an injured person with a chest injury that would allow timely recognition of purulent complications in the post-traumatic period. The results of the examination of patients with intra-pleural complications of thoracic injury using the spiral computed tomography method, which in dynamics allows objectively documenting the dynamics of the pathological process, evaluating the effectiveness of treatment, timely diagnose secondary complications, are presented.

KEY WORDS: thoracic trauma, internal pleural complications, diagnosis

ДІАГНОСТИКА ЗАПАЛЬНИХ ВНУТРІШНЬО ПЛЕВРАЛЬНИХ УСКЛАДНЕНЬ ТОРАКАЛЬНОЇ ТРАВМИ

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Не специфічність клінічних ознак посттравматичних гнійних торакальних ускладнень свідчить про значущість інструментальних методів діагностики. Немає загальноприйнятого алгоритму обстеження постраждалих з ушкодженнями грудей, який дозволив би своєчасно розпізнати гнійні ускладнення в посттравматичному періоді. Представлені результати обстеження пацієнтів з внутрішньо плевральними ускладненнями торакальної травми за допомогою методу спіральної комп'ютерної томографії яка в динаміці дозволяє об'єктивно документувати динаміку патологічного процесу, оцінити ефективність лікування, своєчасно діагностувати вторинні ускладнення.

КЛЮЧОВІ СЛОВА: торакальна травма, внутрішньо плевральні ускладнення, діагностика

ДИАГНОСТИКА ВОСПАЛИТЕЛЬНЫХ ВНУТРИ ПЛЕВРАЛЬНЫХ ОСЛОЖНЕНИЙ ТОРАКАЛЬНОЙ ТРАВМЫ

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Не специфичность клинических признаков посттравматических гнойных торакальных осложнений свидетельствует о значимости инструментальных методов диагностики. Нет общепринятого алгоритма обследования пострадавшего с повреждения груди, который позволил бы своевременно распознать гнойные осложнения в посттравматический период. Представлены результаты обследования пациентов с внутри плевральными осложнениями торакальной травмы с помощью метода спиральной компьютерной томографии, которая в динамике позволяет объективно документировать динамику патологического процесса, оценить эффективность лечения, своевременно диагностировать вторичные осложнения.

КЛЮЧЕВЫЕ СЛОВА: торакальная травма, внутренне плевральные осложнения, диагностика

INTRODUCTION

There is an increase in the proportion of breast damages in the structure of injuries in peacetime from 12.5 % in the late 70s of the

20th century to 25 % at present [1]. Severe chest injury is the cause of both early mortality due to bleeding and hypoxia, and late mortality associated with the development of septic complications and multiple organ failure [2].

Purulent process due to thoracic trauma may develop as in the pleural cavity and the lungs and heart cavity shirt, mediastinum and chest wall [3]. For posttraumatic purulent-inflammatory thoracic complications include the following types of purulent complications: pleural empyema, lung abscess, pericarditis, mediastinitis [4]. Empyema of the pleura – the most common purulent complication, the issues of its diagnosis and treatment are highlighted in the literature quite widely, although most scientific research is based on the analysis of its nontraumatic etiology. According to various data, 7–16 % empyema of the pleura has traumatic etiology [5–6]. However, some other post-traumatic inflammatory complications are devoted to a few publications based on the study of a small number of observations. Post-traumatic lesions are lung abscesses that develop as a result of breast injury, regardless of the nature and severity of the primary lung injury [7].

According to the generalized data, pneumonia develops in 20–40 % of observations after a combined injury [8]. In the event of breast injury, pneumonia develops more often after the gunshot trauma (8–18 %) against the background of blood loss and large hematoma of the lung [5], abscesses of the lungs are found much less frequently – from 0.9 % to 2.8 % [4].

Purulent mediastinitis continues to be one of the most severe forms of generalized surgical infection, worldwide mortality ranges from 23–48 % [4], reaching with anaerobic mediastinitis 68–80 % [6]. High mortality with purulent mediastinitis is connected, first of all, with its later diagnosis. [2, 6].

Diagnosis of pleural empyema in spiral computed tomography (SCT) is based on signs such as heterogeneity of the contents of the pleural cavity with gas inclusions and thickening of the pleura leaves [4]. According to [4], using SCT can also diagnose the phase of development of empyema of the pleura and choose a rational volume of intervention in the onset forms of suppuration.

Important information can be obtained from SCT in patients with purulent pulmonary complications [1]. Significant difficulties are the differential diagnosis between the hematoma of the lung and progressive pneumonia on the background of hematomas in the period from 1 to 3 weeks after the closed injury. Similar difficulties are noted in the differentiation of inflammatory changes in the lungs from their damage in case of chest injury [6–7]. SCT – a picture of mediastinitis, according to some data [6], allows to differentiate the nature of the inflammation of cell mediastinum.

Thus, the analysis of domestic and foreign literature suggests that the frequency of post-traumatic purulent thoracic complications has no tendency to decrease, the lethality remains at a very high level. The problem of prediction, diagnosis and treatment of purulent complications of chest damage is not sufficiently studied.

Not specificity of clinical signs of post-traumatic purulent thoracic complications testifies to the importance of instrumental diagnostic methods. Until now, chest X-ray is the most common method of diagnosing chest lesions. The literature data indicate that there is no comparative analysis of the sensitivity of such X-ray methods as ultrasound diagnosis (USD), computer tomography (CT), depending on the nature of the injury and complications. There is no generally accepted algorithm for examination of victims with chest injuries, which would allow timely recognition of purulent complications in the post-traumatic period.

OBJECTIVE

Improve the outcome of treatment for injuries with injuries and closed chest injury by improving the methods of early diagnosis of inflammatory intra-pleural complications.

MATERIALS AND METHODS

The results of the examination of patients with intra-pleural complications of thoracic trauma with the help of the SCT method are presented.

In the empyema of the pleura I–II phase, 47 victims were examined, including after chest injury (CI) – (18) and closed chest trauma (CCT) – (29). 42 patients who had pulmonary suppuration, including after injury (7) and CCT (35), were also investigated at

different times after chest injury. In all observations of pulmonary suppuration after injury, the primary SCT study was performed at the stage of development of purulent process.

At mediastinitis, 27 patients suffered from SCT on different occasions (from day one to four months) after injury (17) and closed injury (10). In all 17 examined patients with mediastinitis after wounds, inflammatory changes of fibrous mediastinum of general or restricted nature were detected. Phlegmon mediastinum was diagnosed in 10 patients early in the post trauma period.

RESULTS AND DISCUSSION

At empyema of pleura I-II phases in all SCT observations there was a heterogeneity of pathological intra pleural content, its density varied in a wide range, from –30 to +60 ODN. The presence of small inclusions of gas density in the contents of the pleural cavity was found in 91.4 %, the horizontal level of the fluid – 12.3 %. In the vast majority of patients, the inequality of the contours of the pleura leaves with their thickening was detected (92.7 %). The nature of the changes in the leaves of the pleura depended on the prescription of the pathological process until the time of the study, the thickness of the parietal leaf varied within 3-11 mm, visceral – 2–7 mm.

According to the SCT, the pleural empyema (EP) was diagnosed in 43 patients, the sensitivity of the method was 91.4 % (90.7 % for injuries and 91.7 % for CCT). Mistakenly negative results of SCT were noted in 5 observations of EP, including 2 – after wounds and 3 – after CCT.

The absence of small gas inclusions in the contents of the pleural cavity was the main cause of diagnostic errors. In these observations, the diagnosis was verified with puncture (4) or autopsy (1).

Despite the high sensitivity of the method of SCT in the diagnosis of purulent process, in 7 observations noted the difficulty in determining the exact localization of the pathological process. So in 6 patients, according to SCT, it was not possible to differentiate the EP from the lung abscess. The diagnosis is clarified only with X-ray contrast study after draining purulent foci.

In one observation, the subpleural chronic abscess of the chest wall, which led to the development of osteomyelitis of the ribs, was considered as a pleural empyema in the SCT series. X-ray contrasting pleurography also confirmed the presence of a restricted EP.

The sensitivity of the SCT method to pleural empyema was 91.4 %, including 90.7 % for injuries and 91.7 % for closed trauma.

In all observations of pulmonary suppuration after injury, the primary SCT study was performed at the stage of development of purulent process. The pathological process was more often localized in the upper lobe (5), less frequently in the lower (2), but in no observation did not coincide with the anatomical boundaries of segments or lung particle. In three patients, examined at 5–12 days after injury, areas of irregular shape with lesions from 2 to 4 segments of lung, heterogeneous density from -36 to 52 ODN with small inclusions of gas density and increased density of adjacent pulmonary tissue were detected. These changes were treated as abstinent pneumonia with multiple destruction cavities. Two of them in the lung inflammation zone visualized the course of the wound canal in the thickness of the lower lobe. In all three observations, the pathological content in the pleural cavity was visualized, including that which is not differentiated from the pulmonary changes in one of them.

In the study of dynamics (18–28 days), including after the additional drainage of the pleural cavity (2), a decrease in the zone of inflammatory changes with the formation of rounded ones, including multiple (2) cavities with a horizontal fluid level, was noted. In 4 patients, examined for 12–52 days after breast injury, rounded forms of single cavities with a density of gas and liquid (horizontal level) were detected, with a total volume of 15 to 256 cm^3. The walls of the cavity had a value of soft tissue density, thickness from 3 to 8 mm, perifocally marked increase in the density of lung tissue. Such changes indicated that there were solid abscesses in the lungs. Drainage bronchi were visualized in three observations. The pathological process was localized within the 2 to 3 segments of the lung.

In the case of closed chest injury, the primary SCT was performed for 1–2 days of hospitalization in 8 patients, later than 5 days in 27. All patients who were examined from

the first day after CCT, according to SCT, lung changes in the form of polymorphic areas of density increase from 256 to 38 ODN with fuzzy contours, irregular shape, drainage, and with a clear visualization of the bronchial lumen that was considered as a hemorrhagic hemorrhage or a lung injury. In 5 of these, lung tissue ruptures with the formation of cavities in the thickness of the lung tissues filled with liquid density of blood and (or) gas were visualized on the background of the lung. At studying in the dynamics after 7–10 days about the accession of the inflammatory process was indicated by the lack of regression of the slaughtering centers (5) or the growth of infiltrative changes in the lungs, which is not characteristic of the uncomplicated course of the pathological process.

In the remaining 27 patients who were first examined in the stage of development of inflammatory complications, judging by the amount of primary lung damage was not possible. Nevertheless, in 8 of them, thin-walled cavity formation with liquid and (or) gaseous contents was revealed, indicating the traumatic nature of the formations in the lungs. The diagnosis of pulmonary suppuration according to SCT was confirmed in 32 out of 35 patients examined for 12–30 days after CCT, the sensitivity of the method was 91.4 %.

SKT signs of abstinence pneumonia (15) were characterized by the presence of polymorphic zones of heterogeneity of the pulmonary tissue with values of soft tissue density and multiple inclusion of round or irregular shape with values of density of liquid and gas. The prevalence of the destructive process varied from 2 to 7 segments, in 3 observations, it was of two-sided nature.

In 17 victims, a single lung abscess was found – a rounded form of formation with distinct unequal contours of a heterogeneous structure with small inclusions of gas density or a horizontal level in the total volume from 139 to 580 cm^3. The walls of the cavity had a value of the density of soft tissues, their thickness varied from 6 to 9 mm.

The data obtained in the SCT study (the nature of suppuration, the amount and localization of abscesses, proximity to the chest wall, the degree of adequacy of natural drainage in the bronchus) were the basis of the therapeutic tactic in patients with purulent pulmonary complications.

CT false negative results were obtained in three observations during examination of 13–18 days after injury. In the first observation, hematoma was detected in the lung (7 cm in diameter) on the background of a rounded hemothorax in a volume of 1200 ml and compression of the lungs. In two other observations, lung abscesses were not diagnosed against a background of a limited multi-chamber EP and were visualized only on a re-study after drainage of the pleural cavity. In general, in the case of SCT, the complexity of differentiation of intra-pulmonary and intra-pleural content was noted in 6 observations of pulmonary suppuration after CCT.

Thus, the sensitivity of the method in the diagnosis of purulent pulmonary complications was 92.9 %, including 100 % in wounds and 91.4 % in CCT.

In all 17 examined patients with mediastinitis after wounds, inflammatory changes of fibrous mediastinum of general or restricted nature were detected. The phlegmon of mediastinum was diagnosed in 10 patients early in the aftermath of the trauma in the form of a zone with values of the density of the fluid of irregular shape, with fuzzy contours, heterogeneous structure due to areas with soft tissue density and 6 observations with gas values. In 2 observations, inflammatory changes were localized throughout the anterior mediastinum, in 5 – in the upper parts of the anterior and posterior mediastinum, and in 3 - in the posterior and anterior mediastinum throughout (total mediastinitis). Three patients with combined wounds in the neck and breast, along with inflammatory changes in the mediastinum, also revealed a neck phlegmon in the form of the lack of differentiation of the fiber pretraheal, peripheral and around the vascular spaces, increasing its density to the values of the fluid and inclusion of sites with soft tissue density and gas.

Mediastinal abscess was detected in two injured (after 35 and 60 days after injury) in the form of formation with liquid density values, rounded form, with clear contours, heterogeneous structure due to areas with soft tissue (capsule) densities in the periphery.

In one case, the formation was located in the upper parts of the anterior mediastinum, in the other – in the lower parts, squeezing the right chambers of the heart. Infiltration of mediastinum was observed in 5 patients, in the dynamics with signs of suppuration in the form of a zone with values of soft tissue density, irregular shape, with fuzzy contours, heterogeneous structure due to sites with values of density of the fluid.

In two cases, infiltrate was localized in the lower parts of the anterior mediastinum, in the other two – in the upper parts of the anterior mediastinum and one in the lower parts of the posterior mediastinum. In three cases, destructive changes in the bone structure of the breast, adjacent to the infiltrate, were discovered.

In 8 out of 10 examined patients with mediastinitis after closed chest trauma during the initial CT study found a broken sternum and retrosternal hematoma in a zone of increased density, irregular shape, with fairly sharp contours. In the study of the dynamics noted an increase in the volume of the hematoma and the emergence of heterogeneity of its structure at the expense of areas with values of density of the fluid and gas sections, which was a sign of hematoma suppuration. In two observations of the closed trauma, destructive changes of the sternoclux joint with the presence of infiltrate in the adjacent parts of the anterior mediastinum in the form of a zone with values of the density of soft tissues of irregular shape, with fuzzy contours, were diagnosed.

Sensitivity of SCT in relation to purulent mediastinitis, regardless of the nature of the damage, was 100 %.

CONCLUSION

Thus, the analysis of the results of the study indicates the need for a comprehensive examination of suspected post-traumatic intra pleural thoracic complications.

In the presence of any manifestations of systemic inflammatory response in the post-traumatic period in patients with chest lesions, regardless of the results of the X-ray examination, ultrasound examination and computed tomography of the chest should be performed, which allows to detect the accumulation of pathological contents in the pleural and pericardial cavity, to assess the nature changes in the pulmonary tissue, mediastinum, thoracic wall and decide on the drainage of lesions fester.

At the same time, for the correct interpretation of some changes in spiral computed tomography, including the lung abscess that developed in the background of pulmonary hemorrhages and mediastinitis against the background of hemorrhages in the mediastinum and pneumomediastinum, it is necessary to compare them with the baseline data. This testifies to the necessity of performing an early spiral computed tomographic examination in patients with chest lesions in the presence of any focal changes on the review X-ray.

Spiral computer tomography of the chest in dynamics allows to objectively document the dynamics of the pathological process, evaluate the effectiveness of treatment, timely diagnose secondary complications.

The application of this algorithm allows to diagnose the entire spectrum of intra-pleural complications in the early stages and to avoid diagnostic errors.

PROSPECTS FOR FUTURE STUDIES

Detection of the developmental frequency and structure of inflammatory intra-pleural complications of thoracic trauma. Study of distribution and localization of purulent process depending on the nature of damage to the chest. Setting the factors that significantly contribute to the development of septic complications after injury and closed chest trauma. Conducting a comparative evaluation of X-ray methods for diagnosing inflammatory complications of thoracic trauma and developing a diagnostic algorithm.

Thus, the issue of prediction, diagnosis and treatment of inflammatory complications of thoracic trauma is largely unresolved, which is the basis for further research.

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CANDIDA ALBICANS AND STAPHYLOCOCCUS AUREUS CO-INFECTION IN MICE AFTER ANTIBIOTIC-INDUCED DYSBIOSIS

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Microbial interactions in Staphylococcus aureus–Candida albicans dual-species biofilms is a relevant research topic given the significant contribution of these microorganisms to hospital-acquired infections. Therefore, the purpose of our investigation was to study the interaction of opportunistic C. albicans and S. aureus in vivo and in vitro, both with the participation of normal microflora and in mice with antibacterial dysbiosis. The study of mentioned interactions was carried out on 100 white male mice weighing approximately 18 grams in vivo and using smears prepared from the grown mixed cultures of C. albicans and S. aureus and the Japan JEM 1400 transmission electron microscope for the purpose of electron microscopic study of microorganisms in vitro. Healthy mice forming control groups and mice with antibiotic-induced dysbiosis (after introduction of vancomycin, gentamicin, ampicillin) were divided into groups to create a mono- and associative infection: I group was given 1×10^7 CFU of C. albicans, II group – 1×10^8 CFU of S. aureus, and III group – a mixture of specified concentrations of C. albicans and S. aureus in the same proportion. Microorganisms causing monoinfection were being isolated from the body of animals treated with antibiotics till the end of the experiments in large quantities unlike in case of the healthy mice. Co-inoculation of these microbes in the same dose to animals (co-infection), which were injected with antibiotics, turned out to be fatal for them, whereas an adhesive bond was seen between the cells of C. albicans vs. S. aureus in vitro.

As can be seen, such bacterial-fungal co-infection reduce substantially the effectiveness of antibiotic therapy and the likelihood of successful treatment and can not be ignored when choosing the appropriate treatment.

KEY WORDS: C. albicans, S. aureus, colonization, interaction

CANDIDA ALBICANS ТА STAPHYLOCOCCUS AUREUS КО-ІНФЕКЦІЯ У МИШЕЙ ПІСЛЯ АНТИБІОТИКО-ІНДУКОВАНОГО ДИСБІОЗУ

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Мікробні взаємодії в біоплівках двох видів мікроорганізмів, Staphylococcus aureus і Candida albicans, є актуальною темою дослідження, враховуючи значний внесок останніх у розвиток внутрішньолікарняних інфекцій. Тому метою нашого дослідження стало вивчення взаємодії опортуністичних C. albicans і S. aureus in vivo та in vitro як за участю нормальної мікрофлори, так і у мишей з антибактеріальним дисбіозом. Вивчення вищенаведених взаємодій проводили на 100 білих самцях мишей вагою близько 18 г in vivo і in vitro з використанням мазків, отриманих з вирощуваних змішаних культур C. albicans і S. aureus, і японського трансмісійного електронного мікроскопа JEM 1400 з метою електронно-мікроскопічного дослідження мікроорганізмів. Здорові миші, якы сформулювали контрольні групи, і миші з викликаним антибіотиками дисбіозом (після введення ванкоміцину, гентаміцину, ампіциліну) були розділені на групи для створення моно- і асоціативної інфекції: групі I вводили 1×10^7 КУО C. albicans, II групі – 1×10^8 КУО S. aureus і III групі – суміш зазначених концентрацій C. albicans і S. aureus в тій же пропорції. Мікроорганізми, що спричиняли моноінфекцію, виділялися з організму тварин, які отримували антибіотики до кінця експерименту в великих кількостях, на відміну від таких у здорових мишей. Спільна інокуляція цих мікробів в тій же дозі тваринам (коінфекція), яким вводили антибіотики, виявилася для них фатальною, тоді як in vitro був помітний клітинний зв'язок між клітинами C. albicans vs. S. aureus.

Як бачимо, така бактеріально-грибкова коінфекция істотно знижує ефективність антибактеріальної терапії і ймовірність успішного лікування, та не може бути проігнорована при виборі відповідного лікування.

КЛЮЧОВІ СЛОВА: C. albicans, S. aureus, колонізація, взаємодія

CANDIDA ALBICANS И STAPHYLOCOCCUS AUREUS КО-ИНФЕКЦИЯ У МЫШЕЙ ПОСЛЕ АНТИБИОТИКО-ИНДУЦИРОВАННОГО ДИСБИОЗА

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Микробные взаимодействия в биопленках двух видов микроорганизмов, Staphylococcus aureus и Candida albicans, являются актуальной темой исследования, учитывая значительный вклад последних в развитие внутрибольничных инфекций. Поэтому, целью нашего исследования стало изучение взаимодействия оппортунистических C. albicans и S. aureus in vivo и in vitro как с участием нормальной микрофлоры, так и у мышей с дисбиозом. Изучение упомянутых взаимодействий проводили на 100 белых самцах мышей весом около 18 г in vivo и in vitro с использованием мазков, полученных из смешанных культур C. albicans и S. aureus. Приготовленные препараты изучали под трансмиссионным электронным микроскопом JEM 1400 производства Японии. Здоровые мыши контрольных групп, и мыши с вызванным антибиотиками дисбиозом (после введения ванкомицина, гентамицина, ампициллина) были разделены на группы для создания моно- и ассоциативной инфекции: группе I вводили 1×10^7 КОЕ C. albicans, II группе – 1×10^8 КОЕ S. aureus и III группе – смесь указанных концентраций C. albicans и S. aureus в той же пропорции. Микроорганизмы, вызывающие моноинфекцию, выделялись из организма животных, получавших антибиотики до конца эксперимента в больших количествах, в отличие от таковых у здоровых мышей. Совместная инокуляция этих микробов (коинфекция) в той же дозе животным, которым вводили антибиотики, оказалась для них фатальной, тогда как in vitro была замечена клеточная связь между клетками C. albicans vs. S. aureus.

Такая бактериально-грибковая коинфекция существенно снижает эффективность антибактериальной терапии и вероятность успешного лечения, и не может быть проигнорирована при выборе соответствующего лечения.

КЛЮЧЕВЫЕ СЛОВА: C. albicans, S. aureus, колонизация, коинфекция

INTRODUCTION

Beneficial or harmful interactions between C. albicans i S. aureus for the host can both develop in a healthy organism and in the case of illness [1–3]. Microbial communities are known to be functionally diverse microorganisms localized in certain places and long-lastingly interacting with each other. The microorganisms included in these communities are in certain symbiotic relationships, influencing the biological properties of each other, stimulating and/or inhibiting growth and reproduction [4–6]. Such relationships contribute to the worsening of the course of the disease by strengthening the virulence of the causative agents of the disease in the human body [7–9]. Specifically, exometabolites of S. aureus affect the phospholipase activity of C. albicans, thereby increasing their aggressiveness, contribute to the development of antibiotic resistance and activation of other features [10–13]. At the same time, C. albicans also stimulate some bacterial infections [9, 14]. There is the scientific evidence of the diverse influence of C. albicans on the structural mechanism of the population of the main causative agents of nosocomial infections. In case of immunosuppressive animals, C. albicans can cause an increase and reproduction of highly virulent strains in populations of E. coli, S. aureus that are resistant to antibacterial drugs and their anti-lactoferrin and antilysocyme activity [15–16].

Thus, microbial associates play a certain role in the pathogenesis of infectious diseases: the dynamics of the isolation of associates may change, their prolonged stay in the body (in chronic form or as carriage) may also affect the ineffectiveness of antibiotic therapy. All these issues have not been studied enough, and their study is of practical importance, since they have been of significance in the diagnosis and treatment of associative infections.

OBJECTIVE

Purpose of the study was to research on the interaction of opportunistic C. albicans and S. aureus most frequently encountered in infectious pathologies in vivo and in vitro conditions, both with the participation of normal microflora, and in mice with antibiotic-induced dysbacteriosis, and to study their electron microscopic features.

MATERIALS AND METHODS

The strains of C. albicans and S. aureus used in the experiments were isolated from patients who applied to the private laboratory of the Department of Microbiology and Immunology of the Azerbaijan Medical University, and then were identified by morphological, cultural, and enzymatic features. Suspensions of 48-h culture of C. albicans in the amount of 1×10^7 CFU/ml and 1×10^5 CFU/ml, and prepared from the 24-h culture of S. aureus in the amount of 1×10^8 CFU/ml and 1×10^5 CFU/ml were used in the experiments.

In vivo experiments were carried out on 100 white male mice weighing approximately 18 grams. 0.20 t mg/ml vancomycin, 0.40 t mg/ml gentamicin, 0.50 t mg/ml ampicillin per 100 grams of weight were administered to mice orally in a dose of 0.1 ml for 5 days. They were used in order to prevent the possible influence of normal microbiota of mice on C. albicans and S. aureus. Healthy animals and mice with antibiotic-induced dysbacteriosis were divided into groups to create a mono- and associative infection: I group was given 1×10^7 CFU of C. albicans, II group –1×10^8 CFU of S. aureus, and III group – a mixture of specified concentrations of C. albicans and S .aureus in the same proportion. C. albicans were administered in the same amounts in the first control group of healthy mice, S. aureus – in another.

Infected mice were opened after 1, 3, 7, and 10 days according to generally accepted rules by preparing a homogenate from visceral organs (stomach, small and large intestine, liver, spleen, kidney) and cultured on Saburo's medium with gentamicin and egg-yolk salt agar. The resulting colonies were counted based on the weight of 1 gram.

The obtained figures were subjected to a variation row, the average number of each row (M) and the error (m) were calculated and indicated in log10. The difference between the indicators of the groups was established by means of Mann-Whitney statistical accuracy ($p \leq 0.05$).

The in vitro experiments were carried out in test tubes with sugar broth, where 0.1 ml from a concentration of 1×10^5 CFU of C. albicans and S. aureus were transferred and incubated at 37°C. Smears were prepared from the suspension on the 1, 3, 5. 7, 11, and 15 days of the experiment, and inoculated on solid nutrient media with sugar agar, Saburo medium. Smears were prepared from the grown mixed cultures, and then stained using the Gram method.

Concentrations of 1×10^7 and 10^8 CFU/ml were used for the purpose of electron microscopic study of microorganisms. The mixture of microorganisms was kept at 37°C for 48 hours, then centrifuged, and the blocks of microorganisms were prepared using accepted protocols. The obtained materials were then cut with diamond knives in an EM UC 7 -LEİCA microtome into plates with a thickness of 60 nm. Sections were stained with uranyl acetate and mercuric citrate. Ready-made preparations were studied using the Japan JEM 1400 transmission electron microscope.

The studies were conducted in the Electron Microscope Laboratory under the AMU headed by Professor E. Gasymov (with financial support from the Science Development Foundation under the President of the Republic of Azerbaijan. Grant No. EIF-2011-1 (3) -82 / 44/3-M-6).

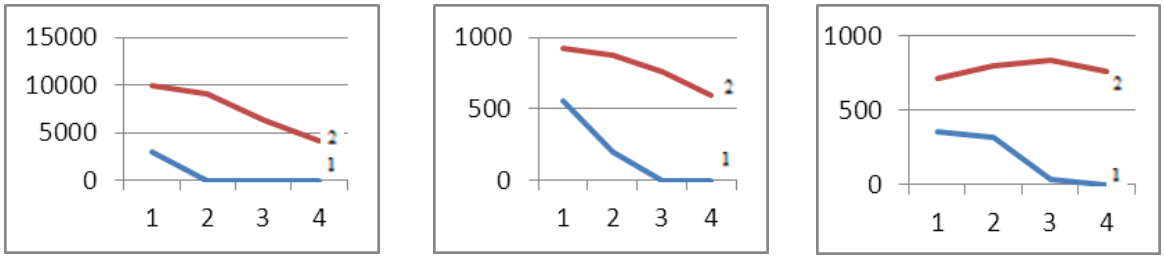
RESULTS AND DISCUSSION

C. albicans and S. aureus were isolated only from the digestive tract of both groups of animals, injected with cultures of these microorganisms. C. albicans was isolated from the stomach of healthy mice in the amount of 2.81 ± 0.19 CFU on the first day of the experiment, on the second day – in the amount of 2.40 ± 0.98 CFU; 2.92 ± 0.80 CFU were isolated from animals that were given antibiotics on the first day of the experiment, 2.83 ± 0.54 CFU – on the third day, 2.72 ± 0.27 CFU – on the seventh day, and $2.64 \pm 0{,}45$ CFU – on the tenth day (fig. 1 (1)).

C. albicans was found in the small intestine of healthy animals only on the first day and on the second day of the experiment in the amount of 2.75 ± 0.03 CFU and 2.3 ± 0.45 CFU, respectively. C. albicans isolated from the small intestine of animals that were given antibiotics in an amount of 2.96 ± 0.27 CFU on the first day, 2.94 ± 0.80 CFU on the third day, , 2.88 ± 0.15 CFU on the seventh day, and in the amount of 2.78 ± 0.35 CFU on the tenth day (fig. 1 (2)).

C. albicans was found in the large intestine of healthy mice on the first day in an amount of 2.56 ± 0.71 CFU, on the third day in an amount of 2.51 ± 0.15 CFU, on the seventh day it was detected only in the colon in an insignificant amount ($1,6 \pm 0.45$ CFU), and on the tenth day

microbes were not detected at all. Fungi were isolated from the colon in animals receiving antibiotics on the first day of observation in the amount of 2.86 ± 0.54 CFU, on the third day – 2.90 ± 0.05 CFU, on the seventh day – 2.92 ± 0.18 CFU and on tenth day – in the amount of 2.88 ± 0.06 CFU.



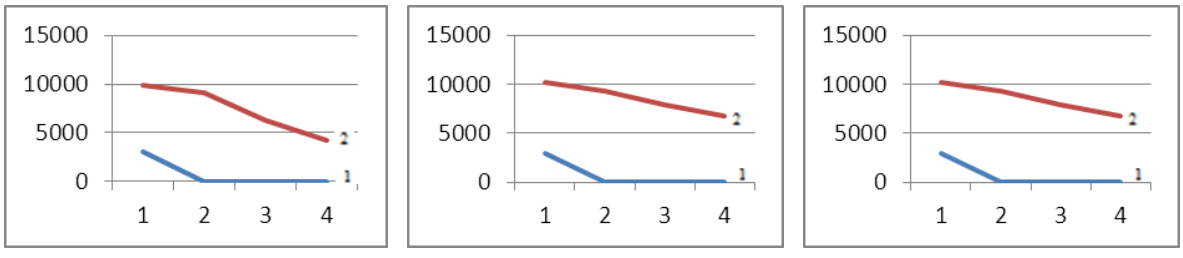
Stomach Small intestine Colon

Fig. 1. Detection of C. albicans (CFU) in case of mono-infection in healthy mice (1) and in animals that were administered antibiotics (2) in the stomach, small and large intestines
Abscissa: 1 – the first day, 2 – the third day, 3 – the seventh day, 4 – the tenth day. *Ordinate*: indicates CFU

S. aureus was found in the stomach of healthy animals only on the first day of the experiment (3.48 ± 0.43 CFU). In contrast, microbes were excreted from animals that were given antibiotics until the end of the experiment (3.99 ± 0.88 CFU on the first day, 3.96 ± 0.71 CFU on the third day, 3.80 ± 0.55 CFU on the seventh day, 3.62 ± 0.25 CFU on the tenth day) (fig. 2 (1)).

S. aureus was isolated from the small intestine of healthy animals only on the first day of the experiment (3.47 ± 0.25 CFU), whereas it was found in animals that were given antibiotics, on the first day in an amount of 4.1 ± 0.71 CFU, on the third day – 3.97 ± 1.12 CFU, on the seventh day – 3.90 ± 0.83 CFU, and on the tenth day – 3.83 ± 0.35 CFU (fig. 2 (2)).

Staphylococci were detected in the large intestine of healthy animals only on the first and second days of the experiment (2.92 ± 0.05 CFU). In contrast, they were found in animals that were given antibiotics, in an amount of 4.02 ± 0.84 CFU on the first day, 4.01 ± 0.88 CFU on the third day, 3.92 ± 0.91 CFU on the seventh day, and in the amount of 3.87 ± 0.81 CFU on the tenth day (fig.2 (3)).



Stomach Small intestine Colon

Fig. 2. Detection of S. aureus (CFU) in case of mono-infection in healthy mice (1) and in animals that were administered antibiotics (2) in the stomach, small and large intestines
Abscissa: 1 – the first day, 2 – the third day, 3 – the seventh day, 4 – the tenth day. *Ordinate*: indicates CFU

Oral administration with intervals of C. albicans and S. aureus simultaneously to animals that were given antibiotics in the first 24 and 48 hours caused the death of 60 % and 100 % of the animals, respectively. The microbial load of both types of microorganisms in the organs of dead animals exceeded to a significant degree such load in the control groups ($p \leq 0.05$). Microbes were found not only in the gastrointestinal tract of animals, they also colonized the liver, spleen and kidneys. The results of the experiments are shown in table.

Table

Microbial load in case of co-infection, caused by C. albicans and S. aureus of white antibiotic-treated mice (log10)

	I day		II day	
	C. albicans (CFU/gr)	S. aureus (CFU/gr)	C. albicans (CFU/gr)	S. aureus (CFU/gr)
Stomach	3,03 ± 0,25	4,2 ± 0,55	3,12 ± 0,13	4,29 ± 0,80
Small intestine	3,06 ± 0,17	4,06 ± 0,69	3,08 ± 0,05	4,26 ± 0,76
Colon	3 ± 0,13	4,07 ± 0,88	3,03 ± 0,13	4,19 ± 0,65
Liver	2,55 ± 0,15	3,61 ± 0,43	2,75 ± 0,65	3,74 ± 0,59
Spleen	2,51 ± 0,17	3,48 ± 0,35	2,68 ± 0,05	3,59 ± 0,25
Kidney	2,61 ± 0,35	3,62 ± 0,65	2,83 ± 0,07	3,60 ± 0,35

The study of the relationship between C. albicans vs. S. aureus in vitro showed once again the existence of a relationship between these two microorganisms that can be seen in smears prepared at different times from a mixture of microbes (fig. 3), where there is an accumulation of staphylococci around Candida fungi (fig. 3, A). You can also observe that staphylococci are distributed in insignificant amounts in the field of view where there are no cells of fungi. And staphylococci are arranged in small clusters and chains in places where cells of fungi are not detected. (fig. 3, B). Thus, staphylococci are more intense and with large groups, forming a classical cluster of bunches, are located around the cells of Candida fungi (fig. 3, A–B).

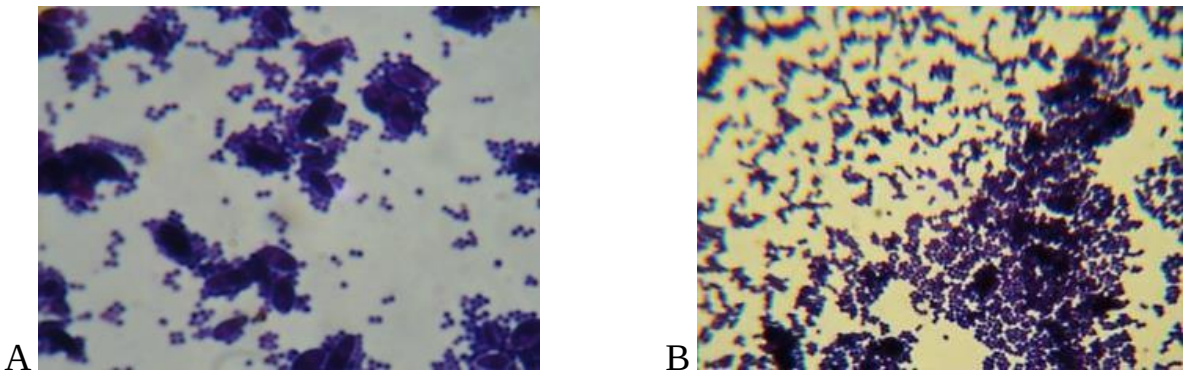


Fig. 3. Smears prepared from the mixed cultures of C. albicans and S. aureus (Gram stain)

We provide electron microscopic images for the purpose of introducing some clarity on the relationship between these microorganisms.

One can notice the attraction between the cells of staphylococci and fungi in the figure 4, these attractions are hardly noticeable (indicated by the arrow in figure 4.A) at the beginning of the experiments, and more clearly visible (fig. 4 B–C) as the cells approach each other; the formation of bridges between the cells (fig. 4 D–F) is also noticeable. Thus, an adhesive bond is formed between the cells of C. albicans vs. S. aureus. Reproduction of staphylococci adhered to the surface of fungal cells can also be viewed (fig. 4 C). 2–3 cells of staphylococci can accumulate on the surface of a single cell of the fungus (fig. 4 F).

Normal microflora of healthy organism contributes to the elimination of pathogens immediately after their introduction to the large extent along with other protection mechanisms. That is why, cultures of C. albicans and S. aureus, orally administered to healthy mice, were isolated from the digestive tract only in the initial days of the experiment. These microorganisms were also isolated mainly from the digestive tract, where the amount of host-gut microbiota was gradually decreased, in animals with antibiotic-induced dysbacteriosis. But the

studied microorganisms in the body of these mice were isolated in larger quantities unlike the body of healthy animals, and moreover, until the end of the experiment. However their number gradually decreased by the tenth day. Signs of the disease were observed from the first days of the experiment in animals that were injected with antibiotics.

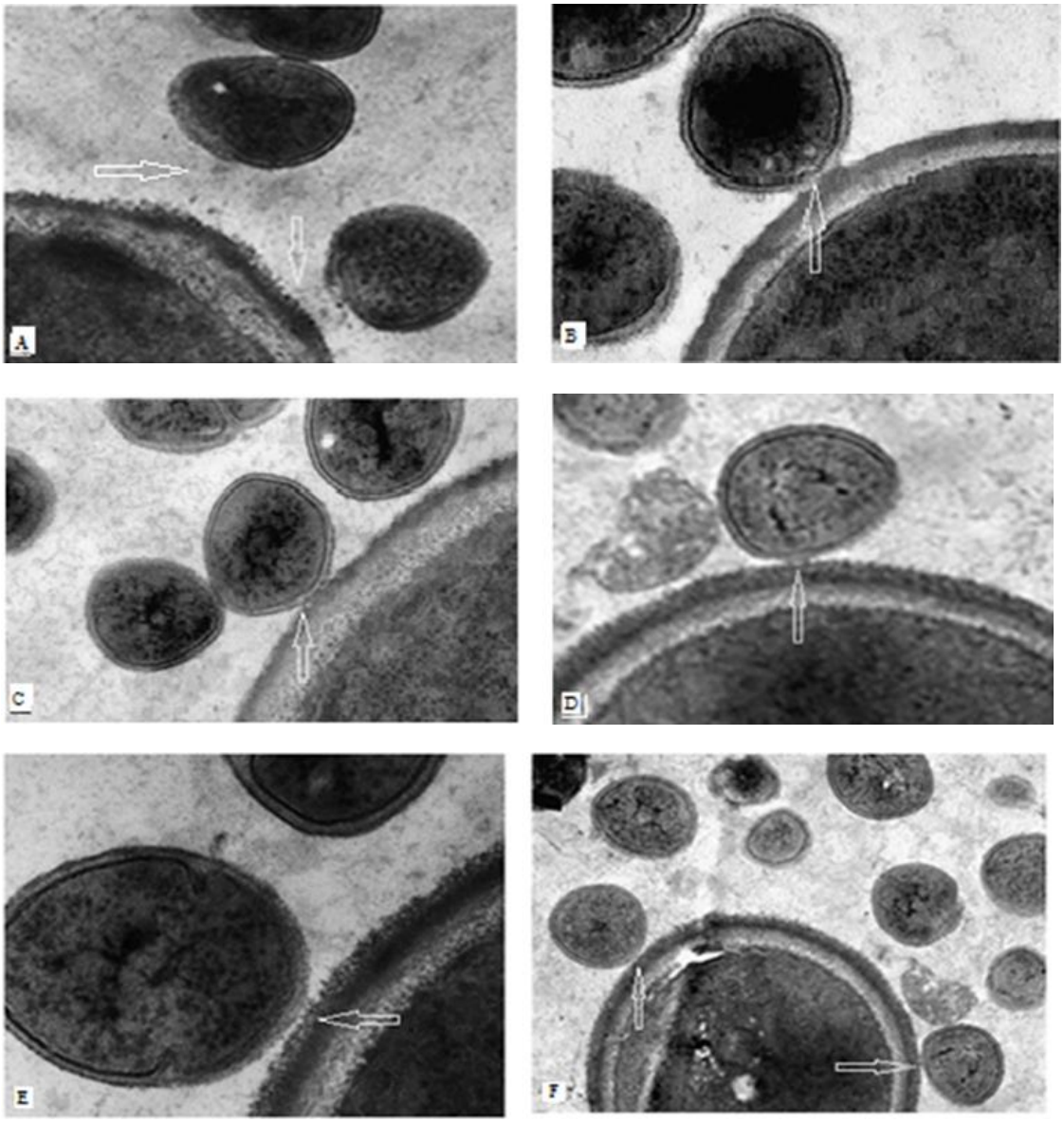


Fig. 4. Electron-microscopic image of the relationship between cells of C. albicans and S. aureus (arrows indicate adhesion)

Despite the significant decrease in normal microflora that was observed in animals administered with antibiotics, there was a gradual restoration of the composition of the microflora after cessation of the introduction of antibiotics. Detection of C. albicans and S. aureus for a long time (throughout the experiment) in the digestive tract of animals that were injected with antibiotics, in large quantities (CFU), was due to a decrease in the number of native microflora. The gradual restoration of normal microflora was accompanied by a gradual decrease in these microorganisms. Thus, signs of the disease were observed in mono-infections caused by C. albicans and S. aureus, both in healthy animals and in those who were given antibiotics, but no deaths were observed. There were no signs of major changes in the internal organs. However, co-infection induced by the Candida-Staphylococcus association in animals with dysbiosis caused

by antibiotics was the most difficult – accompanied by high rates of development and a high degree of mortality. Namely, 100 % of the animals died within the first 48 hours during co-infection. An autopsy of the dead animals showed an increase in the quantity of both microorganisms in the digestive tract, liver, kidney and spleen. Such results show the marked synergistic effect between C. albicans and S. aureus. Namely, when staphylococci are the only source of infection, a low percentage of dead animals are present, and co-infection with C. albicans at the same time increases the percentage of dead animals and the microbial burden in the internal organs, which coincides with the results of similar studies [9, 17]. On the basis of the data obtained, the causes of death of the mice are the mutual reinforcement of the physiological properties of the associates and reduction of the nonspecific protective system of the microorganism through the developed dysbacteriosis on the other hand. Microbial products have a synergistic effect in association, influencing on the growth and reproduction of bacteria, on the expression of pathogenicity factors [18]. The main factor ensuring the microbial pathogen's ability to cause an infection with severe flow is the amplification of the bacteria virulence under the influence of fungi.

In vitro studies also allowed establishing the dependence of staphylococci on Candida fungi. The results obtained and electron-microscopic images confirm the adhesive interaction between the cells of C. albicans and S. aureus, which led to an intensive multiplication of staphylococci on the surface of the fungi and formation of a multilayer film. As can be seen, S. aureus forms a film with the participation of C. albicans in the absence of the ability to form separately a film in the serum, and reproduce in the form of microcolonies based on the biofilm of C. albicans.

The complex interaction between fungi and staphylococci that arose under certain conditions is due to the presence of certain receptors [19–20]. The C. albicans agglutinin-like sequences (ALS) and specific surface glycoproteins in mixed microbial associations are important for co-adhesion [21].

CONCLUSIONS

The obtained results showed that the relationship between these microorganisms is ambiguous. Polymicrobial infection even nowadays remains a significant predictor of the patient's prognosis deterioration, reducing the effectiveness of antibiotic therapy and likelihood of successful treatment [22–23]. Various interactions arise under conditions of mutual enhancement of virulence in the animal organism, under conditions of quantity and time. Therefore it is especially important and necessary to take into account these data in case of the treatment of polymicrobial aetiology infections. The treatment strategy should not only be directed against a single pathogen, but also aim to destroy the microbial association.

PROSPECTS FOR FUTURE STUDIES

It remains relevant to study the effect of various therapeutic approaches in order to improve the effect on the fungus-bacterium association depending on the localization of the infection. Given the antibiotic resistance of individual bacteria, which significantly increases with cooperation with various representatives of the fungal flora, it makes sense to search for new methods of exposure, along with medical management. Further exploration of changes in the activity of various pathogens during the co-infection can make a significant contribution in this direction.

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UNIFFERENTIATED CONNECTIVE TISSUE DYSPLASIA AS A POTENTIAL PREDICTOR OF ARTERIAL HYPERTENSION DEVELOPMENT IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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The article presents the results of studies of markers of connective tissue dysplasia and the content of the main fibroblast growth factor in blood plasma in patients with type 2 diabetes. The presence of significant correlations between the studied parameters with the occurrence and progression of hypertension in patients with type 2 diabetes is established. A regression model for predicting the development of arterial hypertension is proposed in patients with diabetes mellitus.

KEY WORDS: arterial hypertension, type 2 diabetes mellitus, undifferentiated connective tissue dysplasia, basic fibroblast growth factor, prediction

НЕДИФЕРЕНЦІЙОВАНА ДИСПЛАЗІЯ СПОЛУЧНОЇ ТКАНИНИ ЯК ПОТЕНЦІЙНИЙ ПРЕДИКТОР РОЗВИТКУ АРТЕРІАЛЬНОЇ ГІПЕРТЕНЗІЇ У ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ 2 ТИПУ

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У статті наведено результати досліджень маркерів дисплазії сполучної тканини та вмісту основного фактору росту фібробластів в плазмі крові у хворих на цукровий діабет 2 типу. Встановлено наявність достовірних кореляцій між вивчаємими показниками з виникненням та прогресування артеріальної гіпертензії у хворих на цукровий діабет 2 типу. Запропоновано регресійну модель для прогнозування розвитку артеріальної гіпертензії у хворих на цукровий діабет.

КЛЮЧОВІ СЛОВА: артеріальна гіпертензія, цукровий діабет 2 типу, недиференційована дисплазія сполучної тканини, основний фактор росту фібробластів, прогнозування

НЕДИФФЕРЕНЦИРОВАННАЯ ДИСПЛАЗИЯ СОЕДИНИТЕЛЬНОЙ ТКАНИ КАК ПОТЕНЦИАЛЬНЫЙ ПРЕДИКТОР РАЗВИТИЯ АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИИ У БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ 2 ТИПА

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В статье приведены результаты исследований маркеров дисплазии соединительной ткани и содержания основного фактора роста фибробластов в плазме крови у больных сахарным диабетом 2 типа. Установлено наличие достоверных корреляций между изучаемыми показателями с возникновением и прогрессирования артериальной гипертензии у больных сахарным диабетом 2 типа. Предложено регрессионную модель для прогнозирования развития артериальной гипертензии у больных сахарным диабетом.

КЛЮЧЕВЫЕ СЛОВА: артериальная гипертензия, сахарный диабет 2 типа, недифференцированная дисплазия соединительной ткани, основной фактор роста фибробластов, прогнозирование

INTRODUCTION

Arterial hypertension (AG) is one of the most common cardiovascular diseases that

afflicts more than 1.5 billion people in the world, with almost 45 % of them being not aware of the presence of high blood pressure (BP) [1]. Hypertension is often manifested in combination with another pathology, in particular, with diabetes mellitus (DM), obesity, coronary heart disease (CHD), chronic kidney disease (CKD), heart failure (CH), and others [2–3]. The most often combination is hypertension and diabetes. The prevalence of hypertension among patients with diabetes is more than 60 %. Such a comorbidity significantly increases the risk of severe cardiovascular complications [4–5]. The presence of hypertension in patients with type 1 diabetes is associated with an increase in the frequency of severe stages of diabetic nephropathy and retinopathy; on the other hand, diabetes significantly increases the risk of emergence and developing hypertension, and hypertension is associated with a high risk of insulin resistance and metabolic abnormalities [6]. When combined with hypertension and diabetes, the risk of CHD developing increases by 2–4 times, stroke – 2–3 times, visual loss – 10–25 times, renal insufficiency – 15–20 times, gangrene of the lower extremities – 20 times [7].

Mechanisms of damage of many organs and systems in case of diabetes and hypertension are discussed by many researchers. Endothelial dysfunction due to metabolic disorders in diabetes or long-term BP increase lead to a breach of vasodilatation, thickening of the basement membrane of microvessels, metabolic disorders in extracellular matrix and initiating of large vessels atherosclerotic lesions [8–9]. Disturbance of lipid metabolism is essential as trigger factor, in addition to hyperglycemia. The immediate damaging factors may be the end products of glycolysis (AGE), products of lipid peroxidation, increased activity of angiotensin II, proinflammatory cytokines, leukocyte adhesion factors, activation of protein kinase C and others [10]. Increase of AGE, accumulation of circulating fatty acids, products of oxidative stress and dysfunction of the endothelium lead to accelerated apoptosis, reducing angiogenesis and cardiac remodeling violation [11].

S. Shakya et al. (2015) believe that the main target of hyperglycemia is hyaluronic-containing glycocalyx, located in the microvascular endothelium. Its damage leads to an increase in the adhesive properties of leukocytes and causes the formation of proinflammatory cytokines. In turn, this leads to the further development of oxidative stress and the progression of endothelial disorders with an increase in the proinflammatory cytokines release. Such a proinflammatory condition affects the function of functionally active cells (pericytes, smooth muscle cells, fibroblasts), which worsens reparative processes in vessels and tissues, including angiogenesis [12]. One of the mechanisms of the pathological effect of hyperglycemia is also thought to be the increase in the activity of prophylactic factors, in particular, TGF-β, which is a modulator of proteoglycan synthesis in the extracellular matrix [12–13]. is The increase in excretion of type IV collagen in patients with diabetes, with diabetic nephropathy and nephropathy of another genesis serves as an evidence of connective tissue metabolic abnormalities role in the extracellular matrix [14–15].

These data indicate that the target of pathological processes taking place in hypertension and diabetes is connective tissue. On the other hand, the presence of feedback is increasingly considered – the role of metabolic disorders of connective tissue in the emergence of another, in particular, vascular pathology. It is known that the connective tissue, which constitutes more than 50 % of the human body mass, carries out not only the supporting-skeleton, structural-forming, protective and reparative functions in the whole organism, but also takes part in almost all processes of metabolism. Pathological activation or inhibition of the activity of cellular elements of the connective tissue and changes in the structure of the basic substance can be the basis for the development and progression of the pathological process, the emergence of its complications and the appearance of comorbid pathology [16–17].

At this time, the disease, the characteristic feature of which is the development of connective tissue disorders, are defined as connective tissue dysplasia (CTD). The genetically determined differentiated variants of CTD are known that are Ehlers-Danloss, Marfan, Sticker's syndrome, and variants of CTD with clinical manifestations that are not

included in the clinical picture of hereditary pathology – undifferentiated CND (UCTD). Nowadays clinical variants of UCTD include numerical diseases with the presence of neurological, skeletal, cardiac, vascular, visceral, visual and other manifestations [16, 18]. The prevalence of CTD is rather high in the population, and single phenotypic symptoms are found in almost every fourth person [16, 19]. It is believed that the presence of CTD contributes to the development of another pathology. In particular, it has been established that diabetic neuropathy and nephropathy in children and adolescents with type 1 diabetes occur earlier and have severe course of disease in the presence of signs of connective tissue dysplasia [20].

Taking into account the value of the connective tissue in ensuring of the normal structure of all organs and tissues and in the processes of tissue remodeling under conditions of pathology, it is well-founded to study the role of UCTD in the development of hypertension, in particular, in patients with type 2 diabetes.

OBJECTIVE

The purpose of the study is to study the possibility of using the clinical signs of UCTD as predictors of the development of arterial hypertension in patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

The study was performed on the basis of the endocrinology department of the Kharkiv Regional Clinical Hospital. The study included 90 patients aged from 35 to 45 years who were on treatment during the period of 2016–2018 years with an established diagnosis of type 2 diabetes mellitus, lasting no more than 10 years.

All patients have undergone a complex general clinical, laboratory and instrumental examination in accordance to the Order of the Ministry of Health of Ukraine No. 1118 dated 21.12.2012 «Unified clinical protocol of primary and secondary (specialized) medical aid. Type 2 diabetes mellitus». The diagnosis of hypertension was carried out in accordance to the Order of the Ministry of Health of Ukraine No. 384 dated 24.05.2012, «Arterial Hypertension: An Updated and Adapted Clinical Invention based on Evidence». Diagnosis of UCTD was carried out through a comprehensive examination and evaluation of the presence of internal (visceral) and external (skeletal, skin, articular) signs according to T. I. Kadurina (2009) [15].

Phenotypic (visceral and/or skeletal) signs of UCTD were found in 48 patients (Group I) among the patients included in the study and 42 patients had no signs of UCTD (Group II). The control group consisted of 20 practically healthy people – donors with an average age of 36.3 ± 3.1 years.

The study of the concentration of the basic fibroblast growth factor 2 (FGF2) in blood plasma was carried out by immunoassay using a Quantikine (Human FGF basic immunoassay) reagent kit manufactured by R&D Systems, Inc. (USA) on the semi-automatic analyzer ImmunoChem 2100 in the Department of Experimental Pharmacology and Toxicology of the the SI «V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine».

The obtained results were processed using the package of statistical software PSSR (an open-source program that does not need a license). Quantitative data is given in the form of M ± SD (average and standard deviation of the mean) for normal data distribution or Me [Q_{25}–Q_{75}] (median – 25th and 75th quarters) with abnormal data distribution. The normality of distribution was determined by the Kolmagorov-Smirnov criterion, amended by Liliefors. Quantitative indices with normal distribution were compared using Student's t-test, with an abnormal distribution – according to the Mann-Whitney criterion. The frequency of quality indicators was performed using criterion $\chi 2$. Correlation analysis performed according to Pearson criteria.

RESULTS AND DISCUSSION

It was found that the average duration of the disease did not differ significantly and was in the 1st group (5.1 ± 2.3 years), in the 2nd group it was 4.2 ± 2.6 years ($p = 0.108$ per t-criterion) according to the analysis results of the peculiarities of the course of diabetes. There was 37 (77.1 %) patients with a duration of diabetes up to 5 years in Group I and 29 (69.0 %) in group II, 11 (22.9 %) patients with a duration of disease from 5 to 10 years in group I, 13 (31.0 %) – in the 2nd group ($\chi 2 = 0.740$, $p = 0.390$) (tab. 1).

Table 1

Features of diabetes mellitus based on the presence of UCTD

Indicator	Group I (n = 48)	Group II (n = 42)	p
Duration of DM, years	5,1 ± 2,3	4,2 ± 2,6	0,108[1]
Duration of DM: up to 5 years 5–10 years old	 37 (77,1 %) 11 (22,9 %)	 29 (69,0 %) 13 (31,0 %)	0,390[2]
Diabetic nephropathy – Microalbuminuria	38 (79,2 %) 17 (35,4 %)	20 (47,6 %) 6 (14,3 %)	0,002[2] 0,040[2]
Diabetic retinopathy	25 (52,1 %)	16 (38,1 %)	0,184[2]
Diabetic neuropathy	41 (85,4 %)	30 (71,4 %)	0,105[2]

Notes: 1 – the significance of the difference based on the t-criterion. 2 – the significance of the difference by criterion χ2. 3 – the significance by the Mann-Whitney criterion

Diabetic nephropathy was a frequent complication of diabetes in the analyzed sample of patients. It was diagnosed in 38 (79.2 %) patients in group I, in 20 (47.6 %) patients in II group ($\chi 2 = 9{,}729$, $p = 0{,}002$). While microalbuminuria (MA) was observed in 17 (35.4 %) patients in group I and in 6 (14.3 %) patients in group II ($\chi 2 = 4{,}205$; $p = 0{,}040$).

Thus, there is more severe course of diabetes with more frequent development of diabetic nephropathy with MA, as well as with the tendency to increase the incidence of diabetic neuropathy and retinopathy in patients with the signs of the UCTD.

The most common accompanying pathology in the analyzed group of patients was hypertension, which was detected in 45 (93.7 %) patients in group I, including: stage 1 hypertension – in 25 (52.1 %), stage 2 hypertension – in 17 (35.4%) and stage 3 hypertension – in 3 (6.3 %) patients. Hypertension was detected in 22 (52.4 %) patients in the II group including: 16 (38,1 %) patients with stage 1 hypertension, 5 (11,9 %) – with stage 2 hypertension, 1 (2.4 %) – with stage 3 hypertension ($\chi 2 = 21.783$; $p < 0.001$) (fig. 1).

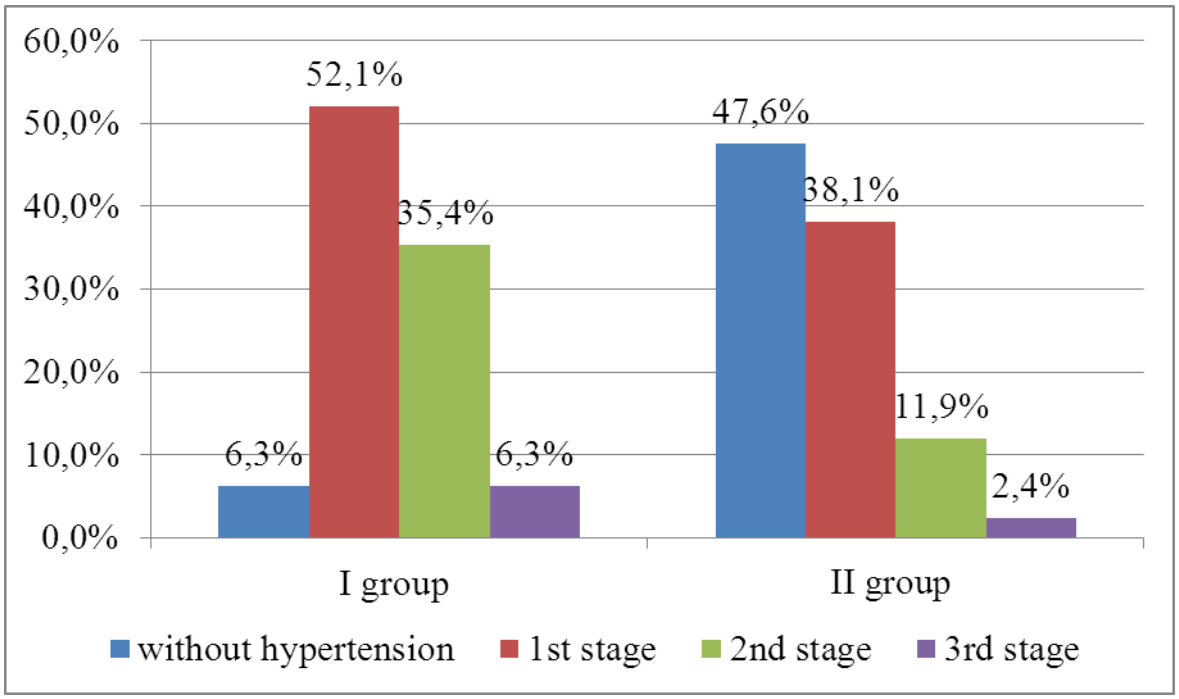

Fig. 1. Frequency and severity of hypertension in I and II groups of patients

Certain patterns have been identified depending on the duration of diabetes as a result of the analysis of the frequency and severity of hypertension. No differences in the frequency of hypertension of varying stages depending on the duration of diabetes was detected in group I (with the presence of UCTD) ($\chi 2$ = 1,603; p = 0,659). On the contrary, clear dependence of hypertension stages on the duration of diabetes can be traced in patients of II group (without phenotypic signs of UCTD) – the frequency of hypertension itself and 2nd and 3rd stages significantly increases ($\chi 2$ = 17,961; p < 0,001) in patients with a duration of diabetes from 5 to 10 years in comparison with patients whose disease lasted less than 5 years old. These patterns are confirmed by the results of the correlation analysis: a very weak positive correlation was found at the level r_S = 0.091 (p = 0.538) in the I group and there was a significant positive correlation at r_S = 0.522 (p < 0.001) in the II group.

The comparison of frequencies of UCTD cases was performed depending on the presence of visceral and/or skeletal signs for a more detailed study of the effect of UCTD on the development of hypertension in patients with type 2 diabetes. Only visceral signs of UCTD were detected in 31 patients in the Ia group, visceral and skeletal symptoms were detected in 17 patients who were Ib group (fig. 2).

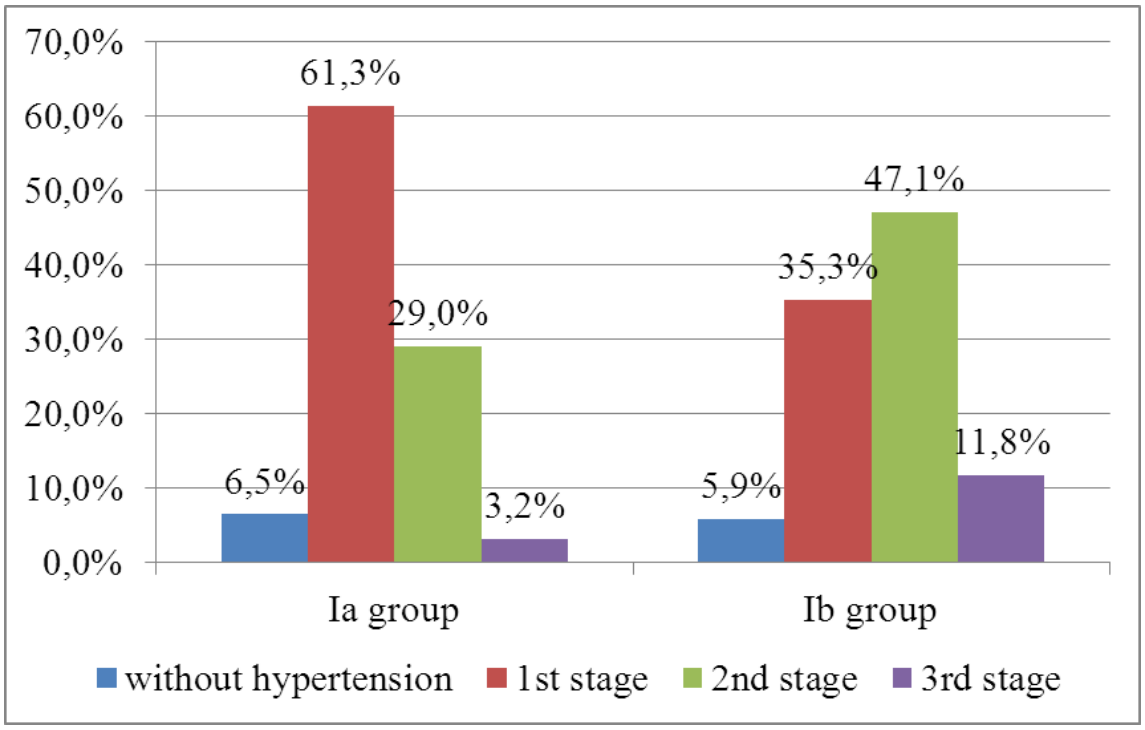

Fig. 2. Frequency of hypertension depending on the type of UCTD

Hypertension was found almost with the same frequency in both groups, but its structure differed based on stages. Stage 1 hypertension was detected in 19 (61.3 %) patients in the Ia group, stage 2 – in 9 (29.0 %) patients, stage 3 – in 1 (3.2 %). In Ib group, stage 1 hypertension was established in 6 (35.3 %) patients, stage 2 – in 8 (47.1 %), stage 3 – in 2 (11.8 %). That is, an insignificant tendency to the development of more severe stages of hypertension due to the increase in number of phenotypic signs of UCTD, in particular skeletal anomalies, is traced ($\chi 2$ = 3.718; p = 0,294).

The results of studies of FGF2 content in blood plasma are shown in tab. 2. All patients with diabetes included in the study have significantly higher median of the content of FGF2 than in the control (p < 0.05 by the Mann-Whitney criterion). The content of FGF2 was significantly higher in group I than such of controls or in group II (p < 0.05 according to the Mann-Whitney criterion in both comparisons). No significant differences were observed between subgroups Ia and Ib,

but there was a tendency for FGF2 to increase in patients of Ib group (with the presence of visceral and skeletal symptoms of UCTD).

Table 2

FGF2 content in the blood of patients with type 2 diabetes and in control group (pg/ml)

Groups	Me [Q_{25}; Q_{75}]	Min	Max
All patients (n = 90)	28,1 [18,7; 34,2][1]	7,4	63,2
I group (n = 48)	30,8 [21,6; 39,9][1,2]	12,8	63,2
Ia group (n = 31)	30,3 [22,6; 39,7][1,2]	12,8	56,2
Ib group (n = 17)	31,4 [20,5; 47,6][1,2]	16,2	63,2
II group (n = 42)	22,1 [16,2; 29,3][1]	7,7	41,2
Control group (n = 20)	3,6 [1,9; 9,0]	0,8	19,6

Notes: 1 – the differences are significant compared to the control (p < 0,05 according to the Mann-Whitney criterion). 2 – the differences are significant compared to the second group (p < 0,05 according to the Mann-Whitney criterion)

A correlation analysis of the FGF2 level in blood was performed separately in groups I and II (parametric Pearson method), which resulted in significant differences in the strength of the correlation relationships between the groups (tab. 3).

Table 3

Results of correlation analysis of FGF2 content and other indicators

Indicators	I group		II group	
	r	p	*r*	p
Age, years	0,107	0,227	0,649	< 0,001
Duration of DM, years	0,059	0,877	0,357	0,020
Hypertension (0 – no, 1–3 st.)	0,564	0,001	0,413	0,006
Diabetic nephropathy	0,206	0,159	0,372	0,015

Interesting differences have been identified as a result of the correlation analysis of FGF2 content and other indicators. The correlation of FGF2 depending on age and duration of diabetes mellitus was absent in group I – $r = 0.107$ and $r = 0.059$, respectively ($p > 0.05$). On the contrary, a significant strong positive correlation was found between FGF2 and age ($r = 0.649$, $p < 0.001$) and duration of diabetes ($r = 0.357$, $p = 0.02$) in group II. These patterns are shown in fig. 3, 4

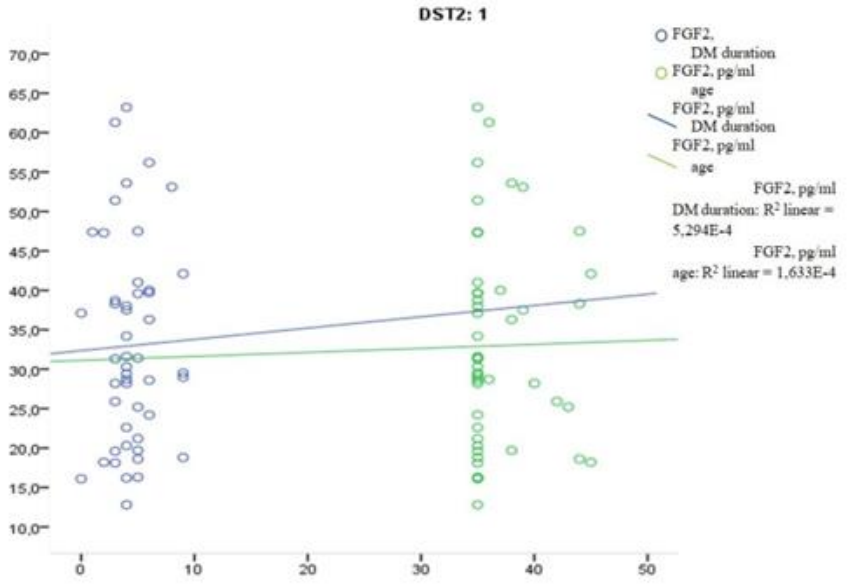

Fig. 3. Diagram of scattering of FGF2 dependence on age and duration of diabetes in I groups of patients

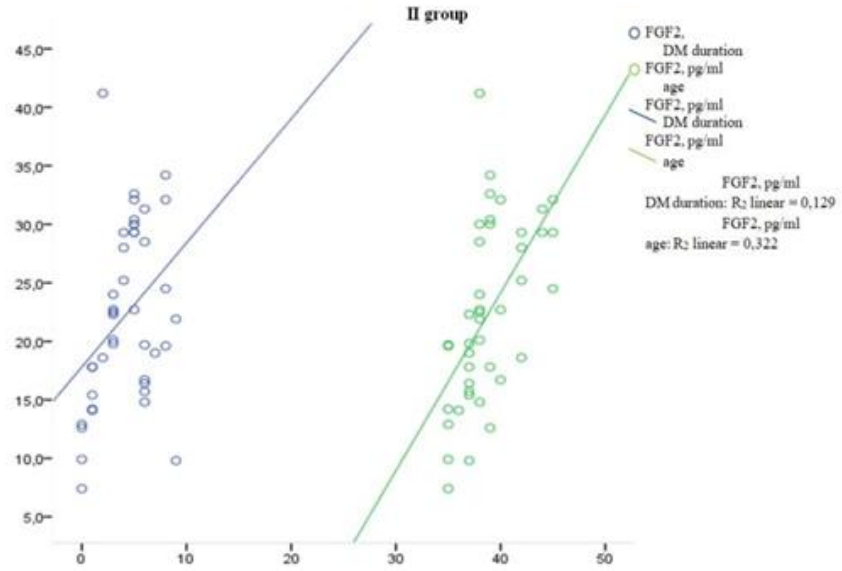

Fig. 4. Diagram of scattering of FGF2 dependence on age and duration of diabetes in II groups of patients

Data suggests that the content of FGF2 increases with age and during the course of the disease in patients with DM type 2. This dependence is absent in the presence of UCTD in patients with DM type 2, which can be explained by the initial increase in the content of FGF2 on the background of metabolic disorders of the connective tissue.

Another interesting regularity is the presence of a statistically significant correlation between the content of FGF2 in patients with diabetes with the presence and severity of hypertension in both groups – r = 0.564 (p = 0.001) and r = 0.413 (p = 0.006) respectively in I and II groups.

Also, there was no significant positive correlation with the presence of diabetic nephropathy – r = 0.206 (p = 0.159) in patients with UCTD (group I). The correlation with the presence of diabetic nephropathy was significantly positive r = 0.372 (p = 0.015) in patients of the II group (without evidence of UCTD). In addition, the presence and severity of hypertension and diabetic nephropathy had correlations among themselves: in group I there was a significant positive correlation – r = 0.451 (p = 0.001), and in the second group there was also a significant positive correlation r = 0.506 (p = 001).

Thus, the presence of UCTD and increased content of FGF2 are associated with the development and progression of hypertension in patients with DM type 2, suggesting the possibility of using these indicators as predictors of hypertension. But for studying this possibility, dynamic observation for a long period since the debut of the DM is necessary. It is also needed to take into account the fairly high cost of immuno-enzymatic analysis of the content of FGF2. On the other hand, the content of FGF2 is closely related to the UCTD, therefore, the possibility of using clinical signs of UCTD to predict the development of hypertension in patients with type 2 diabetes has been tested.

To this end, an analysis of the written discharge reports after a previous inpatient treatment (from two to five years ago) was performed in patients with a duration of type 2 diabetes for at least 2 years. Such discharge reports were available in 78 patients.

The method of binary logistic regression (BLR) is used for the analysis of the possibilities of using the UCTD signs as predictors of hypertension. The patients included in this analysis were divided into two groups:

– I group – patients with DM type 2, in whom hypertension was not diagnosed during the previous hospitalizations and wasn't found during the last hospitalization, as well as patients who have no differences in degree of hypertension during the previous and last hospitalization – 38 patients;

– II group – patients with DM type 2, in whom hypertension was not diagnosed during the previous hospitalization, but during their last hospitalization hypertension was detected, as well as patients whose hypertension during the last hospitalization increased in comparison with the previous one – 40 patients.

Groups are encoded in the order scale: I group – «0»; II group – «1».

In the logistics analysis, two indicators were introduced:

– presence of signs of UCTD, encoded as the sum of individual visceral and/or skeletal symptoms – x_1.

– duration of DM type 2 in years – x_2. This indicator was introduced due to the influence of the duration of diabetes in patients with type 2 diabetes, regardless of other indicators.

According to the results of the analysis, the following data was obtained using the BLR (tab. 4).

All regression coefficients of the indicators included in the equation are reliable, with the value of significance <0.05 for all indices and constant as an evidenced, while the regression coefficient of UCTD has a very high significance < 0.001 and significantly higher than the duration of the DM. This indicates a rather high predictive value of UCTD in the development of hypertension.

Table 4

Results of regression analysis of the hypertension development or progression in patients with type 2 diabetes

Indicator	B	S.E.	Wald Statistics	Sign. (p)
x_1	2,970	0,689	18,578	0,000
x_2	0,470	0,204	5,320	0,021
Constant	-4,520	1,367	10,927	0,001

Notes: x_1 - signs of UCTD; x_2 - duration of type 2 diabetes; B - coefficient of regression B; S.E. - standard error of the regression coefficient; p - level of significance of the regression coefficient.

The obtained data allow us to calculate the value of z, which can be presented in this analysis as:

$$z = x_1 \cdot 2{,}970 + x_2 \cdot 0{,}470 + (-4{,}520) \quad (1)$$

Further calculation of the hypertension development or progression probability of is carried out according to the formula:

$$P = \frac{1}{1+e^{-z}} \quad (2)$$

The greater final value of P is, the greater likelihood of hypertension or its progression in patients with DM type 2. The critical value, which determines the patient with risk of hypertension, is 0.5.

While checking the predictive ability of the generated equation to predict the onset or progression of hypertension, the following data are established (tab. 5)

Table 5

Table of classification of observed and predicted values

Observed value		Estimated value		
		Development of hypertension		Percentage of correct
		0 (no)	1 (yes)	
Development of hypertension	0 (no)	34	4	89,5
	1 (yes)	5	35	87,5
Total percentage				88,5

Notes. Distinguishing value = 0.500

The data obtained indicate that the predictive value of the negative outcome is 89.5 %, the predictive value of the positive result is 87.5 %, and the overall predictability is 88.5 %. In 34 (87.2 %) cases out of the 39 patients who had no development or

progression of hypertension, patients received a correct prediction that characterizes the specificity of the BLR equation. A correct prognosis was obtained as well in 35 (89.7 %) cases out of the 39 patients who had hypertension or progression that characterizes the sensitivity of the BLR equation.

Sufficiently high values of predictability, specificity and sensitivity suggest that this method can be used to predict the onset or progression of hypertension in patients with type 2 diabetes and to identify patients who require active prophylaxis of hypertension, even in the absence of other cardiovascular risks.

CONCLUSIONS

1. The development of hypertension in patients with DM type 2 in combination with UCTD occurs earlier, is almost independent of the duration of diabetes, more pronounced hypertension is observed in patients with visceral and skeletal symptoms of UCTD. This is evidence of the role of UCTD in the pathogenesis of hypertension in patients with type 2 diabetes.

2. Essential role in the emergence and progression of hypertension in patients with DM type 2 is played by metabolic disorders of the connective tissue that manifests as clinical signs of UCTD. FGF2, which content is increased in patients with visceral and/or skeletal symptoms of UCTD, is involved in its pathogenesis.

3. The regression model of the prediction of hypertension in patients with DM type 2 with an assessment of the presence of UCTD signs and the duration of diabetes has a high prognosticity, specificity and sensitivity, which makes it possible to apply this method in clinical practice to determine patients with high risk of development of hypertension.

PERSPECTIVES FOR FUTURE STUDIES

The data limitation is the age group (from 35 to 45 years) and the duration of diabetes (less than 10 years). In addition, the forecasting results were obtained based on the analysis of retrospective data. More evidence may be obtained in a prospective study involving more patients with more extensive inclusion criteria.

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CONDITION OF LIPID PEROXIDE OXIDATION AND ANTIOXIDANT SYSTEM IN PATIENTS WITH INFECTIOUS MONONUCLEOSIS

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The indicators of the activity of lipid peroxidation and the antioxidant system were studied in dynamics in 158 patients with infectious mononucleosis depending on the severity of the clinical course of the disease.

It is proved that lipid peroxidation is significantly activated in patients with infectious mononucleosis as the severity of the disease increases and therefore increases the oxidative activity of blood plasma, the concentration of dyne conjugates and malondialdehyde in the blood, decreases the activity of antioxidant enzymes of erythrocytes (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase), glutathione peroxidase and glutathione reductase activity in plasma and lowering the concentration of total and reduced glutathione in the blood, as well as reduce the antioxidant activity of blood plasma and erythrocytes.

KEY WORDS: infectious mononucleosis, lipid peroxides, antioxidant system, catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, dyne conjugates, malonic dialdehyde, glutathione

СТАН ПЕРЕКИСНОГО ОКИСЛЕННЯ ЛІПІДІВ ТА АНТИОКСИДАНТНОЇ СИСТЕМИ У ХВОРИХ НА ІНФЕКЦІЙНИЙ МОНОНУКЛЕОЗ

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У 158 хворих на інфекційний мононуклеоз вивчені в динаміці показники активності перекисного окислення ліпідів і антиоксидантної системи в залежності від тяжкості клінічного перебігу захворювання.

Доведено, що у хворих на інфекційний мононуклеоз протягом наростання тяжкості захворювання достовірно активується перекисне окислення ліпідів, в зв'язку з чим підвищуються окислювальна активність плазми крові, концентрація в крові дієнових кон'югатів і малонового діальдегіду, відбувається ослаблення активності антиоксидантної системи, що асоційоване зі зниженням активності антиокислювальних ферментів еритроцитів (каталази, супероксиддисмутази, глутатіонпероксидази, глутатіонредуктази), активності глутатіонпероксидази і глутатіонредуктази в плазмі крові і зниженням концентрації загального та відновленого глутатіону в крові, а також зниженням антиоксидантної активності плазми крові та еритроцитів.

КЛЮЧОВІ СЛОВА: інфекційний мононуклеоз, перекиси ліпідів, антиоксидантна система, каталаза, супероксиддисмутаза, глутатіонтероксидаза, глутатіонредуктаза, дієнові кон'югати, малоновий діальдегід, глутатіон

СОСТОЯНИЕ ПЕРЕКИСНОГО ОКИСЛЕНИЯ ЛИПИДОВ И АНТИОКСИДАНТНОЙ СИСТЕМЫ У БОЛЬНЫХ ИНФЕКЦИОННЫМ МОНОНУКЛЕОЗОМ

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У 158 больных инфекционным мононуклеозом изучены в динамике показатели активности перекисного окисления липидов и антиоксидантной системы в зависимости от тяжести клинического течения заболевания.

Доказано, что у больных инфекционным мононуклеозом по мере нарастания тяжести заболевания достоверно активируется перекисное окисление липидов, в связи с чем повышаются окислительная активность плазмы крови, концентрация в крови диеновых коньюгатов и малонового диальдегида, происходит ослабление активности антиоксидантной системы, что ассоциировано со снижением активности антиокислительных ферментов эритроцитов (каталазы, супероксиддисмутазы, глутатионпероксидазы, глутатионредуктазы), активности глутатионпероксидазы и глутатионредуктазы в плазме крови и понижением концентрации общего и восстановленного глутатиона в крови, а также снижением антиокислительной активности плазмы крови и эритроцитов.

КЛЮЧЕВЫЕ СЛОВА: инфекционный мононуклеоз, перекиси липидов, антиоксидантная система, каталаза, супероксиддисмутаза, глутатионтероксидаза, глутатионредуктаза, диеновые коньюгаты, малоновый диальдегид, глутатион

INTRODUCTION

Infectious mononucleosis (IM) is a widespread disease of childhood, which has been increasingly diagnosed among adults recently. The etiological factor of myocardial infarction is Epstein-Barr virus (EBV) in most cases, which is characterized by a discrete symptom complex followed by damage of the immune system, namely, the lifetime persistence of the virus in B lymphocytes. EBV is a lymphotropic agent that causes the development of lymphoproliferative syndromes and immune deficiency [1–3].

Other clinical manifestations are possible in addition to the triad of symptoms – lymphadenopathy, tonsillitis, hepatosplenomegaly in case of IM, that are associated with heart damage (myocardium, pericardium, coronary arteries), central and peripheral nervous system (meningitis, meningoencephalitis), kidney (nephritis), pancreatitis orchites and others [4–6].

Previous studies have shown that activation of lipid peroxidation, which is accompanied by the formation of primary and secondary lipid hydroperoxides with toxic properties, plays an important role in the pathogenesis of cell destruction of various organs [7–9]. However, the processes of free-radical lipid oxidation and antioxidant protection have been poorly understood in patients with IM, and the data obtained are contradictory.

OBJECTIVE

The aim of the work was to study the state of lipid peroxidation and antioxidant system of the disease in patients with various forms of severity of IM in the dynamics.

MATERIALS AND METHODS

158 patients with IM (80 women and 78 men) aged from 18 to 32 years were examined. IM was mild in 58 patients, in moderately severe form in 56 patients and 44 patients had severe form. The work was performed at the Department of General and Clinical Immunology and Allergology, Medical Faculty, V. N. Karazin Kharkiv National University on the basis of the Regional Clinical Infectious Diseases Hospital in Kharkiv within the framework of the research theme of the department «Study of the role of immune, autoimmune and metabolic disorders in the pathogenesis and consequences of the infectious process caused by herpes viruses», state registration № 0112U005911. The study is open, controlled, conducted in accordance with the Declaration of Helsinki and the principles of GCP and approved by LEC [10].

All patients underwent a complete clinical, haematological, biochemical and immunelogical examination.

The diagnosis of IM was established on the basis of the clinical picture of the disease, taking into account epidemiological analysis

data and confirmed by detecting EBV DNA in serum and saliva of patients with EBV, also studied the serological markers of EBV that are immunoglobulin's (IgM, IgG) to capsid (VCA), to the nuclear (NA) and early (EA) antigens by means of enzyme linked immunosorbent assay (ELISA) using the IBL (Germany) and Vector-Best (RF) kits, detection of EBV DNA by blood polymerase chain reaction (PCR).

A comprehensive study of patients included the study of peripheral blood with the detection of atypical mononuclear cells, the activity of alanine, aspartic transaminases, the content of bilirubin fractions in the blood. The state of lipid peroxidation (LPO) in patients with IM was appreciated according to the content of dyne conjugates (DC), malondialdehyde (MDA), the serum total oxidative activity (TOA). The state of the antioxidant system was assessed according to the determination of the serum and erythrocytes total antioxidant activity (TAA), the activity of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase of erythrocytes, total glutathione, oxidized and reduced serum glutathione. Examination of patients was carried out in the first days upon admission to the clinic, then on the 5th, 10th, 20th and 30th days of treatment. The control group consisted of 30 healthy people aged from 18 to 30 years, in whom the indicators of oxidative and antioxidant activity of blood plasma and erythrocytes were determined.

All patients received standard therapy that included anti-viral medications; valaciclovir 1000 mg 3 times daily, detoxification therapy (reosorbilact, 5 % glucose solution), hepatoprotectors, antipyretic drugs, antihistamines.

Statistical processing of the research results was carried out using the Statistika 6.0 for Windows (Stat Soft Inc., USA) on a personal computer with a Pentium II Celeron 850 PPGA processor. These results are presented in an article with assessing the reliability of differences by the Student's criterion at $p < 0.05$. The non-parametric Spearman method of correlation analysis was used to balance the relationship between various biochemical parameters.

RESULTS

The clinical picture of IM was typical in most patients. Patients complained of general weakness, headache, myalgia, arthralgia, sore throat when swallowing, sweating, and fever up to 38–39.6 ° C, which persisted for 15 to 30 days and often longer during the early days of the disease. Typical changes specific for IM were detected in the clinical analysis of blood, leukopenia was detected more often at the beginning of the disease, which was later replaced by leucocytosis ($9–10 \times 10^9$/l), the content of mononuclear lymphocytes and monocytes significantly increased, reaching 50–80 % of the total number of leukocytes. The detection of atypical mononuclear was a characteristic sign of IM in the blood (20 % or more). The duration of preservation of wide plasma lymphocytes averaged around 2–3 weeks, sometimes up to 2 months, the ESR remained within the normal range or slightly increased in this category of patients.

An ultrasound examination revealed an enlarged liver, spleen, peribronchial and peritracheal lymph nodes.

Anterior and posterior cervical, submandibular and axillary lymph nodes were enlarged in all patients with IM.

Cytolytic syndrome was diagnosed in 43 (76.3 %) patients with moderate-severe course of IM and in 30 (88.7 %) patients with severe course, which proceeded with an increase of direct bilirubin in blood. The level of direct bilirubin increased at an average of 15.0 ± 0.9 μmol/l ($p < 0.01$), the ALT activity – 1.85 ± 0.09 mmol/l, ACT – 1.12 ± 0.07 mmol/l ($p < 0.01$) in patients with a moderate-severe course of IM. In patients with severe course of IM, the level of direct bilirubin in the blood increased on average to 23.9 ± 1.1 μmol/l ($p < 0.01$), ALT activity – to $2.24 \pm 0, 1$ mmol/l ($p < 0.01$), ACT activity – to 1.35 ± 0.08 mmol/l ($p < 0.01$), which indicated liver involvement in the pathological process.

The content of direct bilirubin, the activity of ALT and ACT in blood in patients with IM with a mild course did not significantly differ from those in the control group.

Direct bilirubin content, ALT and ACT activity in blood normalized closer to the 20th day in the course of treatment in patients with moderate-severe IM, whereas in case of severe course – to the 30th day, and often later.

The results of the study of indicators of the oxidative and antioxidant systems in patients with IM are presented in table, from which it follows that TOA of blood plasma ($p < 0.01$), concentration in blood primary (DC) and secondary (MDA) lipid hydroperoxides ($p < 0.01$) increase significantly in accordance

with the severity of the disease in patients with IM. A high direct correlation was found ($r = 0.65$; $r = 0.69$) between the blood levels of DC and MDA, on the one hand, and the overall oxidative activity of blood plasma, on the other hand, which indicates the important role of lipid hydroperoxides in increasing the oxidative activity of blood plasma in these patients with IM.

The activation of lipid peroxidation in patients with IM of a mild course was accompanied by a statistically significant decrease in the TAA of blood plasma and erythrocytes ($p < 0.001$), a decrease in the activity of erythrocyte antioxidant enzymes – catalase ($p < 0.001$), superoxide dismutase ($p < 0.001$), glutathione peroxidase ($p < 0,01$), an increase in activity of plasma glutathione reductase ($p < 0.001$), reduced glutathione ($p < 0.001$), which indicates an increase in the activity of the glutathione system, which is involved in the inactivation of lipid hydroperoxides in the blood and tissues [11–13].

As the clinical, immunological and haematological parameters improved while treating patients with IM with a mild course, the indicators of the LPO decreased and the activity levels of the antioxidant system increased, and by the 20th day of treatment the concentration of DC in the blood decreased to 1.4 ± 0, MDA on average 03 μmol/l ($p < 0.001$), MDA on average up to 0.40 ± 0.03 μmol/l ($p < 0.001$) and TOA of blood plasma on average to 3.75 ± 0.4 % ($p < 0.001$). The plasma antioxidant activity was statistically significantly increased by an average of 6.6 ± 0.8 % and of erythrocytes by an average of 39.6 ± 1.6 % ($p < 0.001$), the activity of catalase increased on average to 66.4 ± 2,0 mmol/s.mg protein ($p < 0.001$) in erythrocytes, erythrocyte superoxide dismutase, on average, up to 62.2 ± 2.1 units/mg ($p < 0.001$), glutathione peroxidase, on average, up to 180.5 ± 9.0 μmol/s.mg protein ($p < 0.001$), blood plasma glutathione peroxidase, on average, up to 4.9 ± 0.13 mkkat/l ($p < 0.05$), plasma glugate reductase, on average, up to 2.1 ± 0.03 mkkat/l ($p < 0.05$), erythrocyte glutathione reductase, on average, up to 76.5 ± 2.5 μmol/s.mg protein ($p < 0, 05$), the concentration of total glutathione in blood did not significantly change ($p > 0.05$), the content of oxidized glutathione in blood decreased, on average, to 55.6 ± 6.4 mmol/l ($p < 0.05$), and the level of reduced glutathione increased, on average, to 974.8 ± 7.3 μmol/l ($p > 0.05$) and did not significantly differ from the corresponding indicators of the control group.

Analysis of the obtained data (tab. 1) showed that severity of the disease increases in parallel with the increase of the activity of lipid peroxidation and the decrease in the activity of the antioxidant system in patients with IM, that manifests in an increase in the TOA of blood plasma ($p < 0.001$) , the concentration of DC ($p < 0.001$) and MDA ($p < 0.001$), and in a decrease of the TAA of plasma ($p < 0.001$) and erythrocytes ($p < 0.001$), the actioxidative activity of erythrocyte enzymes – catalase ($p < 0.001$), superoxide dismutase ($p < 0.001$), glutathione peroxidase ($p <0.001$), glutathione reductase ($p < 0.001$); plasma glutathione peroxidase and plasma glutathione reductase ($p < 0.01$), the concentration of total and reduced glutathione in blood ($p < 0.001$) and the content of oxidized glutathione in blood ($p < 0.001$), which indicates a pronounced imbalance between the processes of radical oxidation of lipids and antioxidant protection.

Table

Indicators of lipid peroxidation and antioxidant systems in patients with IM (M ± m)

Indicator	Control (n=30)	Mild IM (n=58)	Moderate-severe IM (n=56)	Severe IM (n=44)
Total oxidative activity of plasma, %	4,3 ± 0,3	6,7 ± 0,45; p < 0,001	9,3 ± 0,52; p < 0,001	12,7 ± 0,75; p < 0,001
Dyne conjugates, μmol/l	1,2 ± 0,02	2,4 ± 0,03; p < 0,001	3,7 ± 0,06; p < 0,001	5,1 ± 0,07; p < 0,001
Malonic dialdehyde, moll/l	0,35 ± 0,015	0,8 ± 0,03; p < 0,001	2,2 ± 0,04; p < 0,001	2,8 ± 0,04; p < 0,001
Total antioxidant activity of plasma, %	8,2 ± 0,4	6,3 ± 0,08; p < 0,001	5,4 ± 0,65; p < 0,001	3,6 ± 0,05; p < 0,001
Total antioxidant activity of red blood cells, %	41,5 ± 1,2	34,2 ± 0,9; p < 0,001	22,7 ± 1,0; p < 0,001	11,4 ± 0,8; p < 0,001

Continuation of the table

Erythrocyte catalase activity, mmoll/sg protein	67,2 ± 2,6	50,4 ± 1,7; p < 0,001	42,6 ± 1,2; p < 0,001	30,4 ± 1,1; p < 0,001
Erythrocyte superoxide dismutase activity, units/mg Hb	63,8 ± 2,3	53,4 ± 1,8; p < 0,001	43,7 ± 1,8; p < 0,001	36,2 ± 1,5; p < 0,001
Total blood glutathione, mmol/l	1076 ± 8,2	990,0 ± 9,4; p < 0,05	735,6 ± 8,6; p < 0,001	564,7 ± 6,4; p < 0,001
Oxidized blood glutathione, mmol/l	50,4 ± 4,7	86,5 ± 5,2; p < 0,001	215,6 ± 4,6; p < 0,001	254,5 ± 3,9; p < 0,001
Reduced blood glutathione, mmol/l	940,5 ± 8,3	990,0 ± 8,2; p < 0,001	524,7 ± 6,0; p < 0,001	326,3 ± 3,6; p < 0,001
Erythrocyte glutathione peroxidase activity, μmol/sg protein	183,8 ± 5,6	159,4 ± 6,1; p > 0,05	91,6 ± 5,2; p < 0,001	62,7 ± 4,5; p < 0,001
Plasma glutathione peroxidase activity, mkkat/l	3,9 ± 0,15	3,1 ± 0,13; p < 0,01	1,85 ± 0,02; p < 0,001	0,9 ± 0,02; p < 0,001
Glutathione reductase plasma activity, mkkat/l	2,0 ± 0,02	2,9 ± 0,03; p < 0,001	1,6 ± 0,01; p < 0,001	0,9 ± 0,01; p < 0,001
Erythrocyte glutathione reductase activity, μmol/sg protein	73,7 ± 2,6	96,5 ± 2,0; p < 0,001	62,7 ± 1,9; p < 0,001	53,7 ± 1,9; p < 0,001

Note: Reliability of differences p compared with the control group.

The activity of lipid peroxidation decreases and the activity of the anti-peroxide system increases while conducting antiviral therapy in patients with moderate-severe and severe course of IM, however, the full normalization of the activity indicators of these systems was not observed by the 30th day of the disease, which is consistent with the data of other authors [14], and indicates a severe violation of antioxidant protection in patients with severe and moderate-severe forms of IM, which may be associated with adverse outcomes of IM.

DISCUSSION

Lipid peroxidation is one of the most important biological processes that constantly occurs in the human body and is actively involved in the processes of adaptation, anti-infective protection, removal of endo- and exotoxins, tumour cells and destroyed tissues, regulation of vascular tone, permeability of cell membranes and the vascular wall, haemostasis, etc. [13, 15–16]. Under normal conditions, LPO flows continuously inside all cells at an extremely low level, providing regulation of the structure and function of cell membranes [15]. The most reactive in this regard cells are of the monocyte-macrophage system of the blood, Kupffer cells of the liver, alveolar macrophages, macrophages of connective tissue, Langerhans cells, osteoclasts, glial astrocytes, which activated when exposed to infection, antibodies, aggregated Ig, leptins, C-reactive protein, complement components [17].

EBV in patients with IM leaves an enveloped antigen on the cell membrane, modifying it by penetrating B lymphocytes, which leads to activation of NK cells and cytotoxic T lymphocytes [18–20], and destruction of infected cells. As a result of cytolysis, cell degradation products enter the bloodstream, which are phagocyted by the means of neutrophilic granulocytes and macrophages.

Granulocyte lysosomes contain the enzyme NAD, NAD-P-oxidase, which is activated during phagocytosis and catalyses the recovery process of molecular oxygen with the formation of oxygen with an unpaired electron (O^-). The intracellular superoxide dismutase (SOD) enzyme binds this oxygen with hydrogen producing hydrogen peroxide (H_2O_2), which is then cleaved by catalase and glutathione peroxidase with the formation of hydroxide (HO^-) and hydroperoxide (HO_2^-) radicals, which inactivate glutathione. At the same time, glutathione is converted to the oxidized form, and under the influence of the glutathione reductase, it turns into reduced glutathione and provides binding of lipid hydroxides [21–23]. Glutathione peroxidase and glutathione transferase are able to restore the hydroperoxy groups of oxidized phospholipids directly in the cell membranes without prior hydrolysis by phospholipase or free fatty acids.

Consequently, glutathione and enzymes of its metabolism are one of the most important universal defence mechanisms and the central link of the homeostatic system of the body, they play a primary role in the formation of the body's resistance to aggressive factors [24–25].

Glutathione is formed in the liver and contains glutamic acid, cysteine and glycine. Glutathione including functional group of SH participates in oxidation-reduction reactions, being a donor of hydrogen, passing from reduced to the oxidized form.

The antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione) maintain free radical oxidation at a safe level under the normal conditions, they restore syngeneic oxygen creating less active forms, and also suppress the formation of HO^-, HO_2^-, H_2O_2 and destroy lipid hydroxides formed in excess amount [26].

The LPO system is well balanced and functions on the basis of feedback, since LPO products inhibit the activity of antioxidant enzymes. However, activation of LPO is accompanied by increased activity of glutathione reductase and the concentration of reduced glutathione in the blood in patients with mild IM, which connects the primary and secondary hydroxide lipids, thereby significantly accelerating the process of the activity antioxidant enzymes recovery (catalase, superoxide dismutase, glutathione peroxidase), blood plasma antioxidant activity and red blood cells.

The activation of the LPO leads to a deep suppression of the antiperoxide system activity, and its normalization proceeds at a slower rate over a long period in patients with IM of severe and moderate-severe course.

We found a decrease in the activity of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase in plasma and erythrocytes, the concentration of total and reduced glutathione in the blood of IM patients with severe and moderate-severe course, that indicates a deep disruption of the functioning of various parts of the antioxidant system and contributes to the increased formation of active forms of oxygen, hydroxide and hydroperoxide radicals, hydrogen peroxide in cells of the antioxidant system causing oxidation of unsaturated fatty acids of cell membrane and the formation of primary and secondary lipid hydroperoxides, which have a toxic effect not only on EBV, but also on other cellular structures.

Lipid peroxides are highly reactive and can suppress the activity of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase, cause the degradation of SH-containing compounds, depolymerisation of DNA, induce vasospasm, increase the permeability of cell membranes, endothelium damage, enhance platelet aggregation [27].

Oxidized glutathione accumulates in the tissues of patients with severe and moderate-severe IM as a result of a decrease in the activity of glutathione reductase, and also has toxic properties. Oxidized glutathione inactivates membrane ATPase, hexokinase, glucose-6-dehydrogenase, inhibits phosphorylation and nuclear synthesis of RNA, inhibits protein synthesis due to the formation of protein-thiol-glutathione crosslinks [28].

If the systems of generation and inactivation of free radicals are not balanced, the ordered structure of the monooxygenase complex can be destroyed, and its components can attack the polyunsaturated fatty acids of the surrounding membranes in the absence of a normal substrate.

In this case, the formation of toxic lipid peroxides is possible, and subsequently – secondary products of LPO, which disrupt the functional state of the liver that plays an important role in the processes of detoxification in various pathological conditions. Glutathione, glutathione peroxidase, glutathione reductase and cytochrome P-450 are formed in hepatocytes and involved in inactivating of primary and secondary lipid hydroperoxides. As a result of the toxic effect of active forms of oxygen and lipid hydroperoxides in the liver, blood circulation is disturbed and hypoxia of hepatocytes develops, lipid peroxidation is activated in cell membranes and the activity of antioxidant enzymes and cytochrome P-450 is reduced. These processes result in hepatocytes destruction, which is accompanied by an increase in direct bilirubin, the activity of ACT and ALT in blood. A high direct correlation was found between the indicators of plasma TOA, direct bilirubin concentration, ALT and ACT activity in the blood ($r = 0.62$; $r = 0.64$; $r = 0.62$), which indicates the important role of the LPO products in the development of cytolytic syndrome in patients with severe and moderate-severe course of IM.

Our study showed that, the process of lipid peroxidation increases and the activity of the antioxidant system decreases in parallel with the severity of the disease in patients during the acute period of the IM. Deficiency of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase), reduction of their activity and concentration of total glutathione in the blood of patients with IM lead to the breakdown of protective mechanisms and, as a result, to increased LPO-free radical chain process, which uncontrolled growth causes irreversible damage to the membranes of various cells underlying visceropathy in patients with IM.

Consequently, the level of adaptive capacity of antioxidant enzymes decreases in patients with IM. It may be a result of primary or secondary enzymopathies due to the toxic effect of reactive forms of oxygen and lipid hydroperoxides on enzymes. Our findings confirm the expediency of using antioxidants in the treatment of patients with IM.

CONCLUSIONS

1. Lipid peroxidation significantly increase and the activity of the antioxidant system decrease in patients with infectious mononucleosis in the acute period of the disease, as evidenced by the increased concentration of dyne conjugates, malonic dialdehyde and total oxidative plasma activity in the blood, reduced activity of the total antioxidant activity of plasma and erythrocytes, the decrease in the activity of antioxidant enzymes of erythrocytes catalase, superoxide dismutase, glutathione peroxidase, glutathione peroxidase and glutathione reductase of blood plasma, the decrease in the concentration of glutathione in the blood.

2. The most pronounced disorders of lipid peroxidation and antioxidant system activity were found in patients with moderate-severe and severe infectious mononucleosis.

3. The development of cytolytic syndrome in infectious mononucleosis is associated with the action of reactive oxygen forms species and lipid hydroperoxide.

PROSPECTS FOR FUTURE RESEARCHES

It seems to be appropriate to study further clinical course of IM after using various antioxidants in addition to existing therapy to test the ability to suppress viral replication and to define the most effective compound.

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FEATURES OF CYTOKINE STATUS IN PATIENTS WITH CHRONIC EBV-INFECTIONS

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Infections caused by EBV are the most common and occupy an important place in the structure of herpes aetiology diseases.

The purpose of this work was to study the characteristics of the cytokine status in patients with chronic EBV infection, depending on the level of viral replication.

We examined 78 patients with chronic EBV infection, the main clinical manifestations of which were various immunopathological and immunodeficiency states: Group I – with low, Group II – with medium, Group III - with a high degree of viral replication. The Tiff method was used using the Vector-Best reagent kits (Novosibirsk, Russia) to study the cytokine profile in the serum of patients with EBV infection. The determination of alpha and gamma fractions of serum interferon was carried out using the ELISA method by means of the ProCon IF2 plus reagent kit manufactured by Protein Contour LLC (St. Petersburg, Russia).

As a result of a study of the cytokine status in patients with chronic EBV infection, it was found that in all three groups there was a significant increase in both pro-inflammatory (IL-1β, IL-6, TNF-α) and anti-inflammatory cytokines (IL-10, IL 4, TGFβ1). However, anti-inflammatory cytokinemia was more compensated in group I patients compared with patients in groups II and III. A decrease in IFN-α and IFN-γ was detected in all patients with chronic EBV infection while studying the interferon status. A correlation was found between the level of viral replication and a decrease in the level of IFN-α and IFN-γ.

The identified features of the cytokine status in patients with chronic EBV infection can be used to optimize therapy and help develop a differentiated approach to the immunocorrection of these patients, depending on the level of viral replication.

KEY WORDS: interferon, interleukins, cytokines, chronic EBV infection, viral replication level

ОСОБЛИВОСТІ ЦИТОКІНОВОГО СТАТУСУ У ХВОРИХ НА ХРОНІЧНУ ВЕБ-ІНФЕКЦІЮ

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Інфекції викликані ВЕБ є найбільш поширеними і займають важливе місце в структурі захворювань герпесвірусной етіології.

Метою даної роботи було вивчити особливості цитокінового статусу у хворих на хронічну ВЕБ-інфекцію, в залежності від рівня вірусної реплікації.

Нами було обстежено 78 пацієнтів з хронічною ВЕБ-інфекцією, основними клінічними проявами в яких були різні іммунопатологичні і імунодефіцитні стани: I група – з низьким ступенем вірусної реплікації, II група – із середнім ступенем вірусної реплікації, III група – з високим ступенем вірусної реплікації. Для дослідження цитокінового профілю в сироватці крові хворих на ВЕБ-інфекцію використовували метод тІФА з використанням наборів реагентів ЗАТ «Вектор-Бест» (Новосибірськ, Росія). Визначення фракцій сироваткового інтерферону альфа та гамма здійснювали за допомогою твердофазного імуноферментного методу з використанням набору реагентів ProCon IF2 plus виробництва ТОВ «Протеїновий контур» (Санкт-Петербург, Росія).

За результатами дослідження цитокінового статусу у хворих на хронічну ВЕБ-інфекцію було виявлено, що в усіх трьох групах відзначалося суттєве підвищення як прозапальних (ІЛ-1β, ІЛ-6, ФНП-α), так і протизапальних цитокінів (ІЛ-10, ІЛ-4, ТФРβ1). Однак, протизапальна цитокінемія була у пацієнтів І групи більш компенсованою у порівнянні з пацієнтами ІІ та ІІІ груп. При досліджені інтерферонового статусу було виявлено зниження ІФН-α та ІФН-γ в усіх хворих на хронічну ВЕБ-інфекцію. Було виявлено кореляційний зв'язок між рівнем реплікації вірусу та зниженням рівнем ІФН-α та ІФН-γ. Виявлені особливості цитокінового статусу у хворих хронічною ВЕБ-інфекцією, в залежності від рівня вірусної реплікації, можуть бути використані для оптимізації терапії та допоможуть розробити диференційований підхід до імунокорекції таких пацієнтів.

КЛЮЧОВІ СЛОВА: інтерферон, інтерлейкіни, цитокіни, хронічна ВЕБ-інфекція, рівень реплікація вірусу

ОСОБЕННОСТИ ЦИТОКИНОВОГО СТАТУСА У БОЛЬНЫХ ХРОНИЧЕСКОЙ ВЕБ-ИНФЕКЦИЕЙ

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Инфекции, вызванные ВЭБ, являются наиболее распространенными и занимают важное место в структуре заболеваний герпесвирусной этиологии.

Целью данной работы было изучить особенности цитокинового статуса у больных хронической ВЭБ-инфекцией, в зависимости от уровня вирусной репликации.

Нами было обследовано 78 пациентов с хронической ВЭБ-инфекцией, основными клиническими проявлениями у которых были разные иммунопатологические и иммунодефицитные состояния: I группа – с низкой, II группа – со средней, III группа – с высокой степенью вирусной репликации. Для исследования цитокинового профиля в сыворотке крови больных ВЭБ-инфекцией использовали метод Тифа с использованием наборов реагентов ЗАО «Вектор-Бест» (Новосибирск, Россия). Определение фракций сывороточного интерферона альфа и гамма осуществляли с помощью твердофазного иммуноферментного метода с использованием набора реагентов ProCon IF2 plus производства ООО «Протеиновый контур» (Санкт-Петербург, Россия).

В результате исследования цитокинового статуса у больных хронической ВЭБ-инфекцией было выявлено, что во всех трех группах отмечалось существенное повышение как провоспалительных (ИЛ-1β, ИЛ-6, ФНО-α), так и противовоспалительных цитокинов (ИЛ-10, ИЛ 4, ТФРβ1). Однако противовоспалительная цитокинемия была у пациентов I группы более компенсированной по сравнению с пациентами II и III групп. При исследовании интерферонового статуса было выявлено снижение ИФН-α и ИФН-γ у всех больных хронической ВЭБ-инфекцией. Была обнаружена корреляционная связь между уровнем репликации вируса и снижением уровнем ИФН-α и ИФН-γ.

Выявленные особенности цитокинового статуса у больных хронической ВЭБ-инфекцией, в зависимости от уровня вирусной репликации, могут быть использованы для оптимизации терапии и помогут разработать дифференцированный подход к иммунокоррекции таких пациентов.

КЛЮЧЕВЫЕ СЛОВА: интерферон, интерлейкины, цитокины, хроническая ВЭБ-инфекция, уровень репликация вируса

INTRODUCTION

Every year, the impact of adverse environmental factors increases on the body and, particularly on the immune system. In the modern world, infectious diseases occupy a leading place in human pathology [1]. Among the numerous factors that directly affect the immune system, special attention is paid to herpesvirus infections. Nowadays there is a tendency for the diseases caused by herpesviruses to spread widely [2–3].

The Epstein-Barr virus (EBV) belongs to the Herpesviridae family. Infections caused by this virus are the most widespread and occupy an important place in the structure of herpesvirus aetiology diseases. This is due to the high degree of infection of the population

all over the world, as specific antibodies to this virus are found in almost 95 % of the adult population [4–5]. Active proliferation of the virus in all organs and systems that have lymphoid tissue, leads to structural changes, which has a negative impact on the whole body [6]. EBV has multiple mechanisms of immunosuppression and protection from the host's immune response that can lead to the formation of a chronic viral infection and immunological disorders deepening [7]. It is also established that EBV violates the mechanisms of the immune response, suppresses the production of interferons (INFs), blocks the mechanisms of apoptosis. A secondary immunodeficiency is formed on the basis of these disorders, that promotes the development of autoimmune and tumour processes in genetically predisposed individuals. The study of diseases caused by EBV is relevant due to the life-span persistence of the virus in the human body, the potential for oncogenicity, and the ability to reactivate autoimmune diseases, to damage the heart, kidneys, joints, and others [2, 8–9].

Data obtained from many studies suggest a violation of the interferon chain of immunity while herpesvirus infections. Low content of these cytokines does not provide sufficient antiviral effect. In this regard, the study of indicators of immunity provided by interferons becomes particularly relevant in patients with chronic EBV infection [10].

Non-specific factors of protection, including interferons are the first line response while infecting the human body and they induce the increase of the body's resistance. The course and effects of the disease depend on the rate of INF system involvement in the process of INF antiviral protection. The untimely or reduced production of its own IFN, as a rule, leads to the chronization of the WEB infection. Interferon-alpha (IFN-α) is the most active in the early phase of the EBV infection, when even the EBV antigens do not spred beyond the cell, and products of specific antibodies are not possible. α-IFN prevents the spread of EBV by providing the antiviral function from the site of penetration into the body to distant organs and systems.

Interferon gamma (IFN-γ) is an indicator of activity of the cellular immunity. It also has an antiproliferative effect, which is relevant in case of EBV infection, which is an antigen of many tumours and neoplasia. However it is known that EBV is capable of suppressing the IFN-γ synthesis and thus suppressing cellular immunity, creating conditions for its lifelong persistence [11].

OBJECTIVE

The purpose of this work was to study the cytokine status features in patients with chronic EBV infection, depending on the level of viral replication.

MATERIALS AND METHODS

We examined 78 patients with chronic EBV infection, the main clinical manifestations of which were various immunopathological and immunodeficient conditions. All patients in the main group were divided into 3 groups according to the level of viral replication: Group I – with low degree of viral replication, composed of 25 people (11 men and 14 women); Group II – with an average degree of viral replication, included 27 patients (12 men and 15 women); Group III – with a high degree of viral replication, consisted of 26 patients (12 men and 14 women). The age of patients ranged from 19 to 57 years (mean age was 33 years ± 11.7 years). The control group consisted of 20 practically healthy people aged from 20 to 35 years.

The work was performed at the Department of General and Clinical Immunology and Allergology of the Medical Faculty of the V. N. Karazin Kharkov National University and clinical bases of the department: Regional Clinical Infectious Hospital in Kharkiv and the Clinical Hospital «City Polyclinic № 6», as well as on the basis of the SI «I. I. Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine» in the period of 2014–2018 in the framework of the research topic: «The study of the role of immune, autoimmune and metabolic disorders in pathogenesis and the consequences of the infection process caused by herpesviruses», state registration number 0112U005911. The analysis of samples and their technical implementation was carried out in the clinical diagnostic laboratory of the Regional Clinical Infectious Hospital, part of the analyses were carried out in the laboratories «Virolos» and «Analitika». The TIFA method was used by means of the

reagent kits of CJSC Vector-Best (Novosibirsk, Russia) using the manufacturer's instructions to investigate the cytokine profile in the blood serum of patients with EBV infection. Determination of alpha and gamma fractions of serum interferons was performed using a solid phase immunoassay method by means of the set of ProCon IF2 plus reagents manufactured by the Protein Contour LLC (St. Petersburg, Russia).

The statistical processing of the results of the study was conducted using the STATISTICA 10.0 statistical software package.

RESULTS AND DISCUSSION

IFN-α level was reduced in patients with chronic WEB infection with different levels of virus replication in all studied groups compared with the control group (tab. 1).

Table 1

The content of IFN in serum in patients with chronic WEB infection

Indicator	I group	II group	III group	Control group	p
IFN-α, pg/ml	30,3 ± 1,7	27,5 ± 1,9	25,6 ± 1,8	32,4 ± 2,3	p < 0,001
IFN-γ, pg/ml	37,1 ± 1,8	35,3 ± 1,6	25,4 ± 1,7	38,2 ± 1,6	p < 0,001

Thus, IFN-α was 30.3 ± 1.7 g/ml in I group patients, in group II – 27.5 ± 1.9 g/ml, in group III – 25.6 ± 1.8 g/ml. Thus, there was a correlation between the level of viral replication and a decrease in the level of IFN-α in chronic EBV infection. Possibly, EBV conversion from a latent state and its replication (reactivation of EBV infection) occurred under favourable conditions. A significant decrease in IFN-α and, as a consequence, a significant inhibition of antiviral defence, contributed to the accumulation of an active infectious agent that was actively multiplying.

According to many studies, in the majority of patients with acute EBV infection, a significant increase in IFN-γ levels is usually observed in comparison with relatively healthy people. This indicates cellular immunity level and its predominance over the humoral link. Probably, therefore, in many cases, the acute form of the EBV infection ends with a rapid and, as a rule, complete recovery [12–13].

Among our patients with chronic forms of EBV infection, IFN-γ was decreased in all studied groups compared with the control group. Thus, IFN-γ was 37.1 ± 1.8 pg/ml in group I patients, in group II patients – 35.3 ± 1.6 pg/ml, in group III patients – 25.4 ± 1.7 pg/ml. So, it is probably the IFN-γ deficiency that led to protracted forms of infection with possible latent (for several years), or recurrent course. Probably the decrease in the content of IFN-γ may be considered as one of the indicators of prolonged EBV infection.

It was found an increase in all indicators in all three groups (I, II, III) compared to the control group during the studying of the content of cytokines in blood serum of patients with chronic EBV infection, but these changes, had their own characteristics due to the level of replication of the virus (tab. 2). A higher anti-inflammatory cytokinemia and a deeper cytokine imbalance were recorded in patients of groups II and III compared with patients in group I.

Table 2

The content of the main serum cytokines in patients with chronic EBV

Indicator	I group	II group	III group	Control group	p
IL-1β, pg/ml	59,1 ± 2,1	56,4 ± 2,4	56,3 ± 2,3	29,5 ± 1,9	p < 0,001
IL-6, pg/ml	91,2 ± 2,1	100,3 ± 2,7	104,4 ± 3,6	39,2 ± 1,7	p < 0,001
TNF-α, pg/ml	52,8 ± 2,4	62,4 ± 2,2	65,7 ± 2,8	32,6 ± 1,8	p < 0,001
IL-2, pg/ml	78,1 ± 2,5	68,4 ± 2,4	68,3 ± 2,6	40,2 ± 2,1	p < 0,001
IL-10, pg/ml	91,3 ± 1,8	88,3 ± 2,1	88,2 ± 1,9	19,1 ± 1,5	p < 0,001
IL-4, pg/ml	80,3 ± 2,3	75,6 ± 2,6	75,5 ± 2,2	21,2 ± 1,7	p < 0,001
TGFβ1, pg/ml	172,4 ± 3,1	166,5 ± 2,9	166,3 ± 2,2	58,3 ± 2,2	p < 0,001

Thus, there was a more pronounced increase in IL-1β (59.1 ± 2.1 pg/ml) in case of the group I than in patients in groups II and III (56.4 ± 2.6 pg/ml and 56.3 ± 2.3 pg/ml, respectively) in comparison with the control group (29.5 ± 1.9 pg/ml).

It was found during the study that IL-6 increase was observed in all patients with chronic EBV infection in comparison with the control group, but this indicator increased to a greater extent in patients of groups II and III (100.3 ± 2.7 pg/ml and 104.4 ± 3.6 kg/ml, respectively). IL-6 was at 91.2 ± 2.1 pg/ml in patients of group I, while in the control group it was 39.2 ± 1.7 pg/ml.

The level of TNF-α was elevated in all patients with chronic EBV infection compared to the control group, but it was higher in patients of groups II and III (62.4 ± 2.2 g/ml and 65.7 ± 2.8 g/ml in accordance). In patients of Group I, the FNP-α was 52.8 ± 2.4 pg/ml and in the control group it was 32.6 ± 1.8 pg/ml.

It is known that IL-2 is an important factor in the activation of immune cells and the development of a full-fledged immune response [14–16]. IL-2 levels were also elevated in all patients with chronic EBV infection, but this increase was not as significant as an increase in IL-1, IL-6 and FNP-α levels. Thus, IL-2 levels were higher (78.1 ± 2.5 pg/ml) in group I patients than in patients of groups II and III (68.4 ± 2.4 pg/ml and 68.3 ± 2.6 pg/ml, respectively). In the control group, IL-2 was at 40.2 ± 2.1 pg/ml.

Concentration of IL-10 in serum was elevated in all patients with chronic EBV infection compared to control group. However, IL-10 levels increased to a greater extent (91.3 ± 1.8 pg/ml) in group I patients, compared with patients in groups II and III (88.3 ± 2.1 pg/ml and 88.2 ± 1.9 pg/ml, respectively). In the control group, this indicator was 19.1 ± 1.5 pg/ml.

The level of IL-4 was elevated in all patients with chronic EBV infection compared to the control group, but it reached much higher figures (80.3 ± 2.3 g/ml) in group I patients, compared to patients of groups II and III (75.6 ± 2,6 pg/ml and 75.5 ± 2.2 pg/ml, respectively). In the control group, this figure was 21.2 ± 1.7 pg/ml.

The concentration of TGFβ1 was elevated in all patients with chronic EBV infection compared to the control group, but in patients of group I it level increased greater (172.4 ± 3.1 pg/ml), compared to patients of groups II and III (166.5 ± 2.9 μg/ml and 166.3 ± 2.2 μg/ml, respectively). This indicator was 58.3 ± 2.2 pg/ml in the control group.

Thus, anti-inflammatory cytokinemia was more compensated in patients of group I, compared with patients of groups II and III, as indicated by the ratio of proinflammatory and anti-inflammatory cytokines. It is known that anti-inflammatory cytokines are involved in limiting the inflammatory response, inhibit the secretion of proinflammatory cytokines, and reduce the damaging effect of inflammation on tissues [17–20].

CONCLUSIONS

Thus, our study of cytokine status in patients with chronic EBV infection showed that a significant increase in all three groups in both proinflammatory (IL-1β, IL-6, FNP-α) and anti-inflammatory cytokines (IL-10, IL-4, TFRβ1). However, the following features were revealed depending on the level of viral replication: elevation of IL-1β, IL-10, IL-4, TFRβ1 to a greater extent was observed in patients of group I, compared to patients in groups II and III, in which IL-6, FNP-α prevailed. Thus, anti-inflammatory cytokinemia was more compensated in patients group I compared to patients of groups II and III, as shown by the ratio of proinflammatory and anti-inflammatory cytokines. IL-2 levels were also elevated in all patients with chronic EBV infection, but this increase was not as significant as the increase in other cytokines levels, which in turn affected the process of activation of immune-competent cells and prevented the formation of a complete immune response.

IFN-α and IFN-γ levels were decreased in all patients with chronic EBV infection when interferon status was studied. A correlation was found between the level of viral replication and a decrease in IFN-α and IFN-γ levels. A significant decrease in IFN-α contributed to significant inhibition of antiviral defence, which in turn effected the accumulation of an active infectious agent that was actively multiplying. The IFN-γ deficiency did not allow forming a sufficient immune response and contributed to the development of a chronic form of infection. Probably the decrease in the content of IFN-γ may be considered as one of the indicators of

the prolong course of EBV infection and the development of chronic forms of EBV infection.

PROSPECTS FOR FUTURE STUDIES

The revealed features of cytokine status in patients with chronic EBV infection can be used to optimize therapy depending on the level of viral replication and will help to develop a differentiated approach to immune correction of such patients.

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MODERN FEATURES OF CHICKEN POX COURSE IN ADULTS

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The article presents the epidemiological and clinical data of the modern course of chickenpox. A high incidence among adults with a bright manifestation of the clinical picture was shown. Recurrent episodes of chicken pox were marked.

KEY WORDS: VZV, chicken pox, epidemic process, clinical picture, adults

СУЧАСНІ ОСОБЛИВОСТІ ПЕРЕБІГУ ВІТРЯНОЇ ВІСПИ У ДОРОСЛИХ

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У статті представлені епідеміологічні та клінічні дані сучасного перебігу вітряної віспи. Показана висока захворюваність серед дорослого населення з яскравою маніфестацією клінічної картини. Відзначено повторні епізоди вітряної віспи.

КЛЮЧОВІ СЛОВА: VZV, вітряна віспа, епідемічний процес, клініка, дорослі

СОВРЕМЕННЫЕ ОСОБЕННОСТИ ТЕЧЕНИЯ ВЕТРЯНОЙ ОСПЫ У ВЗРОСЛЫХ

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В статье представлены эпидемиологические и клинические данные современного течения ветряной оспы. Показана высокая заболеваемость среди взрослого населения с яркой манифестацией клинической картины. Отмечены повторные эпизоды ветряной оспы.

КЛЮЧЕВЫЕ СЛОВА: VZV, ветряная оспа, эпидемический процесс, клиника, взрослые

INTRODUCTION

Chickenpox is a disease with global prevalence. 80–90 million cases of chickenpox are registered worldwide every year. The incidence of varicella remains stably high with small variations over the years and is determined by the presence of acquired immunity in the population. In the European Region, chickenpox is also characterized by a high intensity of the epidemic process. The incidence rates were: in Spain – 2.5–5.5 %, France – 1000–1350 %, Slovenia – 770.0 %, in Latvia – 277.9 %, in Estonia – 580.8 %, the Netherlands – 253.5 % [1]. In addition, in the last decade there has been a tendency to an increase in the incidence rate in Ukraine [2]. According to experts, in the near future in Ukraine there may be a large-scale epidemic of chickenpox [3].

The current epidemic process of chickenpox is characterized by the tendency of «maturity» of the infection, especially among the urban population. Every year from 5 to 6 % of cases are diseases among adults. Sero-epidemiological studies show regional differences in the proportion of susceptible adults [4]. Some studies has shown that there was a remarkable increase in the incidence rate of chickenpox in middle and older age groups till 2014 and before chickenpox and shingles vaccination [5–7]. Varicella zoster has also been associated with elevated risk for cerebrovascular disease and contributes to vasculopathy that is especially important matter in adults [8–9]. Despite the low incidence of chickenpox in adults, the risk of severe

complications and even death is 10–20 times higher than in children. Most often the disease is benign, but in 1 out of 50 cases of the disease there are complications, the most severe are pneumonia and encephalitis among them. The incidence of encephalitis, which is predominantly manifested by cerebral ataxia, is about 1 in 4000 cases of chicken pox. Pneumonia can be both primary viral and bacterial in nature [10–11].

The significance of chickenpox is also determined by the presence of a chronic recurrent form of the infection – shingles, the frequency of which is about 70 per 100,000 people who have had chickenpox. The Varicella-Zoster virus (VZV) is epitheliotropic and neurotropic, but it is also possible to generalize the process with damage to the internal organs: liver, lungs, and gastrointestinal tract [12–14]. The most important risk factors associated with the severity of varicella, generalization of the process and death are age and impaired function of the immune system [15–17].

OBJECTIVE

The aim of this study is to assess the increase in the chickenpox incidence and the current course of the disease among the adult population of the Kharkiv region on the basis of hospitalization data from 2015 to 2018 years.

MATERIALS AND METHODS

There were 174 patients with diagnosed chickenpox under our supervision, who were hospitalized in the Regional Clinical Infectious Diseases Hospital during the period from 2015 to 2018. The median age of the examined patients was 23 ± 5.8 years. Gender analysis showed the prevalence of males – 106 (61 %) men versus 68 (39 %) women. This distribution among the adult population is explained by varicella outbreaks among military personnel living in barracks – 42 (24 %) cases. The diagnosis was established on the basis of clinical, epidemiological data and was confirmed by serological and molecular genetic techniques. Serological studies were performed by the method of enzyme-linked immunosorbent assay (ELISA) with the detection of specific antibodies, IgM (qualitative) and IgG (quantitative), to the antigens of the VZV. Molecular genetic studies of samples for the detection of presence of VZV DNA were carried out by PCR with hybridization-fluorescence detection of results in real time.

RESULTS AND DISCUSSION

162 (93.1 ± 3.8 %) patients among the 174 observed had contact with chickenpox patients. In particular, 15 people had contact with sick children (9.2 ± 0.4 %), at the work place – 21 people (12.9 ± 4.6 %), 138 patients (85.2 ± 5.9 %) were hospitalized not only according to clinical, but also epidemiological indications: persons living in hostels and military personnel hospitalized from barracks where varicella outbreaks were recorded; in 12 cases (6.9 ± 0.4 %), no direct contact with patients with chicken pox was detected. Thus, the majority of patients had direct contact with patients with chickenpox during the height of the disease, that is, during the period of the greatest infectiousness of patients. At the same time, an important role belongs to close contact (accommodation in hostels, barracks). Recurrent chickenpox disease was diagnosed in 12 (6.9 %) patients, the interval between these episodes ranged from 5 to 12 years. In this group of patients the clinical course of the disease did not have any features. There was a characteristic winter-spring seasonality of chicken pox with a maximum frequency of seeking medical help in March, December, January (fig. 1).

Chickenpox in adults is characterized by a longer prodromal period, if it is 24-72 hours in children, then in adults it can reach 7-10 days, thereby making it difficult to timely diagnose and increasing the risk of infection of contact persons. The prodromal period in our patients lasted for 2.5 ± 1.5 days and was characterized by an increase in temperature of 38.7 ± 1.3 ºC, general weakness, decreased performance, lethargy, and drowsiness in all patients. An increase in body temperature was observed on the first day of the disease – in 108 (62.3 ± 5.7 %) patients. However, this symptom appeared on the second day of the disease in 41 (23.6 ± 4.7 %) patients, on the 3–4th day of the disease in 17 (9.8 ± 2.7 %), and it was short-term for one day in 8 (4, 6 ± 0.7 %) patients (temperature rise to 37.2 ± 0.5 ºC). Clinical manifestations of the prodromal period are presented in fig. 2.

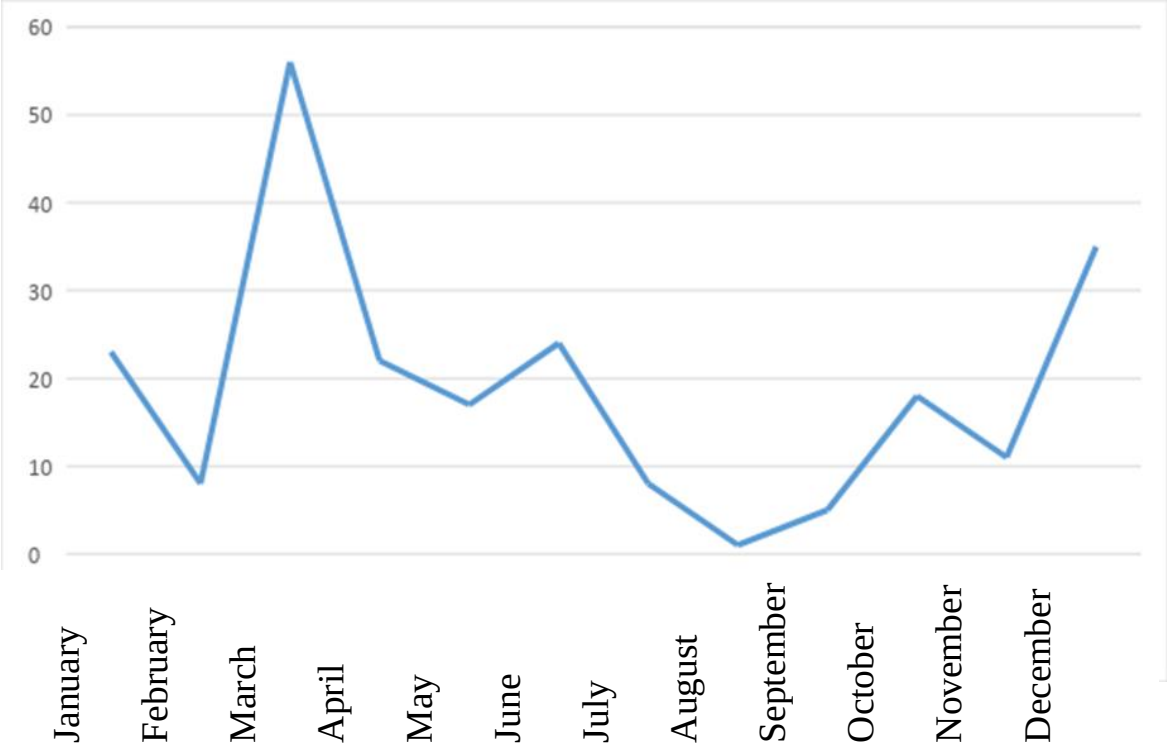

Fig. 1. Dynamics of incidence of chickenpox depending on seasonality

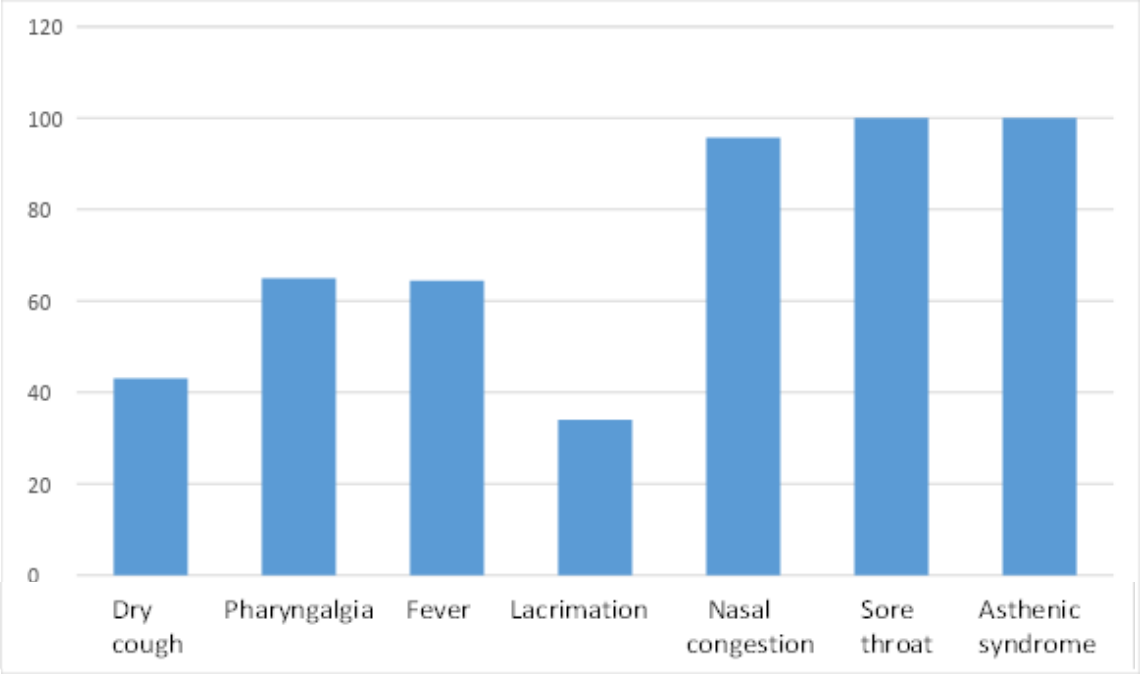

Fig. 2. Clinical manifestations of the chickenpox prodromal period in adults

Hospitalization of 139 (80.1 ± 5.0 %) patients was carried out during the rash on the 2–3rd day of illness. However, hospitalization of 6 (12.8 ± 4.0 %) patients was noted and in the later stages of the disease – on the 4–5th day. The period of rash began predominantly with the appearance of several spotty-papullosis elements, which were localized on the face and scalp (48.5 %), in the ear areas (22.8 %) or simultaneously on the scalp and behind the ear (28.5 %). In the next 1–4 days, the rash began to spread to the neck, torso,

upper and lower extremities. At the same time, the rash was scanty on the lower limbs in 131 (75.3 ± 5.2 %) patients. Within a few hours, the rash acquired the character of vesicles with transparent contents. These rashes were characterized by the phenomenon of pouring in addition, while in every second patient the rash was accompanied by skin itch. The rash became polymorphic – spotty-papular-vesicular on the 3–4th day from the beginning of the appearance of the first elements, then gradually began to dry out with the formation of crusts, which completely fell away on the 7th–12th day. The rash was abundant in 45 (25.9 ± 5.7 %) patients. A distinctive feature of the rash was rapid and abundant pustulization, which was observed in almost half of the patients (54.3 ± 5.9 %). Pustalization was accompanied by a significant (39–40 °C) rise in temperature, and in 14 (20 %) patients pyoderma was so pronounced that it required additional antibacterial therapy, and in 7 cases the disease had severe coarse of the disease associated with the severity of pyoderma. It should also be noted that the period of rash was accompanied by intoxication and astheno-vegetative syndromes: severe headache, malaise and weakness in 100 % of patients. The febrile period lasted 6.0 ± 2.0 days in uncomplicated cases, with the development of complications that continued to 9.0 ± 2.0 days. Usually, the temperature returned to normal with the end of the period of pouring. Catarrhal phenomena were observed in all patients at the height of the disease. So, the mucosa of the oropharynx was vividly hyperaemic in 84 (48.3 ± 5.2 %) patients, moderate hyperaemia was observed in 58 (33.3 ± 4.6 %), and weak – in 32 (18.4 ± 5.9 %) patients, at the same time the granularity of the tonsils, palatine arches and posterior pharyngeal wall was observed in all patients. The exanthema was accompanied by exanthema on the oral mucosa, mainly in the palatal arches and the soft palate, in 80 (45.6 ± 5.8 %) patients. Exanthema initially had the appearance of bright pink papules, and then turned into bubbles, which quickly opened to form sores, covered with a white and yellow patina. Stomatitis developed, causing severe pain while eating. Rashes were observed on the conjunctiva in 63 patients (36.2 ± 5.6 %), which was accompanied by the development of scleritis and conjunctivitis during the 3–5 days period of the rash. The disease was accompanied by lymphadenopathy with an increase in the cervical (57.1 ± 5.9 %) and occipital (42.8 ± 5.9 %) lymph nodes in 161 (92.5 ± 3.1 %) patients.

Etiotropic therapy included acyclovir (85 % of cases) both in case of primary infection and in repeated cases of the disease. Antibacterial drugs (third generation of cephalosporins, penicillins, macrolides) were prescribed in case of the additional bacterial complications.

CONCLUSIONS

The modern course of varicella in Ukraine is characterized by an increase in the incidence among the adult population. Infection is manifested by a long prodromal period, a pronounced intoxication syndrome, lymphadenopathy, and an abundant polymorphic rash. At the same time, the rash had a pustular character in 54.3 % of patients; exanthema was observed with scleritis and conjunctivitis in 45 % of cases. There are repeated cases of chickenpox, the interval between these episodes ranged from 5 to 12 years.

PERSPECTIVES FOR FUTURE STUDIES

Taking into account the epidemiological situation in the country it remains relevant to study main features of the course of chickenpox in individuals against the background of pulmonary tuberculosis, depending on the age and duration of the underlying disease.

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PARAMETERS OF THE HEMODYNAMIC AFTER ABLATION ATRIAL FIBRILLATION AND/OR FLUTTER DEPENDING ON THE FUNCTIONAL CLASS OF CHRONIC HEART FAILURE

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Evaluated parameters of the hemodynamic before and after ablation atrial fibrillation and/or flutter depending on the functional class of chronic heart failure in 74 patients. It was found that patients with the I functional class of chronic heart failure have significantly lower left atrium size compared to III functional class, which is associated with the better efficiency of the radiofrequency ablation in the remote period. Patients with the I and III functional class of chronic heart failure are having increasement of QTc duration in acute period of radiofrequency ablation that could be used as an independent predictor of arrhythmia recurrence. Patients with the I functional class chronic heart failure who failed drug therapy for atrial fibrillation and/or flutter alternative treatment in the form of the radiofrequency ablation should be considered as choice therapy.

KEY WORDS: parameters of the hemodynamic, atrial fibrillation, radiofrequency ablation, functional class, heart failure

ПОКАЗНИКИ ГЕМОДИНАМІКИ ПІСЛЯ АБЛЯЦІЇ ФІБРИЛЯЦІЇ ТА/АБО ТРІПОТІННЯ ПЕРЕДСЕРДЬ В ЗАЛЕЖНОСТІ ВІД ФУНКЦІОНАЛЬНОГО КЛАСУ ХРОНІЧНОЇ СЕРЦЕВОЇ НЕДОСТАТНОСТІ

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Вивчені показники гемодинаміки до та після абляції при фібриляції та/або тріпотінні передсердь в залежності від функціонального класу хронічної серцевої недостатності у 74 хворих. Виявлено, що пацієнти I функціонального класу хронічної серцевої недостатності мають значно нижчий розмір лівого передсердя у порівнянні з III функціональним класом, що пов'язано з кращою ефективністю радіочастотної абляції у віддалений період. У пацієнтів I та III функціонального класу хронічної серцевої недостатності спостерігається збільшення тривалості QTc в гострому періоді радіочастотної абляції, що може використовуватися як незалежний предиктор рецидиву аритмії.

КЛЮЧОВІ СЛОВА: показники гемодинаміки, фібриляція передсердь, радіочастотна абляція, функціональний клас, серцева недостатність

ПОКАЗАТЕЛИ ГЕМОДИНАМИКИ ПОСЛЕ АБЛЯЦИИ ФИБРИЛЛЯЦИИ И/ИЛИ ТРЕПЕТАНИЯ ПРЕДСЕРДИЙ В ЗАВИСИМОСТИ ОТ ФУНКЦИОНАЛЬНОГО КЛАССА ХРОНИЧЕСКОЙ СЕРДЕЧНОЙ НЕДОСТАТОЧНОСТИ

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Изучены показатели гемодинамики до и после абляции при фибрилляции и/или трепетании предсердий в зависимости от функционального класса хронической сердечной недостаточности у 74

пациентов. Выявлено, что пациенты I функционального класса хронической сердечной недостаточности имеют значительно меньший размер левого предсердия по сравнению с III функциональным классом, что связано с лучшей эффективностью радиочастотной абляции в отдаленный период. У пациентов I и III функционального класса хронической сердечной недостаточности наблюдается увеличение продолжительности QTc в остром периоде радиочастотной абляции, которая может использоваться как независимый предиктор рецидива аритмии.

КЛЮЧЕВЫЕ СЛОВА: показатели гемодинамики, фибрилляция предсердий, радиочастотная абляция, функциональный класс, сердечная недостаточность

INTRODUCTION

Dilatation of right and left atrium is a common complication in atrial fibrillation/flutter (AF/AFL), especially in the long-term existence of these arrhythmias, due to a violation of the function of the left ventricle, leading to the development of chronic heart failure (CHF) and its progression according to growth of the functional class (FC). This suggests that AF/AFL can lead to significant morphological and functional changes in the heart [1–3]. Radiofrequency ablation (RFA) of arrhythmias in the right or left atrium is effective for the strategy of rhythm control, disappears or significantly reduces the number of arrhythmia paroxysms, which positively affects hemodynamic parameters of the heart, especially the size of the atrium, that is, it affects the risk of progression of CHF [3–6].

It seems expedient to study hemodynamic parameters in patients with AF/AFL and CHF in the early postoperative period of RFA depending on FC CHF to determine which of them can affect the progression of CHF.

OBJECTIVE

To evaluate parameters of the hemodynamic after ablation atrial fibrillation and/or flutter depending on the functional class of chronic heart failure.

MATERIALS AND METHODS

On the basis SI «Zaycev V. T. Institute of General and Urgent Surgery NAMS of Ukraine», Kharkiv, Ukraine 74 patients were evaluated after RFA of AF/AFL at age 60.6 ± 7.05 ($(M \pm sd)$) (44 men and 30 women). Patients were divided into groups based on FC CHF (according to the New York Heart Association (NYHA)): 23 patients with CHF I FC, 32 – II FC, 19 – III FC. Patients IV FC CHF were absent.

Evaluated: heart rate (HR), pulse, systolic and diastolic blood pressure (SBP and DBP, respectively); electrocardiographic (ECG) characteristics – QRS, QTc; echocardiographic (ECHO) parameters: ejection fraction of left ventricle (LVEF), left ventricle end-diastolic diameter (LVED), left ventricle end-systolic diameter (LVES), left and right atrium size (LA and RA, respectively). For measuring the duration of the QT interval and subsequent calculation of QTc, the ECG was recorded on the computerized cardiologist Cardiolab + (HAI-Medica). The length of the corrected QT (QTc) was calculated using the Bazett formula: QTc = QT/(RR^0.5), the accuracy of the measurement is 0.5 ms. SBP and DBP were measured by the Korotkov method with the tonometer GAMMA 700k, accuracy of measurement – 1 mm Hg. ECHO study was performed on the apparatus Toshiba Applio 400. To calculate LVEF the Teichholz formula was used: LVEF = ((7 / (2.4+LVED)) •LVED^3-(7/(2.4+ LVES)) • LVES ^3)/((7/(2.4+LVED))•LVED^3))•100%.

Parameters were evaluated in FC CHF groups until and within 3–5 days after RFA.

The obtained data after the formation of the database was processed in Microsoft Excel, SPSS 17.0. For statistical evaluation of the results parametric criteria were used (mean value – M, standard deviation – sd), nonparametric criteria (absolute (n, number)). Reliability of the differences between groups was evaluated using the nonparametric Mann-Whitney U Test. The results were considered reliable at the significance levels $p < 0.05$. The Spirman correlation analysis (nonparametric), which shows the degree (r_s) of the statistical dependence between the observation pairs, where $0 > r_s \leq 0.5$ is a weak link, $0.5 > r_s \leq 0.6$ – moderate, $0.6 > r_s \leq 1$ – strong. The reliability of the obtained correlation coefficients was determined from the table «Standard correlation coefficients» based on the calculation of the number of degrees of freedom, followed by the definition of the critical value of the Spirman rank correlation coefficient, $p < 0.01$.

RESULTS AND DISCUSSION

Table shows hemodynamic parameters in patients with AF/AFL in the acute period after ablation, depending on the functional class of CHF.

Table

Parameters of the hemodynamic after ablation of AF/AFL depending on the FC CHF

Parameter		I FC CHF		II FC CHF		III FC CHF	
		before RFA	After RFA	before RFA	After RFA	before RFA	After RFA
HR (M ± sd, 1/min)		90.4 ± 20.1*	68.34 ± 10.66	74.2 ± 12.6	72.06 ± 21.3	84 ± 12.1*	69.3 ± 11.67
Pulse (M ± sd, 1/min)		85.6 ± 20.3*	68.01 ± 10.66	73.2 ± 12.8	72.08 ± 21.6	82 ± 14.2*	69.01 ± 11.69
Atrerial blood pressure	SBP (M ± sd, mmHg)	130 ± 12.6	128 ± 10.18	125 ± 12.8	124.7 ± 12.08	135 ± 10.6	127.89 ± 13.87
	DBP (M ± sd, mmHg)	82 ± 6.2	82 ± 5.98	80 ± 7.14	80 ± 6.35	81 ± 6.35	82.36 ± 8.39
LVEF (M ± sd, %)		60.8 ± 10.16 **	65.1 ± 10.15 **	60.1 ± 7.02	59.65 ± 7.02	55.23 ± 5.04**	55.73 ± 5.04**
LVED (M ± sd, cm)		5.02 ± 0.51	5 ± 0.51	5.21 ± 0.55	5.20 ± 0.55	5.3 ± 0.82	5.3 ± 0.82
LVES (M ± sd, cm)		3.4 ± 0.51	3.2 ± 0.51	3.5 ± 0.58	3.5 ± 0.53	3.7 ± 0.56	3.7 ± 0.56
LA (M ± sd, cm)		4.2 ± 0.81 †	4.1 ± 0.81 †	4.4 ± 0.47	4.4 ± 0.47	5 ± 0.57†	4.9 ± 0.57†
RA (M ± sd, cm)		4.1 ± 0.75	4.1 ± 0.75	3.86 ± 0.55†	3.77 ± 0.55†	4.5 ± 0.75†	4.5 ± 0.75†
QRS (M ± sd, msec)		91.75 ± 11.03	93.04 ± 16.61	90.7 ± 13.54	92 ± 15.12	97 ± 25.32	96.3 ± 15.31
QTc (M ± sd, msec)		380 ± 36.32*	424 ± 33.37*	406 ± 32.04	407 ± 39.74	398 ± 55.16*	432 ± 32.37*

*M – average value; n – number; sd - standard deviation; * $p < 0,05$ before and after ablation inside of the groups of FC CHF; ** $p < 0,01$ between groups of FC CHF; † $p < 0,05$ between groups FC CHF.*

Parameters as level of SBP and DBP, LVED, LVES, QRS have not significant difference, so FC CHF was not related to these indicators.

In I FC and III FC CHF, HR and pulse were significantly higher before ablation.

I FC CHF was associated with significantly higher LVEF both before and after RFA in comparison with III FC CHF.

LA and RA size were significantly lower in I FC CHF both before and after RFA compared to the III FC.

I FC and III FC CHF were associated with significantly incensement in QT_c duration after RFA, in II FC CHF no such trend has been identified.

Below are correlations (Fig. 1–3a) between different pairs of observations, depending on FC CHF before and after ablation.

Accordingly, levels of communication strength are indicated by three types of lines: weak link – dotted line – – – – – – – –; moderate link – simple line ————————; strong link – thick solid line

On the correlograms I FC CHF (both before and after ablation) were determined the appearance of strong connections between the SBP and DBP ($r_s = 1,0$; $p < 0,01$); y II FC CHF – between LVES and LVEF, LVED ($r_s = 0,7$; $p < 0,01$), was maintained between SBP and DBP ($r_s = 0,62$; $p < 0,01$). Also for III FC CHF the maximum numbers of strong links has been clearly defined, especially new between LVEF and QRS ($r_s = 0,64$; $p < 0,01$), LVES and LVED, RA ($r_s = 0,66$; $p < 0,01$), LA and LVED ($r_s = 0,65$; $p < 0,01$). There were no differences between periods of observation.

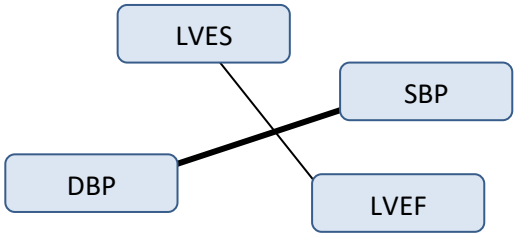

Fig.1. Correlogram for I FC CHF before RFA

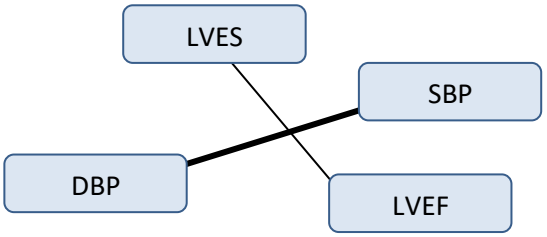

Fig.1a. Correlogram for I FC CHF after RFA

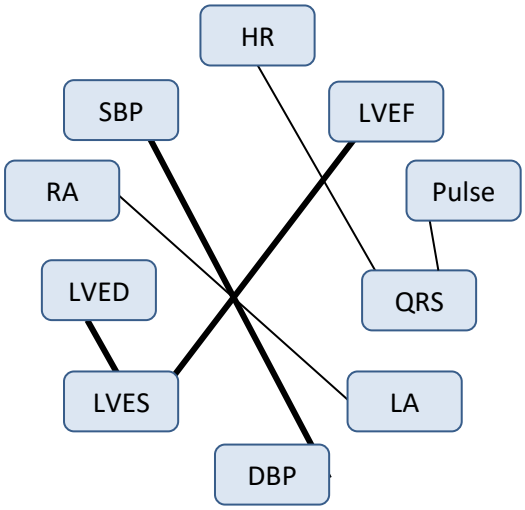

Fig.2. Correlogram for II FC CHF before RFA

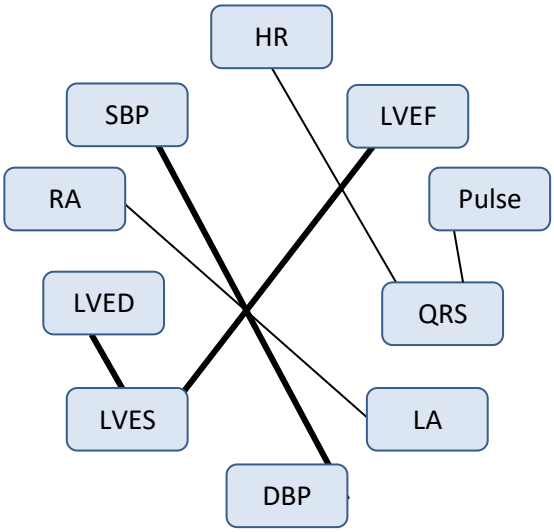

Fig.2a. Correlogram for II FC CHF after RFA

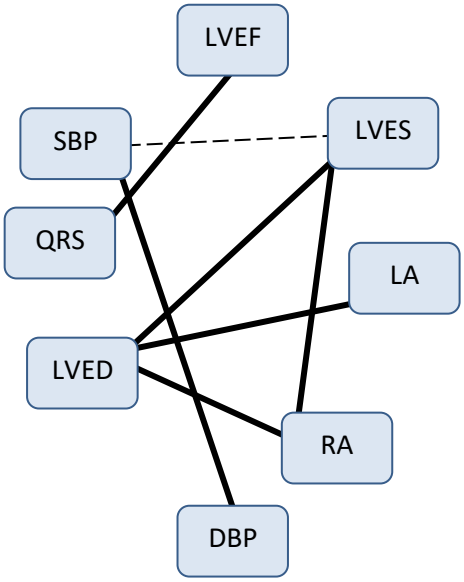

Fig.3. Correlogram for III FC CHF before RFA

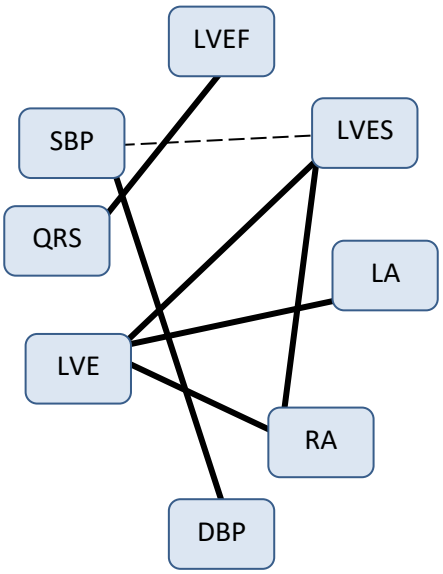

Fig.3a. Correlogram for III FC CHF after RFA

The size of the RA was a characteristic feature of the III FC CHF, which was noted in the study Luong C. and Xiao H. [7–8], as an important indicator for the development of recurrence of AF. Further researches are needed to clarify the impact of these indicators on the efficacy of RFA at various stages after intervention and the risk of CHF progression to a higher FC.

We showed that a smaller LA size was observed in I FC CHF, that was reflected in the study Park J.K. et al. [9–11], where it was shown that a smaller LA size was associated with a better outcome of RFA in the late period, probably due to fewer structural and hemodynamic changes in the heart.

Also, the dilatation of both atriums in the study Xiao H. B. et al. [8, 12–13] was associated with a poor prognosis in patients with AF, which was confirmed in our study: the increase in atrial size was associated with an increase of FC CHF.

QTc interval could used as an independent predictor of arrhythmia recurrence [14–15]. Research of Ning Ma et al. showed that recurrent AF patients had a longer QTc interval than non-recurrent patients. Also, abnormal QT interval predicts AF because it reflects alterations in atrial refractoriness [16].

As a result of the correlation analysis, it was determined that there was a change in the number of correlations and an increase in the correlation between the indicators from I to III FC CHF, which showed a causal relationship, that is, one change led to a mandatory change of another indicator. According to our correlograms, it can be proposed that the dilatation of LA and RA with the background of inadequate treatment of arrhythmia led to the progression of CHF from a clinically insignificant course, such as patients I FC, to III FC CHF due to the dilatation of LV – an increase in LVED. Similarly, in patients with I FC CHF with poorly drug controlled arrhythmia alternative treatment as RFA should be considered as the choice of therapy as early as possible.

There are several limitations to our study. The observation was done in the acute period RFA, so it is not yet possible to reliably talk about the degree of RFA efficiency, however, parameters such as a size of the LA, RA, LVEF, duration QTc are required further monitoring in the delayed period and can serve as reliable predictors of arrhythmia.

CONCLUSIONS

1. The levels of heart rate and pulse decreases in I and III functional class of the chronic heart failure in the acute period radiofrequency ablation; systolic and diastolic blood pressure, QRS, left ventricle end-diastolic and left ventricle end-systolic diameter do not change after radiofrequency ablation.
2. Patient I and III functional class of the chronic heart failure are having increasement of QTc duration in acute period of radiofrequency ablation that could be used as an independent predictor of arrhythmia recurrence.
3. Patients with I functional class of the chronic heart failure have significantly lower left atrium size compared to III functional class of the chronic heart failure, which is associated with the best outcome of effectiveness of radiofrequency ablation in the late period.

PROSPECTS FOR FUTURE STUDIES

It is expedient to study the hemodynamics and features of the treatment of chronic heart failure, depending on the functional class in patients at various stages after interventional interventions for fibrillation and atrial flutter.

The research was carried out within the framework of the research work «Pharmacological and interventional approaches to the treatment of patients with heart rhythm disorders and arterial hypertension» (state registration number 0116U000973).

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Clinical case

UDC: 616.12-008.4

ANGINA PECTORIS AND MYOCARDIAL ISCHEMIA IN THE ABSENCE OF OBSTRUCTIVE CORONARY ARTERY DISEASE: CLINICAL CASE

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Our clinical case shows patient with worsening clinical signs of angina without obstructive lesions of coronary artery which requires further control. Recommendations for lifestyle modification as well as further treatment tactics are described.

KEY WORDS: Cardiac syndrome X, angina, ischemia, microvascular disfunctional

СТЕНОКАРДІЯ ТА ІШЕМІЯ МІОКАРДУ ЗА ВІДСУТНОСТІ ОБСТРУКТИВНОЇ ХВОРОБИ КОРОНАРНИХ АРТЕРІЙ: КЛІНІЧНИЙ ВИПАДОК

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Наш клінічний випадок свідчить про погіршення клінічних ознак стенокардії без обструктивних уражень коронарної артерії, що вимагає подальшого контролю. Описані рекомендації з модифікації способу життя, а також подальша тактика лікування.

КЛЮЧОВІ СЛОВА: кардіальний синдром Х, ішемія, мікроваскулярна дисфункція

СТЕНОКАРДИЯ И ИШЕМИЯ МИОКАРДА ПРИ ОТСУТСТВИИ ОБСТРУКТИВНОЙ БОЛЕЗНИ КОРОНАРНЫХ АРТЕРИЙ: КЛИНИЧЕСКИЙ СЛУЧАЙ

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Наш клинический случай показывает пациента с ухудшением клинических признаков стенокардии без обструктивных поражений коронарной артерии, что требует дальнейшего контроля. Описаны рекомендации по модификации образа жизни, а также дальнейшая тактика лечения.

КЛЮЧЕВЫЕ СЛОВА: кардиальный синдром Х, ишемия, микроваскулярная дисфункция

INTRODUCTION

Cardiac syndrome X (CSX, microvascular angina) is a pathological condition characterized by the presence of signs of myocardial ischemia (typical angina-like chest pain with evidence of myocardial ischemia: ST segment depression ≥ 1.5 mm [0.15 mV] for more than 1 minute, during 48-hour ECG monitoring) in the absence of flow-limiting stenosis on coronary angiography and spasm of the epicardial coronary arteries during coronary angiography. The cost of case management of patients with chest pain and no obstructive CAD is not cheap as a result of challenges in diagnosis and treatment. [1–2]. Most patients with cardiac syndrome X are postmenopausal women [3–5]. However, the «female-pattern» terminology may soon be irrelevant [6]. CSX is likely to be multifactorial in these patients and

it is conceivable that risk factors such as hypertension, hypercholesterolemia, diabetes mellitus and smoking can contribute to its development. Additional factors such as abnormal pain perception may contribute to the pathogenesis of chest pain in patients with angina pectoris and normal coronary angiograms [7].

CLINICAL CASE

The patient C., a man born in 1959, was admitted to the clinical base of internal medicine department in Railway Clinical Hospital № 1 of «HC» JSC «Ukrzaliznytsia» in December, 11.10.17 with complaints of pressing pain behind the sternum radiating to the back with moderate severity of physical exertion, not relieved by nitro-glycerine, shortness of breath when rising to the 3rd floor, periodic numbness of the extremities, high blood pressure periodically to 150/90 mm Hg.

ANAMNESIS MORBI

In May 2017, patient was admitted at hospital for treatment of arterial hypertension, and from that time the pressing pain behind the sternum began to disturb. In June 2017, patient was hospitalized at The Endocrinology department for treatment of diabetes mellitus. There was performed ECG recording, ischemia was detected in the form of ST-segment depression; treadmill test was positive (ischemia detected). It was recommended a diet and patient received bisoprolol 5 mg 1 time per day, valsartan 40 mg 1 time per day in the morning, which he is taking now. Regarding the treatment of diabetes, patient takes glibenclamide 5 mg in the morning before breakfast. However, after the treatment, there was no positive dynamics in the patient's condition. Currently, general condition of the patient is worsening during the last 3 weeks in the form of aggravation of the above complaints.

ANAMNESIS VITAE

1989 – appendectomy, 2000 – hymorotomy, since 2006 – diabetes mellitus type II, since 2011 – arterial hypertension. Ex-smoker – for 40 years until May 2017, 1 pack per day). Family history: mother and father - have high blood pressure. Infections, injuries, tuberculosis, sexually transmitted diseases were denied. Allergic history is not burdened.

OBJECTIVE EXAMINATION

General condition is satisfactory; consciousness is clear, emotionally stable. Height – 1.75 m, weight – 83 kg, BMI – 27.13 kg/m^2 (overweight = 25.1 – 30 kg/m^2).

Skin is pale-pink, without any scars. There is symmetrical oedema of the lower extremities, up to the middle third of the leg, aggravated in the evening, not passing after a night rest. Peripheral lymph nodes are not palpable, on palpation of the thyroid gland painless. Signs of eyelid retraction, periorbital oedema, proptosis are absent.

Respiratory system: on percussion – normal percussion sound above both lungs, on auscultation– symmetrical decreased vesicular breathing. RR= 20/min.

Cardiovascular system: heart borders extended to the left on 2 cm of midclavicular line, HR =70 bpm, regular. Ps= 70 bpm. No pulse deficiency. Auscultation of the heart – heart sounds heart tones are rhythmic, clear. Blood pressure (BP) dextr = 145/88 mm Hg, BP sin = 140/86 mm Hg, (on the background of antihypertensive therapy).

Gastrointestinal system: abdomen is symmetrical, soft, painless, no discrepancies of the abdominal muscles. No visible peristalsis. Liver edge is smooth, painless, palpated 1.5 cm below the costal arch. Spleen and pancreas are not palpable.

Pasternatskiy sign is positive on the right. Urination is free, painless.

LABORATORY AND INSTRUMENTAL TESTS

Tests were conducted according to *the Protocol approved by order of the Ministry of Health of Ukraine from 02.03.16 No. 152*: assessment of the pre-test probability of stable coronary artery disease (CAD), complete blood count, urinalysis, biochemical analysis of blood (potassium, sodium, creatinine, GFR, AlAT, AsAT), lipidogram (total cholesterol, TG, low-density lipoprotein; HDL, high-density lipoprotein), glucose, HbA1c, 12-lead Electrocardiography, echocardiography, treadmill and/or bicycle ergometer (bike) exercise tests, coronary angiography, abdominal ultrasound (additional), X-Ray (additional).

RESULTS OF LABORATORY AND INSTRUMENTAL DIAGNOSIS

Assessment of the pre-test probability of stable CAD: corresponds to medium to high

pretest probability of 66–85 %, non-invasive functional tests with visualization are recommended to confirm the diagnosis.

Complete blood count: normal.

Urinalysis: normal.

Biochemical analysis: all parameters within the normal range.

Fasting glucose test: 8 mmol/l.

HbA1c: 6.53 % (N 4.8–5.9 %).

Lipidogram: total cholesterol 6 mmol / l, TG 2.3 mmol/l.

ECG: HR 79 bpm. Depression of the ST segment > 1 mm in leads II, V4–V5, regional disturbances of ventricular contractility. Diffuse disturbances of repolarization processes, signs of left ventricular hypertrophy (LVH).

Echocardiography: Sclerotic changes of aortic walls, aortic and mitral valves. Dilatation of the ascending aorta. Dilatation of the left atrium. Left ventricular hypertrophy. Mitral regurgitation of the 1nd degree. EF 51 % – 44 (N: 55–78 %).

Abdominal ultrasound: Diffuse changes in the parenchyma of the liver and pancreas without enlargement of them. Thickening of the gallbladder wall. Congestion bile in gallbladder. Right-sided hydrocalycosis. Cyst of the right kidney. Split of pyramid-shaped lobes the left kidney. Kidney microcalculus.

Angiography: Right type of coronary blood flow. Moderate of coronary tortuosity on the coronary blood flow. The left coronary artery - the trunk is not changed, circumflex and left anterior descending coronary artery and its branches is not visible angiographic signs of atherosclerotic lesion. The right coronary artery – *no* plaque was detected.

CLINICAL DIAGNOSIS

Essential arterial hypertension stage II, 1 grade. Hypertensive heart (LVH). The risk is moderate. Ischemic heart disease: microvascular angina (positive stress echoCG 20.06.17). Coronary arteries without obstructive lesions (angiography 12.10.17.). Combined dyslipidaemia. Heart failure with preserved ejection fraction II A stage, NYHA II.

Co-morbidity: Diabetes mellitus type 2, moderate severity, compensation.

OUR RECOMMENDED TREATMENT ACCORDING LAST GUIDELINES

Non-pharmacologic:

✓ Lifestyle modification: adequate physical activity (aerobic exercise for 30 min 3 or more times a week), body weight correction (BMI 18.5 – 24.9 kg/m, waist circumference less than 102 cm in men)

✓ Dietary recommendations – Mediterranean diet:

– decrease sodium intake to 4–6 g/day;

– adequate fluid intake;

– saturated fatty acids should provide less than 10 % of the total energy value of the daily diet, they should be replaced by polyunsaturated fatty acids;

– 200 g of fruit per day (2–3 times);

– 200 g of vegetables per day (2–3 times);

– fish, at least 2 times per week, one of the times – sea fish;

– 30–40 g of dietary fiber per day in the form of whole grain products, vegetables and fruits.

✓ BP monitoring (target level less than 140/90 mm Hg).

✓ Glycemia control.

Treatment strategy:

✓ Perindopril 5 mg once daily continuously, under BP control

✓ Indapamide 2.5 mg once daily continuously

✓ Acetylsalicylic acid 75 mg once daily continuously

✓ Rosuvastatin 40 mg 1 time in the evening for 4 weeks. Control AlAT, AsAT after 4 weeks

✓ Trimetazidine 60 mg per day for 3 months

✓ Kvamatel (famotidine) 20 mg 2 times a day for 14 days

✓ Omega-3 polyunsaturated fatty acids 1000 mg daily for 3 months

✓ Medical therapy of diabetes

PROGNOSIS

Although prognosis is good regarding survival, patients with cardiac syndrome X have an impaired quality of life.

In a recent survey conducted in four large European countries, women reported more severe angina, a higher number of angina attacks per month, and more frequently accompanying symptoms (dyspnoea, arrhythmias/tachycardia), than men. Atypical symptoms were more common for women as well [8].

PREVENTION

Secondary prevention include lifestyle modification; good blood pressure control,

decrease sodium intake, lipid lowering diet, aerobic non strenuous exercises; control of fluid balance and check up for decompensation of heart failure; control of compliance to our medical recommendations.

DISCUSSION

The mechanisms underlying angina pectoris in essential arterial hypertension patents without obstructive coronary artery disease are still largely unknown, but such association doesn't rarely occurs [9–11]. Furthermore, hypertensive patents have a higher likelihood of presenting with features of the metabolic syndrome, e.g., hypertension, dyslipidaemia, obesity and insulin resistance, compared with the general population, which makes the diagnosis and treatment of such comorbid cases more complex and multicomponent [12–13]. Insulin resistance, therefore, may represent an important mechanism for vascular dysfunction in this setting [14–16]. Moreover, cardiac syndrome X is now recognized as a condition that can cause a significant morbidity and increases the risk for CV events [1]

Evidence-based guidelines for treating CSX are still lacking as well as optimal methods of identification of CMD patients although research in this direction continues [1, 17–23]. Therefore, the presentation and study of cases of successful management of patients with this pathology remains relevant.

Our clinical case shows patient with worsening clinical signs of angina without obstructive lesions of coronary artery which requires further control with all the necessary diagnostic methods. This article describes the subsequent management of our patient, which includes both non-drug methods of treatment that cannot be neglected, based on the comorbidity of the disease and the material burden on the patient in general, and medication management.

As a result of our research, our patient needed further correction of the treatment of AH and more accurate diagnosis (and treatment) of angina pectoris and first of all, modification of the lifestyle and reconsideration of the regularity of taking medicines.

CONCLUSION

1. This case is interesting in the development of decompensation (oedema of the lower extremities, reduced tolerance to stress) in a patient with cardiac syndrome X.
2. Management of patents with angina and evidence of myocardial schema on stress testing without obstructive coronary artery disease by angiography (previously referred to as cardiac syndrome X, or CSX) is a challenge.
3. Key points for the clinician include recognition of schema and deployment of guideline-endorsed therapy for angina and reduction of cardiac risk factors.
4. Systemic hypertension is often associated with microvascular angina. Several pathogenic mechanisms have been identified which represent suitable targets for treatment. Microvascular dysfunction needs to be investigated (and treated if present) in patents with systemic hypertension, angina and angiographically normal coronary arteries.

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Review

UDC 617-001.17-089.843

ABDOMINAL COMPARTMENT SYNDROME IN BURN PATIENTS

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Intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) are consistently associated with morbidity and mortality among the critically ill or injured. Thus, avoiding or potentially treating these conditions may improve patient outcomes. Despite a large number of special publications devoted to this problem, very little attention is paid to the ACS in patients with severe burn injuries.

Severe burns have been shown to be a risk factor for developing IAH. Fluid resuscitation practices used in burns management further predispose patients to increase intra-abdominal pressure. The incidence of intra-abdominal hypertension in patients with severe thermal injury is, according to different authors, 57.8–82.6 %. The mortality associated with IAH in severe burns is very high once organ dysfunction occurs.

The purpose of this work is to collect and analyze the problem of abdominal hypertension in burn patients, as well as to draw conclusions on the prevention of this condition and improve the results of treatment of patients with severe burn injury.

KEY WORDS: Abdominal compartment syndrome, Intra-abdominal hypertension, Burns, Fluid resuscitation

АБДОМІНАЛЬНИЙ КОМПАРТМЕНТ СИНДРОМ У ОПІКОВИХ ХВОРИХ

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Внутрішньочеревна гіпертензія і абдомінальний компартмент синдром тісно пов'язані з захворюваністю і смертністю серед критично хворих і уражених. Уникаючи або проводячи адекватне лікування цих потенційно небезпечних для життя станів можна поліпшити результати лікування пацієнтів.

Незважаючи на досить велику кількість спеціальних публікацій, присвячених даній проблемі, дуже мало уваги приділяється абдомінальному компартмент синдрому у хворих з важкою термічною травмою.

У ряді досліджень показано, що важкі опіки є фактором ризику розвитку внутрішньочеревної гіпертензії. Великі обсяги інфузійної терапії, що використовуються при лікуванні важкої опікової травми, додатково привертають пацієнтів до збільшення внутрішньочеревного тиску. Частота розвитку інтраабдомінальної гіпертензії у хворих з тяжкою термічною травмою становить, за даними різних авторів 57,8–82,6 %. Летальність, пов'язана з внутрішньочеревної гіпертензією при великих опіках досить висока після виникнення поліорганної дисфункції.

Мета даної роботи – зібрати і проаналізувати проблему абдомінальної гіпертензії у опікових хворих, а також зробити висновки щодо профілактики даного стану і поліпшенню результатів лікування постраждалих з важкою термічною травмою.

КЛЮЧОВІ СЛОВА: абдомінальний компартмент синдром, інтраадомінальна гіпертензія, опіки, інфузійна терапія

АБДОМИНАЛЬНЫЙ КОМПАРТМЕНТ СИНДРОМ У ОЖОГОВЫХ БОЛЬНЫХ

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Внутрибрюшная гипертензия и абдоминальный компартмент синдром тесно связаны с заболеваемостью и смертностью среди критически больных и пораженных. Избегая или проводя адекватное лечение этих потенциально опасных для жизни состояний можно улучшить результаты лечения пациентов.

Несмотря на достаточно большое количество специальных публикаций, посвященных данной проблеме, очень мало внимания уделяется абдоминальному компартмент синдрому у больных с тяжелой термической травмой.

В ряде исследований показано, что тяжелые ожоги являются фактором риска развития внутрибрюшной гипертензии. Большие объемы инфузионной терапии, используемые при лечении тяжелой ожоговой травмы, дополнительно предрасполагают пациентов к увеличению внутрибрюшного давления. Частота развития интраабдоминальной гипертензии у больных с тяжелой термической травмой составляет, по данным разных авторов 57,8–82,6 %. Летальность, связанная с внутрибрюшной гипертензией при обширных ожогах очень высокая после возникновения полиорганной дисфункции.

Цель данной работы – собрать и проанализировать проблему абдоминальной гипертензии у ожоговых больных, а также сделать выводы по профилактике данного состояния и улучшению результатов лечения пострадавших с тяжелой термической травмой.

КЛЮЧЕВЫЕ СЛОВА: абдоминальный компартмент синдром, интраабдоминальная гипертензия, ожоги, инфузионная терапия

Abdominal compartment syndrome (ACS) is a pathological condition in which organ dysfunction is the result of intra-abdominal hypertension (IAH). It is determined by a steady or repeated increase of intra-abdominal pressure (IAP) over 20 mm Hg. and/or abdominal perfusion pressure (APP) less than 60 mm Hg in combination with newly discovered dysfunction of one system or multiple organ failure [1].

Much good evidence now supports the concept that elevated IAP may impair physiology and organ function by producing the ACS. Complex, adverse physiological consequences of increased IAP develop as the pressure is transmitted to adjacent spaces and cavities, decreasing cardiac output, restricting pulmonary ventilation, diminishing renal function and visceral perfusion, and increasing cerebrospinal pressure [2].

Intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) are associated with increased morbidity and mortality among multiple types of patient populations [3].

The World Society of the Abdominal Compartment Syndrome (WSACS) has published definitions and guidelines for the diagnosis and management of patients with IAH and ACS [4].

Final 2013 consensus definitions of the World Society of the Abdominal Compartment Syndrome:

1. IAP is the steady-state pressure concealed within the abdominal cavity.
2. The reference standard for intermittent IAP measurements is via the bladder with a maximal instillation volume of 25 mL of sterile saline.
3. IAP should be expressed in mmHg and measured at end-expiration in the supine position after ensuring that abdominal muscle contractions are absent and with the transducer zeroed at the level of the midaxillary line.
4. IAP is approximately 5–7 mm Hg in critically ill adults.
5. IAH is defined by a sustained or repeated pathological elevation in IAP ≥ 12 mm Hg.
6. ACS is defined as a sustained IAP > 20 mm Hg (with or without an abdominal perfusion pressure (APP) < 60 mm Hg) that is associated with new organ dysfunction/failure.
7. IAH is graded as follows:

Grade I – IAP 12–15 mm Hg;
Grade II – IAP 16–20 mm Hg;
Grade III – IAP 21–25 mm Hg;

Grade IV – IAP > 25 mm Hg.

8. Primary IAH or ACS is a condition associated with injury or disease in the abdominopelvic region that frequently requires early surgical or interventional radiological intervention.

9. Secondary IAH or ACS refers to conditions that do not originate from the abdominopelvic region.

10. Recurrent IAH or ACS refers to the condition in which IAH or ACS redevelops following previous surgical or medical treatment of primary or secondary IAH or ACS.

11. APP = MAP – IAP (MAP – mean arterial pressure).

12. A polycompartment syndrome is a condition where two or more anatomical compartments have elevated compartmental pressures.

13. Abdominal compliance is a measure of the ease of abdominal expansion, which is determined by the elasticity of the abdominal wall and diaphragm. It should be expressed as the change in intra-abdominal volume per change in IAP.

14. The open abdomen is one that requires a temporary abdominal closure due to the skin and fascia not being closed after laparotomy.

15. Lateralization of the abdominal wall is the phenomenon where the musculature and fascia of the abdominal wall, most exemplified by the rectus abdominus muscles and their enveloping fascia, move laterally away from the midline with time [4].

There are a lot of risk factors for intra-abdominal hypertension and abdominal compartment syndrome. Major burns are one of these factors [4].

It should be noted that an increase in IAP is not always accompanied by the occurrence of ACS. The regularity is known: the higher the IAP and the more factors leading to its increase, the more likely is the development of the ACS [1].

High abdominal pressures lead to several systemic impairments: cephalad movement of the diaphragm leads to cardiac and lung compression, reduced venous return and, subsequently, contributes to hypoxemia, hypercapnia, atelectasis and ventilation-perfusion mismatch. ACS will also compress renal vessels, activating sympathetic drive and the renin-angiotensin system; these effects contribute to a decrease in urine output. Primarily, renal vasoconstriction leads to a significant decrease in urine output, and is typically the first indicator of the onset of ACS – oliguria is noted at IAPs > 15 mm Hg and anuria at IAPs of 30 mm Hg. Reports document a decrease in mesenteric blood flow at 10 mmHg IAP; intestinal mucosa perfusion decreases at 20 mm Hg IAP, and celiac and superior mesenteric artery flow is compromised at IAPs > 40 mm Hg. To further exacerbate the effects on gastric circulation, the increased pressure may compress mesenteric veins, impairing drainage and exacerbating ACS, ultimately leading to further gut hypoperfusion, ischemic bowel, decreased intramural pH and worsening lactic acidosis [5–8]. In the context of tissue injury consistent with severe burn trauma, inflammatory responses can also exacerbate an ischemic bowel. The inflammatory cytokines released will increase capillary permeability, leading to more edema and higher IAP [9]. This is a vicious cycle in which edema results in injury, which in turn worsens edema.

The generalized increase in capillary permeability that occurs in severe burn patients contributes to extensive edema formation and intra-peritoneal accumulation of «third-space» fluid [10].

Capillary leak and third spacing are universal in major burns. In patients with burns of more than 60 % of their body surface area and without abdominal pathology, the pathogenesis for increased IAP is most likely due to massive fluid resuscitation with third spacing and secondary extrinsic compression by burn eschars. «Capillary leak» following shock, with ischemia-reperfusion injury and the release of vasoactive substances and oxygen-derived free radicals increases extracellular volume. Especially when it occurs with associated inhalational injury, delayed resuscitation, and abdominal wall injuries [11–12].

Bowel edema and fluid translocation is further worsened by venous hypertension caused by elevated IAP [13]. This increasing volume in the abdominal cavity, however, is reduced after capillary permeability improves. Therefore, secondary IAH in burn patients generally occurs within 48 hours after injury, during the initial resuscitation period, while ACS usually occurs after the acute phase, during subsequent septic episodes [14–15]. Burn patients are also at risk of tertiary or recurrent ACS any time they require aggressive

resuscitation as, for instance, after any overly aggressive burn excision [10, 13].

IAH/ACS should be suspected in all patients with severe burns. The incidence of IAH in major burn patients is variable in the literature and is associated with the burn area. Patients with > 20 % TBSA burned presented a very high prevalence of IAH. Development of organ failure occurred even at moderately increased values of IAP. In this scenario, monitoring of IAP is the first step for establishing the importance of IAH/ACS in this patient population [16–17]. IAP measurement should therefore be performed every 2 to 4 hours throughout the resuscitation period in burn patients with more than 20 % TBSA [18].

The use of mechanical ventilation is also associated with an increased incidence of IAH and to a worse prognosis in untreated cases [19]. This risk factor is proportional to the severity of respiratory symptoms and the mechanical ventilation requirement.

Malbrain ML at al. [18] believes that IAH will develop in most (if not all) severely burned patients, and may contribute to early mortality [18]. A recent systematic review showed that the prevalence of ACS and IAH in severely burned patients is 4.1−16.6 % and 64.7−74.5 %, respectively [20]. The risk of ACS is higher in burned patients with a higher percentage of total body surface area (TBSA) burned; however, patients with a lower burned TBSA may develop IAH/ACS as well [15]. ACS typically occurs when resuscitation volumes are greater than 275 mL/kg during the first 24 hours or TBSA burned is larger than 60% [21–22]. Patients with severe burn injuries greater than 60 % of TBSA, associated inhalational injuries, delayed resuscitation, and intra-abdominal injuries are at the highest risk of developing IAH and ACS [23]. The mortality rate of patients developing ACS is 50−84 %, even when treated [18, 24].

The effects of IAH/ACS in patients with severe burns are multifactorial. Raised IAP can lead to organ dysfunction and can affect all organ systems. The use of excessive fluid resuscitation in combination with increased capillary permeability as a result of the systemic inflammatory response to burn injury makes these patients particularly vulnerable to the development of IAH and ACS and cardiovascular, respiratory, and renal system dysfunction [15]. In severe burn patients, the kidneys are especially vulnerable to elevated IAP-related injury [25].

Talizin TB at al. evaluated the frequency of intra-abdominal hypertension in major burn patients and its association with the occurrence of acute kidney injury (AKI) [25]. A total of 46 patients were analyzed. Of these, 38 patients developed IAH (82.6 %), thirty-two patients (69.9 %) developed acute kidney injury. The median time to development of acute kidney injury was 3 days. The individual analysis of risk factors for acute kidney injury indicated an association with intra-abdominal hypertension, use of glycopeptides, use of vasopressors [25].

The use of nephrotoxic drugs, such as glycopeptides, is associated with direct kidney injury and the consequent dysfunction of this organ. Changing organic perfusion in the case of circulatory instability, as evidenced in the literature, is a risk factor for kidney injury [26]. The IAH patient also presents hemodynamic changes with impaired renal perfusion [20]. An association between AKI and higher 30-day mortality in intensive care patients has been found [25].

Since an elevated IAP affects renal blood flow, urinary output is an unreliable index of the preload and intravascular volume resulting in the loss of an important physiologic parameter.

Moreover, ACS as well as abdominal decompression for ACS increases susceptibility to multiple organ dysfunction syndrome (MODS) for severe burn patients and may also induce acute lung injury [18].

One should pay attention to the fact that IAH/ACS might occur in patients without circumferential 3rd degree burns of their trunk. Burn patients with smoke inhalation may also be at risk of fluid sequestration [21].

It is fundamental to: 1) recognize IAP and ACS; 2) resuscitate effectively; and 3) prevent the development IAP-induced end-organ dysfunction and failure [27].

The WSACS medical management algorithm for IAH/ACS is based on five treatment options: 1) evacuation of intra-luminal contents; 2) evacuation of intra-abdominal space occupying lesions; 3) improvement of abdominal wall compliance; 4) optimization of fluid administration; 5) optimisation of systemic and regional perfusion [4].

According to WSACS recommendations if patient has IAP ≥ 12 mm Hg medical

management to reduce IAP should be started. If IAP > 20 mm Hg and new organ dysfunction/failure is presented, patient's IAH/ACS is refractory to medical management. Strongly consider surgical abdominal decompression (GRADE 1D) [4].

But management of ACS with decompressive laparotomies is associated with significant morbility and mortality ranging from 50 % to 100 % [28].

Thus, the main thing is the prevention of ACS. Key to the prevention of ACS is the early recognition and treatment of IAH [29–30].

Many burn physicians lack awareness of the deleterious effects of raised IAP and do not regularly measure it [29].

Resuscitation in the very first hours after a burn is a key point in the treatment of severe burn shock [31]. Judicious use of fluids and avoidance of fluid over-resuscitation is the key element in the prevention of secondary ACS. Moreover, the choice of resuscitation fluid among critically ill patients with burns may have a clinical importance [22, 32].

There is no perfect resuscitation protocol and studies have demonstrated that patients frequently receive larger amounts of fluids than required a patient. This condition recently recognized as «fluid creep», a phenomenon which may also be attributed to «opioid creep».

Fluid creep is an iatrogenic phenomenon resulting from misuse of the originally described approaches to crystalloid resuscitation. It is associated with massive edema and compartment syndromes (orbital, abdominal, and extremity compartment syndrome) [18, 33–35].

It is currently unknown whether the syndrome is an iatrogenic consequence of excessive fluid resuscitation or an unavoidable sequelae of the primary injury. A recent systematic review of severely burned patients concluded that the fluid resuscitation volume was directly responsible for the development of ACS. It exacerbates splanchnic edema leading to an increase in gut permeability, bacterial translocation, and increased intra-abdominal pressure. Resuscitation-related ACS is associated with a mortality of 97 % when burn size is greater than 60 % TBSA [12, 36–37].

Groups of burn patients that have been identified in whom resuscitation requirements are usually greater than the parkland Formula predictions include patients with inhalation injuries, electrical burns, those with additional injuries, patients with high alcohol or drug intake, and those in whom resuscitation was delayed. To avoid «fluid creep», the resuscitation formulas have to be used only as indicators for the initial fluid resuscitation rate. This rate must be adjusted according to several parameters, the most important and most frequently used being urine output. According to a survey of the American Burn Association and the International Society of Burn Injuries, 94.9 % of respondents use urine output as the main indicator of successful infusion therapy [38]. This parameter should not be allowed to exceed the recommended hourly urine output range of 0.5 to 1 ml/kg/h [27]. But in overhydrated severely burned patients, a decreased urine output may reflect overresuscitation and the onset of abdominal compartment syndrome [35].

For patients with severe burn injury, it is necessary to strive to restore microcirculation in the shortest possible time, using the minimum amount of fluid necessary to maintain the physiological functions of the body. Both insufficient and excessive amount of injected fluid leads to the dysfunction of organs and tissues, the development of multiple organ failure (MOF).

Ivy has identified 250 ml/kg of volume administration within the first 24 hours as a risk factor for ACS [21]. Regular calculation of the Ivy index will identify patients at risk of developing ACS. However, in the eventuality of a thick abdominal eschar, abdominal distention is restricted, thus the critical point of increased IAP is reached with lesser increase in intra-abdominal volume and IAH and ACS may occur with lesser fluid resuscitation volumes [10, 39].

Now novel resuscitation strategies in burn patients to avoid IAH/ACS are evolving. Recent evidence supports the use of hypertontic sodium chloride solution and colloids enabling less overall fluid volume resuscitation. Despite efforts to minimize fluid administration many patients end up grossly fluid overloaded leading to IAH and ACS [22, 40].

Randomized studies have shown that hypertonic lactated saline (HLS) or plasma-based resuscitation requires less fluid and is associated with a lower risk of IAH and ACS. On the other hand, isotonic resuscitation was associated with a 3.5-fold increased risk for developing IAH. [22].

Treatment of burns with a hypertonic solution reduced the secretion of cytokines by cardiomyocytes, decreased their sensitivity to the action of lipopolysaccharides against cytokine secretion, and improved pumping function [41].

Some authors add colloids to their resuscitation regimen within the first 24h to reduce the total resuscitation volumes. However, this remains a debatable issue even though there is growing evidence of its usefulness. Despite some reservation concerning the use of albumin in the early phases of burn resuscitation, recent work demonstrated a decreased mortality rate.

The use of only salt solutions can be limited in cases where dehydration does not reach the stage of reducing the volume of circulating blood.

If dehydration progresses to the stage of intravascular space reduction, then early administration of colloids is necessary. And later, saline solutions can be assigned to rehydrate the interstitial space. It should be noted that dehydration of the vascular space occurs after interstitial dehydration, and the injected salt solutions will immediately move to the interstitial space before filling the vascular sector.

Low molecular weight dextrans (dextran 40), native plasma, hydroxyethyl starch 130/0.4 are recommended as colloids. Also, glucose and fructose solutions are included in the burn shock infusion therapy.

The ratio of colloids, crystalloids, salt-free drugs in patients with severe and extremely severe thermal injury is an average of 1: 1: 1, but is corrected according to the state of the particular patient. The order of their administration depends on the hemodynamic parameters, especially the central venous pressure [42].

There is also growing evidence that vitamin C supplementation, in the early post-burn period, seems to decrease the needed fluid volumes.

A pronounced inflammatory response in severe burn injury contributes to the release of free oxygen radicals, which further impair the microcirculation and contribute to the development of interstitial edema [43].

Oxidative tissue damage as assessed by increased myeloperoxidase (MPO) activity, lipid peroxidation, and decreased levels of glutathione levels in intestinal and hepatic tissue plays an important role in progression from IAH to ACS. However, reperfusion of decompressed tissue induces a more prominent injury compared to ischaemia itself. Reperfusion promotes generation of various reactive oxygen metabolites via activated neutrophils that cause increased microvascular permeability, interstitial oedema, impaired vasoregulation, inflammatory cell infiltration, and parenchymal cell dysfunction and necrosis [44].

Therefore, antioxidants, prescribed in burn shock, binding free radicals, reduce vascular permeability, improve the course of burn disease, prevent the development of complications, reduce damage to internal organs [45].

Tanaka et al. found that adjuvant high dose ascorbic acid (66 mg/kg/h for 24h), administered during the first 24h after thermal injury, significantly decreased the amount of fluid given compared to the control (patients who received vitamin C required infusions of 3 ml/% of burn /kg, while patients who received one Ringer's solution lactate, required 5.5 ml/% of burn/kg of solutions per day [46].

High-dose vitamin C treatment (bolus 66 mg/kg and maintenance dose 33 mg/kg/hr) reduces endothelial damage to sham burn levels, whereas half the dose is inefficient. High-dose vitamin C should be considered for parenteral treatment in every burn patient [12].

Octreotide, a synthetic somatostatin analogue, has been shown to improve the reperfusion-induced oxidative damage in rats with ACS by reducing levels of MPO activity and malondialdehyde and increasing levels of glutathione when given before decompression. Therefore, octreotide might ultimately be shown to have a therapeutic role as a reperfusion injury-limiting agent among patients with IAH and ACS [47].

So, resuscitation of patients with severe burn injuries should be aimed at the early restoration of the circulating blood volume and microcirculation using a minimum number of solutions. This helps to prevent IAH, the development of the ACS, MOF in patients with severe burn injury.

Non-operative and percutaneous interventions may be applied before surgical decompression is considered. Nasogastric decompression, the use of neuromuscular blocking agents, prokinetic agents, enemas, or colonic decompression, the removal of excess

fluid by ultrasound-guided percutaneous drainage, or by a combination of continuous veno-venous hemofiltration (CVVH) with ultrafiltration and/or diuretics, are simple and possibly effective tools to reduce IAP [3, 44].

Cheatham et al. [48] showed in 62 patients with IAH/ACS treated with percutaneous catheter decompression (PCD) versus traditional open abdominal decompression (OAD) that both techniques were equally effective. Successful PCD therapy was associated with either fluid drainage above 1,000 mL or a decrease in IAP of > 9 mm Hg in the first four hours post decompression. PCD appears to be most effective in patients with secondary ACS due to massive fluid resuscitation in burns. Latenser et al. [49] showed that PCD reduced IAP and prevented ACS in 55 % of burned patients . PCD is a relatively simple technique, cost effective and less invasive then OAD. Bedside ultrasonography to identify intraperitoneal fluid or blood is necessary [44].

Circumferential abdominal burn eschars might also lead to ACS by producing a tourniquet effect. At bedside, urgent decompressive escharotomy of the abdominal wall is a safe surgical procedure that provides rapid relief of intra-abdominal pressure. It improves ventilation, hemodynamic parameters, and oxygen metabolism and can decrease morbidity and mortality [27]. An escharotomy of the trunk to improve abdominal wall compliance should be performed early, especially in the presence of 3rd degree burns [18].

The open abdomen in trauma and non-trauma patients has been proposed to be effective in preventing or treating deranged physiology in patients with severe injuries or critical illness when no other perceived options exist. Its use, however, remains controversial as it is resource consuming and represents a non-anatomic situation with the potential for severe adverse effects [50].

Although a midline laparotomy may make wound management more difficult in abdominal burn patients, it remains very effective in reducing IAP.

Regardless of surgical decompression, it is important to continue to measure IAP postoperatively in order to recognize recurrent IAH and/or ACS. The presence of abdominal burns may pose specific challenges to the management of the open abdomen with regard to infectious complications. The presence of significant protein loss via an open abdomen needs to be considered [51]. Early enteral and/or parenteral nutrition is of the utmost importance in these hypercatabolic patients, although recent literature results may advocate the opposite in ICU patients with ACS [52–53]. However, strong emphasis needs to be placed on the tremendous morbidity and high mortality of an open abdomen in patients with burns [18]. Its use, therefore, should only be considered in patients who would most benefit from it [50].

CONCLUSIONS

Intra-abdominal hypertension is a frequent complication in severe burn patients requiring massive fluid resuscitation. Development of ACS in burn patients is associated with high mortality. Prevention, early detection and proper management may avoid this usually fatal complication. Fluid resuscitation volume is directly responsible for the development of ACS in severe burned patients. Thus, optimal fluid resuscitation can be the best prevention of IAH and ACS.

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AUTOLOGOUS PLATELET-RICH PLASMA: A REVIEW OF SCIENTIFIC ARTICLES ON THE STUDY OF EFFICIENCY IN TREATMENT OF ANDROGENETIC ALOPECIA IN MEN AND WOMEN

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This article is an analysis of scientific publications that reflect the experience of using autologous platelet-rich plasma in patients with androgenic alopecia (AGA), a multifactorial genetically predisposed disease caused by the influence of exogenous or endogenous triggers responsible for the clinical manifestations of pathology. Attention to this topic is due to the significant prevalence of AGA, as well as unsatisfactory results in achieving a positive therapeutic effect.

KEY WORDS: androgenic alopecia, autologous platelet-rich plasma, trichology, immunology

АУТОЛОГІЧНА ЗБАГАЧЕНА ТРОМБОЦИТАМИ ПЛАЗМА: ОГЛЯД НАУКОВИХ СТАТЕЙ ЩОДО ВИВЧЕННЯ ЭФЕКТИВНОСТІ В ЛІКУВАННІ АНДРОГЕНЕТИЧНОЇ АЛОПЕЦІЇ У ЧОЛОВІКІВ ТА ЖІНОК

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Дана стаття являє собою аналіз наукових публікацій, що відображають досвід застосування аутологічної збагаченої тромбоцитами плазми у пацієнтів з андрогенною алопецією (АГА) – мультифакторіальним захворюванням с генетичною схильністю, зумовлене впливом екзогенних або ендогенних тригерів, відповідальних за клінічні прояви патології. Увага до цієї теми зумовлена значною поширеністю АГА, а також незадовільними результатами у досягненні позитивного терапевтичного ефекту.

КЛЮЧОВІ СЛОВА: андрогенна алопеція, аутологічна збагачена тромбоцитами плазма, трихологія, імунологія

АУТОЛОГИЧНАЯ ОБОГАЩЕННАЯ ТРОМБОЦИТАМИ ПЛАЗМА: ОБЗОР НАУЧНЫХ СТАТЕЙ ОБ ИЗУЧЕНИИ ЭФФЕКТИВНОСТИ В ЛЕЧЕНИИ АНДРОГЕНЕТИЧЕСКОЙ АЛОПЕЦИИ У МУЖЧИН И ЖЕНЩИН

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Данная статья представляет собой анализ научных публикаций, которые отражают опыт применения аутологичной обогащенной тромбоцитами плазмы у пациентов с андрогенная алопецией (АГА) – мультифакториальном заболевании с генетической предрасположенностью, обусловленное влиянием экзогенных или эндогенных триггеров, ответственных за клинические проявления патологии. Внимание к этой теме обусловлено значительной распространенностью АГА, а также неудовлетворительными результатами в достижении положительного терапевтического эффекта.

КЛЮЧЕВЫЕ СЛОВА: андрогенная алопеция, аутологичная обогащенная тромбоцитами плазма, трихология, иммунология

INTRODUCTION

Currently, most researchers consider androgenic alopecia (AGA) as organ-specific

segmental accelerated aging of hair follicles that occurs in individuals with a genetic predisposition due to the influence of exogenous or endogenous triggers that are responsible for the clinical manifestations of the disease with the leading role of endocrine disorders [1]. Significant progress has been made in understanding the basic elements of the metabolism of the participating androgens with a clearly expressed genetic component. However, clinical practice has shown that simply blocking androgens does not give the desired results [2]. Clinical and research progress helped to identify several non-androgenic cofactors of complex etiology of AGA, which became a prerequisite for the development of new therapeutic strategies, among which the use of autologous platelet-rich plasma (PRP) is one of the most promising. The published data of fundamental and clinical studies on the effectiveness of the use of PRP in the treatment of AGA will be analyzed in this article.

EFFECTS OF GROWTH FACTORS ON THE HAIR FOLLICLE

Hair follicles are located at the intersection of complex neuroendocrine and immune regulation, actualizing both local and systemic influence of numerous endogenous and exogenous factors, genome and epigenome. The information we know probably comprises a small amount of what future discoveries will bring. Increased awareness of the various signaling pathways involved in hair growth may further lead to the discovery of new targets for drug exposure.

Thus, it was demonstrated in researches Botchkarev et al. (Botchkarev V. A. et al, 2003) [3] that the transition from the telogen to anagen phase is associated with the activation of the Shh, Wnt/beta-catenin/ Lef-1 and Stat3 signaling pathways. A huge number of signal transduction pathways (Shh, Wnt, etc.) and growth factors (BMP, FGF, HGF, IGF, PDGF, SCF, etc.) are activated in the anagen phase in the epithelium and mesenchyme, which should be well coordinated for hair formation.

It should be noted that the same factors can have a completely opposite effect on the cells in the follicle in different phases of the cycle. Modern research has opened up an enormous potential for developing of methods of correcting and managing the hair growth cycle with the help of biologically active substances, growth factors and hormones and in fact revolutionized this direction.

Growth factors – polypeptides with a molecular weight of 5–50 kDa, combined into a group of trophic regulatory substances. Like hormones, these factors have a wide range of biological effects on many cells – they stimulate or inhibit mitogenesis, chemotaxis, differentiation. Unlike hormones, growth factors are produced by unspecialized cells located in all tissues as a rule, and possess endocrine, paracrine and autocrine action.

Growth factors – a link between the expression of the body genes and the environment – play an important role in regulating the life of the hair follicle. Currently, it has been established that such factors as EGF, TGF-β, IGF-1, HGF, KGF, VEGF are involved in the regulation of the hair growth cycle [4].

The localization of receptors for a number of growth factors in various parts of the hair follicle was demonstrated by immunohistochemistry and in situ hybridization. The dermal papilla cells demonstrated the highest immunogenic reactivity against FGF-7, IGF, HGF and VEGF, which play a key role in the regulation of follicle growth. Initially it was expected that the use of growth factors in vivo will allow having a stimulating effect on hair growth. Nevertheless, an interesting feature was revealed during the research: the effect of growth factors on the hair follicle depends on the nature of the implementation of their action.

It was found that growth factors such as EGF, TGF-a, FGF-1. FGF-2, slowed hair growth. A suppressive effect was also observed in TGF-β in tissue culture. This group of growth factors was characterized by an *autocrine* mechanism of action.

At the same time, it was demonstrated that subcutaneous injections of FGF-7, IGF-1, HGF paracrine growth factors stimulated the growth of the hair follicle and modulated the hair cycle in vivo. This effect is probably related to the paracrine nature of the realization of these growth factors synthesized in the hair papilla. The reason why only paracrine growth factors have a stimulating effect remains not fully clarified.

PLATELET-RICH PLASMA

The use of autologous PLR as a method indirectly stimulating the work of dermal papilla cells implies large therapeutic

possibilities. The dermal papilla and connective tissue shell are formed from the same progenitor cells as the fibroblasts in the interfollicular dermis, but their gene expression profile and biological functions are radically different [5–6]. If interfollicular fibroblasts promote the growth and differentiation of the overlying epithelial cells (keratinocytes), the dermal papilla and connective tissue shell play the main role in regulating hair growth. The method is based on the concept of PRP as a natural source of signaling molecules that have a paracrine effect on other cells. The main functions of platelets in restoring damaged tissue are modulation of inflammation through the interaction of innate immune cells, regulation of angiogenesis, and stimulation of cell migration and proliferation.

PRP, isolated from whole blood, is characterized by the presence of growth factors and stimulating mediators. PRP is an autologous platelet preparation in concentrated plasma, with a platelet concentration exceeding the physiological one. Activated α-platelet granules produce numerous growth factors, including follistatin, KGF, VEGF, EGF, IGF. These growth factors appear to stimulate cell proliferation and differentiation. It was found that PRP has a beneficial effect on bone transplants performed in maxillofacial, orthopedic surgery and cardiac surgery. Recently, there has been an upsurge of interest in the use of PRP in dermatology, for example, in tissue regeneration, wound healing, fatty tissue transplantation, and for the rejuvenating effects on the skin, suggestions have been made to use PRP as a new treatment for AGA.

THE RESULTS OF THE STUDY OF THE PRP EFFECTIVENESS IN THE TREATMENT OF AGA

The possible effect of PRP on hair growth has been studied in vitro and in vivo in mice. The actual mechanisms of action on the hair follicle remain controversial: PRP activates the proliferation of dermal papilla cells and prevents apoptosis, which provokes an increase in Akt and Bcl-2 expression levels *in vitro*. In addition, PRP is involved in the formation of the hair epithelium and the differentiation of stem cells into cells of the hair follicle. Increasing the expression level of FGR-7 prolongs the anagen phase in the hair growth cycle.

In 2006, Ubel et al. [7] reported about a new experience of using PRP in the treatment of male-type AGA. The authors of this study demonstrated that the treatment of follicular units using PRP before transplantation contributed to an increase in hair growth and density. After this study, several studies were conducted to investigate the possibilities of using PRP for the treatment of AGA.

These results became a motive for further research, and since 2006 a number of articles have been published describing the experience of using PRP in the treatment of AGA. Part of the work noted a positive effect from the described technique, part – a negative one.

Researches with a positive result

In 2009, Ggeso et al. [8] published the results of a pilot research on studying the effect of direct injection of PRP into the scalp skin. The study involved 10 people, the evaluation of the results was carried out 4 and 8 months after treatment. An increase in the average diameter of the hair shafts was recorded by 9.7 % at 4 months and by 6.1 % at 8 months in the group of patients receiving PRP. In the control group of patients who did not receive PRP, there was a decrease in the average diameter of the hair shafts by 2.8 % after 4 months and by 3.5 % after 8 months.

In 2010, E. Betsi et al. [9] conducted a pilot study of the clinical effectiveness of PRP injections in the treatment of alopecia. The study involved 42 patients – 8 women diagnosed with telogen effluvium and 34 men diagnosed with AGA. Evaluation of the results of the study before and after treatment was carried out using a hair tension test and survey photographs. There was a significant decrease in the number of hair falling out, a significant increase in volume and improvement in hair quality after treatment. Then the researchers noted that more obvious improvements were observed in patients with a history of up to two years; the results were worse for men with 6-7th stage of baldness based on the Hamilton-Norwood scale.

Anitua at al. [10] conducted a blind study of the use of plasma enriched with growth factors in 19 patients with AGA in 2017. Objective methods of assessment using computerized phototrichogram evaluated the result by the following parameters: 1) hair density; 2) diameter; 3) the ratio of terminal hair to vellus hair; 4) thinned/normal/dense hair rods among terminal follicles; 5) independent clinical

assessment of observations (degree of improvement according to macro-photography); 6) epidermal thickness, perivascular inflammatory infiltrate, the amount of epidermal cords, the ratio of terminal hair to miniaturized, the number of collagen, reticular and elastic fibers (according to 3 mm puncture biopsy); 7) proliferation of epidermal/follicular cells, newly formed blood vessels and the presence of stem cell niches in the bulge zone (according to immunohistochemistry). During the study, the method of single centrifugation was used, the obtained PRP was additionally activated by platelet growth factor to stimulate the release of growth factors and morphogens from the obtained material; centrifuging parameters: frequency – 580 revolutions per minute, time – 8 minutes; platelet enrichment ratio x2 ± 0.3; blood volume 18 ml; PRP volume – 3–4 ml. Patients received 5 intradermal injections in areas of hair loss: 1–4 procedures with an interval of 1 month, 5th procedure – 7 months from the start of the study. Evaluation of the obtained results was made after 1 year from the beginning of the experiment, a positive improvement was noted for all 7 evaluation parameters. Subjective assessment data: 85 % of patients noted an improvement in the quality and density of hair; 65 % of respondents noted an increase in hair density.

In 2016, a randomized, placebo-controlled, blind study was conducted by Alves at al. [11] on 25 patients, only 22 of which have fully completed the trials (11 men aged from 18 to 65 years with stage II-V of AGA; 11 women aged from 18 to 86 with stage I – III. Subjects were divided into 2 groups: group A, which was administered 3 ml of PRP in the right half of the head and 3 ml of saline (placebo) in the left half; and group B, which received the same solution in both halves of the head. Parameters for evaluating the obtained results (using a photo-trichogram and a survey photo): 1) hair in the anagen phase (%); 2) hair in the telogen phase (%); 3) anagen/telogen ratio; 4) density of the hair; 5) density of the terminal hair; 6) counting the amount of hair. The method of single centrifugation was used during the study, the obtained PRP was additionally activated with 0.15 ml of 10 % calcium chloride; centrifuging parameters: centrifugal acceleration – 460 g, time – 8 minutes; platelet enrichment ratio ×3; blood volume 18 ml; PRP volume – 3 ml. Anesthesia was not used. Three injection sessions (0.15 ml/cm²) were carried out, at an interval of 1 month, in four rounded sections of 1×1 cm of the frontal and occipital part of the head (marked with a medical tattoo). Evaluation of the results was carried out on the 3rd and 6th month, an improvement in the mean values in the anagen/telogen ratio, hair thickness, terminal hair thickness were observed in the areas of PRP administration relative to the initial state, compared with placebo, where only improvement in the average hair thickness was observed . For the first time, the authors were able to detect the relationship between the number of anagenic hair and the age of patients > 40 years old, the onset of AGA ≥ 25 years, the density of hair and the male sex, age ≤ 40 years, a positive family history of AGA and the duration of the disease > 10 years. The authors concluded that the use of PRP had a positive effect on the state of AGA and could be considered as adjuvant therapy for the treatment of this disease.

In 2015, data was obtained from a randomized, placebo-controlled, blind study by Gentile et al. [12] on 23 patients, of which only 20 fully completed the tests (20 men aged from 19 to 63 years with AGA stage IIa-IV). One-half of the affected scalp was injected with PRP injections, the second – with saline. Parameters for assessing the obtained results (1–3 using a computerized photo trichogram and a survey photo): 1) counting the amount of hair and total hair density; 2) density of terminal hair; 3) epidermal thickness and density of hair follicles (according to the data of 3 mm puncture biopsy); 4) the proliferation of keratinocytes and small blood vessels around the hair follicles (according to immunohistochemistry); 5) AGA relapse. The study used 2 methods: 1) cascade-Selphyl-Esforax system; 2) the platelet lipotransfer system (PRL platelet-rich lipotransfert system) obtained PPR was further activated by Ca^{2+}; centrifuging parameters were: 1. centrifugal acceleration – 1100 g; 2. frequency – 1200 revolutions per minute; time: 1,2 – 8 minutes; blood volume: 1. 18 ml; 2. 60 ml.; PRP volume: 1. 9 ml; 2. 20 ml. During the study, 3 sessions of injections were conducted at an interval of 30 days, without the use of anesthesia, after treatment of the skin with 70 % alcohol, using the method of intradermal injections (0.1 ml/cm²). The observation period for patients was 2 years (with the assessment of the condition at the beginning of the experiment, at

2, 6, 12, 16 and 23 months after the first procedure). A significant improvement in all estimation parameters was noted.

In 2014, a randomized, placebo-controlled, blind study was conducted by Cervelli at al. [13] in 10 patients (10 men aged 20–52 years with stage IIa-IV AGA). Parameters for evaluating the obtained results (1–4 using a computer phototrichogram and a survey photo): 1) counting the number of hairs; 2) density of hair; 3) density of terminal hair; 4) epidermal thickness and density of hair follicles (according to data of 3 mm puncture biopsy); 5) the percentage of Ki67 + keratinocytes and the density of the network of blood vessels (according to immunohistochemistry). During the study, the Cascade-Selphyl-Esforax centrifugation system was used, the resulting PRT was additionally activated by Ca^{2+}; centrifuging parameters were: centrifugal acceleration – 1100 g; time: 10 minutes; blood volume: 18 ml; PRP volume: 9 ml. 3 sessions of injections were performed during the study, at an interval of 1 month, without the use of anesthesia, after treatment of the skin with 70 % alcohol, using the method of intradermal injections (0.1 ml/cm²). The period of observation of patients was 1 year (with the assessment of the state at the beginning of the experiment, at 14 weeks, 6 and 12 months after the first procedure). A significant improvement in all estimation parameters was noted.

In 2015, Singhal et al. [14] conducted a placebo-controlled study to compare PRP with approved drug treatment in 20 patients (16 men aged from 25 to 32 years, 4 women aged from 32 to 35). The following parameters were evaluated: 1) hair tension test; 2) hair growth, hair volume, hair quality (method of review photos). The method of double centrifugation was used during the study, the obtained PRP was additionally activated with calcium chloride (in the ratio 9:1); centrifugation parameters were: frequency: 1. 1500 revolutions per minute, 2. 2500 revolutions per minute; time: 1. 6 minutes, 2. 15 minutes; blood volume 20 ml; PRP volume – 8–12 ml. 4 sessions of injections were conducted, at an interval of 2–3 weeks. Injections were carried out by the nappage method, after the skin surface was treated with alcohol and povidone-iodine. The observation period for patients was 3 months (at weekly intervals). A significant improvement in all estimated parameters was noted. For comparison, patients who were offered drug treatment did not notice an improvement in the results of the hair tension test and hair growth in general.

The study by Gupta et al [15] was conducted on 30 patients (30 men aged from 25 to 35 years, with stage III–VII) in 2017. The following parameters were evaluated: 1) hair density (CapilliCare trichoscan); 2) hair diameter (CapilliCare trichoscan); 3) independent clinical evaluation of observations (overview macrophotography). The method of double centrifugation was used during the study. 6 injections were performed at an interval of 15 days, patients were monitored for 6 months. A mezoroller was used before the injections, after which the PRP was injected into the area of the crown (10 cm from the glabella). A significant improvement in all estimation parameters was noted.

Study Schiavone et al [16] in 2014 were conducted on 64 patients (42 males, mean age 28 years, with AGA stages II-V; 22 females, average age 32 years old, with AGA stage I-II). The following parameters were evaluated: counting the number of hair and hair thickness using a 15-point scale evaluation Jaeschke, clinical changes (macrophotography studied by two independent observers). The study used the method of double centrifugation (GPS III Platelet Separation System, single centrifugation at the first procedure, a two-fold – in 3 months). Plasma protein concentrate was added to the obtained PRP (1st procedure: 6-8 ml PRP with 3–4 ml). 2 injections were performed at an interval of 3 months; patients were monitored for 6 months. Locally irritating effect achieved after applying local anesthesia (1 % Xylocaine with epinephrine 1:100,000) with skalproller exposure with needles length of 1.0 mm in order to enhance injected platelet activation, punctures were made at intervals of 1 cm. As a result, the positive effects of the procedures were obtained.

The study by Gkini et al [17] was conducted on 22 patients, of whom only 20 completed the tests completely (18 men, aged from 24 to 72 years, with AGA stage II-5A, 2 women, aged from 58 to 72 years, with AGA stage I) in 2014. The following parameters were evaluated: 1) hair tension test; 2) density and quality of hair (dermatoscopic photomicrography and macrophotography). Centrifugation was carried out using the RegenA-PRPCentri (Regenlab) system using a single centrifugation method. Calcium gluconate was used as an activator

(0.1 ml to 0.9 ml PRP in the ratio 1:9). Centrifugation parameters were: centrifugal acceleration – 1500 g, time – 5 minutes; platelet enrichment × 5.8; blood volume 16 ml; PRP volume – 6 ml. There were 3 sessions of procedures (plus 1 procedure to enhance the effect) and at an interval of 21 days (3 procedures at an interval of 21 days plus 1 procedure after 6 months from the beginning), the observation of patients lasted 1 year. Injections (0.05–0.1 ml/cm²) were injected according to the nappage technique into the affected areas to a depth of 1.5–2.5 mm. Positive results were noted for all parameters studied. Subjective assessment data: 85 % of patients noted an improvement in the quality and thickness of hair, 65 % – an increase in hair density.

Also in 2014, another study by Khatu et al. [18] was conducted on 11 patients (11 men, aged from 20 to 40 years, with stages II–IV of AGA). The following parameters were evaluated: 1) hair tension test; 2) counting the amount of hair (Trichoscan); 3) hair loss (clinical examination data, macroscopic photographs). The study used the method of double centrifugation (Manual Double Spin). Calcium gluconate (in the ratio 1:9) was used as an activator. Centrifugation parameters were: frequency: 1. 1500 revolutions per minute; 2. 2500 revolutions per minute; time: 1. 6 minutes, 2. 15 minutes; blood volume 20 ml; PRP volume – 2–3 ml. Conducted 4 procedures at a 2-week interval, the observation of patients lasted 12 weeks. After treating the skin with cetavlon, alcohol and povidone-iodine, anesthetic cream was applied to the skin. Injections were administered by the nappage method. As a result, the positive effects of the procedures were seen in all parameters of evaluation.

One of the earliest studies is a placebo-controlled trial by Takikawa et al. [19] in 2011. The experiment involved 26 patients (16 men and 10 women aged from 28 to 59 years). The following parameters were evaluated: 1) average amount of hair (digital and dermatoscopic image); 2) data of cross sections of hair (digital and dermatoscopic image); 3) epidermal thickness, the number of collagen fibers, blood vessels around the hair follicles (according to the 4 mm puncture biopsy). PRP was used with microparticles of dalteparin and protamine (daltepatin and protamine microparticles – D/P MPs). The study used the method of double centrifugation (Manual Double Spin). Parameters of centrifugation were: frequency: 1. 1700 revolutions per minute, 2. 3000 revolutions per minute; time: 1. 15 minutes, 2. 5 minutes; platelet enrichment × 6; blood volume 15 ml; PRP volume – 3 ml. 5 procedures were performed at a 2–3 week interval, the observation of patients lasted 12 weeks. Subcutaneous injections (3 ml) were made into selected 1x1 cm zones located at a certain distance from the tip of the nose and auricle. A positive effect from the administration of PRPs with D/P MPs was noted, and in the administration of simply PRPs zones.

Researches with a negative result

2 studies were published in 2016, during which there was not observed significant improvement while using of PRT: Puig et al. [20] and the study of Mapar et al. [21].

The first double, blind, randomized, placebo controlled, multicenter study involved 26 female patients, AGA stage II. Patients were divided into two groups: 15 women were included in the group where PRP was used, 11 – in the placebo group. The following parameters were evaluated: 1) counting the amount of hair (photo method); 2) counting hair mass index (Cohen HairCheck system). Centrifugation was performed using the Angel PRP system (Cytomedix), platelet enrichment ratio ×2.75–3.4; blood volume 60 ml; PRP volume – 10 ml. Patients received a single subcutaneous injection of PRP or placebo with the following evaluation of results after 26 weeks. Anesthesia used included 2 % lidocaine and 0.5 % bupivacaine. No significant changes were recorded at the 26th week, although patients who were administered PRP, noted a slower hair loss, improved hair density, easiness of hair styling, which was not noted by patients from the placebo group.

A second randomized, placebo-controlled, blind study was conducted with the participation of 19 men, of whom only 17 completed the tests completely (17 men aged from 24 to 45 years with stage IV-VI of AGA). The following parameters were evaluated (using a magnifying glass): 1) counting the number of terminal hairs; 2) counting the amount of vellus hair. The method of double centrifugation (Tubex PRP (Moohan Enterprise)) was used in the study. Calcium gluconate (0.1 ml to 1 ml of PRP) was used as an activator. Centrifugation parameters were:

frequency: 1. 3000 revolutions per minute, 2. 3300 revolutions per minute; time: 1. 6 minutes, 2. 3 minutes; platelet enrichment ratio x3; blood volume 9 ml; PRP volume – 1.5 ml. Square areas on the scalp 2.5×2.5 cm in size at a distance of at least 3 cm from each other, marked with tattoos were selected randomly, 2 injection sessions were conducted at intervals of 1 month. Evaluation of the results was carried out on the 1, 3 and 6 months after the first procedure, no significant changes were observed regarding the initial parameters.

CONCLUSIONS

Positive results were obtained during the 12 clinical studies among the conducted ones, no positive dynamics were observed in the end of other 2 studies. It can be noted that different methods and parameters of centrifugation were used to perform these experiments: PRP used differ or PRP activators were not used at all, variations were observed in methods for evaluating the obtained results, the multiplicity of procedures and intervals between them, the procedure technique (using a mezoroller, anesthetics, injections, etc.). Studies with a negative result were characterized by the smallest number of procedures performed (1 or 2 procedures), and methods for evaluating the results cannot be called sufficiently reliable (a magnifying glass).

It is obvious that the use of PRP has a positive effect on the state of AGA and can be used with approved methods of this disease treatment. However, the published results of only a small number of clinical trials on the effectiveness of PRP for hair growth cannot be considered objective. In addition, there is no published agreed protocol for the standard use of PRP, and the use of growth factors and stimulants in the expression of their genes still requires comparative evaluation with preparations approved for the treatment of hair. Thus, the method of PRP using can be considered as a method of treating AGA, however, it is necessary to continue research for further study and standardization of this technique.

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ENDOGENOUS RETROVIRUSES AS GENETIC MODULES THAT SHAPE THE GENOME REGULATORY NETWORKS DURING EVOLUTION

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Endogenous retroviruses (ERV) are the descendants of exogenous retroviruses that integrated into the germ cells genome, fixed and became inheritable. ERVs have evolved transcriptional enhancers and promoters that allow their replication in a wide range of tissue. Because ERVs comprise the regulatory elements it could be assume that ERVs capable to shape and reshape genomic regulatory networks by inserting their promoters and enhancers in new genomic loci upon retrotransposition. Thus retroransposition events can build new regulatory regions and lead to a new pattern of gene activation in the cell.

In this review we summarize evidence which revealed that ERVs provide a plethora of novel gene regulatory elements, including tissue specific promoters and enhancers for protein-coding genes or long noncoding RNAs in a wide range of cell types. The accumulated findings support the hypothesis that the ERVs have rewired the gene regulatory networks and act as a major source of genomic regulatory innovation during evolution.

KEY WORDS: endogenous retroviruses, enhancer, promoter, lncRNA, regulatory networks, R-operon, mobile elements, genome evolution

ЕНДОГЕННІ РЕТРОВІРУСИ ЯК ГЕНЕТИЧНІ МОДУЛІ, ЩО ФОРМУЮТЬ РЕГУЛЯТОРНІ МЕРЕЖІ ВПРОДОВЖ ЕВОЛЮЦІЇ

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Ендогенні ретровіруси (ERV) є нащадками екзогенних ретровірусів, які впровадилися в геном статевих клітин і стали передаватися у спадок. Впродовж еволюції ERVs ретровіруси придбали енхансери і промотори, які дозволили їм експресуватися у різних тканинах. Оскільки ERVs мають регуляторні елементи, то можна припустити, що вони здатні форматувати і переформовувати регуляторні мережі генома за допомогою впровадження промоторів і енхансерів у нові локуси генома при ретротранспозиції.

У огляді зібрані дані, які підтверджують роль ERVs ретровірусів у формуванні ряду енхансерів і промоторів генів, що кодують білки, і генів, які кодують молекули довгих некодуємих РНК у різних тканинах. У огляді також підсумовуванні докази ролі ERVs у переформатуванні регуляторних мереж. Ці дані підтверджують припущення, згідно якому ERVs ретровіруси сприяють виникненню нових регуляторних програм і геномних інновацій впродовж еволюції.

КЛЮЧОВІ СЛОВА: ендогенні ретровіруси, енхансери, промотори, регуляторні програми, гени довгої некодуємої РНК, R- оперон, мобільні елементи, еволюція генома

ЭНДОГЕННЫЕ РЕТРОВИРУСЫ КАК ГЕНЕТИЧЕСКИЕ МОДУЛИ, ФОРМИРУЮЩИЕ РЕГУЛЯТОРНЫЕ СЕТИ В ТЕЧЕНИЕ ЭВОЛЮЦИИ

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Эндогенные ретровирусы (ERV) являются потомками экзогенных ретровирусов, которые внедрились в геном половых клеток и стали передаваться по наследству. В течение эволюции ERV ретровирусы приобрели энхансеры и промоторы, которые позволили им экспрессироваться в различных тканях. Поскольку ERV обладают регуляторными элементами, то можно предположить, что они способны форматировать и переформатировать регуляторные сети генома с помощью внедрения промоторов и энхансеров в новые локусы генома при ретротранспозиции.

В обзоре собраны данные, которые подтверждают роль ERV ретровирусов в формировании ряда энхансеров и промоторов генов, кодирующих белки, и генов, кодирующих молекулы длинных некодирующих РНК, в различных тканях. В обзоре также суммированы доказательства роли ERV в перефоматировании регуляторных сетей. Эти данные подтверждают предположение, согласно которому ERV ретровирусы способствуют возникновению новых регуляторных программ и геномных инноваций в течение эволюции.

КЛЮЧЕВЫЕ СЛОВА: эндогенные ретровирусы, энхансеры, промоторы, регуляторные программы, гены длинной некодирующей РНК, R-оперон, мобильные элементы, эволюция генома

INTRODUCTION

It becomes more and more obvious that animals and plants live in symbiosis with microorganisms. And not only with bacteria and the simplest, but also with viruses. Understanding the possibility of symbiotic relations with viruses came at the very last time, before that they were considered only as parasites. The reason for the one-sided assessment of the role of viruses is the difficulty in studying the laws underlying the symbiosis, the secrecy of such laws from the eyes of researchers. However, as the relationship between viruses and animals has been studied, it has come to realize that parasitic relationships leading to the development of pathologies are an exception rather a rule in the relationship between viruses and organisms. In most cases, co-operative relationships are established between them [1].

Integration of viral genomes, including genomes of RNA viruses, into the host genomes occurs with an unexpectedly high frequency [2]. Representatives of a number of RNA and DNA viruses were found in the vertebrate and human genome, among them Ebola virus, filoviruses, coronaviruses, circoviruses, hepadnaviruses and parvoviruses [3–7]. Integration mechanisms are the introduction of retroviruses using reverse transcriptase, sometimes accompanied by recombination with other viral sequences [8], and also, possibly, insertion into the genome during theDNA breaks repairing using the mechanism of joining non-homologous ends [9].

Viruses participate along with bacteria in the horizontal transfer of genetic material, including mobile elements between organisms, which play a big role in adaptation of organisms and evolution to the external environment [10].

Viruses don’t only carry mobile elements, but can also contribute to the emergence of new mobile elements. Thus, new DNA transposons can arise as a result of merges of transposons and DNA-containing viruses. The mechanism of such a fusion is recombination.

Exogenous retroviruses, while integrating into the genome during evolution, transformed into one of the fractions of mobile elements, called endogenous retroviruses (ERV).

Mobile elements can change the genome both actively and passively. ERV, transferring genes and cis-regulatory elements, change the genome actively. Mobile elements contribute passively to ectopic recombination and, accordingly, to the occurrence of duplications, deletions or karyotypic rearrangements [11].

Changing the genome, mobile elements can facilitate and accelerate evolution [12–13]. The presence of mobile elements in the genomes is a certain risk. It reduces somewhat the current

adaptation of the organism, but gives advantages in the future. Species containing many mobile elements in the genome acquire a certain evolutionary potential, and if the environment, the habits of the animal or the habitat change, in case of an animal colliding with some challenges, they have a tool for rapid genome change and response to challenges.

Indeed, ERVs played a huge role in the formation of vertebrate genomes, and primarily in the formation of regulatory elements of the genome and the reformatting of genetic regulatory programs in the formation of species, which will be discussed in detail in the article. Emergence of the placenta in mammals is the example of evolutionary innovation, in the origin of which endogenous retroviruses played an important role [13].

Thus, mobile elements formed by retroviruses or acquired due to horizontal transport with the help of viruses are a tool of rapid evolutionary changes or saltation that contradict the dominant ideas about gradual changes in the genome.

FORMATION OF VERTEBRATE GENOMES OF BY ENDOGENOUS RETROVIRUSES

Mobile elements have largely formed a fraction of the repeating sequences of the genome. In vertebrate genomes, the proportion of the repeating sequences is from 1.2 % in primitive fish to 38 % in reptiles [11]. The genome of birds contains the relatively low number of repeats about 6–12 %. At least one third of the genome in mammals is formed by repeating elements, and the genome of some primates is half composed of repeating sequences [11]. According to modern estimates, 69 % of the human genome is repetitive genetic elements [14].

In general, at least in vertebrates, the relationship between the number of repeating sequences and the complexity of the organism is traced. The more evolutionarily developed the species are, the more their genome contains non-coding proteins of genes, including repetitive DNA [11].

Repeating sequences of vertebrates are largely formed by mobile elements, including ERVs. During the evolution of vertebrates, exogenous retroviruses were implanted many times in germinal cells and transmitted to the offspring, becoming ERVs [15]. All placental mammals contain endogenous retroviruses. They played a large role in the formation of the placenta of mammals as was noted above [13].

Currently, it has been possible to observe the process of endogenization of retroviruses in Australian koalas. This species was transported under threat of extinction to the islands in the early 20th century. On the islands, coals were infected with marmoset retroviruses and gibbon leukaemia virus. Many of them died, but some survived and gained stability. Retroviruses were introduced into germinal cells in surviving koalas and viruses were transmitted to offspring [16].

ERVs compose 8 % of the human genome [17]. In comparison, all sequences that encode proteins make up about 1.5 % of the human genome [17]. Complete ERVs encode a group-specific protein Gag, Pro protease, Pol Polymerase and occasionally envelope protein. Long terminal repeats (LTRs) flank ERVs on both sides. LTR elements are necessary for replication of the retrovirus and contain cis-regulatory sequences which transcription factors specifically bind with, as well as promoters which transcription starts from. The length of the long terminal repeats is about 1000 bp. Recombination between 5 'and 3' LTR sequences of endogenous retroviruses results in the formation of single LTRs, 577,000 of which were detected in the human genome [18]. 90 % of ERVs are single LTR elements.

The role of retroviruses in genome formation is determined by the fact that they contain a wide variety of regulatory elements. ERVs are potential sources of enhancers, alternative promoters, splice sites [19], and sites for polyadenylation [20]. So about 40 transcription factors can bind to LTR elements and regulate the transcription of human ERV type K (HERVK) [21]. The presence of regulatory elements creates a potential for tissue-specific expression of the retroviruses themselves, as well as for reformatting the expression of genes of the host genome.

Today, it is not known whether the ERVs contain retroviruses cis-regulatory elements that allow them to interact with trans-regulatory factors of the cell, prior to insertion into the genome, or they acquire them after the introduction. At least in some cases it was possible to show that cis-regulatory elements existed in retroviruses before they were introduced into the genome. So, elements of mice containing the regulatory modules RLTR9B2, RLTR9D and RLTR9E inherited

these modules and the ability to regulate gene expression from progenitor retroviruses prior to their introduction into the genome [22].

TISSUE-SPECIFIC ACTIVATION OF ENDOGENOUS RETROVIRUSES

For a long time it was believed that mobile elements are epigenetically suppressed and therefore cannot play an active role in the regulation of gene expression. However, a number of data allowed overcoming this error.

First, mobile elements and endogenous retroviruses among them form tissue specifically DNAase I sensitive regions. These regions differ of about 100 times based on sensitivity to DNase I. DNase I sensitive regions are chromatin regions with an open chromatin configuration, that is functionally active regions. Scientists have constructed a map of DNase I sensitive regions of the genomes of a number of human cell lines [23]. About 2.9 million DNase I sensitive regions in total were found in the human genome. Approximately 3 % of the DNase sensitive regions are located in the start site region of the transcription of the gene-encoding genes, but lies within 2.5 kb from the point at which the 5 % of transcriptions start. The remaining 95 % of DNase I sensitive regions are located at a great distance from the starting transcription areas in introns and in intergenic regions. The formation of DNase I sensitivity of sites located at large distances is largely tissue-specific.

44 % of DNase I sensitive regions are located in mobile elements. Moreover, if we consider primate-specific sensitive regions of DNase I sensitive regions, then this value reaches 63 % [24]. DNase I sensitive regions are mostly concentrated in long terminal repeats of LTR endogenous retroviruses. Mapping of DNase sensitive sites in normal, embryonic and cancer cells has shown that up to 80% of ERVs in the human genome form tissue specifically DNase I sensitive regions with an open chromatin structure [24]. Tissue-specificity is determined by cis-regulatory sequences of LTR elements. The formation of an open chromatin structure by LTR elements is often associated with the expression of neighbouring genetic loci [24].

Secondly, it was previously thought that mobile elements inactivity is to a large extent due to the hypermethylation of their DNA sequences. However, the study of methylation of 928 subfamilies of mobile elements in embryonic and terminally differentiated human tissues showed that the DNA of these elements is tissue-specific and specific with respect to the kind of mobile element being hypomethylated [25]. Certain classes of ERVs were mostly tissue specifically hypomethylated. We studied the hypomethylation of mobile elements in only 4 types of cells. About 10 % of the studied TE subfamilies are hypomethylated in these tissues. However, if we study more cells, it is likely that a significantly greater percentage of mobile elements are tissue-specific hypomethylated.

A significant part of the genes among located close to the hypomethylated tissue-specific mobile elements is composed of genes encoding proteins necessary for this type of tissue, and gene expression correlates with the hypomethylation of nearby mobile elements. Moreover, hypomethylation is accompanied by the acquiring of a typical epigenetic marker of enhancers by these areas. Many of the hypomethylated mobile elements do have enhancer activity, which is detected by the reporter method. Also, many of these sequences have binding sites to transcription factors that are specific to the respective tissues. Therefore, hypomethylated sequences of mobile elements can potentially function as enhancers. Nevertheless, this is not proven.

Thirdly, an LTR element contains cis elements, and often even clusters of cis-elements which tissue regulatory transcription factors binding with. The total number of DNA fragments came from human endogenous retroviruses is estimated to be 717,778. Approximately (~15 %) of the 110,000 fragments contain at least one binding site with a transcription factor [26]. According to recent estimates, the human genome contains 794,972 binding sites with 97 transcription factors [27].

On the average, about 20% of binding sites with 26 regulatory transcription factors are located in mobile elements, mainly in LTR elements in human and mouse genomes. Some of the mobile elements formed 5 %, and the other 40 % of the overall number of all binding sites with a certain transcription factor [28]. Binding sites with transcription factors have an open chromatin structure in the LTR elements, and therefore DNase sensitivity, they are hypomethylated and contain epigenetic modifications of histones typical for enhancers. On the average, 66 % of the binding sites of transcription factors with mobile elements are formed tissue specifically [28].

Three main clusters can be distinguished among LTR elements of human ERV (HERV) based on binding patterns with transcription factors [27]. The first class includes LTR elements associated with transcription factors of pluripotency (SOX2, POU5F1 and NANOG). For example, evolutionarily young LTR7 elements transcribed actively in pluripotent cells and they are enriched with binding sites with SOX2, POU5F1 and KLF4 transcription factors of pluripotency. The second class includes LTR elements that bind to factors expressed in the embryonic ectoderm and embryonic mesoderm (GATA4 / 6, SOX17 and FOXA1 / 2), and the third class elements bind to hematopoietic transcription factors (SPI1 (PU1), GATA1 / 2 and TAL1). There is also a group of HERV containing a binding site with CTCF factors, i.e. cis sequences capable of forming insulators and topological domains [27].

Binding sites with transcription factors are not only tissue-specific, but also species-specific. Up to 25 % of all binding sites in embryonic stem cells of humans and mice with key transcription factors of pluripotency OCT4 and NANOG came from mobile elements specific to these species, including HERV [29].

More than 98 % of 132,197 0 binding sites with 26 transcription factors localized in the mobile elements of the human genome, are absent in the genome of mice [28]. At the same time, there are also conservative binding sites. In all likelihood, species-specificity of cis-regulatory elements arises from the introduction and amplification of specific mobile elements, which occurred after the separation of the two lines leading to the appearance of human and mice. Another interesting phenomenon is that there is an expansion of species-specific binding sites with transcription factors in both genomes.

Finally, ERVs can not only contain epigenetic markers of active chromatin and bind tissue-specifically to transcription factors, but can also be expressed tissue-specifically and even in response to environmental conditions. Tissue-specificity of ERVs expression is confirmed by data obtained from the ENCODE program, as well as from the number of other studies [30]. Thousands of retroviral sequences are specifically activated in cells, especially embryonic cells, cancer cells, as well as in response to various stimuli [31].

ERVs of the human genome are expressed in oocytes, zygotes, 2–8-cell embryos, morulae and blastocysts, as well as in embryonic stem cells. To the greatest extent they are expressed at the stage of development of oocytes to 4-cell embryos [32]. The expression of ERVs is reduced from the stage of the 8-cell embryo. ERVs are expressed stage specifically, and also specific for differentiated cell populations that arise among blastocysts. The majority of ERV elements expressed during the listed stages of embryonic development are not activated in the tissues of an adult organism. Specific cis-regulatory sequences of LTR elements determine the stage specificity of expression of ERV.

In B lymphocytes, expression of endogenous human and mouse retroviruses is activated by stimulation of cell proliferation in vitro and in vivo, as well as in chronic diseases, including B cell lymphoma. A small number of LTR elements, which are constantly activated in stimulated B cells, are detected, with one of the Xmv45 retroviruses being expressed in a larger amount than the rest of them combined. The expression of a large number ERVs is at the same time activated during B cell transformation [33].

Thus, LTR elements acquire DNAase I sensitivity in a tissue-specific manner, become hypomethylated, acquire epigenetic histone modifications typical for transcriptionally active loci and enhancers, bind to transcription factors and even are transcribed [24–25, 28]. Together, these data indicate the biochemical activity of cis-regulatory elements of ERVs and suggest that they are the source of regulatory sequences of the genome.

However, these data are insufficient to state that cis-regulatory elements of ERVs really control the expression of protein-coding genes. For example, cis-regulating binding sites can serve as a buffer for transcription factors, landing sites from which transcription factors begin to scan DNA in search of an attachment site. At the same time, species-specific ERVs capable of tissue-specific activation are an excellent tool for creating new regulatory elements and genetic programs in the evolution process. Experimental data confirm the role of endogenous retroviruses in the formation of alternative promoters and enhancers, as well as genes of long non-coding RNAs and the role in the reformatting of regulatory networks [29–33].

ENDOGENOUS RETROVIRUSES AS A SOURCE OF REGULATORY ELEMENTS AND NON-CODING RNA GENES

When cis elements of the LTR intrude close to the genes, the sequences of ERVs retroviruses can form alternative promoters, thereby increasing the number of gene isoforms, while the gene can acquire new tissue-specific expression [34–38]. At the moment, a small number of cases of the alternative promoters 'formation by ERVs have been well studied.

A classic example is the acquisition of the ability to express in the human salivary glands a gene that encodes the enzyme amylase. It acquired this ability due to the insertion of the LTR element and the formation of an LTR alternative promoter [34].

The human gene encoding the B3GALT5 metabolism factor is expressed in many differentiated cells, but the primate-specific alternative promoter formed by LTR element is used in cells of the large intestine [35].

Prolactin is not produced by the uterus of a number of mammals, such as rabbits, dogs, pigs and armadillos. At the same time, it is produced during pregnancy by the uterus of the primates, mice and elephants. Regulation of the expression of the gene encoding the precursor of prolactin in the uterus has evolved in mentioned mammals. The alternative gene promoter contains the DNA transposon in humans and spider monkeys, as well as the MER39 retrovirus, and the mouse alternative promoter originated from the MER77 endogenous retrovirus [36].

The NAIP gene encodes an inhibitory apoptosis of neuronal proteins. The NAIP gene is regulated by a variety of promoters both in humans and in mice that do not coincide between them. LTR elements of ERVs in the human genome have formed an alternative gene promoter that allows the gene to be expressed in testicles [37]. Rodents contain several copies of the NAIP gene. The main constitutive promoter of these genes is formed by LTR elements of the ORR1E ERV. In addition, the MT-C ERVs formed a minor promoter of two copies of the NAIP gene.

The gene that encodes the erythroid transcription of Pu. 1 mice factor has an alternative promoter. The promoter is formed by the LTR element of the ORR1A0 retrovirus located in the intron of the Pu.1 gene [38]. A chimeric transcript of Pu.2 is formed when expressed from this promoter. It induces erythroid differentiation in vitro.

Thus, cis-regulatory sequences of LTR elements of ERVs initiate the synthesis of transcripts from gene-encoding genes in addition to its own synthesis [34, 36, 38]. Transcripts formed are chimeric RNA molecules by structure. Chimeric transcripts contain sequences of retroviruses at the 5 'end. The rest of the transcripts are identical to the sequences of the corresponding genes.

ERV are potentially able to quickly reformat regulatory networks possessing the ability to move in the genome, and on the other hand, being introduced near the genes and supplying them with alternative promoters. Indeed, ERVs in the evolution participated species-specific and tissue-specific in the formation of regulatory programs, which will be discussed below.

At the same time, ERVs retroviruses can intrude at large distances from genes encoding proteins and form distal regulatory elements – enhancers.

LTR9 element located at a distance of 40–70 kb upstream of human gamma and beta globin genes forms an enhancer that activates the expression of the β-globin gene in transgenic mice [39]. Moreover, even hypermethylated ERV9 LTR possesses enhancer activity since in vivo deletion of LTR by CRISPR-cas9 method suppresses gene activity more than 50 % [40].

MaLR LTR is an enhancer that controls the expression of the proopiomelanocortin Pomc gene in the pituitary and hypothalamus of mammalian [41]. And the enhancer, which results from the MaLR of LTR element, provides about 80 % of the Pomc gene expression [42].

The tissue-specific enhancer hsERVPRODH formed by the ERV controls the transcription of the PRODH gene in the hippocampus. The gene encodes proline dehydrogenase and, apparently, participates in the synthesis of neurotransmitters in the central nervous system [43]. Expression of the gene is necessary for the normal functioning of the central nervous system. The enhancer activity of hsERVPRODH is manifested in the hypomethylated state during the attachment of the transcription factor SOX2.

The enhancer can be formed by co-opting the regulatory sequences of several mobile elements [44].

So, for example, the region originating from AmnSINE1 of the non-autonomous retrotransposon does not have enhancer activity, but nevertheless preserves its conservatism in the platypus and human genome. However, the integration of the DNA of the transposon allows the acquisition of a binding site with the transcription factor Msx1 after the single-pass divergence. The endogenous retrovirus MER117 is introduced at the next stage after the masculine divergence, resulting in the formation of a modern enhancer [44].

There are reasons to believe that many enhancers have been formed in this way. Indeed, in the human genome 54 (8.6 %) out of 626 conservative AmnSINE1 loci are associated with other evolutionarily conserved mobile elements, among which LTR-containing ERVs and DNA transposons. Such sites are potential enhancers, however, this has not been proved yet.

Data obtained from the ENCODE project showed that at least 75 % of the genome is transcribed with RNA formation, despite the fact that the protein-encoding DNA sequences make up only 1.5 % [45]. These data were obtained by studying of 15 cell lines. Therefore, the data is understated and in fact the percentage can be even higher. As already discussed, most of the genome repeating sequences, including ERV, are actively transcribed [46].

A significant portion of the transcribed RNA is formed by long non-coding RNA (lncRNA). LncRNAs are molecules whose length is above 200 bp. According to the NONCODE database, there were 96,308 genes encoding lncRNA and 172216 lncRNA transcripts in 2018 [47].

The genes encoding lncRNA and mRNA are similar in size and structure. The transcription of lncRNA starts from promoters that contain binding sites with transcription factors and epigenetic markers typical for transcriptionally active genes [45]. The lncRNAs are mainly transcribed by RNA polymerase II. The lncRNA molecules contain a cap at the 5 'end and are polyadenylated at the 3' end and are characterized by alternative splicing [45].

The tissue-specificity of expression is more characteristic for lncRNA genes than for protein-encoding genes. LncRNA genes are expressed not only tissue-specifically, but, apparently, each cell contains its unique set of lncRNA molecules [48].

lncRNAs are not a homogeneous class of molecules, but a mixture of molecules with different biochemical mechanisms of action and function. The ability to interact complementarily with DNA, modular organization and alternative splicing allow lncRNA molecules to function as address epigenetic modulators, which, deliver epigenetic information to the right place in response to external actions and the existing metabolic situation in the cell.

Mobile elements play an important role in the occurrence of long non-coding RNA in vertebrates from fish to humans [49, 50]. ERVs played the main role in the formation of lncRNA genes in mice and humans, and DNA transposon played such role in the genome of zebra fish. Mobile elements formed largely the primate genes encoding lncRNA as well as. Thus, mobile elements were detected in 83 % of 9241 lncRNA molecules and accounted for 42 % of the total sequence of all human lncRNAs [49]. ERVs appear to be the main factor contributing to the emergence of new lncRNA genes due to their ability to species-specific incorporation into genomes and spread in them, as well as due to regulatory sequences presence in them. Genes of human lncRNA are enriched by the sequences of ERV1, ERVL-MaLR, ERVL and ERVK retroviruses.

ERV insertion could diversify the already existing lncRNA genes in some cases during the evolution. At the same time, in other cases, new lncRNAs appeared as a result of the ERV insertion. Apparently, the presence of cis-regulatory elements and tissue-specific transcription were the main properties that allowed retroviruses to form new lncRNA genes during evolution [50].

Indeed, ERVs are located mainly in the region of 5 'ends of lncRNA transcripts in a sense orientation, i. e. in a position that allows LTR elements to initiate transcription and regulate it. For example, transcription of the lncRNARoR gene in human embryonic stem cells is controlled by the cis-regulatory sequences of the LTR7/HERVH element that bind to OCT4, NANOG, and SOX2 transcription factors [50].

A new class of lncRNA has recently been discovered, which is called chromatin enriched RNA (cheRNA). Almost all genes encoding completely cheRNA are formed by retrotransposons, including ERV.

Most cheRNA molecules interact with RNA polymerase II and remain bound to chromatin by transcription or stopping transcription [51].

In general, cheRNA genes are expressed tissue-specifically. Proximity to the genes encoding the expressed cheRNA cells in this type of cell is most accurately combined with the transcriptional activity of the protein-encoding genes. Moreover, such proximity is more often associated with the transcriptional activity of protein-encoding genes than the expression of long non-coding RNAs of other classes and even the transcription of enhancers in this type of cells. Deletion of several cheRNA molecules resulted in a significant, about 75 % reduction in the transcription of a nearby lying gene. Together, these data indicate that cheRNA acts as a transcriptional activator. However, it is not clear if the transcription of these loci itself causes such an effect, or synthesized RNA molecules are needed.

A number of data suggest that a rapid species-specific formation of new lncRNA genes occurs due to mobile elements during evolution [50, 52]. A significant portion of the genes of human lncRNA arouse recently, apparently due to the activity of mobile elements. Indeed, 40 % of lncRNA containing the mobile elements are specific for primates [50].

Very interesting data were obtained during the study of tomatoes. Comparison of the lncRNAs of two tomato species Solanum lycopersicum and Solanumpimpinellifolium showed that a small part 6.7 %, (24 of 353) of lncRNA molecules appeared to be common for both species [52]. And only less than 0.4 % of lncRNAs are common for all sequenced genomes of tomatoes and potatoes. Apparently, the appearance of lncRNA genes is associated with mobile elements in the genomes of two species of tomato, since 85 % of Lycopersicon-specific lncRNA molecules contain mobile elements.

Thus, ERVs can regulate gene activity, not only by forming enhancers and alternative promoters, but also by species-specifically creating genes of lncRNA. LncRNA promoters are formed thanks to retroviruses, as well as tissue-specific regulatory networks controlling the expression of lncRNA genes. LncRNA, regulate the transcription of protein-coding genes in turn, including the transcription of adjacent genes. And, finally, mobile elements, which ERVs are among, can cause rapid evolutionary changes of lncRNA genes.

REFORMATTING REGULATORY NETWORKS BY ERVS

Changes in genetic regulatory programs underlie phenotypic differences between species and within the species. However, the mechanisms of changes in regulatory networks in evolution are poorly understood. Extremely intriguing is the regulatory networks feature to change quickly and consistently. In order for the genome to acquire a certain set of regulatory elements for coordinated regulation, a number of corresponding single mutations must appear in those regulatory regions of the genome that regulate the corresponding genes. So that is necessary that a multiplicity of identical mutations arose in a variety of regions of the genome in a short evolutionary time interval. At the same time, several implementations of mobile elements containing regulatory elements in several regions of the genome are needed to form a new regulatory network.

LTR elements of ERVs contain not only cis-regulatory elements, but also cis-regulatory modules, which significantly expands their potentialities in the formation of new regulatory networks [22]. Cis-regulatory modules are a set of cis-regulatory sequences binding transcription factors that co-regulate the activity of target genes. Thus, ERVs containing single cis-regulatory elements and clusters of binding sites with transcription factors are a good natural tool for rapid and consistent changes in regulatory chains.

One can imagine at least three ways in which ERVs form and reformat regulatory networks. First, they can distribute alternative promoters in the genome. Secondly, they can contribute to the acquisition of new enhancers by multiple genes. And thirdly, they can form networks of tissue-specific and species-specific expressed lncRNA genes.

The spread of alternative promoters by ERVs is confirmed by a variety of data. Synthesis of about 6–30 % transcripts containing cap at the 5 'end isolated from various embryonic and differentiated cells of mice and humans starts with mobile elements [53]. These transcripts are mainly tissue-specific.

Expression of a number of ERVs is activated in mature oocytes and at the stage of the double cellular embryo of mice, but with further

development of the embryo their expression is suppressed. Chimeric transcripts are synthesized when LTR elements are activated in mature ovum, their transcription starts from alternative promoters formed by MaLR and ERVK retrovirus families [54]. It was possible to detect more than 500 chimeric transcripts, in the formation of which 307 protein coding genes take part, using sequencing RNA sequences of cells isolated from a population of mouse embryonic stem cells and corresponding to cells of a 2-cell embryo. Synthesis of chimeric transcripts begins with LTR elements of the retroviruses of the MERVL family and extends beyond the retrovirus, including genes [55].

When the mouse 2–4-cell embryos 259 transcript were sequenced, the relationship between activation of LTR elements of ERVs and a temporary and strong increase in the transcription of adjacent genes were confirmed. LTR elements regulating significantly the transcription of neighbouring genes have been enriched by binding sites with home box containing transcription factors [56]. As it is known, home box-containing transcription factors are key regulators of morphogenesis in embryonic development.

However, the question arises whether chimeric transcripts has the function. In, firstly, the fact that more than 90 out of the 626 chimeric transcripts synthesized in mouse embryonic stem cells retain the open reading frame proves their functional significance [55].

Secondly, genes encoding critical for the differentiation of early embryonic cells transcription factors GATA and TEAD, use LTR as alternative promoters [55].

The promoter of the Dicer1 gene is the promoter containing the CpGIsland in most cells, and the promoter in mouse oocytes is the LTR element of the ERVs – MaLR [57]. Deletion of an alternative promoter reduces the expression of the Dicer1 gene in oocytes and causes infertility [58].

Thirdly, cultured mouse embryonic cells that express transcripts from LTR sequences and cells that do not express transcripts from LTR elements have different phenotypes [55].

Regulatory networks that are altered by ERV-derived enhancers are found in cells of tissues associated with sexual reproduction, in embryonic stem cells and on the early stages of embryogenesis, in erythroblasts and in terminally differentiated liver cells and the immune system.

The cis-regulatory element of the MaLR retroviruses binds to the Tbx6 transcription factor. Expression of at least four genes, whose enhancers are formed by MaLR LTR, significantly decreases in mice deficient in Tbx6 transcription factor [59]. Tbx6 regulates gene expression during early embryogenesis.

Mice placenta cells used ERVs for species-specific reformatting of the regulation of gene expression. A class of retroviruses RLTR13D5 was detected by revealing the profile of epigenetic markers and binding sites with the transcription factors of stem cells of trophoblasts in mice and rats. It forms a significant part of active in the placenta enhancers [60]. These ERVs retroviruses contain binding sites with transcription factors Eomes, Cdx2 and Elf5, which play key roleintrophoblast regulatory networks. Ehancers made by retroviruses have formed a network consisting of hundreds of elements, and control the synthesis of one-third of all placental specific transcripts [60].

However, the RLTR13 family of ERV, which formed a regulatory network of trophoblast stem cells, is specific for mice. Even in rats, most mouse enhancers formed by the ERVs of the RLTR13 family are absent.

LTR elements played a significant role in the formation of new regulatory networks that evolved in monkeys in the liver.

Indeed, 77.1 % of the cis-regulatory elements specific for monkeys and virtually all the cis-regulatory elements those are specific for the human genome, cis-regulatory elements of the liver overlap with retrotransposons [61]. Regulatory activity of a number of elements containing retrotransposons was confirmed by studying the activity of synthesized consensus sequences in cultured liver cells HepG2 using a luciferase reporter assay.

The LTR elements of ERVs and the SVA retrotransposons containing retroviral elements contributed to the greatest extent to the formation of regulatory programs that appeared at the last stages of the evolution of regulatory programs. At the same time, only 16.0 % of evolutionarily conserved cis-regulatory sequences contain mobile elements. Thus, cortical regulatory programs that ensure the identity of liver cells persist throughout the evolution of primates, while peripheral

regulatory programs are rapidly evolving with the help of ERV.

The formation of a line-specific and tissue-specific regulatory network activated by interferon is the most rigorously demonstrated example of the participation of ERVs in the reformatting of regulatory networks by the formation of new enhancers.

Researchers used the CRISPR-Cas9 method for deletion part of ERVs specific for primates of the MER41 family containing cis elements which transcription factors activated by interferon bind to [62]. The genes were no longer regulated by interferon as a result of the deletion, their expression decreased, which manifested among other things in the alteration of various phenotypic signs, among which decreased inflammatory response in response to infection. Thus it was possible to show that the human genome contains 962 ERVs of the MER41 family, which bind to the transcription factors STAT1 and IRF1 in at least one type of cells. Together, these data show that the MER41 elements have formed a regulatory network controlled by interferon.

MER41 contains a tandem sequence that binds to the transcription factor STAT1. The same sequence contains MER41-related retroviruses of lemurous, bats, carnivores and artiodactyls. Therefore, it can be assumed that related ERVs form interferon-induced enhancers in different mammalian species. Indeed, the consensus sequence of MER41-like LTR elements of dogs and cows shows activity in the luciferase reporter assay in the HeLa cell line in response to induction by interferon.

Mice do not have a MER41 family of retroviruses, but endogenous gamma-retroviruses RLTR30B specific for mice also formed enhancers controlled by interferon. Methods of bioinformatics allowed revealing the connection between RLTR30B elements and genome loci containing the immune response genes. Consequently, two different families of endogenous retroviruses formed convergent interferon-regulated immune response programs in two mammalian species: humans and mice.

Next ERV have formed regulatory networks consisting of lncRNA genes.

LTR elements of mice ERVs control the transcription of lncRNA genes in the post-mitotic phase of the cell cycle of spermatocytes and round spermatids [63]. LTR elements have formed tissue-specific lncRNA promoters, thus creating a regulatory network that allows them to regulate the expression of lncRNA genes in spermatogenesis. Interestingly, a small part of the transcript elements initiated with ERVs encodes the open reading frames, which allow the peptides synthesis.

LTR elements of the ERV1 form regulatory sequences that control the tissue-specific expression of lncRNA in human testes [64].

The synthesis of transcripts starts from a number of HERV expressed in oocytes, zygotes, 2–8 cell embryos, morulae and blastocysts, as well as in embryonic stem cells, and extends beyond the retrovirus. Then, RNA molecules are formed as a result of splicing containing exons of non-retroviral nature. For example, 95 % of the elements of the MLT2A1 family of retroviruses encode such RNA molecules. Most of the non-retroviral exons are unannotated and, apparently, form a non-coding lncRNA [32].

The lncRNA genes containing human H retroviruses of the HERVH family are specifically expressed in embryonic stem cells and induced pluripotent stem cells. Expression of HERVH containing lncRNA genes is necessary to maintain pluripotency in cells [49]. 127 lncRNA genes are transcribed in the embryonic stem cells, containing the LTR7 elements of the HERVH in the sense orientation near the sites of the beginning of transcription. LTR elements of HERVH containing lncPHK genes bind with OCT4 and NANOG pluripotency transcription factors [49].

Another method by which endogenous retroviruses can form regulatory networks is found in erythroblasts, which ERV9 human retroviruses have formed a regulatory network in, creating both enhancers, and lncRNA genes [65].

lncRNA transcribed from LTR retrotransposons of ERV9 activates the transcription of key erythroid genes and modulates erythropoiesis ex vivo. Theoretically, ERV9 lncRNA can regulate the transcription of key erythropoiesis genes by acting in cis or in trans, diffusing from the site of synthesis to the target gene, which can be located on another chromosome. To understand the mechanism of action of ERV9 lncRNA, the synthesized transcripts were analysed before and after the global deletion or locus-specific deletion of ERV9 lncRNA in human erythroblasts containing ~ 4000 copies of ERV9 LTR and in mice erythroblasts containing one

transgenic copy of the primate specific ERV9 LTR in the locus, which encodes the beta-haemoglobin gene.

As a result, it was shown that ERV9 lncRNA, synthesized from the ERV9 LTR element, which controls the transcription of the beta-globin gene, remains associated with LTR and interacts with transcription factors and polymerase II, forming an enhancer complex. The enhancer complex interacts with the downstream gene promoter, thereby activating transcription.

In ERV9 erythroblasts, lncRNA is transcribed from many of 4000 copies of ERV9 retrovirus, stabilizes the enhancer complex and activates the transcription of a number of genes in cis, including key erythropoiesis genes, including haemoglobin genes, as well as genes encoding the transcription factors of erythropoiesis KLF1 and CCNDBP1.

So, the regulatory networks of early embryonic tissues, pluripotent embryonic cells, liver cells, erythroblasts, interferon-induced gamma genes are reformatted species-specifically by endogenous retroviruses. These data support the hypothesis that ERVs are used during evolution for species-specific reformatting of regulatory programs. Moreover, ERVs are convergent used by various species to form regulatory networks induced by gamma interferon. Examples of convergent use of ERVs for the formation of promoters were given earlier. These examples of completely amazing convergence remain a mystery of evolution. Is the use of mobile elements for similar regulatory programs creation in different species of animals due to chance? Or as Shapiro writes in his article: the elements responding to some as-yet-to-be-defined regulatory process that guides the adaptive integration of newly established regulatory signals? To answer this question, it is necessary to calculate the probability according to which such a regulatory network can form in evolution, based on the assumption of chance. On the other hand, there is data currently accumulating according to which the introduction of mobile elements is not a random process, but occurs localized in time and space [11]. It is interesting that another type of retrotransposon, long LINE intersperse elements, are activated in neurons during differentiation [66]. The sites for introducing LINE elements in neurons are not accidental. They are predominantly localized in enhancers actively transcribed in the neurons genes [67]. Moreover, it is assumed that LINE elements are implanted into double DNA ruptures that are formed in genes and actively transcribed in terminally differentiated neurons. In other words, the localization of retrotransposon introduction sites is determined by the functional activity of neurons [68].

HYPOTHESIS OF R-OPERON

It is well known that homologous DNA sequences have the ability to recognize and interact with each other. The interaction between homologous sequences will inevitably affect the location of DNA molecules in the core space. Therefore, a model has been proposed, according to which repeated homologous elements of the genome form and/or stabilize the specific spatial structure of both interphase chromatin and mitotic chromosomes [69–70].

According to the model, when interphase chromatin is laid, an association occurs between the homologous mobile elements resulting in the formation of homologous pairs, which then form a repetition assembly (RA). The organization of interphase chromatin is the basis for formation of mitotic chromosomes. More and more homologous pairs establish contact with each other during the laying of mitotic chromosomes. The chromatin filaments gradually become denser, forming mitotic chromosomes accordingly. Thus, repeating elements form the skeleton of interphase chromatin and mitotic chromosomes.

However, the question arises whether the localization of repetitions, mainly formed by mobile elements, is primary. Whether it is the driver of the process of laying interphase chromosomes or just a consequence of chromatin packing.

The following data are further indication of the assumption of the primacy of the interaction between homologous mobile elements

Firstly, it is well established that chromatin has the ability to localize in loci with homologous DNA sequences [71–73].

Secondly, it has been studied the interchromosomal contacts of various families of repeats in the genomes of human embryonic stem cells, drosophilas and mice, and also in three human cell lines to test this hypothesis and built a global picture of the spatial organization of repeats in chromosomes [72]. The degree of localization of repeats formed by

different families of mobile elements, including LTR-containing ERV, DNA transposons, short interspersed elements (SINE) and long interspersed elements (LINE) was quantitatively evaluated.

All families of mobile elements contained subfamilies prone to localization in nuclear space. That is, the formation of clusters in three-dimensional space turned out to be a common feature of mobile elements genomes of different organisms.

It was possible to show the conservatism of the organization of synthetic blocks in the nuclear space of mice and humans. Synthetic regions containing a similar set of mobile elements form similar spatial contacts in the genomes of mice and humans.

The most frequently collocated mobile elements in space are evolutionarily more ancient and contain binding sites with transcription factors. The presence of cis-regulatory sequences in the localizing elements indicates the possibility of regulating the formation of three-dimensional contacts between mobile elements by transcription factors, and, consequently, by the environmental conditions and the metabolism of the cell.

Together, these data suggest that contacts between retrotransposons are not a passive consequence of chromatin packing, but actively influence the architecture of interphase chromatin. These data are in good agreement with the hypothesis of the R-operon [73]. According to this hypothesis, the eukaryotic genome forms structurally functional domains called organized repeats of operons (R-operons) in the nucleus by means of homologous interactions between mobile elements. Each R-operon consists of associated mobile elements and adjacent gene-encoding protein cells.

Representations of repetitive R-operons significantly expand the possibilities of specific regulation of genes and cooperation between genes. What does it happen due to?

Firstly, genes located at large distances in the linear genome in R-operons are in contact with each other in the three-dimensional nucleus space and thus can be regulated by a set of those cis-regulatory elements and the transcription factors that bind to and regulate each of these genes. Indeed, it has been shown that enhancers can regulate the work of genes in trans while approaching genes in space [74–76]. Thus, the R-operons formed by repetition assemblies are structures that enable co-operative gene regulation by a set of those cis-elements that control the activity of each of the genes that are part of regulon.

Secondly, genes possessing the same cis-elements can coexist with different repetitions and be part of various R-operons accordingly.

Thirdly, the transcriptional domains formed in such way are dynamic. Due to the dissociation of repeating assemblies, old R-operons can disappear, and new domains with a different combination of repeats and genes due to the association of repetitions can form.

The dynamics of the formation of homologous sequences pairs can be influenced by the concentration of ions in the cell, the expression of proteins necessary for homologous pairing, the pattern of epigenetic modifications of histones and mobile elements, and the activity of mobile elements regulated by transcription factors.

Transcription factors specifically interacting with regulatory elements of ERVs can facilitate the establishment of contacts between mobile elements that is confirmed experimentally. Thus, HERV possessing binding sites with transcriptional regulators such as NANOG and OCT4 are localized in human embryonic stem cells. However, localization disappears in cells in which the expression of transcription factors is suppressed [74].

Transcription itself can lead to the establishment of contacts between repetitive sequences. A number of experimental data confirms the assumption about the existence of transcription factories – nuclei regions in which transcription occurs and which can contain up to hundreds of simultaneously operating RNA polymerase molecules [77]. But many ERVs are transcribed in a cell and accordingly can be localized in transcription factories. In the process of differentiation and in response to external conditions, the pattern of transcribed ERVs can change, and accordingly the contacts between them will change.

Indeed, according to a number of studies, the expression of retroviruses in cells of even one type varies considerably. This is due to epigenetic mechanisms [78–79]. Thus, R-operons will be formed not only tissue-specifically, but even specifically for each cell.

The regulation of association and dissociation of repetition assemblies is a mechanism of coordinated changes in gene activity in response to changes in cell

metabolism and promotes the emergence of new combinations of expressed genes in response to changing environmental conditions. Therefore co-expression of genes coordinated with the help of R-operons expands essentially the possibility for emergence of new temporary cooperation between genes dependent on the specific context that has developed in the cell.

CONCLUSION

Summarizing the data, we can note the following. ERVs contain regulatory elements or clusters of elements, by means of which they can be tissue-activated and transcribed. ERVs participated in the reformatting of regulatory networks and in the creation of gene-specific non-coding RNA forming binding sites with transcription factors and spreading them inside the genome. During the evolution, different ERVs were injecting into the genomes of different species, but in a number of cases they were used by genomes to solve similar problems and reformat similar regulatory programs. Therefore, it can be assumed that the participation of ERVs in the formation of regulatory networks obeys certain laws and is not completely random.

In cells, due to homologous interactions, ERVs can form regulatory R-operons, which provide an additional way for the disintegration of old and emerging new gene associations in response to cell-building conditions and provide a level of genome plasticity that was previously difficult to imagine.

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PATTERN-RECOGNIZING RECEPTORS AND THE INNATE IMMUNE RESPONSE TO VIRAL INFECTION

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The innate immune response to viral pathogens is crucial in mobilizing defensive reactions of an organism during the development of an acute viral infection. Cells of the innate immunity system detect viral antigens due to genetically programmed pattern-recognition receptors (PRRs), which are located either on the cell surface or inside the certain intracellular components. These image-recognizing receptors include Toll-like receptors (TLRs), retinoic acid-inducible gene I-like receptors (RIG-I-like receptors), nucleotide oligomerization domain-like receptors (NOD-like receptors), also known as NACHT, LRR and PYD domains of the protein, and cytosolic DNA sensors. The trigger mechanisms for these receptors are viral proteins, and nucleic acids serve as activators. The presence of PRRs that are responsible for the determination of viral antigens in cellular components allows the cells of innate immunity to recognize a wide range of viral agents that replicate in various cellular structures, and develop an immune response to them. This article summarizes the disparate data presented in modern English literature on the role of PRRs and the associated signaling pathways. Understanding the recognition of viral pathogens required triggering a cascade of cytokine and interferon production provides insights into how viruses activate the signal paths of PRRs and the effect of the interaction of viral antigens and these receptors on the formation of the antiviral immune response.

KEY WORDS: pattern-recognition receptors, Toll-like receptors, RIG-I-like receptors

ПАТЕРН-РОЗПІЗНАВАЛЬНІ РЕЦЕПТОРИ ТА ПРИРОДЖЕНА ІМУНОЛОГІЧНА ВІДПОВІДЬ НА ВІРУСНУ ІНФЕКЦІЮ

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Вирішальне значення в мобілізації захисних реакцій організму протягом розвитку гострої вірусної інфекції має вроджена імунологічна відповідь на вірусні патогени. Клітини системи вродженого імунітету виявляють вірусні антигени за допомогою генетично запрограмованого патерн-розпізнавання рецепторів (PRRs), які розташовані або на поверхні клітини, або всередині певних внутрішньоклітинних компонентів. Ці образ-розпізнавальні рецептори включають Toll-подібні рецептори (TLRs), RIG-I-подібні рецептори (RLRs), NOD-подібні рецептори, також відомі як домени NACHT, LRR та PYD білків, та цитозольні ДНК-сенсори. Пусковим механізмом для цих рецепторів є вірусні протеїни, а активаторами слугують нуклеїнові кислоти. Наявність PRRs, що відповідають за визначення вірусних антигенів у клітинних компонентах, дає клітинам природженого імунітету можливість розпізнавати широкий спектр вірусних агентів, що реплікуються в різних клітинних структурах, і виробляти імунологічну відповідь на них. В даній статті узагальнено розрізнені дані, які представлені в сучасній англомовній літературі, щодо ролі PRRs та пов'язаних з ними сигнальних шляхів. Розуміння розпізнавання вірусних патогенів, необхідних для запуску каскаду продукції цитокинів і інтерферонів, дозволяє збагнути, як віруси активізують сигнальні шляхи PRRs і як впливає взаємодія вірусних антигенів і цих рецепторів на формування противірусної імунної відповіді.

КЛЮЧОВІ СЛОВА: патерн-розпізнавальні рецептори, Toll-подібні рецептори, RIG-I-подібні рецептори

ПАТЕРН-РАСПОЗНАЮЩИЕ РЕЦЕПТОРЫ И ВРОЖДЕННЫЙ ИММУННЫЙ ОТВЕТ НА ВИРУСНУЮ ИНФЕКЦИЮ

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Решающее значение в мобилизации защитных реакций организма во время развития острой вирусной инфекции имеет врожденный иммунный ответ на вирусные патогены. Клетки системы врожденного иммунитета обнаруживают вирусные антигены благодаря генетически запрограммированным образ-распознающим рецепторам (PRRs), которые располагаются либо на поверхности клетки, либо внутри определенных внутриклеточных компонентов. Эти образ-распознающие рецепторы включают в себя Toll-подобные рецепторы (TLRs), RIG-I-подобные рецепторы (RLRs), NOD-подобные рецепторы, известные также как NACHT, LRR и PYD домены белка, и цитозольные сенсоры ДНК. Пусковым механизмом для этих рецепторов являются вирусные протеины, а активаторами служат нуклеиновые кислоты. Наличие PRRs, отвечающих за определение вирусных антигенов в клеточных компонентах, дает клеткам врожденного иммунитета возможность распознать широкий спектр вирусных агентов, реплицирующихся в различных клеточных структурах, и выработать в отношении их иммунный ответ. В данной статье обобщены разрозненные данные, представленные в современной англоязычной литературе, относительно роли PRRs и связанных с ними сигнальных путей. Понимание распознавания вирусных патогенов, необходимых для запуска каскада продукции цитокинов и интерферонов, позволяет получить понимание того, как вирусы активируют сигнальные пути образ-распознающих рецепторов и какое влияние взаимодействие вирусных антигенов и этих рецепторов оказывает на формирование противовирусного иммунного ответа.

КЛЮЧЕВЫЕ СЛОВА: образ-распознающие рецепторы, Toll-подобные рецепторы, RIG-I-подобные рецепторы

INTRODUCTION

Cells of the innate immune system use pattern-recognition receptors (PRRs) that identify pathogen-associated molecular patterns (PAMPs) located on the surface of viral cells and differ from those of the host cell to identify viral pathogens. The ability to identify nucleic acids has become a major component of the antimicrobial link of the immune system. A wide range of pathogens are identified by recognizing their genome or nucleic acids that accumulate during the replication of viruses. PRRs are activated in response to viral molecules such as 5′-triphosphate RNA, as well as viral DNA, which is determined by sensory elements located in the cytoplasm.

The main PRRs are Toll-like receptors (TLRs). They are type 1 transmembrane proteins providing communication between the plasma membrane and endosomal vesicles. The main function of TLRs is the detection of PAMPs in the extracellular space. Receptors located on the plasma membrane are involved in the detection of hydrophobic lipids and proteins, and receptors located in endosomes are able to detect nucleic acids. Such a division allows cells of the innate immune system to identify components of the viral envelope located on the cell surface, such as components of the fusion mechanism, and nucleic acids located in the endosomes. Entering the cytoplasm viral components enter the area monitored by RIG-I-like receptors, NOD-like receptors and cytosolic DNA sensors, such as members of the AIM2 family. Similar to TLRs, RIG-I-like receptors and cytosolic DNA sensors regulate the expression of transcription factors necessary for the production of interferons and cytokines. And NOD-like receptors and members of the AIM2 family, by contrast, activate the process of maturation of IL-1β and IL-18 by activating caspase 1. Induction of immature forms of IL-1β and IL-18 occurs through the activation of TLRs signaling pathways, and NOD-like receptors serve as a kind of «control mechanism» that regulates and activates the release of these powerful effectors. Many PRRs are involved in the activation of the adaptive immune system by enhancing expression of the major histocompatibility complex class II and stimulating the expression of co-stimulating molecules CD40, CD80 and CD86 in addition to the release of pro-inflammatory components.

TOLL-LIKE RECEPTORS

10 types of TLRs have been identified in humans, and 13 types – in mice, 9 of them (TLRs of types 1–9) are identical. TLRs of 1, 2, 4, 5 and 6 types (TPRs 1, 2, 4, 5, 6) are located on the cytoplasmic membrane, and TLRs 3, 7, 8 and 9 are endosomal. All TLRs have a common structure and consist of extracellular repeats rich in leucine and the Toll/Interleukin-1 Receptor (TIR) cytoplasmic domain [1]. These receptors transmit a signal differentiating the adapter proteins Mal (MyD88 adapter-like), also known as TIR domain-containing adapter proteins (TIRAPs) and Myeloid differentiation primary response gene 88 (MyD88), gene for primary myeloid differentiation 88, and/or TIR-domain-containing adapter inducing interferon-β (TRIF) and Trif-related adaptor molecule (TRAM) [1]. Adapters initiate the launch of signaling cascades, culminating in activation of the nuclear factor κB (NF-κB), mitogen-activated protein kinase (MAPK) and interferon regulatory factors 1, 3, 5 and 7 (INF-1, -3, -5 and -7) [2]. The combination of these transcription factors promotes not only the expression of interferons, cytokines and chemokines, but also affects the maturation and survival of cells.

Toll-like receptor signaling pathways

All TLRs, apart from TLR 3, require the presence of MyD88 for their activation [3–4].

TLR 3 is not capable of MyD88 capturing and interacts with the TRIF through the adapter protein. TRIF has the ability to directly bind TRAF6 and induce NF-κB along a path similar to MyD88. TRIF is also capable of involving the receptor-interacting protein-1 (RIP-1) in the process in contrast to MyD88. RIP-1 interacts with TRAF6 that leads to powerful activation of NF-κB. TRAF3 is the third protein attracted to TRIF. It binds with TANK-binding kinase-1 (TBK1) and IKKi and is a necessary component of the production process of interferon type 1. This allows them to undergo a dimerization procedure and penetrate the nucleus, where they interact with NF-κB and activator protein 1 (AP-1), that in turn leads to the transcription of the target gene. The study, which included children with non-functioning MyD88 proteins, showed that patients with this pathology are predisposed to develop recurrent pyogenic bacterial infections [5]. Patients with IRAK-4 deficiency and with a defect of UNC-93B1, a protein that is involved in the transport of TLRs 3, 7, 8, and 9 into endosomes, had an increased susceptibility to the herpes simplex virus type 1 with predominant brain damage [6–7]. Peripheral blood mononuclear cells and fibroblasts obtained from these patients demonstrated a decrease in type 1 interferon activity in response to the introduction of HSV-1, accompanied by enhancement of viral replication [7].

Expression and activity of Toll-like receptors

The severity of the inflammatory reaction caused by viral PAMPs depends on the following factors.

1. Cellular expression of TLRs varies depending on cell type. It is known that macrophages express a large number of TLRs 2 and 4, while plasmacytoid dendritic cells (pDCs) mainly produce TLRs 7 and 9 [1];
2. The expression level also varies between species; for example, TLR 9, expressed in the human body by just a few cell types, is well represented in mice;
3. The reaction to identical viral PAMPs can vary between cell types, both in the nature of the produced effector molecules and in the response kinetics.

INTRACELLULAR NUCLEIC ACID SENSORS

TLRs play an important role in the detection of viral PAMPs that present both on the surface of cells and in endosomes. The identification of additional mechanisms of antiviral protection has revealed many classes of innate sensors that play an important role in the purification of viruses that replicate and locate in the cytosol. Specialized classes of cytosolic nucleic acid sensors, called RIG-I like receptors (RLRs), are capable of recognizing intracellular RNA that penetrates into the cytosol during virus introduction or accumulates during viral replication, as well as DNA that is inside cytosol.

The RLRs family includes three DExD/H box RNA helicases: retinoic acid-inducible gene (RIG-I), melanoma differentiation-associated gene 5 (MDA-5), and LGP-2 [8–11].

RIG-1 and MDA-5 consist of N-terminal caspase activation and recruitment domains (CARDs), the following helicase RNA DExD/H box domain, which has ATP-ase activity, and the C-terminal repressor domain. RIG-I controlled by its regulatory domains is

inactive in the cytoplasm in the absence of pathogenic activation. Conformational changes occur in RIG-1, when viruses enter the body, RIG-1 dimerizes as a result [12]. The activated multimeric form of RIG-1 or MDA5 interacts with the mitochondrial antiviral-signaling protein (MAVS) located on the outer mitochondrial membrane. MAVS activates IKK-related kinase after capturing RIG-I or MDA5, resulting in the transcription of interferons type 1. Also, MAVS also activates NF-kB by recruiting a tumor necrosis factor receptor type 1-associated death domain protein (TRADD), the FAS-associated protein with the death domain (FADD), caspase-8 and caspase-10 [13–16]. LGP-2 does not contain N-terminal DARK domains, but consists only of the helicase RNA domains. It is assumed that it acts as a negative regulator of other RLRs [9, 12].

Recognition of RNA RIG-like receptors

RLRs are important components of antiviral protection for many types of cells, including fibroblasts, epithelial cells and normal dendritic cells. Studies have shown that only MDA-5 is responsible for the production of interferon through the stimulating of polyI: C [17–18]. RIG-I does not have the ability to recognize the 5'PPP-ssRNA of the host cell; they use the 5 'end of the transcript to recognize the virus RNA and the host cell. In contrast, MDA-5 uses not the 5 'end of the transcript, but the length of the RNA sequence for recognition of the virus RNA and the host cell; long dsRNAs are usually absent in the host cell, and, thus, act as a ligand for MDA-5. RIG-I is also able to recognize short dsRNA, which is a by-product of viral replication, in addition to recognizing 5'-triphosphate RNA.

RIG-I is involved in the recognition of vesicular stomatitis virus (VSV), rabies virus, Newcastle disease virus, respiratory syncytial virus, measles virus, influenza A and B viruses, hepatitis C virus (HCV), Japanese encephalitis virus and Ebola virus [18-19]. MDA-5 is involved in the recognition of CMV, Theiler's encephalomyelitis virus and Mengo virus [20]. All of these viruses do not contain 5'-triphosphate RNA, but are capable of producing long dsRNA, that provides additional evidence that MDA-5 distinguishes RNA based on sequence length, rather than 5'-triphosphate. It is proven that Dengue virus, West Nile virus and reovirus transmit signals through the use of a combination of RIG-I and MDA-5.

DDX3

A recent study described the participation of another representative of the DExD/H box family of RNA helicase, DDX3, in the antiviral response. It was found that the K7 protein of the measles virus inhibits the induction of INFβ through the binding to DDX3, which in turn led to the discovery of the positive role of DDX3 in activating the RLR signaling pathway. DDX3 binds to polyI: C and viral RNA penetrating into the cytosol and binds to MAVS/IPS-1, whereby it takes part in activating the production of INFβ, enhances RNA recognition, forming a complex with RIG-I and MAVS that induces interferon production.

DNA cytosolic sensors

Scientists knew that pathogen DNA is capable of activating fibroblasts and stimulating the production of IFN type 1 even before the discovery of TLR 9.

Cytosolic recognition of DNA and RNA leads to activation of TBK1, IRF-3 and production of IFN type 1. However, signaling pathways connecting upstream DNA and TBK1 sensors are currently poorly understood. TBK1 interacts with DDX3, a DEAD box RNA helicase, which regulates the transcription of IFNβ through the IRF-3.

DAI

DNA-dependent activator of IFN-regulatory factors (DAI) was one of the first cytosolic DNA sensors detected. It consists of two related domains capable of recognizing the left-twisted Z-forms of DNA and its B-forms. DAI increased the dose-dependent production of IFN type 1in the L929 cell culture during the exogenous expression after stimulation of the B and Z forms of DNA. Similarly, turning off DAI by means of ssRNA disrupts the production of type 1 IFN in response to DNA, 45 bp interferon-stimulating DNA (ISD) of Listeria and herpes viruses, as well as HSV-1. It was also found that the production of IFN type 1 also depends on DAI during the CMV introducing. These results suggest that the role of DAI may be specific for each individual cell type, it plays an excessive role in probing of cytoplasmic DNA, and that other sensors must be needed to induce these responses.

Pol III RNA and LRRFIP1

Viral RNAs trigger the production of IFN type 1 by activating RIG-I. It has been proven that the B-form of dsDNA in human cells can

also induce the production of INFβ in a way that depends on the adapter molecule RIG-I MAVS [21–22]. DNA with a high content of antibodies is transcribed into 5'-pp RNA using RNA polymerase II, which in turn activates RIG-I [21].

Another regulator of DNA-controlled innate immune signal transmission, a leucine-rich repeat flightless-interacting protein 1 (LRRFIP1) has recently been described in addition to DAI and Pol III RNA. It inhibits the production of IFN 1 type induced by bacteria. Turning LRRFIP1 off inhibits the production of IFN in response to polyI: C stimulation, synthetic DNA, poly (dG:dC) and poly (dA:dT) stimulation, involving LRRFIP1 in the recognition of dsRNA, as well as B-and Z-forms of dsDNA.

IFI16

IFI16 was identified as a DNA-binding protein that interacts with dsDNA through the process of analyzing the immune responses to these dsDNA regions obtained from the HSV-1 genome. IFI16 is a member of the PyHIN protein family (containing the pyrin and HIN200 domains). The PHYIN family includes 4 representatives: IFIX, IFI16, MNDA and AIM2. They all contain one or more HIN200 domains that recognize DNA, as well as the pyrin domain. Turning IFI16 or p204 (a member of the PYHIN family of mice) off leads to a decrease in the intensity of the IFNβ response to these dsDNA. IFI 16 is localized in the nucleus, and in the cytosolic cellules of macrophages. IFI16 pooling is required for IFNβ production in response to DNA. Turning IFI16 and its homologue of p204 in mice off in mRNA leads to a decrease in the activation of IRF3 and NF-κB, and also leads to the IFNβ gene induction in cells once infected with HSV-1.

DDX9 and 36

DExD/H box RNA helicase – DHX9 and DHX36 are found in plasmacytoid dendritic cells. Activation of DHX9 leads to activation of IRF-7 and increased production of IFNα, and activation of DHX36 leads to activation of NF-κB and increased production of IL-6 and TNFα. Turning DHX9 and DHX36 off in mRNA inhibits cytokine production in response to DNA-containing HSV-1, while the response to an RNA-containing influenza A virus remains unchanged.

Inflammasomes, their types

The recognition of viral DNA is associated with the transcriptional induction of IFN type 1 and other pro-inflammatory cytokines, as well as with the launch of caspase-1-dependent maturation of pro-inflammatory cytokines IL-1β and IL-18. IL-1β is involved in the recruitment of innate immunity cells, T-lymphocyte activation and fever induction, and IL-18 increases the cytolytic activity and production of IFNγ by natural killer cells (NK cells), and also affects the recruitment and activation of neutrophils.

The production of IL-1β is controlled at the level of transcription, translation, maturation and secretion. Many cellular stimuli, including TLRs ligands, activate the transcription of pro-forms of IL-1β and IL-18. Maturation (i.e. cleavage) of pro-IL-1β and pro-IL-18 is catalyzed by cysteine protease of caspase-1. The activity of inflammatory caspase-1 is controlled by a large complex called «inflammasomes protein complex». Then active caspase-1 cleaves pro-IL-1β and pro-IL-18.

Inflammasomes AIM2 recognize their own and foreign cytosolic dsDNA, including viral DNA, through the HIN 200 domain. DNA recognition provokes the assembly of inflammasomes complexes. Upon DNA binding, AIM2 undergoes oligomerization and binds apoptosis-associated speck-like protein (ASC) through the interaction of pyrin-pyrin homotypic domains, which in turn recruits pro-caspase 1. Inflammasomes and AIM2 are an integral component of the innate recognition of DNA-containing viruses, CMV, as well as Francisella tularensis and Listeria monocytogenes.

NLRP 3 inflammasomes play an important role in the formation of a response to RNA-containing viruses, adenoviruses, and DNA-containing viruses. NLRP3 deficiency weakens the normal response of IL-1β and IL-18 to the influenza virus and is associated with a decrease in cell recruitment of the innate immune system [23].

CONCLUSIONS AND PROSPECTS FOR FUTURE STUDIES

The understanding of how the innate immune system detects viruses and triggers a cascade of antiviral reactions has increased significantly over the past decade. The discovery of Toll-like receptors and nucleic

acids led to the discovery of various cytosolic RNA and DNA receptors and their downstream signaling pathways. However, many cytosolic sensors play an excess role in the detection of viruses. Such excessive protection strategies have evolved to deal with the evasion mechanisms of detection inherent in viruses. Determining the function of newly identified pattern-recognizing receptors in the immune defense against viral infection is an important step in understanding of their unique or auxiliary contribution to the pathogenesis of viral protection.

The mechanisms of nucleic acid sensors operation, the aim of which is to distinguish their own nucleic acids from the nucleic acids of viruses, require clarification, as well as the process of recognition of viral RNA and DNA, which are becoming available for pattern-recognition receptors.

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