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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

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გამოიცემა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტთან
თანამშრომლობითა და მისი პატრონაჟით

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

ТБИЛИСИ - НЬЮ-ЙОРК

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board and The International Academy of Sciences, Education, Industry and Arts (U.S.A.) since 1994. **GMN** carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

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GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები, რეცენზიები.

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При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

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2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

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Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

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რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

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5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

Содержание:

НАУКА

IMPROVING THE PERFORMANCE OF SMALL AMPUTATIONS IN COMPLICATED FORMS OF DIABETIC FOOT

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Diabetes mellitus (DM) is one of the most widespread and socially significant diseases in the world. Currently, according to the world statistics based on appealability to a doctor, there are 366 million people with diabetes (about 7% of the world population), with about half of all patients being of employment age. With the rapid spread of the disease, experts of the World Diabetes Federation predict that number of diabetic patients by 2030 will increase by 1.5 times and reach 552 million people, i.e., will affect every 10th inhabitant of our planet.

Diabetic foot syndrome - one of the most complex, in terms of pathogenesis, and severe complications of diabetes. This syndrome is characterized by pathological changes of the peripheral nervous system, arterial and microcirculatory vessels with osteoarthropathy occurrence. Against the background of these complex changes in the tissues of the lower extremities occur ulcerative necrotic processes up to gangrene [1,2]. As a result during the most pronounced and widespread processes it is necessary to use the most traumatic surgical method of treatment - lower limb amputations. Herewith the mortality from amputations reaches 50%.

In St. Petersburg according to the Center for Diabetic Foot (31,000 patients with DFS are observed in the center) in 2011 have been performed 628 amputations, 53% of them - high amputations [4]. The indications for amputation are: progressive gangrene of the foot, chronic osteomyelitis with extensive bone destruction, extensive soft tissue defects, damage of support ability of the foot without the possibility of its restoration, critical ischemia of lower extremities of IIIB-IV degree.

In diabetic patients, amputations are also performed routinely, in order to change the configuration of the foot with formation of overpressure points (and tropic ulcer development), during chronic osteomyelitis of bones of the foot, or the presence of extensive superficial defect of soft tissues [2]. It should be noted that the preferred ones are sparing amputations at foot level, since the five-year survival rate in patients undergoing high amputation is 28 to 32% and the risk of contralateral amputation within 2 years is 40-50% [9].

High amputations include amputations at the level of hip and thigh. Taking into account the distal type of vascular lesions and atrophic changes of the soft tissues, most often amputations on the lower leg are performed in the upper third with the formation of posterior musculocutaneous flap. In case of severe general condition of the patient are performed guillotine amputations at the hip level, when limb amputation is performed without cutting out flaps, hemostasis and wounds suturing. Sparing foot amputations include amputation of toes, transmetatarsal amputation of the foot, Lisfranc and Chopart amputation. Often operations are characterized by atypical amputations, combining a combination of operational techniques. [6,8].

Selection of the amputation level is determined by the level of arterial bed damage. Under modern conditions in the first stage of a comprehensive treatment of the patient with purulent-necrotic processes in the lower extremities, it is efficient to recover the main arterial blood supply of the lower extremities. Surgery on the arteries of the lower extremities may be divided into: traditional (open) and endovascular.

During affection of the iliac-femoral and femoral-popliteal, can be performed bypass surgery or the prosthetic repair of the corresponding area of main artery. The selection of such surgical manipulation is effective for patients with a proximal affection of the arterial bed of the lower extremities, i.e. with atherosclerotic stenosis and occlusion in combination with distal angiopathy due to the presence of diabetes mellitus. On the other hand, bypass surgery in patients with diabetic foot syndrome and presence of peripheral arterial disease are ineffective - increased volume of the main flow meets the resistance of rigid microvasculature and modified veins similar to capillary types of the lower extremities [7].

Nowadays, the most effective treatment of macroangiopathy in patients with diabetic foot syndrome are endovascular methods. In case of vascular lesions below the femoral artery, which is characteristic to peripheral diabetic macroangiopathy, the operation of choice is transluminal balloon angioplasty. In the presence of purulent-necrotic destruction focus on the foot, revascularization is performed taking into account angiosomal zones of blood supply to the foot. Despite the abundance of anastomoses between the distal portions of the arteries of the foot, clinical observations indicate that macroangiopathy leads to their failure.

Angiosomy concept allows emergency revascularization of arteries of causal zone. Thus the elimination of occlusion in the proximal portion of the supplying vessel by anterograde (from the femoral vessels), as well as by retrograde method during catheterization process of the vessels of the foot. From the standpoint of angiosomal concepts, it is necessary to restore blood flow, either directly from the main supply vessel, or by collateral recovery path [1,4].

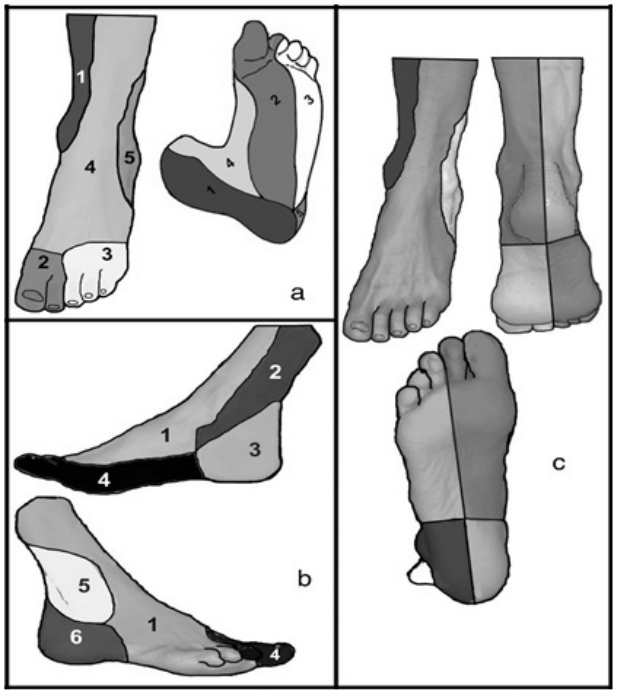

Fig. 1. Arterial blood supply scheme according to angiosomal theory

The reason for this study was the idea of the need to perform surgical procedures, performed for the sanation of purulent-necrotic process in the lower limbs, only in well-perfused tissues in order to prevent the recurrence of inflammatory-necrotic complications.

The aim - a comparative assessment of the effectiveness of typical and atypical amputations at the foot in patients with a complicated course of diabetic foot syndrome (DFS).

Material and methods. The object of the study were patients with diabetes mellitus (DM) type 2 with purulent-necrotic complications DFS, treated in the surgical department of St. Petersburg Cadet Corps, N.I.Pirogov National Medical-Surgical center for the period of 2012-2015. In the studied group were enrolled 68 patients who underwent atypical intervention considering angiosomal blood supply to tissues of the foot. In 47 patients in the control group were performed typical surgical interventions at the foot level (exarticulation of the fingers from the medialorlateral side, "claw" resection of 2-4 toes, transmetatarsal amputation by Sharp, exarticulation by Lisfranc and Chopart). The study and control groups were matched by sex, age, course of DFS and severity of complications.

Objective study with the evaluation of the local status, laboratory tests (blood count, basic coagulation, carbohydrate metabolism indices) and duplex ultrasound study of vessels of the lower extremities. In all patients have been performed angiography. Only in patients admitted with the acute purulent processes in the lower extremities, surgery in the form of opening, drainage of purulent focus was performed on an emergency basis before angiography. In other cases, the individual characteristics of circulatory disorders of the lower limbs determined the choice of tactics of surgical treatment. In 76% and 46%, respectively for each group after angiography was performed balloon angioplasty to recover the main blood supply.

Results and their discussion. For the evaluation of treatment results the following parameters were studied: duration of hospital treatment, the frequency of repeated surgical interventions due to recurrence of local destructive process, the ability of independent movement without using means of support. In the study group, the average duration of hospital treatment was 14,4±2,3. In the control group - 18,2±3,1. Duration of hospitalization differed significantly ($p<0.05$), however a large group of patients (31and 18 respectively) were re-hospitalized within the period from 2 to 6 weeks after discharge for the purpose of autodermoplasty to close the surgical wound. We believe that the technology of performance of both typical and atypical interventions in many cases means open conductions of post-surgical wound. Hospitalization duration for wound plastic did not exceed 8 days in all cases.

Significant differences between the terms of healing of postoperative wounds and wound surfaces after reconstructive interventions have not been revealed, which may indicate the correct choice of the scope and method of operation, as well as the indications for secondary wound closure. Have been manifested a marked difference in the number of repeated operations performed for recurrent purulent-necrotic process - 2 in the study group and 9 in the control group. Repeated operations were needed in cases of development of destructive process before healing of the original wound. Recurrence of purulent-necrotic events was the major cause of increase of duration of the inability of independent movement - 6,3±1,2 and 13,1±3,4 days, respectively ($p <0.05$). To explain these results, we see a leading role of sufficient blood supply to paravulnar tissues in the healing of surgical wounds in patients with diabetic foot syndrome.

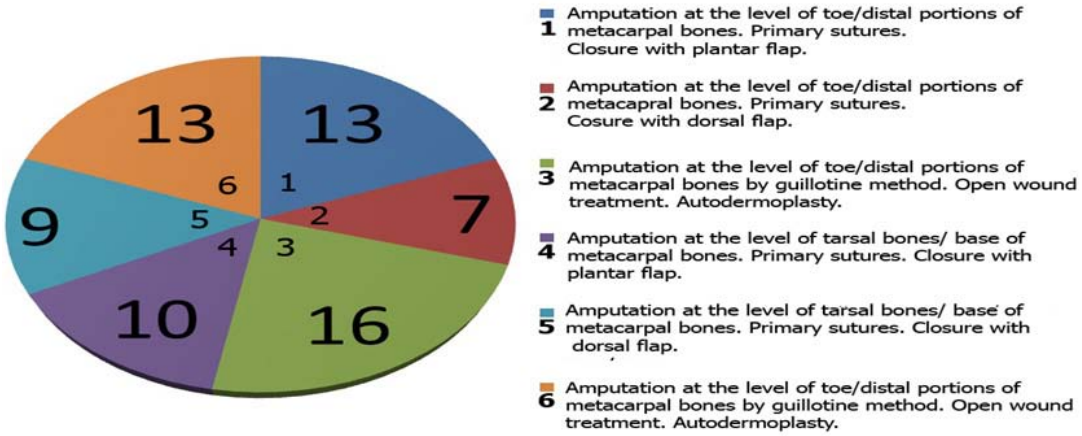

Fig. 2. Atypical operations on the lower extremities, performed in patients of the study group

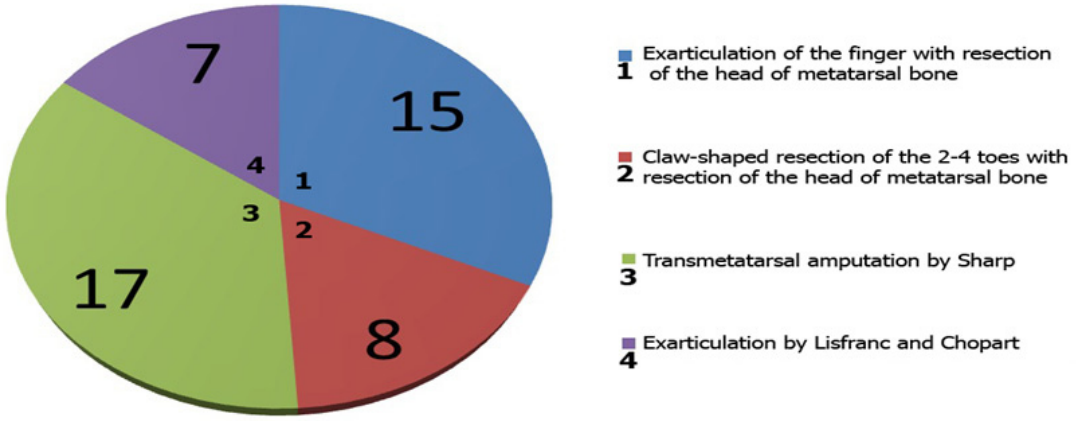

Fig. 3. Typical operations on the lower extremities, performed in patients of the control group

Conclusions. 1. In patients with purulent-necrotic complications of diabetic foot syndrome preoperative angiography is indicated and, if necessary to perform angioplasty before surgical intervention on the tissues of the foot.
2. Application of the principle of angiosomal blood supply on the basis of ultrasonography and angiography data, allows you to select the most rational strategy of surgical treatment.
3. The use of atypical method of amputations in the feet after preoperative examination allows to reduce the number of postoperative complications, reduces the duration of hospital treatment and improves the postoperative mobility and ability for independent movement of patients.

REFERENCE

1. Аметов А.С., Соловьёва О.Л. Нарушения в системе гемостаза при сахарном диабете и пути их коррекции при назначении комбинированной терапии диабетоном МВ и метформином. Сахарный диабет 2007; 3: 33-39.
2. Диагностика и лечение хронических заболеваний вен: рекомендации Ассоциации флебологов России. М.: 2013; 109.
3. Капустин С.И. Молекулярно-генетические аспекты венозного тромбоэмболизма. Автореф. дисс. д-ра биол. наук. СПб.: 2007; 36.
4. Мухин Н.А. Синдром Гудпасчера: особенности патогенеза, подходы к лечению. Клиническая нефрология 2009; 4: 37-44.
5. Николаев А.Я., Осипов Е.В., Кцоева С.А. Биохимия инсулинзависимого сахарного диабета. СПб.: 2009; 114.
6. Российские клинические рекомендации по диагностике и лечению хронических заболеваний вен нижних конечностей. Флебология 2009; 3(3): 52.
7. Benigni J.P., Allaert F.A., Virkus A. Изучение эффективности и переносимости компрессионного комплекта для лечения язвенных поражений нижних конечностей венозного происхождения (перевод с англ.) Флебология 2010; 1: 49-58.
8. Baker J.C. et al. Diabetic musculoskeletal complications and their imaging mimics. Radiographics. 2012; 32(7): 1959-74.

SUMMARY

IMPROVING THE PERFORMANCE OF SMALL AMPUTATIONS IN COMPLICATED FORMS OF DIABETIC FOOT

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The aim of the study was comparative assessment of the effectiveness of typical and atypical amputations at the level of footstep in patients with the most complicated course of the diabetic foot syndrome (DFS).

The patients suffering from diabetes mellitus type 2 and purulo-necrotic complications of the DFS, treated in the surgical department of the № 1 Pirogov National Surgical and Medical Center of the Sankt Petersburg Clinical Complex were investigated. The study group included 68 patients who underwent atypical surgical interventions taking into account blood flow angiosomes of footstep tissues. Operative interventions at the level of footstep were carried out to 47 patients of the control group. Obligatory angiography was performed in all patients. Operative intervention was carried out under emergency conditions before conduction of angiography only to those patients who were admitted with acute purulent processes in the area of lower extremities. In all other cases individual peculiarities of blood circulation disorder in lower extremities determined the choice of tactics of operative treatment. In 76% and 46% correspondingly, for each group after angiography balloon angioplasty was carried out for reconstruction of the main blood flow. The average duration of the in-patient treatment in study group was 14,4±2,3. In the control group – 18,2±3,1.

Conduction of post-operative angiography and when necessary angioplasty in the footstep tissues is prescribed before operative intervention to patients with purulo-necrotic complications of DFS. Application of the principle of angiosome blood flow based on the data of duplex sonography of arteries and angiography enables to choose the most rational tactics of the operative treatment. 3. Application of atypical technique of amputations in the area of footstep after postoperative complications enables to reduce the length of inpatient treatment and to improve postoperative mobility and ability for independent movement of patients.

Keywords: diabetic foot syndrome, varicose vein disease of the lower limbs, lower limbs vein pathology.

РЕЗЮМЕ

СОВЕРШЕНСТВОВАНИЕ ТЕХНИКИ ВЫПОЛНЕНИЯ «МАЛЫХ» АМПУТАЦИЙ ПРИ ОСЛОЖНЕННЫХ ФОРМАХ СИНДРОМА ДИАБЕТИЧЕСКОЙ СТОПЫ

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Целью исследования явилась сравнительная оценка эффективности типичных и атипичных ампутаций на уровне стопы у больных с осложненным течением синдрома диабетической стопы.

Наблюдались 68 больных сахарным диабетом (СД) 2 типа с гнойно-некротическими осложнениями синдрома диабетической стопы (СДС), которые проходили лечение в хирургическом отделении СПбКК НМХЦ им. Н.И. Пирогова. Контрольную группу составили 47 пациентов, которым проводились типичные оперативные вмешательства на уровне стопы. Всем пациентам в обязательном порядке выполнялась ангиография. Только больным, поступившим с острыми гнойными процессами в области нижних конечностей, оперативное вмешательство в виде вскрытия, дренирования гнойного очага проводилось в экстренном порядке до проведения ангиографии. В остальных случаях индивидуальные особенности нарушения кровообращения нижних конечностей определяли выбор тактики оперативного лечения. В 76% и 46% соответственно для каждой группы после ангиографии выполнялась баллонная ангиопластика для восстановления магистрального кровоснабжения. В исследуемой группе средняя продолжительность стационарного лечения составила 14,4±2,3 дня, в контрольной группе – 18,2±3,1.

Больным с гнойно-некротическими осложнениями синдрома диабетической стопы показано проведение предоперационной ангиографии и при необходимости ангиопластики перед выполнением оперативного вмешательства на тканях стоп. Применение принципа ангиосомного кровоснабжения на основании данных ультразвуковой допплерографии артерий и ангиогра-

фии позволяет выбрать наиболее рациональную тактику оперативного лечения. Применение атипичной техники ампутаций в области стоп после предоперационного обследования позволяет снизить количество послеоперационных осложнений, сократить длительность стационарного лечения, улучшить послеоперационную мобильность и способность к самостоятельному передвижению пациентов.

რეზიუმე

“მცირე“ ამპუტაციის ტექნიკის სრულყოფა
დიაბეტური ტერფის სინდრომის გართულებული ფორმების დროს

[1]თ. გვაზავა, [2]გ. სმირნოვი, [2]ვ. პეტროვა,
[2]ა. რემეზოვი, [1]ვ. აკიმოვი

[1]ი.ი. მეჩნიკოვის სახ. ჩრდილო-დასავლეთის სახელმწიფო სამედიცინო უნივერსიტეტი,
სანკტ-პეტერბურგი; [2]სანკტ-პეტერბურგის სახელმწიფო უნივერსტეტი, რუსეთი

კვლევის მიზანს წარმოადგენდა ტერფის ტიპობრივი და ატიპიური ამპუტაციის ეფექტურობის შედარებითი შეფასება დიაბეტური ტერფის სინდრომის გართულებული მიმდინარეობის დროს.

დიაბეტური ტერფის სინდრომის ჩირქოვან-ნეკროზული გართულებების მქონე პაციენტებში ტერფის ქსოვილებზე ქირურგიულ ჩარევამდე ნაჩვენებია ოპერაციისწინა ანგიოგრაფიის ჩატარება და,ასევე,საჭიროებისას - ანგიოპლასტიკის წარმოება.

არტერიების ულტრაბგერითი დოპლეროგრაფიის და ანგიოგრაფიის მონაცემების საფუძველზე ანგიოსომური სისხლმომარაგების პრინციპის გამოყენება იძლევა ოპერაციული ჩარევის ყველაზე რაციონალური ტაქტიკის შერჩევის საშუალებას. ამპუტაციის ატიპური ტექნიკის გამოყენება ოპერაციისწინა გამოკვლევის შემდეგ ამცირებს ოპერაციისშემდგომ გართულებებს და სტაციონარული მკურნალობის ხანგრძლივობას, აუმჯობესებს პაციენტების ოპერაციისშემდგომ მობილობას და მათი დამოუკიდებელი გადაადგილების უნარს.

A CASE OF GIANT FECALOMA IN A 24-YEAR-OLD WOMAN

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Fecaloma is defined as a mass of inspissated feces accumulated in the colon or rectum that is much harder in consistency than impacted feces [4]. They are hard, laminated, and calcified fecal masses that sometimes may mimic carcinoma. Fecalomas are usually found in the rectosigmoid region, and rarely in the more proximal colon. Chronic constipation is one of the most common causes of fecaloma in adults. The feces initially accumulate, then stagnate and get impacted due to coprostasis, expand and deform the intestine, and develop into a large tumor like masses. Fecalomas, often giant ones have been described in Hirschsprung’s disease, psychiatric patients, neglected elderly or bedridden patients, Chagas disease, inflammatory and neoplastic conditions, and in patients suffering from idiopathic chronic constipation [2,5].

Softeners used by rectal route, oral mineral and olive oil usage are appropriate treatment methods for mild and moderate obstructions due to fecaloma. However, when medical treatment is unsuccessful and multiple fecalomas are present, surgery is required [5]. We present a 24 year old woman with giant fecaloma that developed consequent to chronic constipation, could not managed by medical treatment and underwent to the surgical treatment.

Material and methods. *Case Presentation.* A 24-year old mentally healthy married female with history of constipation andmeteorism from childhood was admitted at our hospital. She gave the history of 1 bowel movement every third-fifth day with passage of hard stools only with using laxatives. Patient was complaining on periodic severe pain in abdomen, especially during last 6 months. Patient

had no history of fever, anorexia, nausea, vomiting, blood in stools, weight loss or previous hospitalizations. No urinary complaints were present. Therefore, she was pregnant two times at 22 and 23 years old age, without successful delivery. Both times, pregnancy was interrupted by the doctors, because of scalp defects, namely triangular head. Genetic analyzes were performed to exclude fetal trisomy 18, therefore genetic abnormalities in fetus, as well as in parents were not confirmed [7].

Examination revealed a normal temperature, supine blood pressure of 120/70 mm Hg, pulse rate of 78 bpm, a respiratory rate of 18 breaths/min. She weighed 52 kg with good overall nourishment.Abdominal palpation revealed hard, fixed, non-painful mass with smooth surface in the hypo and mesogastrium with sizes 25.0x25.0 cm.There was no hepatosplenomegaly. Bowel sound appeared normal. Per rectal examination was not informative. No major physical malformations were notable during the examination. Rest of the systemic examination was unremarkable.Routine laboratory blood tests were: hemoglobin 140 g/L, WBC $5/10^9$L , platelets $179/10^9$L, HCT-41.8% and ESR of 4 mm per hour. The renal functions and serum electrolytes were normal at creatinine of 0.78 mg/dl, serum Na 142.4 mmol/L, serum K 4.04 mmol/L, urinalysis and liver function tests were normal. Her thyroid function was normal, and her results were negative for toxoplasma, rubella virus, herpes simplex virus infection and for tumor markers (α-fetal protein, carcinoembrionic antigen and carbohydrate antigen 125); and, positive for cytomegalovirus.

A pelvic ultrasound revealed a large, solid dense tumor occupying the pelvic region. To classify the nature of the tumor, we performed abdominal and pelvic magnetic resonaance imaging (MRI) (Fig. 1,2). Study demonstrated a large mass consistent with megarectum and megasigmoid, namely dilated lumen of upper one third of rectum and recto-sigmoid angle up to 12 cm. Uterus, ovaries and urinary bladder were without pathology. There were not any pathologically enlarged lymph nodes in abdomen. Patient was undergone to the sigmoidoscopy, where on the distance of 15 cm from the anus appeared markedly dilated, atonic sigmoid colon filled with hard fecal stone. Irrigoscopy revealed enlarged sigmoid colon, namely was increased its length and width. In the distal part of sigmoid colon appeared big, movable filling defect with sizes 12x12.5 cm, most likely coprolith. Proximally from it were seen residual fecal masses.

After discussing the case in a multidisciplinary meeting with Gastroemterologist, General Surgeon and Radiologist, was taken into account anamnesis of chronic constipation, insufficient effect of laxatives and rectal enemas, interrupted pregnancies, patient underwent to the surgical treatment. Was performed a lower midline incision of abdomen. Intraoperative inspection of abdominal cavity showed dilated and lengthened sigmoid and distal part of descending colon with spreading on recto-sigmoid angle (Fig. 3). Inside the colon was easily palpable two giant fecalomas, with sizes (25.0x15.0x15.0 cm). Distally from the dilated colon there was not appeared stenosis, which is highly characteristic for the Hirschsprung's disease. Therefore, most like cause of the fecaloma should be idiopathic megacolon. Damaged parts of the colon were removed surgically and descendo-rectostomywas performed. After surgery removed parts of descending colon, sigmoid colon and recto-sigmoid angle were sent for pathomorphological study. Postoperative period went without complications and intrahospital stay was 7 days. Histopathological study showed that surgically taken material from different parts of damaged colon is corresponding to the changes characteristic for idiopathic megacolon.

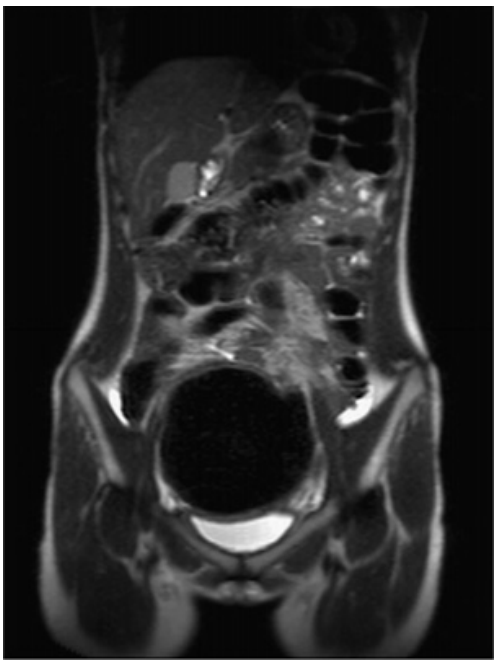

Fig. 1. Abdominal MRI

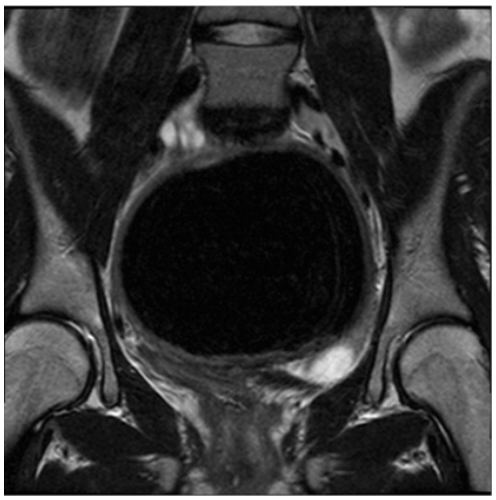

Fig. 2. Pelvic MRI

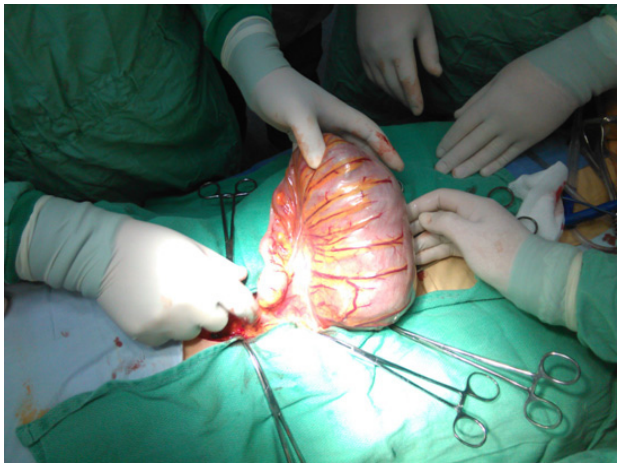

Fig. 3. Operative photograph showing a large sigmoid colon

Results and their discussion. Fecaloma is very uncommon condition and an extreme manifestation of fecal impaction. Fecalomas are formed due to prolonged retention of intracolonic fecal residue which gets organized with time to form a well-formed, hard intraluminal fecal mass [1].Calcification can also be seen. Distal parts of colon, namely sigmoid colon and rectum are the most common sites for fecalomas [3,8]. Fecalomas present as abdominal masses mimicking neoplasm's and can cause local complications by compressing the adjacent anatomical structures, the ureters, urinary bladder, uterus, vagina etc. Occasionally life threatening complications and surgical catastrophes like stercoral perforations, intestinal obstruction, anuria, hydronephrosis, bladder rupture, peritonitis, and septicemia may occur [1]. Most of the fecal impactions are successfully treated by conservative methods such as laxatives, suppositories and trans-rectal enemas. Often it is necessary to dislodge hard stools by manual disimpaction, finger fracture method and digital evacuation. A surgical intervention for uncomplicated fecal impaction is rarely needed [1,6]. When conservative measures fail or when potentially serious complication supervenes, surgical intervention is essential to prevent mortality. Surgical procedure involves either exploratory laparotomy or laparoscopy followed by removal of fecalomas and resection of the involved colonic segment. It is important to keep a close follow up, prescribe stool softeners and educate the patient about proper dietary habits and involve the patient in regular toilet training sessions.

We report a very rare case of idiopathic megacolon with a giant fecaloma in a woman of childbearing age. Fecaloma should be considered in the differential diagnosis of patients presenting with chronic constipation and abdominal mass. Severe chronic constipation should be keenly investigated and should be aggressively approached by appropriate medical, endoscopic and surgical management to prevent significant complications. In case of failure of medical treatment, surgical interventions should be planned. After discharge, patient should maintain on proper diet, medical treatment and regular toilet training. According to the literature, which is quite old, pregnancy can be carried out normally in a patient with this rare pathology. Therefore, we recommend that further investigations are mandatory to delineate guidelines for clinical management of megacolonespecially in women of childbearing age.

REFERENCES

1. Aiyappan SK, Ranga U, Samraj A, Rajan SC, Veeraiyan S. A case of fecaloma. Indian J Surg. 2013; 75: 323-324.
2. Akinci E., Karamercan A., Coşkun F. Radiopaque fecaloma which mimic foreign body. Journal of Academic Emergency Medicine 2013;12:164-166.
3. Caiazzo P, De Martino C, Del Vecchio G, Di Lascio P, Marasco M, Laviani F, et al. Megacolon for a giant faecaloma with unlucky outcome: case report and review of the literature. Ann ItalChir. 2013; 84: 319-322.
4. Garisto JD, Campillo L, Edwards E, Harbour M, Ermocilla R. Giant fecaloma in a 12-year-old-boy: a case report. Cases Journal. 2009; 2: 127.
5. Gupta M., Aggarwal P., SinghR., Lehl S. Case of giant fecaloma in a 32-year-old woman. Austin J Clin Case Rep 2014;1(4):1-2.
6. Kim KH, Kim YS, SeoGS, Choi CS, Choi SC. A case of fecaloma resulting in the rectosigmoidmegacolon. Korean J GastrointestMotil 2007; 13: 81-85.
7. Loevner L.A. Head and Neck MRI, An Issue of Magnetic Resonance Imaging Clinics. Elsevier Health Sciences, Sep 13, 2012.
8. Soyder A., Ozgun H. Giant Fecaloma in 16-year old boy: case report. KolonRektum Hast Derg 2012;22:160-163.

SUMMARY

A CASE OF GIANT FECALOMA IN A 24-YEAR-OLD WOMAN

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Chronic constipation is a very common complaint at outpatient clinics. It can progress to fecal impaction, and rarely to fecalomas if not managed promptly. Fecaloma is characterized by a hardened large mass of feces frequently localized in sigmoid colon and rectum and is difficult to discharge. Fecaliths, stagnating andhardening by time, may cause intestinal obstruction,ulcer development and colonic wall perforation. We present the case of a 24-year-old woman whoadmitted to our hospital with complaints of severe constipation with 1 bowel movement every third-fifth day with passage of hard stools only with using laxatives and meteorism. This is a rare case of fecalomas and megacolon, when conservative measures were absolutely ineffective

and surgical treatment was needed. Therefore, diagnosis of fecaloma must be considered in patients presenting with chronic constipation and abdominal mass.Further investigations are mandatory to delineate guidelines for clinical management of megacolonespecially in women of childbearing age.

Keywords: fecaloma, megacolon, surgical treatment.

РЕЗЮМЕ

КЛИНИЧЕСКИЙ СЛУЧАЙ ГИГАНТСКОЙ ФЕКАЛОМЫ У 24-ЛЕТНЕЙ ЖЕНЩИНЫ

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Хронический запор является распространенной жалобой в амбулаторно-поликлинических учреждениях, часто прогрессирует до каловых закупорок и, в случае неадекватного лечения, превращается в фекалому. Фекалома характеризуется скоплением и затвердением большой массы фекалий, чаще всего локализованных в сигмовидной и прямой кишке, которые трудно вывести из организма. Постепенно происходит стагнация и упрочнение фекальных камней, которые вызывают кишечную непроходимость, развитие язв и перфорацию кишечника. Авторами представлен случай 24-летней женщины, которая поступила в больницу с жалобами на метеоризм и тяжелые запоры с 1 дефекацией на каждый третий-пятый день, с прохождением жесткого стула только с помощью слабительных веществ. Поставлен диагноз мегаколон и фекалома. Консервативное лечение в данном случае оказалось неэффективным, возникла необходимость хирургического лечения. Таким образом, у пациентов с хроническими запорами и плотными образованиями в брюшной полости следует рассматривать диагноз фекаломы. Дальнейшие исследования являются обязательными для уточнения тактики клинического ведения пациентов с мегаколоном, особенно женщин детородного возраста.

რეზიუმე

გიგანტური ფეკალომის შემთხვევა 24-წლის ქალში

კ. ზურაბიშვილი, ა. რეხვიაშვილი, მ. სახამბერიძე, კ. წიკლაური

წმინდა მიქაელ მთავარანგელოზის სახ. მრავალპროფილიანი კლინიკური საავადმყოფო, თბილისი, საქართველო

ქრონიკული ყაბზობა წარმოადგენს ამბულატორულ დაწესებულებებში მიმართვიანობის ხშირ მიზეზს და მოითხოვს დაუყოვნებელ მკურნალობას. წინააღმდეგ შემთხვევაში მდგომარეობის პროგრესირება განავლის ჩაჭედვას ან ფეკალომის წარმოქმნას იწვევს. ფეკალომა ხასიათდება განავლოვანი მასის დიდი ნაწილის გამკვრივებით, რომელიც, უპირატესად, სიგმოიდურ კოლინჯსა და სწორ ნაწლავში ვითარდება. ნაწლავიდან მისი გარეთ გამოტანა დიდ სიძნელეს წარმოადგენს; დროთა განმავლობაში გამყარებულ განავლოვან ქვებს შეუძლია გამოიწვიოს ნაწლავის ობსტრუქცია, დაწყლულება და კედლის პერფორაცია. ავტორების მიერ აღწერილია 24 წლის ქალის კლინიკური შემთხვევა, რომელმაც კლინიკას მომართა მეტეორიზმის და იშვიათი, 3-5 დღეში ერთჯერადი, არასრულფასოვანი, მხოლოდ საფაღარათოების დახმარებით განხორციელებადი, მკვრივი მასებით გაძნელებული დეფეკაციის გამო. დადგენილია ფეკალომისა და მეგაკოლონის იშვიათი შემთხვევა, რომლის კონსერვატიული მკურნალობა უშედეგო აღმოჩნდა და დადგინდა ქირურგიული ჩარევის აუცილებლობა. ფეკალომის დიაგნოზი უნდა გამოირიცხოს პაციენტებში ქრონიკული ყაბზობით და მუცელში პალპირებადი სიმსივნური წარმონაქმნით.

REPRODUCTIVE HEALTH PATTERNS: GEORGIA VERSUS AUSTRALIA

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Abortion and contraception are alternative means of achieving the certain level of fertility. Thus, fertility, abortion, and contraception are fundamentally related: Electing to give birth to a child at a time of a woman's choosing has only been possible with the knowledge and use of contraception before pregnancy and/or the decision and access to terminate a pregnancy after conception. Accordingly, abortion rates are higher in societies with smaller family preferences, with low contraceptive prevalence or the use of ineffective methods, and in societies with a high propensity to rely on induced abortion [1]. As societies have moved through the fertility transition, they have experienced a substitution from traditional contraceptives to modern ones, and the use of to abortion has declined as more effective and user-friendly contraception has been made available. This general model of the inverse relationship between contraception and induced abortion has been observed in many countries of the developed world. In this review, we compare fertility, abortion and contraception trends in two examples of developing (Georgia) and developed (Australia) country.

Fertility

The total fertility rate (TFR) in Georgia has been declining since the 1970s. Official estimates show the TFR fell from around 2.3 children per woman to about 1.6 in 1993 [12] and since then it has been fairly flat. RHS 2005 in Georgia [8] found the TFR to be 1.6 and this appears to be best estimate through 2002-2005. The total fertility rate (TFR) of 1.6 children per woman in 2002-2005 was only slightly lower than the TFR in 1996-1999 (1.7 births per woman) and for the period 2007-2010 TFR rose to 2.0 birth per woman [9]. This is the highest rate recorded for Georgian women since the 1970s and is 25% increased in comparison to the preceding five years. Georgian women continue to marry early (median age at first marriage was 21.6 years in 2005) and overwhelmingly report having their first sexual experience after marriage. Most women complete childbearing at an early age. The median age at the birth of their first child is 23 years, and fertility reaches peak levels among women at age 20 to 29 years. The preference among women for small families is reflected not only in their below replacement level fertility (2.1 babies per fertile woman) and high abortion rates, but also in the commonly stated desire for no more children. Because they achieve their desired family size early and the divorce rate is low (6%), most Georgian women spend the majority of their reproductive years (generally 20-25 years) in a union and in need of effective contraception to prevent unintended pregnancies.

Similar trends have been observed in Australia over the last decade. Australia's fertility rate has been falling steadily since the baby boom of the immediate post World War II period. Australia's fertility rate is well below the international average of 2.5, and above the average of 1.7 in developed nations. Many developed nations of the world (such as Germany, Italy, Japan, Canada and Korea) have low or very low fertility rates, in most cases well below the replacement level of 2.1 babies per fertile woman and notably lower than Australia. The dominant thinking of demographers was that Australia would follow this trend and could have a fertility rate in the range of 1.5 or 1.6 babies per fertile woman by 2016. However, fertility rates have increased in most parts of Australia since about 2002, which has radically challenged this perspective. The reasons for this upswing in fertility rates are strongly debated. The reasons include: favorable economic conditions including low unemployment rates, leading to more flexible working conditions allowing parents to balance home and work obligations (important for middle and high income families); government income support including the baby bonus and family tax benefits (important for middle and lower income families); a new social paradigm with greater gender equality and greater emphasis on supporting parenting by fathers; delayed fertility in a large cohort of women leading to higher rates for a period of time (but these rates are likely to decrease in the coming years again). While 297,900 babies were born in 2011, the highest number on record, new Australian Bureau of Statistics figures [4] show the TFR sits at 1.89 babies per woman last year, virtually unchanged from 2009. The changing demographic of Australian Society suggests that certain population groups have a much higher TFR whilst other segments are shrinking dramatically. Bolstered by strong economic conditions and government policies such as the baby bonus, the nation's fertility rallied from a low of 1.73 babies per woman in 2001 to a 30-year high of 1.96 in 2008. However, a second consecutive year of lower fertility means Australia is still falling short of the replacement level (of 2.1 babies per woman of reproductive age) and must continue to rely on migration to increase its population. The main reason is that women continue to wait longer before having children.

Georgian Society would be described as perhaps largely homogenous with strong cultural influences delaying first sexual debut and remaining monogamous with a low divorce rate in childbearing years. Australia is considered to be be more of a Cosmopolitan society with segments of the population with high fertlity rates; in particular indiginous aboriginal people and imigrants from India, Africa, South

East Asia and the Middle East. Strong cultural influences control age of marriage, divorce rates and contraception acceptability. The majority of Australians would be generational European with a much younger age of first sexual intercourse and a high divorce tolerance and liberal Western sexual behaviour and attitudes which require more extensive contraception use and awareness, but when it fails a higher demand for abortion services. As the mix of cultures proliferates these demographic changes impact on the composite "Australian" statistics.

Abortion

According to a 2012 analysis by the Guttmacher Institute and the World Health Organization [13] after declining substantially over the last couple of decades (from 35 to 29 per 1000 women aged 15-44) the worldwide abortion rate has now stalled. Contraceptive use in developing countries has cut the number of maternal deaths by 44% (about 270,000 deaths averted in 2008) but could prevent 73% if the full demand for birth control was met. Georgian Reproductive Health surveys conducted during the last two decades years showed that in the year 2000 Georgia had its' highest reported total abortion rate (TAR) - 3.7 abortions per woman [7]. During the same period, Georgia also had the lowest proportion of women, 25 % using contraception. Georgia RHS survey 2005 showed that contraceptive prevalence was 28 %, an increase caused by greater use of modern contraceptive methods [8]. The use of effective modern methods of contraception has increased from 25 to 28 percent and abortion rates have decreased from 3.7 to 3.1 abortions per woman. Between 2005 and 2010, the abortion rate dropped significantly to 1.6 abortions per woman (95% CI 1.5-1.8), a 48% decline from 3.1, or 57% from 3.7. More than one-half of Georgian women obtaining abortions in 2007–2010 were aged 25–29 (102 abortions per 1,000 women) and 30–34 (83 abortions per 1,000 women). The third highest age specific abortion rate, contributing to 25% of the TIAR, occurred among women aged 35–39. The main reasons given for choosing abortion included: desire to stop childbearing (51%), desire to space the next birth (18%), and socioeconomic circumstances that prevented the family from supporting another child (20%) [9].

IIn Australia, the law surrounding abortion is defined by each state. Abortions can be carried out legally everywhere in the country to protect the health of a woman, though the precise definition of this differs depending on the state or territory. In 2008, The Abortion Law Reform Act [10] decriminalized termination of pregnancy and set out guidelines for when abortion can take place. Australia's abortion rate is reasonably low compared to Georgia. An estimated 80,000 - 90,000 surgical abortions are performed in Australia each year [3]. This equates to approximately 250 per day, or one abortion for every 2.8 live births. One in three Australian women will have an abortion in their lifetime. The estimated rate for Australia in 2003 was around 19.7 abortions per 1000 women aged between 15 and 44. The highest abortion rate was at age 20-24. However, the exact numbers of abortion are extremely difficult to obtain because there is no routinely collected national abortion statistics. Many are done in the private sector and this may account for another 20 000 per year. These are not included in the statistics as for privacy reasons women may not claim the governmental rebate so true numbers are not accurate. International rates range from 7.7 in Germany to 90 in Eastern Europe, with a world average of 33-37 abortions per 1000 women.

Contraception

Contraceptive use in Georgia, as mentioned above, is relatively low, as shown by the high percentage of women who have never used any contraceptive method (58%) and the high percentage of women not currently using contraception (72 %) [9]. However the use of modern contraceptive methods rose sharply, from 20% in 1999 to 35% in 2010. For the first time, the prevalence of modern methods exceeded the prevalence of traditional methods, which had declined. As a result, the contraceptive prevalence rate for married women increased from 41% in 1999 to 45% in 2005 and 53% in 2010. Almost two-thirds (65%) of married women have a potential demand for contraception, including 52% who already use a method and 12% whose demand has yet to be satisfied (i.e. they have an unmet need for some contraceptive method). The unmet need for contraception among married women in 2010 is half the level documented in 1999 (12% vs. 24%), mostly as a result of increased use of modern methods. Need rises with rural residence, low education, larger families, and poor wealth quintiles. Among current users (52%), 18% use traditional methods, which are subject to high failure rates and subsequent abortions. When these are added to the unmet need group (12%) the total need for modern methods is 30%, nearly a third of all married women. Among all current contraceptive users, 26% were using the condom (14% out of 53%), followed by 25% using the IUD (13% out of 53%), 21% using withdrawal (11% out of 53%), 13% using periodic abstinence (7% out of 53%), 7% using the pill (4% out of 53%), 5% using tubal ligation (2.9% out of 53%), and 3% using spermicides (1.5% out of 53%). Between 1999 and 2010, condom use among couples increased 2.5 times (from 6% to 14%) and IUD use increased from 10% to 13%, becoming the first and second most used methods, respectively. Withdrawal and the rhythm method, the leading methods in 1999, became the third and fourth most commonly used methods in 2010 [9]. Pill use, still very low, increased from 2% in 1999 to 4% in 2010, and tubal ligation increased from 2% to 3%. An important component of the newly implemented reproductive health strategy in Georgia is to train health professionals to provide family planning counseling at any point of contact with medical care, including primary health care services.

In contrast, nearly 70% of Australian women of reproductive age are using a contraceptive method. Despite

this, estimates are that over 50% of Australian women have had an unplanned pregnancy. The uptake of LARC (Long-acting reversible contraception) is low in Australia with only 1.2% of women using it [11]. LARC is a public health priority in the UK and US, but there is no clear policy on LARC in Australia. Recent Australian studies have shown that the most popular contraceptive method is the contraceptive pill [14]. Condom use is the next most commonly used, followed by vasectomy and tubal ligation with the rapid increase in the use of the contraceptive pill from its introduction in 1961 through to 1986. Research also describes the very low uptake of the intrauterine device (IUD). Despite the IUD being the most widely used reversible method in the world (17% in France and 21% in Sweden), there are considerable regional differences in its use, with much higher prevalence rates in developing countries. Estimates from Australia show that few women use IUDs: less than 2% of women of reproductive age. Reasons for the low usage include misinformation, a belief that it is unsuitable prior to childbirth and concern about infection. We do know that it does not have any effect on future fertility and there is a small infection risk associated with IUD insertion but it is really confined to the first 20 days and it is a very low risk [5]. The rate of pelvic inflammatory disease is lower with the Levonogestrel IUS than with the copper IUD. Research shows that LARCS improve long-term continuation rates: the Levonogestrel IUS and copper IUDs had similar 24-month continuation rates at 79% and 77% respectively, implants - 69% compared to OCP - 43%, vaginal rings - 41% and depot Medroxyprogesterone acetate - 38% [2].

In terms of hormonal contraception, Levonogestrel/ethinyloestradiol is the most frequently recorded medication, accounting for nearly half of the medications in all age groups. Drospirenone/ethinyloestradiol and cyproterone/ethinyloestradiol were more frequently recorded for 12-34 year-olds than those aged 35-54 years. The reverse was true for LARCs, with medroxyprogesterone and the levonorgestrel intrauterine device being recorded more often for 35-54-year-olds than 12-34-year-olds. Data suggests that the Copper intrauterine and ethinyloestradiol/etonogestrel devices were least often prescribed. For emergency contraception problems, levonogestrel was the most frequently recorded medication [6].

General contraception and emergency contraception were highest among young women (18-24) and declined with a women's age. This could be because older women are more likely to use contraceptive methods that do not require them to regularly fill prescriptions. These lower rates of encounter may also account for increasing levels of unplanned pregnancy and abortion being seen in women in this age group.

The COCs have been found to be the preferred method of contraception before and between pregnancies, but its rate of use decreases with age and with higher numbers of pregnancies. Middle-aged women tend to use longer-term methods of contraception when no further pregnancies are desirable and this strategy requires fewer visits to doctor.

Conclusion

The limitations of our review include the bias of all observational studies. Direct comparison is not possible as it depends heavily on social, cultural and traditional specifics of Georgian and Australian populations and also different time periods. Fertility, abortion and contraception figures for Australia and Georgia tend to reflect the attitude of each country to comprehensive sexuality education and effective contraception rather than the sexual behavior of the people who live in these countries.

Also, a number of immeasurable factors may complicate the analysis and interpretation of the relationship between abortion and contraceptive use. These include the lack of reliable information on contraceptive use among women who are sexually active but not in union and variations in rates of contraceptive failure (either method failures or failures caused by irregular or incorrect use). In addition, a women's motivation to achieve a small family or to time their births more precisely may simply outpace the rise in effective method use. Furthermore, an increase in sexual activity among unmarried people may lead to an increase in unplanned pregnancies and in the demand for abortion. Thus, the level of abortion may remain stable or continue to rise even while contraceptive use rises or remains at a high level.

Further research needs to assess the needs of Georgian women's for modern methods and their knowledge of these methods and their benefits. Survey data show that many Georgian women still dislike most modern methods, know little about them, and believe that they are associated with negative health consequences. In addition, 75 percent of traditional method users believe that the method they are using is equally or more effective than modern contraceptive methods. Research also needs to explore the attitudes and practices of providers to determine whether they advise women to avoid hormonal or other effective methods or if they instill fear in women about the effects of those methods. Finally, research should determine why Georgia continues to have one of the highest documented abortion rates in the world, in spite of sustained efforts to improve access to modern methods of contraception.

Australian medical practitioners could also improve access to contraception, particularly for young women who have higher rates of unintended pregnancy and abortion. Hormonal implants, intrauterine hormones and devices (IUDs) as top tier LARCs offer safe and effective solutions to these problems and should be among first-line contraception options, particularly for younger women, where they surprisingly have tended to be used more as second

or third-line contraceptives. Like any choices, they each have their pros and cons and will appeal to people for different reasons but Australia's main point is that all of these three methods should be offered as first-line contraceptives across all age groups.

REFERENCES

1. Bongarts J., Westoff C. The potential role of contraception in reducing abortion. Stud Fam Plann 2000; 3: 193-202.
2. Hanrahan C. Medical Observer Obstet Gynecol. 2013; 122: 1083-91.
3. http://www.betterhealth.vic.gov.au/bhcv2/bhcarticles.nsf/pages/Abortion_in_Australia. 2008.
4. http://www.who.int/reproductivehealth/publications/unsafe_abortion/induced_abortion . 2012.
5. Kamenev M. ANZJOG Medical Observer. 2012.
6. Mazza D., Harrison C., Taft A. et al. Current contraceptive management in Australian general practice: an analysis of BEACH data. 2012.
7. Georgia Reproductive Health Survey – 2000 NCDC; MoLHSA; UNFPA; UNICEF; USAID.
8. Georgia Reproductive Health Survey - 2005 NCDC; MoLHSA; UNFPA; UNICEF; USAID.
9. Georgia Reproductive Health Survey - 2010 NCDC; MoLHSA; UNFPA; UNICEF; USAID
10. Pratt B. Australian Parliamentary Research Brief no. 9 2004-05. How many abortions are there in Australia? A discussion of abortion statistics, their limitations, and options forimproved statistical collection. 2005.
11. Richters J, Grulich AE, de Visser RO et al. Sex in Australia: contraceptive practices among a representative sample of women. Aust N Z J Public Health 2003; 27: 210-216.
12. Ross J**.** Reproductive Health in Georgia: contraception, abortion and costs. UNFPA, 2009.
13. WHO. World Health Statistics . Geneva, World Health Organization; [Online] 2014.
14. Yusuf F. Siedlecky S. Patterns of contraceptive use in Australia Journal of Biosocial Science 2007; 39: 735-744.

SUMMARY

REPRODUCTIVE HEALTH PATTERNS: GEORGIA VERSUS AUSTRALIA

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The review compares a few reproductive indicators - fertility, abortion and contraception in both a developing (Georgia) and a developed (Australia) country. Fertility, abortion and contraception figures in both countries tend to reflect the attitude and the degree of development of each countries sexual health education and their use of effective contraception. Further research is required to accurately evaluate the need and access of Georgian women to modern methods of family planning and their knowledge of the benefits of modern contraception that can assist to reduce pregnancy termination rate. In Australia better insight is needed on how to facilitate a shift to more efficacious long-term contraceptives across all age groups.

Keywords: reproductive health, Induced abortion, fertility, contraception.

РЕЗЮМЕ

НЕКОТОРЫЕ РЕПРОДУКТИВНЫЕ ПОКАЗАТЕЛИ В ГРУЗИИ И АВСТРАЛИИ

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В обзоре приводятся сравнительные данные некоторых репродуктивных показателей – фертильности, контрацепции и искусственного прерывания беременности в Грузии и Австралии. Репродуктивные показатели отражают отношение и степень развития в каждой стране всесторонних аспектов репродуктивного здоровья и эффективной контрацепции. Необходимо проведение дальнейших исследований с целью оценки нужд женщин репродуктивного возраста для увеличения доступа к современным методам контрацепции с целью снижения частоты прерывания беременности.

რეზიუმე

რეპროდუქციული ჯანმრთელობის ზოგიერთი მაჩვენებელი საქართველოში და ავსტრალიაში

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მიმოხილვაში მოყვანილია საქართველოში და ავსტრალიაში რეპროდუქციული ჯანმრთელობის რამდენიმე მნიშვნელოვანი მაჩვენებლის - ფერტილობის, კონტრაცეპციის და ორსულობის ხე-

ლოვნური შეწყვეტის ანალიზი. ნაჩვენებია ორივე ქვეყნის ტენდენციები და სხვაობა ფერტილობის, კონტრაცეპციის და ორსულობის ხელოვნური შეწყვეტის მაჩვენებლებში. დასახულია შემდგომი გზები ოჯახის დაგეგმარების გაუმჯობესებისათვის, მოსახლეობაში თანამედროვე კონტრაცეპტივების მოხმარების დანერგვისა და ორსულობის შეწყვეტის შემცირების მიზნით.

DISTINCT DRUG RESISTANCE PROFILE OF HIV-1 SUBTYPE A STRAIN CIRCULATING IN GEORGIA

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HIV/AIDS remains the major global public health challenge of modern times, with around 35 million people living with HIV by the end of 2013 [17]. The global HIV epidemic is primarily caused by HIV type 1 (HIV-1), which is characterized by divergent evolution leading to development of multiple subtypes and inter-subtype recombinant forms [14]. This genetic diversity can affect disease progression, response to antiretroviral therapy (ART) and emergence of drug resistance [5,7,18]. The knowledge about subtype-specific responses to currently available antiretroviral drugs is especially important for resource poor countries, where treatment options are limited because of economic constraints. Resistance pathways in different subtypes may affect drug cross-resistance and the potential use of specific second-line regimens, which further narrows limited treatment options in developing countries.

The HIV epidemic in Georgia began in 1989 and as of December 1, 2014 a cumulative 4 646 cases of HIV infection had been reported in the country. For many years, Georgia's HIV epidemic has been driven by injection drug use (IDU) accounting for 50% of total reported cases. Previous studies of HIV-1 subtype epidemiology in Georgia showed predominance of subtype A [6], which is also commonly found in other countries of the Eastern European region [12].

Since 2004, through the Global Fund support Georgia ensured universal access to ART for all patients in need. Provision of ART is governed by the national guidelines and is coordinated by the Infectious Diseases, AIDS and Clinical Immunology Research Center (National AIDS Center). Currently ART is recommended in all patients with CD4 count <500 cells/mm3. The recommended initial regimen consists of two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). A ritonavir (r) boosted protease inhibitor (PI) is recommended in cases when an NNRTI cannot be prescribed. Currently tenofovir + emtricitabine and zidvudine + lamivudine are used for the NRTI component of initial regimen. Efavirenz is the preferred NNRTI with nevirapine being recommended as an alternative to EFV.

As per national guidelines, the standard of ART monitoring relies upon laboratory monitoring of CD4 count, HIV-1 viral load and development of resistance based on a resistance-genotype detection when indicated. Previously we have shown effectiveness of this approach in early identification of patient failing on ART and in improving clinical outcomes in patients with drug resistant viruses.[3, 4] Our earlier works have also suggested that subtype A circulating in Georgia has distinct NNRTI resistance profile, however the generalizability of this finding was limited because of small sample size [6, 16] The objective of the current study was to describe drug resistance profiles among ART-experienced patients in Georgia.

Materials and methods. Study included 193 adult (age ≥ 18 years) HIV patients who experienced virological failure and were found to carry drug resistant strains of HIV-1 based on HIV genotypic resistance testing. Study period was 2005-2013. Subjects were identified through laboratory and medical records. Data on demographic and clinical characteristics were abstracted through chart review.

The virologic failure was defined as plasma HIV-1 RNA level >400 copies/mL 6 months or >50 copies/mL 12 months after commencing treatment in patients that remain on ART, or if patient had viral rebound confirmed by two consecutive measurements following the undetectable plasma HIV-1 RNA levels while on therapy.

Genotypic resistance testing was performed using the TruGene HIV-1 Genotyping Kit (Siemens, Germany) according to the manufacturer's instructions on OpenGene DNA Sequencing System. HIV *pol* gene sequences were examined for the presence of resistance-associated mutations. Stanford HIV Sequence Database (http://hivdb.stanford.edu/) was used for interpretation of resistance data. Mutations listed by the International Antiviral Society-USA were considered.

Multiple alignments were created with CLUSTAL W program [15]. Phylogenetic analyses were conducted using MEGA software version 6.0 [13]. The Neighbor Joining (NJ) method and Kimura two-parameter model with reliability estimated from 1000 bootstrap replicates were used for tree construction. HIV-1 Subtypes were assigned by inferring relatedness of participant sequences to reference HIV-1 sequences obtained from the Los Alamos National Laboratory HIV Sequence Database (http://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html).

Statistical analyses were carried out using SAS 9.2 (SAS Institute, Cary, NC, USA). Standard descriptive statistics were performed to describe patient characteristics. Comparisons were tested using Pearson's chi-square test or Fisher's exact test as appropriate. Significance was defined as p value <0.05.

Results and their discussion. Among 193 HIV patients included in the analysis the median age was 39 years and majority (74%) were men (table 1). More than half (53.4%) of patients were infected through injection drug use and 86.5% had HIV subtype A. Nearly 80% of patients received Efavirenz-based ART regimen, up to 20% of patients were on Nevirapine-based regimen and only one patinets was on Lopinavir-based ART at the time of virologic failure. The most common NRTI backbone was Zidovudine + Lamivudine (45.1%) followed by Abacavir + Lamivudine (22.3%), Tenofovir + Emtricitabine (21.7%) and Stavudine + Lamivudine (10.9%).

A total of 170 (88.1%) patients had virus with dual-class NRTI + NNRTI resistance, virus from one (0.5%) patient had dual-class NRTI + PI resistance, single-class resistance was identified 21 (10.9%) viruses, including 10 (5.2%) cases of NRTI resistance only and 11 (5.7%) cases of NNRTI resistance only. One (0.5%) patient carried the virus with triple-class NRTI + NNRTI + PI resistance.

The most common NRTI mutation was M184V found in 166 (86.0%) viruses, followed by L74V (22.3%, n=43) and K65R (13.5%, n=26) (Table 2). Any thymidine analogue mutation (TAM) was found in 23.3% of samples and 7.8% of viruses carried ≥3 TAMs. The most common TAM was D67N – 12.9%. Comparison of occurrence of NRTI mutations by subtype did not show any statistically significant difference (Table 2).

Table 1. Characteristics of study population

	n=193
Age, median years (IQR)	39 (35-44)
Gender, n (%)	
Men	143 (74.0)
Women	60 (25.9)
Mode of HIV Transmission, n (%)	
Injection drug use	103 (53.4)
Heterosexual contact	77 (39.9)
Male-to-male sex	6 (3.1)
Blood recipient	2 (1.0)
Unknown	5 (2.6)
HIV subtype, n (%)	
A	167 (86.5)
B	21 (10.9)
Other	5 (2.6)
ART regimen, n (%)	
Efavirenz-based regimen	154 (79.8)
Nevirapine-based regimen	38 (19.7)
Lopinavir-based regimen	1 (0.5)

Table 2. HIV-1 reverse transcriptase drug-resistant mutations

	Total (n=193)	**Subtype A (n=167)**	**Subtype non-A (n=26)**	***p* value**
NRTI mutations, n (%)				
M41L	12 (6.2)	11 (6.6)	1 (3.8)	0.99
D67N	25 (12.9)	24 (14.4)	1 (3.8)	0.21
K70R	13 (6.7)	12 (7.2)	1 (3.8)	0.99
L210W	9 (4.7)	9 (5.4)	0 (0.0)	0.61
T215YF	22 (11.4)	18 (10.8)	4 (15.4)	0.51
K219QE	15 (7.8)	13 (7.8)	2 (7.7)	0.99
K65R	26 (13.5)	24 (14.4)	2 (7.7)	0.54
L74V	43 (22.3)	36 (21.6)	7 (26.9)	0.61
Y115F	23 (11.9)	22 (13.2)	1 (3.8)	0.32
M184V	166 (86.0)	146 (87.4)	20 (76.9)	0.22
NNRTI mutations, n (%)				
K101E	61 (31.6)	61 (36.5)	0 (0.0)	<0.0001
K103N	58 (30.1)	40 (24.0)	18 (69.2)	<0.0001
V106M	8 (4.1)	7 (4.2)	1 (3.8)	0.99
V108I	13 (6.7)	11 (6.6)	2 (7.7)	0.69
Y181CI	52 (26.9)	43 (25.7)	9 (34.6)	0.34
G190S	105 (54.4)	104 (62.3)	1 (3.8)	<0.0001
G190A	8 (4.1)	5 (3.0)	3 (11.5)	0.08

The most frequent NNRTI mutation was G190S, found in 105 (54.4%) of samples. Other significant NNRTI mutations included K101E (31.6%, n=61), K103N (30.1%, n=58) and Y181CI (26.9%, n=52). Comparisons showed significant differences in drug resistance profiles between A and non-A subtypes. The prevalence of G190S and K101E was significantly higher in subtype A samples (p<0.0001), while K103N was more frequent in non-A samples (p<0.0001) (table 2). In 69 samples G190S co-occurred with either K101E or Y181C or with both: 39 genotypes G190S/K101E; 10 genotypes G190S/Y181CI and 20 genotypes G190S/K101E/Y181CI.

Two patients had viruses with mutations conferring resistance to PIs. In one case it was D30N mutation, the other case was combination of three mutations – M46I, I47V and L76V.

Our study shows that HIV-1 Subtype A has distinct NNRTI drug resistance profile. This has been first described by our group and recently has been confirmed in larger study from Russia [6, 9, 16]. Similar to our previous reports G190S was the most common NNRTI mutation and the frequency was significantly higher in subtype A samples. This is the main distinction from other subtypes, which tend to select for K103N and Y181CI mutations more frequently [11, 19].

Another important characteristic observed in our study was high frequency of K101E mutation, which was identified only in subtype A samples and which almost exclusively was co-occurring with G190S. This has not been described previously. Also there was significant co-occurrence with Y18ICI. It is unclear whether these NNRTI mutations were selected simultaneously or were accumulated. In all cases co-occurrence limits the use of novel generation NNRTIs in subtype A infected patients with previous exposure to this drug class.

In addition to subtype-based comparisons, differences in frequency of G190S and K101E were also explored by gender, mode of HIV transmission and ART regimen. None of these analyses yielded statistical significance, underscoring that HIV-1 subtype is the only source of difference.

With regard to other drug resistant mutations, as in the rest of the world, M184V appears to be the most frequent drug resistance mutation [1, 8]. Compared to our previous report we observed increase in the frequency of L74V (22.3% in 2014 vs. 14.6% in 2011) and K65R (13.5% in 2014 vs. 4.9% in 2011) [16], which is consistent with increased use of Abacavir and Tenofovir. The frequency of TAMs in our study was lower compared to report from Marconi and colleagues [10], but was similar to that of reported in settings where viral load monitoring is available routinely [2]. This finding once again underscores that routine use of viral load is key for preventing accumulation of resistance mutations. As Zidovudin remains one of the key drugs used

in Georgia, close monitoring for development of TAMs needs to be continued.

The level of PI resistance was low. Only two patients in our study carried the virus with PI mutations. One of these patients had been on Nelfinavir based treatment in early 2000s, when laboratory monitoring capacities were very limited, and the PI resistance was only identified several years after the exposure to the drug. The other patient was on Lopinavir-based ART as part of first line regimen. By the end of 2013 nearly 13% of those on first-line ART were receiving PIs and only one case of drug resistance indicates that PI can be considered for initial treatment in resource-limitted settings.

The study has limitation in terms of sample size, particularly for non-A subtypes with only 26 samples included in the study. This limited statistical power for comparisons and over generalizability. On the other hand we included all cases of documented HIV-1 drug resistance in treated patients and this limitation is not due to sampling bias. Despite this limitation study provides important information for clinicians and program managers.

Our study further confirms that HIV-1 subtype A has distinct drug resistance profile and G190S appears to be its favored NNRTI mutation. There is little information about the PI drug resistance patterns in subtype A stains. Continued monitoring of HIV-1 drug resistance is warranted to inform HIV treatment programs.

REFERENCES

1. Assoumou L, Descamps D, Yerly S, Dos Santos G, Marcelin AG, Delaugerre C, Morand-Joubert L, Ruffault A, Izopet J, Plantier JC, Pakianather S, Montes B, Chaix ML, Wirden M, Costagliola D, Masquelier B. Prevalence of HIV-1 drug resistance in treated patients with viral load >50 copies/mL in 2009: a French nationwide study. J Antimicrob Chemother. 2013; 68**:**1400-1405.
2. Barth RE, van der Loeff MF, Schuurman R, Hoepelman AI, Wensing AM. Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review. Lancet Infect Dis. 2010; 10**:**155-66.
3. Chkhartishvili N, Dvali N, Gochitashvili N, Sharvadze L, Tsertsvadze T. Successful application of laboratory tools for the detection of HIV drug resistance in routine clinical care in Georgia. Georgian Med News 2008; 12 (165); 16-22.
4. Chkhartishvili N, Sharvadze L, Dvali N, Karchava M, Rukhadze N, Lomtadze M, Chokoshvili O, Tsertsvadze T. Virologic outcomes of second-line antiretroviral therapy in Eastern European country of Georgia. AIDS Res Ther. 2014; 11**:**18.
5. Dolling DI, Dunn DT, Geretti AM, Sabin CA. HIV-1 subtype and virological response to antiretroviral therapy: a confirmatory analysis. Clin Infect Dis. 2013; 56**:**162-3.
6. Dvali N, Parker MM, Chkhartishvili N, Sharvadze L, Gochitashvili N, Abutidze A, Karchava M, DeHovitz JA, Tsertsvadze T. Characterization of HIV-1 subtypes and drug resistance mutations among individuals infected with HIV in Georgia. J Med Virol. 2012; 84**:**1002-8.
7. Easterbrook PJ, Smith M, Mullen J, O'Shea S, Chrystie I, de Ruiter A, Tatt ID, Geretti AM, Zuckerman M. Impact of HIV-1 viral subtype on disease progression and response to antiretroviral therapy. J Int AIDS Soc. 2010; 13**:**4.
8. Hosseinipour MC, Gupta RK, Van Zyl G, Eron JJ, Nachega JB. Emergence of HIV Drug Resistance During First- and Second-Line Antiretroviral Therapy in Resource-Limited Settings. J Infect Dis. 2013; 207**:** S49-S56.
9. Kolomeets AN, Varghese V, Lemey P, Bobkova MR, Shafer RW. A uniquely prevalent nonnucleoside reverse transcriptase inhibitor resistance mutation in Russian subtype A HIV-1 viruses. AIDS. 2014; 28**:** F1-F8.
10. Marconi VC, Sunpath H, Lu Z, Gordon M, Koranteng-Apeagyei K, Hampton J, Carpenter S, Giddy J, Ross D, Holst H, Losina E, Walker Bruce D, Kuritzkes Daniel R. Prevalence of HIV-1 Drug Resistance after Failure of a First Highly Active Antiretroviral Therapy Regimen in KwaZulu Natal, South Africa. Clin Infect Dis. 2008; 46**:**1589-1597.
11. Reuman EC, Rhee SY, Holmes SP, Shafer RW. Constrained patterns of covariation and clustering of HIV-1 non-nucleoside reverse transcriptase inhibitor resistance mutations. J Antimicrob Chemother. 2010; 65**:**1477-1485.
12. Saad MD, Shcherbinskaya AM, Nadai Y, Kruglov YV, Antonenko SV, Lyullchuk MG, Kravchenko ON, Earhart KC, Sanchez JL, Birx DL, Carr JK. Molecular epidemiology of HIV Type 1 in Ukraine: birthplace of an epidemic. AIDS Res Hum Retroviruses. 2006; 22**:**709-14.
13. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013; 30**:**2725-9.
14. Tebit DM, Arts EJ. Tracking a century of global expansion and evolution of HIV to drive understanding and to combat disease. Lancet Infect Dis. 2011; 11**:**45-56.
15. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994; 22**:**4673-80.
16. Tsertsvadze T, Chkhartishvili N, Sharvadze L, Dvali N, Chokoshvili O, Gabunia P, Abutidze A, Nelson K, Dehovitz J, Del Rio C. Outcomes of Universal Access to Antiretroviral Therapy (ART) in Georgia. AIDS Res Treat. 2011; 2011**:**621078.
17. UNAIDS. The Gap Report. UNAIDS, Geneva. 2014.
18. Wainberg MA, Brenner BG. The Impact of HIV Genetic Polymorphisms and Subtype Differences on the Occurrence of Resistance to Antiretroviral Drugs. Mol Biol Int. 2012; 2012**:**256982.
19. Wallis CL, Aga E, Ribaudo H, Saravanan S, Norton M, Stevens W, Kumarasamy N, Bartlett J, Katzenstein D. Drug Susceptibility and Resistance Mutations After First-

Line Failure in Resource Limited Settings. Clin Infect Dis. 2014; 59**:**706-715.

SUMMARY

DISTINCT DRUG RESISTANCE PROFILE OF HIV-1 SUBTYPE A STRAIN CIRCULATING IN GEORGIA

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Emergence of HIV-1 drug resistance limits effectiveness of antiretroviral therapy (ART). Since 2004 Georgia provides free ART to all patients in need. We aimed to evaluate drug resistance patterns of Georgian HIV-1 variants among patients with virologic failure.

Study included adult HIV-1 patients, who experienced virologic failure and were found to carry drug resistant strains based on genotypic resistance testing in 2005-2013. HIV-1 *pol* gene sequences were examined for the presence of resistance-associated mutations. Stanford HIV Sequence Database was used for interpretation of resistance data.

A total 193 patients were included in the study. Among them majority (86.5%) carried subtype A virus and nearly 80% were on Efavirenz-based regimen. The most common nucleoside reverse transcriptase inhibitor (NRTI) mutation was M184V – 86.0% (n=166). The most frequent non-nucleoside reverse transcriptase inhibitor (NNRTI) mutation was G190S, found in 105 (54.4%) of samples. Other significant NNRTI mutations included K101E (31.6%, n=61), K103N (30.1%, n=58) and Y181CI (26.9%, n=52). The prevalence of G190S was 62.3% in subtype A viruses compared to 3.8% in non-A variants ($p<0.0001$). Frequency of K101E was also significantly higher in subtype A (36.5% vs. 0%, $p<0.0001$).). In 69 samples G190S co-occurred with either K101E or Y181C or with both: 39 genotypes G190S/K101E; 10 genotypes G190S/Y181CI and 20 genotypes G190S/K101E/Y181CI.

High prevalence of G190S and K101 mutations suggests subtype A specific response to currently approved first-line NNRTIs. Frequent co-occurrence of G190S with Y181C and K101E may limit the use of novel generation NNRTIs in subtype A infected patients with previous exposure to this drug class.

Keywords: HIV-1, subtype A, drug resistance.

РЕЗЮМЕ

ОТЛИЧИТЕЛЬНЫЙ ПРОФИЛЬ ЛЕКАРСТВЕННОЙ РЕЗИСТЕНТНОСТИ ЦИРКУЛИРУЮЩЕГО В ГРУЗИИ СУБТИПА А ВИЧ-1

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Возникновение лекарственной резистентности ВИЧ-1 ограничивает эффективность антиретровирусной терапии (АРТ). С 2004 г. Грузия предлагает бесплатную АРТ всем пациентам, которые нуждаются в данном лечении.

Целью исследования явилась оценка профиля лекарственной резистентности вариантов ВИЧ-1 у больных с вирусологической неэффективностью.

Исследованы взрослые больные ВИЧ-1, у которых установлена вирусологическая неэффективность и наличие резистентных штаммов вируса на основании теста генотипической резистентности за период 2005-2013 гг. Образцы *pol* гена ВИЧ-1 изучены на наличие мутаций, вызывающих резистентность. База данных «Stanford HIV Sequence Database» была использована для интерпретации данных по резистетности.

В исследование включены 193 пациента. Большинство из них (86,5%) имели субтип А ВИЧ-1 и 80% из них лечились эфавирензом. Наиболее часто встречаемой мутацией, вызывающей резистентность к нуклеозидным ингибиторам обратной транскриптазы (NRTI), была M184V – 86,0% (n=166). На счет ненуклеозидных ингибиторов обратной транскриптазы (NNRTI), наиболее часто выявляемой NNRTI мутацией, явилась G190S, обнаруженная в 105 (54,4%) образцах. Другие значимые NNRTI мутации были K101E (31,6%, n=61), K103N (30,1%, n=58) и Y181CI (26,9%, n=52). Превалентность G190S в образцах субтипа А вируса была 62,3% в сравнении с 3,8% в остальных субтипах вируса ($p<0.0001$). Частота K101E также была значительно выше при субтипе А (36,5% по сравнению с 0%, $p<0,0001$). В 69 образцах G190S выявлена наряду с K101E или Y181C либо с обеими мутациями: 39 генотипов G190S/K101E; 10 генотипов G190S/Y181CI и 20 генотипов G190S/K101E/Y181CI.

Высокая превалентность мутаций G190S и K101E позволяет судить о специфическом ответе со стороны субтипа А ВИЧ-1 на лечение рекомендуемыми пре-

паратами NNRTI первого ряда. Частая сочетаемость G190S с Y181C и K101E может ограничить применение препаратов NNRTI нового покаления среди пациентов, инфицированных субтипом A, которые ранее принимали препараты данного класса.

რეზიუმე

საქართველოში გავრცელებული აივ-1 სუბტიპის წამლებისამდი რეზისტენტობის განსხვავებული პროფილი

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აივ-1 წამლებისადმი რეზისტენტობის განვითარება ზღუდავს ანტირეტროვირუსული (არვ) მკურნალობის ეფექტურობას; 2004 წლიდან საქართველოში ყველა პაციენტი, რომელსაც სჭირდება არვ მკურნალობა იღებს მას. კვლევის მიზანს წარმოადგენდა საქართველოში გავრცელებული აივ-1 ვარიანტების რეზისტენტობის პროფილის შესწავლა ვირუსული უშედეგობის მქონე პაციენტებში.

კვლევაში ჩართული იყო მოზრდილი აივ-1 პაციენტები, რომელთაც აღენიშნათ ვირუსული უშედეგობა და აღმოაჩნდათ წამლებისადმი რეზისტენტული შტამები გენოტიპური რეზისტენტობის ტესტის საფუძველზე 2005-2013 წწ. აივ-1 *pol* გენის ნიმუშები შესწავლილ იქნა რეზისტენტობასთან ასოცირებული მუტაციების არსებობაზე. “Stanford HIV Sequence Database” გამოყენებულ იქნა რეზისტენტობის მონაცემების ინტერპრეტაციისათვის.

კვლევაში ჩართული იყო 193 პაციენტი, მათგან უმრავლესობას (86.5%) აღმოაჩნდა ვირუსის სუბტიპი A და თითქმის 80% იმყოფებოდა ეფავირენზის შემცველ მკურნალობაზე. შებრუნებითი ტრანსკრიპტაზას ნუკლეოზიდის ანალოგის ინჰიბიტორების (NRTI) მიმართ რეზისტენტობის გამომწვევი მუტაციებიდან ყველაზე ხშირი იყო M184V – 86.0% (n=166). შებრუნებითი ტრანსკრიპტაზას არანუკლეოზიდის ანალოგის ინჰიბიტორების (NNRTI) მიმართ რეზისტენტობის გამოწვევი მუტაციებიდან ყველაზე ხშირია G190S – 105 შემთხვევა (54.4%).

სხვა მნიშვნელოვანი NNRTI მუტაციები იყო K101E (31.6%, n=61), K103N (30.1%, n=58) და Y181CI (26.9%, n=52). G190S მუტაციის პრევალენტობამ A სუბტიპის შტამებში შეადგინა 62.3%, ხოლო არა-A სუბტიპის შტამებში - 3.8% ($p<0.0001$). K101E მუტაციის სიხშირე, ასევე, სარწმუნოდ მაღალი იყო A სუბტიპის ნიმუშებში (36.5% და 0%, $p<0.0001$). 69 ნიმუშში G190S მუტაცია ერთდროულად დაფიქსირდა K101E ან Y181C ან ორივესთან ერთად: 39 გენოტიპი G190S/K101E; 10 გენოტიპი G190S/Y181CI და 20 გენოტიპი G190S/K101E/Y181CI.

G190S და K101E მუტაციების მაღალი პრევალენტობა მიუთითებს ამჟამად რეკომენდებული პირველი რიგის NNRTI მედიკამენტების მიმართ სპეციფიკურად A სუბტიპისთვის დამახასიათებელი პასუხის არსებობის შესაძლებლობაზე. G190S და ორი სხვა NNRTI მუტაციის Y181C და K101E ერთდროულად განვითარების მაღალმა სიხშირემ, შესაძლოა შეზღუდოს ახალი თაობის NNRTI მედიკამენტების გამოყენება A სუბტიპის მქონე პაციენტებში, რომელთაც მანამადე ჩატარებული აქვთ მკურნალობა ამ კლასის სხვა მედიკამენტებით.

СРАВНИТЕЛЬНАЯ ЭФФЕКТИВНОСТЬ ИСПОЛЬЗОВАНИЯ БЕТА-АДРЕНОБЛОКАТОРОВ И БЛОКАТОРОВ If-КАНАЛОВ У БОЛЬНЫХ СТАБИЛЬНОЙ СТЕНОКАРДИЕЙ

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Значительные достижения в изучении механизмов патогенеза стенокардии и острых коронарных синдромов явились причиной изменения приоритетов в классификации, диагностике, лечении и профилактике ишемической болезни сердца (ИБС), которая по сей день остается наиболее распространенной причиной смертности в развитых странах. В отличие от острого коронарного синдрома, связанного с разрывом атеросклеротичной бляшки, тромбозом и окклюзией коронарной артерии, причиной стабильной стенокардии чаще является стенозирующее атеросклеротическое поражение коронарного русла с неадекватным кровоснабжением миокарда [5,7]. Ведение больных ИБС – одна из наиболее противоречивых проблем современной кардиологии. В современных терапевтических стандартах довольно четко определена тактика ведения больных острыми коронарными синдромами, хотя благодаря развитию инвазивных технологий расширились возможности «радикальных» реваскуляризационных вмешательств: ангиопластика, стентирование и аортокоронарное шунтирование, которые способны существенным образом повлиять на качество жизни больных острыми и хроническими формами ИБС. Однако указанные вмешательства имеют ряд ограничений, поскольку не способны повлиять на течение атеросклеротического процесса – основную причину ИБС, и не исключают необходимости дальнейшей профилактической терапии. В связи с этим, в клинической практике по сей день является актуальной проблема оптимального медикаментозного лечения хронической ИБС. В 1997 г. появились первые согласованные рекомендации Европейского кардиологического общества по лечению стабильной стенокардии [1,3,6]. Дальнейшая эволюция терапевтических подходов обобщена в рекомендациях Американской коллегии кардиологов и Американской кардиологической ассоциации (ACC/AHA) 2002 г. Кроме того, в последнее время осуществлен ряд широких контролируемых исследований, которые могут повлиять на тактику ведения больных стабильной стенокардией (СС).

Снижение частоты госпитализаций по поводу указанных нозологий является глобальной задачей для системы здравоохранения как по вопросам сохранения трудоспособности пациентов, так и по экономическим затратам на лечение больных. В связи с этим, введение в схемы лечения новых, более современных, основанных на доказательных данных, методов ведения пациентов является актуальной задачей кариологии [2,4,8].

Целью исследования явилось сравнение антиишемического влияния бета-адерноблокаторов (бисопролола, карведилола) и ингибитора натриевых if-каналов ивабрадина у пациентов со стабильной стенокардией II-III функционального класса.

Материал и методы. Обследованы 90 пациентов с СС, которые кроме нитропрепаратов (Кардикет, «Schwarz pharma" в дозе 10-40 (23,56±3,27) мг/сут., аспирина ("Bayer AG" 100 мг/сут.) и розувастатина (Мертенил, "Гедеон Рихтер" 10 мг/сут.) получали бисопролол (Конкор, "Nycomed") в дозе 1,25-7,5 (5,41 ±1,36) мг/сут) – I группа, 30 больных; карведилол (Кориол, "KRKA") в дозе 6,25-12,5 (9,75±1,69) мг/сут – II группа, 30 больных; и ивабрадин (Кораксан, "Servier") в дозе 5-15 (9,81 ±2,13) мг/сут – III группа, 30 больных. Группы пациентов были репрезентативны по возрасту (средний возраст 51,3±5,7 лет), полу, продолжительности заболевания (11,9±3,8 лет). Пациентов обследовали на момент поступления, в период пребывания на стационарном лечении (в среднем 11,7±2,1 дней) и через месяц после назначенного лечения. Проводилось клиническое обследование пациентов, Холтеровское мониторирование электрокардиограммы (ХМ ЭКГ).

Математический анализ полученных результатов осуществляли оценкой среднего значения и стандартной ошибки среднего значения. Достоверность количественных показателей определялась методом контроля «нулевой гипотезы» с использованием t-критерия Стьюдента (достоверными считались результаты с показателем $p<0,05$). Анализ в двух зависимых выборках при нормальном распределении массивов проводился с использованием парного t-критерия Стьюдента. При ненормальном распределении массивов использовался t-критерий Уилкоксона. В двух независимых выборках при нормальном распределении использовался двувыборочный t-критерий Стьюдента, в двух независимых выборках при ненормальном распределении – U-критерий Уилкоксона.

Результаты и их обсуждение. В результате проведенной терапии у всех обследованных пациентов выявлена положительная динамика указанных показателей (таблица 1). В частности, на фоне терапии бисопрололом достигнуто снижение частоты сердечних сокращений (ЧСС) на 10,92%, а карведилолом – на 4,92%. Наибольшее снижение среднесуточной ЧСС - на 19,23% наблюдалось

у пациентов, которым к базисному лечению добавляли ивабрадин. При этом необходимо отметить отсутствие побочных эффектов при коррекции дозы ивабрадина, что является значимым фактом терапии больных СС, особенно с сопутствующей патологией. В отличие от ивабрадина, у двух больных I группы не удалось достичь эффективной дозы препарата вследствие усиления бисопрололом признаков бронхообструкции. Вместе с тем, у одного больного I группы, принимавшего бисопролол, периодически появлялось чувство похолодания нижних конечностей, а у другого – на фоне приема 10 мг бисопролола на 13 день лечения, по данным ЭКГ, зарегистрирована атриовентрикулярная блокада I степени (PQ=0,21 с). В каждом из этих случаев, побочные эффекты были устранены путем уменьшения дозы препарата вдвое, до 5 мг бисопролола. Наименьшую динамику частотных показателей деятельности сердца наблюдали у пациентов II группы, которые в составе базисной терапии принимали карведилол.

Существенное снижение среднесуточной ЧСС на фоне приема ивабрадина уже спустя месяц коррелировало с улучшением качества жизни пациентов. При этом необходимо указать значимую черту терапевтического действия препарата – снижение ЧСС у обследованных пациентов не сопровождалось снижением артериального давления (АД). Так, у пациентов I группы месяц спустя после проведенного лечения систолическое артериальное давление (САД) снизилось на 9,13%, во II группе – на 6,98%, а у пациентов III группы показатели САД оставались практически без изменений (среднесуточный САД до лечения составлял 126,78±3,24 мм рт ст., а спустя месяц после проведенной терапии – 125,3±2,29 мм рт ст), что следует объяснить клинико-фармакологическими свойствами ивабрадина. Препарат снижает ЧСС путем блокирования активируемых гиперполяризацией натриевых if-каналов в синусовом узле, без существенного влияния на трансмембранные потоки K+ и Na+ в кардиомиоцитах и в клетках проводящей системы сердца, а также на продол-

Таблица 1. Динамика показателей ХМ ЭКГ под влиянием проведенного лечения у пациентов со стабильной стенокардией

показатели	Группы обследованных больных					
	I группа (n=30)		II группа (n=30)		III группа (n=30)	
	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения
среднесуточная ЧСС, уд/мин	73,45±3,72	65,43±2,23* **	72,93±2,61	69,33±3,13**	75,56±1,32	61,03±2,26*
максимальная дневная ЧСС, уд/мин	129,87±4,51	107,62±3,67*	133,66±7,91	111,33±3,54*	129,45±5,81	103,8±1,98*
минимальная дневная ЧСС, уд/мин	64,62±5,12	52,54±2,94*	64,82±1,38	55,66±2,21*	65,13±2,17	50,73±0,97*
циркадный индекс,	1,23±0,02	1,25±0,02*	1,22±0,02	1,23±0,02* **	1,22±0,01	1,27±0,01*

*примечание: * - достоверная разница показателей (p<0,05) до и после проведенного лечения в одной группе больных;*
*** - достоверная разница показателей (p<0,05) после проведенного лечения между III группой и другими обследованными группами*

Таблица 2. Динамика показателей ХМ ЭКГ под влиянием проведенного лечения у пациентов со стабильной стенокардией

показатели	Группы обследованных больных					
	I группа (n=30)		II группа (n=30)		III группа (n=30)	
	до лечения	после лечения	до лечения	после лечения	до лечения	после лечения
количество эпизодов БИМ, n	5,05±1,07	1,84±0,81*	4,42±0,93	2,36±1,17*	5,94±1,52	2,98±1,06*
количество эпизодов ББИМ, n	7,72±1,98	2,65±1,05*	6,36±1,07	2,51±1,34*	9,72±1,86	2,52±1,13*
средняя продолжительность БИМ, мин	10,42±1,98	1,65±1,05*	8,36±1,07	2,19±1,48*	8,79±1,63	2,11±098*
средняя продолжительность ББИМ, мин	12,01±4,68	2,27±1,57*	9,20±4,19	1,97±1,76*	10,64±2,12	2,19±0,88*
суммарная продолжительность эпизодов ишемии, мин	20,52±8,90	4,31±1,34*	21,59±8,25	3,11±0,93*	24,08±7,69	3,51±1,56*

*примечание: * - достоверная разница показателей (p<0,05) до и после проведенного лечения в одной группе больных*

жительность интервалов PQ, QT и комплекса QRS, что, в конечном счете, понижает риск развития кардиологических осложнений.

Анализ данных динамики ишемических эпизодов показал, что у пациентов всех групп проведенная терапия почти одинаково снизила частоту ангинозных приступов (таблица 2). По данным ХМ ЭКГ снизилось количество и продолжительность эпизодов ишемии миокарда. На фоне терапии бисопрололом уменьшилась частота приступов стенокардии на 85%, а у 63% – приступы ангинозной боли прекратились. При этом средняя продолжительность эпизодов болевой ишемии миокарда (БИМ) уменьшилась с 10,42±1,98 мин. до 1,65±1,05 мин. ($p<0,05$), а средняя продолжительность безболевой ишемии миокарда (ББИМ) – с 12,01±4,68 мин. до 2,27±1,57 мин. ($p<0,05$). У пациентов, которые принимали карведилол, частота приступов стенокардии уменьшилась на 78%, а у 59% - приступы прекратились. При этом средняя продолжительность БИМ уменьшилась с 8,36±1,07 мин. до 2,19±1,48 мин. ($p<0,05$), а средняя продолжительность приступов ББИМ - с 9,20±4,19 мин. до 1,97±1,76 мин. ($p<0,05$). У пациентов, которые принимали ивабрадин: частота приступов уменьшилась на 76% и у 61% приступы прекратились, а средняя продолжительность БИМ и ББИМ снизилась соответственно с 8,79±1,63 мин. до 2,11±098 мин. ($p<0,05$) и с 10,64±2,12 мин. до 2,19±0,88 мин. ($p<0,05$). Достоверной разницы между полученными результатами после проведенной терапии между обследованными группами не выявлено. Изменения показателей ишемии миокарда по данным ХМ ЭКГ коррелировали с возможным увеличением продолжительности физической нагрузки до появления ангинозной боли и уменьшением потребности в нитропрепаратах (в I группе – на 68%, во II группе – на 72%, в III группе – на 66%). Итак, сравнивая результаты анализа ишемических проявлений в начале и после проведенной терапии, необходимо указать отсутствие статистически достоверных изменений между показателями исследуемых групп в зависимости от варианта терапии, которая свидетельствует о сопоставимых эффектах антиангинальной терапии у бисопролола, карведиола и ивабрадина.

Выводы.

1. Примененные схемы антиангинальной терапии у пациентов с СС являются весьма эффективными. На фоне проведенной терапии отмечалось уменьшение частоты и продолжительности ишемических эпизодов.
2. Статистически достоверных изменений между показателями исследуемых групп в зависимости от варианта терапии не выявлено, что свидетельствует о сопоставимых эффектах антиангинального действия бисопролола, карведилола и ивабрадина.
3. Наиболее эффективное и безопасное снижение ЧСС достигнуто на фоне приема ивабрадина.

ЛИТЕРАТУРА

1. Амосова Е.Н., Сюй Яо, Безродный А. Б. и др. Сравнительная оценка влияния контроля частоты сокращений сердца посредством комбинации ивабрадина и метопролола и монотератии метопрололом на вариабельность ритма сердца и его систолическую функцию у больных, перенесших инфаркт миокарда с зубцом Q, с фракцией выброса левого желудочка менее 45%. Український кардіологічний журнал 2012; 4: 23-30.
2. Колчин Ю.М., Капранова Ю.С. Клиническая эффективность и влияние ивабрадина на показатели систолической функции и ремоделирования левого желудочка у пациентов с постинфарктным кардиосклерозом в сочетании с ожирением и артериальной гипертензией, сохраненной фракцией выброса левого желудочка в условиях жесткого контроля ЧСС: результаты 12-месячного наблюдения. Сердце и сосуды 2013; 2(42): 55-63.
3. Пархоменко А.Н., Лутай Я.М., Иркин О.И. Эффективность и безопасность применения ингибитора If-каналов ивабрадина у больного с острым Q-инфарктом миокарда с синусовой тахикардией на фоне терапии блокаторами бета-адренорецепторов. Укр. мед. часопис. 2012; 1(87): 103-110.
4. Чесникова А.И., Лаврик Е.А., Бедарева И.В. Оценка эффективности применения карведилола и бисопролола у больных с сердечной недостаточностью, перенесших инфаркт миокарда. Кардиоваскулярная терапия и профилактика 2008; 7(3): 68-73.
5. Borer J.S., Tardif J.C. Efficacy of ivabradine, a selective I(f) inhibitor, in patients with chronic stable angina pectoris and diabetes mellitus. Am. J. Cardiol. 2010; 105(1): 29-35.
6. Ceconi C., Freedman S.B. et al. Effect of heart rate reduction by ivabradine on left ventricular remodeling in the echocardiographic substudy of BEAUTIFUL. Int. J. Cardiol. 2011; 146(3): 408-414.
7. Couvreur N., Tissier R., Pons S., et al. Chronic heart rate reduction with ivabradine improves systolic function of the reperfused heart through a dual mechanism involving a direct mechanical effect and a long-term increase in FKBP12/12.6 expression. Eur. Heart. J. 2010; 31(12): 1529-1537.
8. Fasullo S., Cannizzaro S. et al. Comparison of ivabradine versus metoprolol in early phases of reperfused anterior myocardial infarction with impaired left ventricular function: preliminary findings. J. Card. Fail. 2009; 15(10): 856-863.

SUMMARY

THE COMPARATIVE EFFECTIVENESS OF BETA-BLOCKERS AND INHIBITORS OF If-IODIUM USING IN PATIENTS WITH STABLE ANGINA PECTORIS

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The purpose of the study was to evaluate the comparative effectiveness for using of different approaches to anti-

ischemic therapy in patiens with stable angina pectoris Class II-III.

90 patients with chronic form of ischemic heart disease that have been involved in present study along with nitrates, antiplatelet agents and statins received bisoprolol (Group I), carvedilol (Group II) and ivabradine (Group III). General clinical findings were analyzed as well as the results of diurnal ECG monitoring.

As a result of treatment the number and duration of ischemic episodes reliably decreased in patients of all groups.

The most effective and safe decrease in average daily heart rate was achieved in patients taking ivabradine.

No statistically significant differences between values of investigated groups were revealed, that point out about comparable effects of antianginal action of bisoprolol, carvedilol and ivabradine.

Keyworls: stable angina, bisoprolol, carvedilol, ivabradine.

РЕЗЮМЕ

СРАНИТЕЛЬНАЯ ЭФФЕКТИВНОСТЬ ИСПОЛЬЗОВАНИЯ БЕТА-АДРЕНОБЛОКАТОРОВ И БЛОКАТОРОВ If-КАНАЛОВ У БОЛЬНЫХ СТАБИЛЬНОЙ СТЕНОКАРДИЕЙ

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Целью исследования явилась оценка сравнительной эффективности применения различных схем антиишемической терапии у пациентов со стабильной стенокардией II-III функционального класса.

Обследовано 90 больных хроническими формами ишемической болезни сердца, которые кроме нитропрепаратов, антиагрегантов и статинов получали бисопролол (I группа), карведилол (II группа) и ивабрадин (III группа). Проанализированы общеклинические данные, а также результаты суточного мониторирования ЭКГ. После проведенного лечения у пациентов всех групп достоверно снизилось количество и длительность ишемических эпизодов.

Наиболее эффективным и безопасным в плане снижения среднесуточной и среднедневной частоты сердечных сокращений оказался ивабрадин.

Статистически достоверной разницы между вышеупомянутыми показателями исследуемых групп в зависимости от использованной терапии не выявлено, что свидетельствует о сопоставимых эффектах антиангинального действия бисопролола, карведилола и ивабрадина.

რეზიუმე

If-არხების ბლოკატორების და ბეტა-ადრენობლოკატორების შედარებითი ეფექტურობა სტაბილური სტენოკარდიით ავადმყოფებში

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კვლევის მიზანს წარმოადგენდა II-III ფუნქციური კლასის სტაბილური სტენოკარდიით ავადმყოფებში ანტიიშემიური მკურნალობის სხვადასხვა სქემის ეფექტურობის შედარებითი შეფასება.

შესწავლილია 90 ავადმყოფი გულის იშემიური დაავადების ქრონიკული ფორმით, რომელთა მკურნალობის სქემაში, ნიტროპრეპარატების, ანტიაგრეგანტების და სტატინების გარდა, შედიოდა ბისოპროლოლი (I ჯგუფის ავადმყოფები) კარვედილოლი (II ჯგუფის) და ივაბრადინი (III ჯგუფის). გაანალიზებულია ზოგადკლინიკური მაჩვენებლები და. ასევე, ელექტროკარდიოგრამის დღე-ღამური მონიტორინგის შედეგები.

ჩატარებული მკურნალობის შედეგად ყველა ჯგუფის პაციენტებში სარწმუნოდ შემცირდა იშემიური ეპიზოდების რაოდენობა და ხანგრძლივობა.

გულისცემის სიხშირის საშუალო სადღეღამისო და საშუალო დღიური ეფექტური და უსაფრთხო შემცირება მიღებული იქნა ივაბრადინით მკურნალობის ფონზე.

საკვლევი ჯგუფის მაჩვენებლებს შორის სტატისტიკურად სარწმუნო ცვლილებები,დამოკიდებული გამოყენებული თერაპიის ვარიანტზე, არ გამოვლინდა, რაც მიუთითებს ბისოპროლოლის, კარვედილოლის და ივაბრადინის ანტიანგინალური მოქმედების ერთნაირ ეფექტზე.

INFLUENCE OF CIRCADIAN BLOOD PRESSURE PROFILE ON ENDOTHELIAL FUNCTION IN PATIENTS WITH AND WITHOUT ARTERIAL HYPERTENSION

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Hypertension is associated with altered endothelial nitric oxide release in brachial, coronary, brain and renal arteries, which is a risk factor for cardiovascular and cerebrovascular disease. Endothelial dysfunction is considered as the initial step in the pathogenesis of atherosclerosis and plays an important role in hypertension maintenance [12]. Vascular endothelium participates in the regulation of blood flow in response to changes in tissue and organ perfusion requirements. Vasodilatation in response to increased blood flow is known as flow mediated vasodilatation (FMD) [2,3]. Celermajer and colleagues introduced a unique setup to study FMD noninvasively, bases of which is post-ischemic dilatation of the downstream vascular bed. At present, for measurement of endothelial function in a large population is mainly used the ultrasonic method, because of its non-invasive nature, high validity, simple technique, brief duration, its repeatability and low variability [4,11].

Studies have shown that 24-hour ambulatory blood pressure monitoring (ABPM) is a better predictor of subsequent complications than spot measurements of blood pressure (BP) [1,7,10]. In healthy subjects, BP decreases during sleep by 10% to 20% and increases promptly on waking. In hypertensive patients, this normal diurnal BP variation pattern is usually preserved, particularly when there is no target organ damage. Individuals with a non-dipper circadian pattern of BP are at increased risk for cerebrovascular and cardiovascular complications than are individuals with a dipper circadian rhythm [10]. However, little is known about the relationship between the circadian BP rhythm and endothelial function in patients with essential hypertension. Consequently, we have hypothesized, that hypertensive patients with non-dipper circadian BP profile have more deteriorated endothelial function, than those with dipper BP profile.

Materials and methods. 57 outpatients with arterial hypertension (AH), 30 men and 27 women (mean±SD, 51.26±1.94 years) and 17 normotensive subjects, 9 men and 8 women (mean±SD, 51.0±5.41 years) were included in the study. All the individuals underwent anthropometrical measurements, physical examinations, review of their medical histories, off-therapy 24-hour ABPM and vascular doppler-echography with high resolution ultrasound, which was performed to analyze FMD of brachial artery.

AH was defined as an untreated systolic BP over than 140 mmHg in the sitting position after 5 minute of rest on at least three different occasions. The study protocol was approved by the local ethics committee and informed consent for participation was obtained from all the patients. Patients with obesity, age over 60 years and those who were on medication with vasoactive and/or hormonal drugs, hypercholesterolemic subjects, diabetics, smokers, patients with manifested heart failure and ischemic heart disease, Raynaud's phenomenon, cerebrovascular disease, peripheral vascular disease, end stage renal and liver diseases were excluded from the study.

24-Hour ABPM was obtained with TM2420 recorders. Measurements were performed in accordance with The Guideline of the Working Party on Blood Pressure Monitoring of the European Society of Hypertension. ABPM was performed on a working day with the subjects performing usual daily activities. A noninvasive ABPM was attached to the upper left arm. In accordance with the 24-hour BP rhythm, all patients were divided into two groups: dippers and non-dippers. According to the last decisions of "Consensus Conference on Ambulatory Blood Pressure Monitoring", for circadian blood pressure classification we have used systolic blood pressure level [12].

Endothelium-dependent FMD of the brachial artery was investigated by using high resolution (7MHz) ultrasound equipment. FMD test was performed in a blind manner. Investigator did not have clinical information about the patient. All patients were instructed to avoid eating for at least 8 to 12 hours before the study. Subjects were informed that they should not exercise and ingest substances that might affect FMD, such as caffeine, alcohol, high-fat foods and vitamin C or use tobacco for at least 4-6 hours before the study. All studies were performed at 9.30_{AM} in a quiet, air-conditioned room (22^0C to 24^0C). Blood pressure cuff was placed above the antecubital fossa. Recordings were taken two times: baseline – rest image and after reactive hyperemia test (RHtest). The cuff was inflated to at least 50 mmHg above the systolic pressure for 5 minutes to occlude arterial inflow. FMD was calculated with the equation: $FMD = D_1\text{-}D_0 / D_0 \bullet 100\%$; where D_1 is vessel diameter after RHtest and D_0 is initial diameter [5,6].

Statistical management and analyses was performed by using the SPSS 15.0 package (SPSS 15.0 for windows Evaluation Version). Data are presented as mean ± standard deviation. Bivariate correlations were estimated through Pearson's correlation coefficients. $P<0.05$ was considered statistically significant. Between-groups differences were estimated by Mann-Whitney Test. For testing hypothesis, whether dipper/non-dipper circadian blood pressure profiles are independent of normotension/hypertension status, was

Table. Clinical characteristics and brachial artery duplex-scanning data in hypertensive and healthy subjects with different circadian blood pressure pattern

Parameter	Patients with Arterial Hypertension		P	Subjects with normal Blood Pressure		P
	Dippers (n=20)	Non-Dippers (n=37)		Dippers (n=11)	Non-Dippers (n=6)	
Age, years	49.4±3.57	52.27±2.34	0.142	46.72±12.38	54.00±9.25	0.241
Heart rate (beats/min)	78.6±3.68	74.14±2.51	0.059	74.45±4.39	73.33±2.50	0.474
FMD %	11.94±0.95	3.54±1.76	<0.01	13.37±2.22	4.51±5.70	<0.01
Vascular diameter, cm D0	3.76±0.2	4.31±0.16	<0.01	3.83±0.25	4.57±0.64	<0.01
D1	4.21±0.22	4.46±0.17	NS	4.34±0.25	4.75±0.53	NS
ΔD	0.45±0.03	0.15±0.07	<0.01	0.51±0.07	0.18±0.25	<0.01

All results are presented as mean±SD. FMD – Flow mediated vasodilatation. D0 – Initial vascular diameter, D1 – Vascular diameter after RHtest; ΔD – Change of vascular diameter because of RHtest

used χ^2 test for independence. Exact Test method was used for testing hypothesis, whether dipper circadian blood pressure profile is more frequent in normotensive subjects, than in hypertensive patients.

Results and their discussion. The baseline clinical characteristics and data obtained via brachial artery duplex-scanning of the 20 dipper and 37 non-dipper patients with AH and 11 dipper and 6 non-dipper control subjects are summarized in Table. Age of patients with dipper and non-dipper circadian BP profile was not significantly different. Independent from blood pressure level, dipper patients had significantly higher FMD%, as well as absolute change of brachial artery diameter while RHtest. Initial diameter of brachial artery of non-dipper patients was significantly higher in spite of having normotension or hypertension. In the whole study population (n=74), FMD% showed strong negative correlation with 24-hour SBP, DBP and PP (r=-0.475, r=-0.521, r=-0.317; P<0.01, respectively). Average daytime, as well as nighttime SBP, DBP and PP also correlated with the FMD%. Subgroup analyzes in hypertensive patients showed strong negative correlation only between FMD% and nighttime SBP, DBP and PP.

Using χ^2 test for independence appeared, that circadian BP profile (dipper/non-dipper) is not independent from the blood pressure level (P=0.0298). Furthermore, Exact Test method showed, that dipper circadian blood pressure profile is more frequent in normotensives, than it is in hypertensive patients (P = 0.0139).

Our study shows that circadian BP profile is not independent from the blood pressure level. Dipper circadian BP profile is more frequent in normotensive subjects, than in hypertensive patients. Accordingly, we can suggest that high BP somehow acts on circadian BP profile and leads it to non-dipper circadian BP profile. The fact that initial vascular diameter of non-dipper patients was higher than it was in dippers in spite of BP level, gives us an opportunity to suppose, that non-dipper circadian BP profile aggressively acts on vascular wall and is the cause of increased diameter, because of vascular wall muscle hypertrophy, which is accompanied by endothelial dysfunction. Our study confirms that AH is associated with the deterioration of endothelial function. Therefore, it gives an explanation why the elevated BP is associated with increased predisposition to cardiovascular and cerebrovascular morbidity and mortality. Furthermore, our findings might help to identify hypertensive patients who are at increased risk for cardiovascular and cerebrovascular events. Like Anderson et al. [2], Giadoni et al. [6], Hodgson et al. [8], our data show that FMD% is significantly lower in non-dipper subjects.

In the framework of our study we compared the vascular parameters of all 74 dipper and non-dipper subjects from both, normotensive and hypertensive groups. Non-dipper patients, compared with dippers have significantly lower level of endothelium-dependent vasodilatation independent of BP levels. Taken into account all the above said, our group of investigators, like Mordi et al. [9] considers an assessment of endothelial function as an attractive and useful target for modern cardiology.

Our study confirms the presence of disturbed endothelium-dependent vasodilatation in AH. Furthermore, our study confirms that non-dipper circadian BP rhythm is associated with the significant impairment of endothelial function. Consequently, we can suggest that patients with non-dipper circadian BP profile per se could be assessed as a high risk group, which might need permanent supervising for avoiding of future cardiovascular and cerebrovascular complications, which are highly available because of injury of vascular endothelium.

REFERENCES

1. Akasaki Y., Ohishi M. Dipper, non-dipper pattern. Nihon Rinsho 2014; 72(8):1400-1403.

2. Anderson T. Prognostic significance of brachial flow-mediatedvasodilation. Circulation 2007; 115: 2373-2375.
3.Charakida M., de Groot E., Loukogeorgakis S., Khan T., Lüscher T., et al. Variability and reproducibility of flow-mediated dilatation in a multicenter clinical trial. European Heart Journal 2013; 34: 3501-3507.
4. Charakida M., Masi S., Lüscher T., Kastelein J., Deanfield J. Assessment of atherosclerosis: the role of flow-mediated dilatation. European Heart Journal 2010; 31: 2854-2861.
5. Donald A., Halcox J., Charakida M., Storry C., Wallace S et al. Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. Journal of the American College of Cardiology 2008;51:1959-1964.
6. Ghiadoni L., Faita F., Salvetti M., Cordiano C., Biggi A. et al. Assessment of flow-mediated dilation reproducibility: a nationwide multicenter study. Journal of Hypertension 2012; 30: 1399-1405.
7. Head G., Mihailidou A., Duggan K., Beilin L., Berry N. et al. Definition of ambulatory blood pressure targets for diagnosis and treatment of hypertension in relation to clinic blood pressure: prospective cohort study. British Medical Journal 2010; 340: 1104.
8. Hodgson J., Woodman R, Croft K., Ward N., Bondonno C. et al. Relationships of vascular function with measures of ambulatory blood pressure variation. Atherosclerosis 2014; 233(1):48-54.
9. Mordi I., Tzemos N. Is reversal of endothelial dysfunction still an attractive target in modern cardiology? World Journal of Cardiology 2014; 6(8):824-835.
10. Palatini P., Reboldi G., Beilin L, Casiglia E., Eguchi K. et al. Added predictive value of night-time blood pressure variability for cardiovascular events and mortality: the Ambulatory Blood Pressure - International Study. Hypertension 2014; 64(3):487-493.
11. Rekhviashvili A., Tsinamdzgvrishvili B., Chkhetia M, Labakhua G. Relationship of 24-hour blood pressure rhythm with endothelial function and blood rheology. Georgian Medical News 2008; 1(159):21-26.
12. Schnabel R., Schulz A., Wild P., Sinning C., Wilde S. et al. Noninvasive vascular function measurement in the community: cross-sectional relations and comparison of methods. Circulation: Cardiovascular Imaging 2011;4:371-380.

SUMMARY

INFLUENCE OF CIRCADIAN BLOOD PRESSURE PROFILE ON ENDOTHELIAL FUNCTION IN PATIENTS WITH AND WITHOUT ARTERIAL HYPERTENSION

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Little is known about the relationship between the circadian BP rhythm and endothelial function in patients with essential hypertension. Consequently, we have hypothesized, that hypertensive patients with non-dipper circadian BP profile have more deteriorated endothelial function, than those with dipper BP profile. 57 untreated hypertensive patients and 17 normotensive controls were undergone to the anthropometrical measurements, physical examinations, review of their medical histories, 24-hour ABPM and vascular doppler-echography with high resolution ultrasound. Circadian BP profile was not independent from the BP level; namely, dipper profile was more frequent in normotensives. Independent from hypertension, dipper patients had significantly higher FMD%. In the whole study population, FMD showed strong negative correlation with 24-hour SBP, DBP and PP. Our study confirms the presence of disturbed endothelium-dependent vasodilatation in AH. Furthermore, our study showed that non-dipper circadian BP rhythm is associated with the significant impairment of endothelial function. Consequently, we can suggest that patients with non-dipper circadian BP profile could be assessed as a high risk group, which might need permanent supervising for avoiding of future cardiovascular and cerebrovascular complications.

Keywords: arterial hypertension, circadian, endothelial function.

РЕЗЮМЕ

ВОЗДЕЙСТВИЕ ЦИРКАДНОГО ПРОФИЛЯ КРОВЯНОГО ДАВЛЕНИЯ НА ЭНДОТЕЛИАЛЬНУЮ ФУНКЦИЮ У ПАЦИЕНТОВ С АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ И БЕЗ НЕЁ

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Взаимосвязь циркадного профиля артериального давления и эндотелиальной функции у пациентов с эссенциальной гипертензией по сей день недостаточно изучена. Авторы предполагают, что пациенты с гипертензией и с нон-диппер циркадным профилем имеют более выявленные нарушения эндотелиальной

функции, чем пациенты с диппер-профилем кровяного давления. Антропометрические измерения, физикальные обследования, 24-часовый амбулаторный мониторинг артериального давления и допплер-эхо сосудов с ультразвуком высокого разрешения проведены 57 нелеченным гипертензивным (группа исследования) и 17 нормотензивным (группа контроля) пациентам. Проанализированы истории болезни. Выявлено, что циркадный профиль артериального давления зависим от уровня кровяного давления; диппер-профиль установлен чаще у нормотензивных пациентов. В независимости от гипертензии, пациенты с диппер профилем имели значительно высокий показатель поток-опосредованной вазодилатации (FMD%). Как у пациентов с диппер-профилем, так и без него FMD% показал высокий коэффициент корреляции с 24-часовым систолическим, диастолическим и пульсовым давлением. Результаты проведенного исследования подтверждают наличие нарушенной эндотелий-зависимой вазодилатации во время артериальной гипертензии. Более того, выявлено, что нон-диппер циркадный ритм артериального давления взаимосвязан со значительным нарушением функции эндотелия. Следовательно, авторы предполагают, что пациенты с нон-диппер циркадным профилем представляют группу высокого риска, которая нуждается в перманентном контроле для избежания кардиоваскулярных и цереброваскулярных осложнений.

რეზიუმე

არტერიული წნევის ცირკადული პროფილის გავლენა ენდოთელიუმის ფუნქციაზე პაციენტებში ჰიპერტენზიით და მის გარეშე

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ავტორების მიერ შემოთავაზებულია ჰიპოთეზა იმის შესახებ, რომ ჰიპერტენზიულ პაციენტებში ნონ-დიპერ ცირკადული პროფილით, დიპერ პაციენტებთან შედარებით, გაუარესებულია ენდოთელური ფუნქცია. 57 არანამკურნალებ პაციენტს და 17 ნორმოტენზიულ პირს (კონტროლი) ჩაუტარდა ანთროპომეტრული გაზომვები, ფიზიკალური გამოკვლევა, არტერიული წნევის 24-საათიანი ამბულტორიული მონიტორინგი და სისხლძარღვთა დოპლერექოგრაფია მაღალი რეზოლუციის ულტრაბგერით. არტერიული წნევის ცირკადული პროფილი დამოკიდებული იყო წნევის დონეზე; კერძოდ, დიპერ ცირკადული პროფილი უფრო ხშირად ახასიათებდა ნორმოტენზიულ ინდივიდებს. ჰიპერტენზიის არსებობისგან დამოუკიდებლად, დიპერ პაციენტებს გააჩნდათ სარწმუნოდ მაღალი ნაკადდამოკიდებული ვაზოდილატაცია (FMD%). მთლიან საკვლევ პოპულაციაში, ნაკად-დამოკიდებულმა ვაზოდილატაციამ აჩვენა ძლიერი უარყოფითი კორელაცია 24-საათიან სისტოლურ,დიასტოლურ და პულსურ წნევასთან მიმართებაში. კვლევა ადასტურებს გაუარესებული ენდოთელიუმ-დამოკიდებული ვაზოდილატაციის არსებობას ჰიპერტენზიის დროს. ნაჩვენებია,რომ ნონ-დიპერ ცირკადული პროფილი ასოცირებულია ენდოთელიუმის ფუნქციის მნიშვნელოვან გაუარესებასთან. შესაბამისად, პაციენტები ნონ-დიპერ ცირკადული პროფილით უნდა განიხილებოდნენ როგორც მაღალი რისკის ჯგუფი, რომელიც საჭიროებს დაკვირვებას კარდიოვასკულური და ცერებროვასკულური გართულებების თავიდან აცილების მიზნით.

РЕЗИСТЕНТНОСТЬ КАПИЛЛЯРОВ И НЕКОТОРЫЕ ПОКАЗАТЕЛИ СИСТЕМЫ ГЕМОСТАЗА У БОЛЬНЫХ РОЗАЦЕА

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Розацеа (acne rosacea, gutta rosacea, teleangiectasiasis faciei, cuperose) - хроническое, рецидивирующее заболевание кожи лица, имеющее полиэтиологическую природу и характеризующееся стадийным течением. Распространенность розацеа составляет 10% среди всего населения земли [11]. По данным косметологов, эта цифра значительно выше - 20,6% [1,2]. Согласно данным National Rosacea Society [2], около 16 млн. американцев страдают розацеа. Распространенность заболевания имеет прямую корреляцию с его ранней диагностикой [13]. В странах с низким уровнем медицинского обеспечения вероятность достоверности данных о процентном соотношении распространенности заболевания низкая, что связано с поздней диагностикой или ее отсутствием. В странах со средним и высоким качеством оказания медицинской помощи чаще всего пациенты обращаются к врачу при наличии папуло-пустулезной и более тяжелых стадий розацеа, поэтому, с учетом эритематозно-телеангиэктатической стадии, распространенность розацеа значительно выше в популяции. Что касается пола, то данные по этому показателю неоднозначны. Известно, что чаще заболевание встречается у женщин. Однако тяжелые стадии, такие как ринофима, характерны в большей степени для мужчин. Возможно, это связано с более высокой обращаемостью женщин к врачам, так как заболевание отражается на внешнем виде пациента [12]. На основании данных исследований, проведенных с участием 50235 пациентов с розацеа, установлено, что мужчины и женщины страдают этим заболеванием в одинаковой степени [14]. Клинически розацеа проявляется первично возникающей гиперемией лица, отечными папулами, пустулами, телеангиэктазиями, а также гиперплазией сальных желез и соединительной ткани. Этиология розацеа до конца не изучена. В течение нескольких десятилетий некоторыми авторами предлагаются гипотезы, касающиеся этиопатогенетического аспекта данного заболевания. Большинство исследователей существенную роль в патогенезе розацеа отводят сосудистым нарушениям [3,4]. Патология капилляров кожи может быть связана с несколькими факторами, которые объединяет один результат – стойкое расширение сосудов кожи и в последующем стаз крови [6,15], что клинически проявляется эритемой и телеангиэктазиями [7,8]. Последние данные, касающиеся состояния свертывающей системы у больных розацеа свидетельствуют о наличии сдвига функциональной активности системы гемостаза в сторону гиперкоагуляции [10]. Полагают, подобное отклонение гемостаза вызвано гиперпродукцией компонентов каликреин-кининовой системы. С другой стороны, имеются работы [9,13] о риске развития сердечно-сосудистых заболеваний у пациентов с розацеа. В тоже время использование простого манжеточного теста, выполнение которого возможно вне лаборатории, достаточно информативно для диагностики тромбоцитарно-сосудистых нарушений [5].

Целью исследования явилось изучение взаимосвязи ломкости капилляров и некоторых показателей системы гемостаза и липидного спектра у больных розацеа.

Материал и методы. В исследование были включены 50 больных розацеа (30 женщин и 20 мужчин) в возрасте 35-65 лет. Длительность заболевания - 1,5-14 лет. У большинства больных – 30 (60%) давность заболевания составила от 6 до 9 лет. Неоднократное лечение с назначением полноценных комплексных схем лечения, включая краткосрочные курсы антибиотиков или антибактериальных, сосудистых препаратов, антигистаминных, десенсибилизирующих средств и витаминов, не сопровождалось существенным улучшением. Проводилась беседа для определения возможных триггерных факторов. Все пациенты получали консультации смежных специалистов (гастроентеролог, эндокринолог, гинеколог, офтальмолог). Контрольную группу составили 50 практически здоровых лиц, гомогенных с группой сравнения по полу и возрасту. Для определения резистентности капилляров была использована манжеточная (турникетная) проба Румпеля – Лееде, которая информативна для диагностики тромбоцитарно-сосудистых нарушений. Подсчитывалось количество петехий на ограниченном участке кожи ладонной поверхности предплечья, образующихся при дозированном повышении венозного давления. На коже верхней части ладонной поверхности предплечья очерчивали круг диаметром в 5 см. Накладывали на плечо этой же руки манжету сфингометра и в течение 5 мин. поддерживали в ней давление 90 мм рт. ст. Снимали манжету и 5 мин. спустя после восстановления кровообращения в руке подсчитывали число петехий в очерченном круге. Обращали также внимание на размеры кровоизлияний. Нормальной величиной считали число петехий не более 10, диаметром не более 1 мм. При выраженных тромбоцитопениях, тромбоцитопатиях и ангиопатиях количество петехий на той же площади достигает 20 и более, нередко диагностируются кровоизлияния диаметром более 1 мм. При проведении исследований больные розацеа с учетом возраста были разделены на 2 группы: I группа - больные в возрасте от 35 до 45 лет, II группа - больные в возрасте от 45 до 65 лет.

Таблица 1. Некоторые показатели состояния системы гемостаза и манжеточной пробы у больных розацеа в различных возрастных группах

Показатели системы гемостаза	I группа (n=25)	II группа (n=25)	Контрольная группа (n=50)
протромбиновое время, сек.	16,5±4,1	18, 5±4,7*	15,4±3,1
тромбиновое время, сек.	21,5±5,2	24,5±5,1*	19,4±4,8
фибриноген (mg/dl)	280±26*	295±28*	262±22
протромбиновый индекс, %	105-110	115	70-100
манжеточная проба	слабо положительна	положительна	отрицательна

** - p<0,05 - достоверность различий относительно здоровых лиц*

Таблица 2. Сравнительная характеристика исследуемых показателей липидного обмена у больных розацеа и здоровых лиц

Показатели	I группа (n=25)	II группа (n=25)	Контрольная группа (n=50)
ХС холестерин mg/dl	270,5±21,6	290±23,5*	190,6±10,4
ЛПНП, mg/dl	170±15,7	190±18,7*	135,±12,4
ЛПВП, mg/dl	28±4,5*	20±3,5*	45,7±8,9
триглицериды, mg/dl	219±21,5*	250± 24,4*	170,5±11,5

** - p<0,05 - достоверность различий относительно здоровых лиц*

Состояние системы гемостаза оценивали по показателям протромбинового, тромбинового времени. Определяли содержание фибриногена, фибринолитической активности крови, а также протромбиновый индекс. Липидный спектр сыворотки изучали по следующим показателям: общий холестерин, липопротеиды высокой плотности (ЛПВП), липопротеиды низкой плотности (ЛПНП), триглицериды (изучение липидного спектра проводилось на аппарате фирмы « РОШ» INTEGRAM + 400).

Статистическая обработка данных проведена посредством пакета статистических программ Statistic For Windows с вычислением средней арифметической и ее стандартной ошибки (M±m). Различие между сравниваемыми группами считали достоверными при p<0,05.

Результаты и их обсуждение. Результаты манжеточной пробы и некоторые показатели системы гемостаза у больных розацеа представлены в таблице 1.

Из данных таблицы 1 следует, что у больных розацеа четко наблюдается тенденция изменения состояния свертывающей системы крови с проявлением гиперкоагуляции, что более выражено у больных в возрасте от 45 до 65 лет. В целях определения воздействия изменения состояния свертывающей системы на резистентность (ломкость) капилляров у больных розацеа изучено распределение показателей системы гемостаза в зависимости от результатов манжеточной пробы. Результаты исследования у 25 из 50 больных группы наблюдения выявили положительную манжеточную пробу, тогда как в контрольной группе она оказалась слабо положительной лишь в 2 случаях. Достоверной корреляции с показателями липидного обмена не выявлено.

Представленные в таблице 2 данные свидетельствуют об увеличении концентрации общего холестерина в крови у больных розацеа, причем в группе больных в возрасте от 45 до 65 лет изменения более выражены. Аналогичная тенденция прослеживается и в других показателях липидного обмена (увеличение содержания ЛПНП и триглицеридов). На фоне высоких концентраций общего холестерина, триглицеридов, ЛПНП у больных обеих групп имеет место снижение содержания ЛПВП в сыворотке крови. Полученные в результате исследования данные показывают, что липидные нарушения, выявленные у больных розацеа (особенно в возрастной группе от 45 до 65 лет), представлены всеми компонентами наиболее частого варианта атерогенной дислипидемии - «липидной триады»; гипертриглициридемией, низким уровнем ЛПВП и повышенной фракцией липопротеидов низкой плотности. Проведенные исследования подтверждают мнение ученых о возможном риске развития сердечно-сосудистой патологии больных розацеа.

Таким образом, результаты проведенного исследования позволяют заключить, что явления гиперкоагуляции у больных розацеа, с одной стороны, свидетельствуют о протекающих процессах микросвертывания, а с другой - связь с результатами манжеточной пробы может быть использована для прогнозирования тяжести течения дерматоза и возможного риска развития сердечно сосудистой патологии.

ЛИТЕРАТУРА

1. Борисевич И.В. Маркетинговое изучение рынка косметологических услуг в условиях крупного города. Автореф. дисс... канд. мед. наук. СПб: 2006; 157.
2. Интернет-ресурс: http// rosacea.org/ index. php
3. Ключарева С.В., Дубровина А.А. Нарушение микродинамики кожи лица у больных розацеа. Экспериментальная и клиническая дерматокосметология 2011: 5; 6-9.
4. Самоделкина К.А., Короткий Н.Г., Маяцкая Т.В. Современные концепции этиологии и патогенеза розацеа. Клиническая дерматология и венерология 2012; 3: 4-9.
5. Borchgrevink C.F. In: Thrombosis and bleeding disorders. Theory and methods. – Stuttgard: Georg Thieme Verlag. 1971; 429.
6. Crawford G.H., Pelle M.T., James W.D. Rosacea: Etiology, pathogenesis and subtype classification. J Am Acad Dermatol. 2004; 51: 327-341.
7. Del Rosso JQ, Callo RI, Kircik L, Thiboutot D, Baldwin HE, Cohen D. Why is rosacea considered to be an inflammatory disorder? J Drugs Dermatol. 2012; 11,694-700.
8. Diamantis S., Waldorf H.A. Rosacea: clinical presentation and pathophisiology. J Drugs Dermatol. 2006; 5(1): 8-12.
9. Duman N., Ersoy Evans S., Atakan N. Rosacea and cardiovascular risk factors : a case control study. JEADV 2014; 9: 1165-1169.
10. Fimmel S, Abdel-Naser MB, Kutzner H, Kligman AM, Zouboulis CCNew aspects of the pathoge – nesis of rosacea. Drug Discov Today Dis Mech 2008; 5: 103 -111.
11. Gupta A.K, Chaudhry M.M. Rosacea and its managment an overview J Eur Acad Dermatol. Venereol. 2005; 19: 273 -285.
12. Khaled A., Hammami H., Zeglaoi F et al. Rosacea. 244 Tunisian cases. Tunis Med. 2010; 8(88): 597-601.
13. Kimball AB, Wu Y Cardiovascular disease and classic cardiavascular risk factors in patient with psoriasis Int J Dermatol. 2009; 48: 1147-1156.
14. Kyriakis K.P., Palamaras I., Terzoudi S et all. Epidemiologic aspects of rosacea. J Am Acad Dermatol. 2005; 53: 918 -919.
15. Laquer V., Hoang V., Nguyen A. et al. Angiogenesis in cutaneous diseases: part II. J Am Acad Dermatol. 2009; 61(6): 945-58.

SUMMARY

CAPILLARY FRAGILITY AND SOME HEMOSTATIC PARAMETERS IN PATIENTS WITH ROSACEA

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The aim of the study was to investigate the association between capillary fragility and some hemostatic parameters, lipid profile in patients with rosacea. 50 patients (30 women and 20 men) aged 35 to 65 years were under observation. Control group consisted of 50 healthy persons, adequate to comparison group by sex and age.

To determine the resistance of the capillary, Rumpel-Leede cuff (tourniquet test) was used which consists in determining the formation of petechial hemorrhages on the skin in the area of short-term increase in venous pressure. The hemostatic system was evaluated in terms of prothrombin and thrombin time. Content of fibrinogen and fibrinolytic activity of blood were determined also. The serum lipid profile was studied by means of the following parameters: total cholesterol, triglycerides, HDL (high density lipoprotein), LDL (low density lipoproteins).

The survey revealed that in 25 patients the arm cuff test was positive, whereas in the control group, only 2 cases it was weakly positive.

Manifestations of hypercoagulation were found in half of patients with a positive cuff test, almost in half of the patients an increased level of fibrinogen and the reduced fibrinolytic activity in blood serum has been revealed. Significant correlation with lipid metabolism have not been identified. Phenomenon of hypercoagulation in rosacea patients on the one hand suggests the existence of processes of microcoagulation, on the other hand the connection with the results of a cuff test can be used to predict the severity of the dermatosis and the possible risk for developing of cardiovascular disease.

Keywords: rosacea, capillary fragility, hemostatic parameters.

РЕЗЮМЕ

РЕЗИСТЕНТНОСТЬ КАПИЛЛЯРОВ И НЕКОТОРЫЕ ПОКАЗАТЕЛИ СИСТЕМЫ ГЕМОСТАЗА У БОЛЬНЫХ РОЗАЦЕА

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Последние данные, касающиеся состояния свертывающей системы крови у больных розацеа, свидетельствуют о наличии сдвига функциональной активности системы в сторону гиперкоагуляции. Полагают, что подобное отклонение гемостаза вызвано гиперпродукцией компонентов каликреин-кининовой системы. С другой стороны, появились работы о риске развития сердечно-сосудистых заболеваний у пациентов с розацеа. В тоже время использование простого манжеточного теста, выполнение которого возможно вне

лаборатории, весьма информативно для диагностики тромбоцитарно-сосудистых нарушений.

Целью исследования явилось изучение взаимосвязи ломкости капилляров и некоторых показателей системы гемостаза и липидного спектра у больных розацеа.

Наблюдались 50 больных (30 мужчин и 20 женщин) в возрасте 35-65 лет. Контрольную группу составили 50 практически здоровых лиц, гомогенных с группой контроля по полу и возрасту. Для определения резистентности капилляров использована манжеточная (турникетная) проба Румпеля - Лееде, заключающаяся в определении точечных кровоизлияний на коже в области кратковременного повышения венозного давления. Состояние системы гемостаза оценивали по показателям протромбинового, тромбинового времени, содержанию фибриногена, фибринолитической активности. Липидный спектр оценивали по следующим показателям: общий холестерин, триглицериды, липопротеиды высокой плотности, липопротеиды низкой плотности.

Результаты исследования выявили положительную манжеточную пробу у 25 больных группы наблюдения, тогда как в контрольной группе она оказалась слабо положительной лишь в 2 случаях.

Проявления гиперкоагуляции обнаружены у 25 пациентов с положительной манжеточной пробой; почти у половины больных отмечен повышенный уровень фибриногена и снижение фибринолитической активности сыворотки крови. Достоверной корреляции с показателями липидного обмена не выявлено. Явления гиперкоагуляции у больных розацеа с одной стороны свидетельствуют о протекающих процессах микросвертывания, с другой стороны - связь с результатами манжеточной пробы может быть использована для прогнозирования тяжести течения дерматоза и возможного риска развития сердечно-сосудистой патологии.

რეზიუმე

კაპილარების რეზისტენტობა და ჰემოსტაზის სისტემის ზოგიერთი მაჩვენებელი როზაცეათი დაავადებულებში

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კვლევის მიზანს წარმოადგენდა როზაცეათი ავადმყოფებში კაპილარების მსხვრევადობის კავშირის შესწავლა ჰემოსტაზის სისტემის და ლიპიდური სპექტრის ზოგიერთ მაჩვენებელთან.

გამოკვლეულია სხვადასხვა ასაკობრივი ჯგუფის 50 როზაცეათი ავადმყოფი და ასაკით ჰომოგენური 50 პრაქტიკულად ჯანმრთელი პირი (საკონტროლო ჯგუფი). კაპილარების რეზისტენტობის განსაზღვრა ხდებოდა მანჟეტის სინჯით, ჰემოსტაზის სისტემის შეფასება პროთრომბინის, თრომბინის დროთი, პროთრომბინის ინდექსით და ფიბრინოგენის კონცენტრაციით; ლიპიდური სპექტრი შესწავლილია ტრიგლიცერიდების, დაბალი და მაღალი სიმკვრივის ლიპოპროტეიდების, ზოგადი ქოლესტერინის განსაზღვრით. კვლევის შედეგად დადგინდა,რომ საკვლევ ჯგუფში მანჟეტის სინჯი დადებითი იყო 50 პაციენტიდან 35-ში, საკონტროლო ჯგუფში კი მხოლოდ 2 შემთხვევაში იყო სუსტად დადებითი. დადებითი მანჟეტის სინჯით პაციენტთა 50% აღენიშნებოდა ჰიპერკოაგულაციის ნიშნები, კავშირი ლიპიდური სპექტრის დარღვევებთან სარწმუნო არ იყო. ჰიპერკოაგულაციის ნიშნები ადასტურებენ პაციენტების ორგანიზმში მიმდინარე მიკროშედედების პროცესებს. კავშირი მანჟეტის ტესტთან შეიძლება გამოყენებული იყოს დერმატოზის მიმდინარეობის სიმძიმის და გულ-სისხლძარღვთა პათოლოგიის შესაძლო განვითარების პროგნოზირების მიზნით.

EVALUATION OF BLOOD REDOX-BALANCE, NITRIC OXIDE CONTENT AND CCR6 RS3093024 IN THE GENETIC SUSCEPTIBILITY DURING PSORIASIS

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Psoriasis is a common chronic inflammatory disease believed to be caused by a combination of genetic, immunologic and environmental factors. The pathogenesis is mainly related to the persistent activation of T lymphocytes with a pattern of TH-1 and TH-17 cytokine secretion, which leads to the production of high local and systemic levels of interleukin IL-1, IL-6, IL-17, IL-22, IL-23, IFN-γ, TNF-α [10] and oxigen reactive species (ROS) [1]. The redox system, an antioxidant defence mechanism, participates in the regulation of physiological or pathological processes with key role, regulating the reactive oxygen and nitrogen species (ROS, NOS) content. Excess ROS production and activation of inducible NO-sinthase (iNOS) is one major consequence of activated T-cells.

Mature myeloid dendritic cells (DCs) highly infiltrate lesional skin mimicking together with T-cells an ectopic lymphoid structure. Psoriatic skin keratinocytes are themselves able to produce several cytokines, ensuring a persistent interaction with T lymphocytes and chronic inflammation [4,13].

Chemokines receptors and their corresponding chemokine ligands play important roles in T-cell mediated inflammatory diseases as shown by recent evidences providing that CCR6 may play a critical role in the pathogenesis of psoriasis [6].

Psoriatic plaques express high levels of epithelial chemokine 20 (CCL20) and its receptor 6 (CCR6), leading to wide local inflammation. Th17 and DCs both share CCR6, while psoriatic keratinocytes and endothelial cells produce CCL20.In addition to CCL20, human b-defensin 2 (hBD2) can also elicit chemotaxis through CCR6 [12].

It was documented that ROS and NOS signaling pathways via the c-Jun transcription factor, after its deacetylationby SirT1, are involved in T-cell mediated immune responces, formation of chemokine receptor 6 (CCR6) related with intensification of cellular infiltration in the psoriatic plaques. Involvement of CCR6 was found to be essential in activating T-helper 17 cells abd this has been linked to the development of different diseases. Further more inhibiting CCR6 formation resulted in suppressed cellular infiltration with concomitant decrease in oxidative stress [8].

Earlier reports suggest that even before the formation of characteristic psoriatic lesions, fibroblasts in the lesion-free skin of psoriasis patients show signs of increased oxidative damage which may be involved in the abnormal immune reactions leading to the onset of the disease [2,3].

The aim of our study was to evaluate whether this polymorphism of *CCR6* gene and oxidative stress are associated with psoriasis risk in Caucasian population.

Materials and methods. The association of the CCR6 polymorphism in the genetic susceptibility of psoriasis was performed at the Department of Dermatology and Venereology, Policlinico Umberto I of Rome (Italy). 516 participants were enrolled including 127 patients (75 men and 52women) affected with psoriasis and 389 healthy controls (197 men and 192 women). All cases and controls were Caucasians and > 18 years old at the enrolment in the study. Cases and controls were genotyped, using a commercially available assay (Life Technologies, Carlsbad, California, USA) for CCR6 rs3093024 polymorphism. To verify the relations between genotypes and psoriasis risk we evaluated genotype frequencies for each individual DNA polymorphism in both case and control series. Deviations from Hardy-Weinberg equilibrium in controls were assessed using Pearson's chi-square test with one degree of freedom. The association between psoriasis risk and *CCR6* rs3093024 was estimated using unconditional logistic regression after adjustment for age and gender. Then, the analysis was performed with three logistic regression models based on a co-dominant, dominant and recessive inheritance effects. Associations between the polymorphism and clinical features were assessed by using the Chi-square and ANOVA statistics.

II. The possible role of ROS in pathogenesis of psoriasis was carried out in the Department of Dermatology and Venereology in Tbilisi State Medical University. A total of 187 individuals were included in this study, out of these 84 were affected by psoriasis and 103 were healthy controls. Blood redox-status - free nitric oxide (NO) and lipoperoxide ($LOO^{\cdot}$) free radicals were investigated by Electron Paramagnetic Resonance (EPR) Spectroscopic method. For detection of NO and $LOO^{\cdot}$ free radicals in blood the spin–traps diethyldithiocarbamate (DETC) and α-phenyltertbutylnitrone (PBN) (Sigma) were used. Blood antioxidant system (Catalase (CAT) and superoxide dismutase (SOD)) activity was determined by spectrophotometric method.

All values were expressed as Mean ± SD. The results obtained were analyzed statistically using the unpaired student's "t" test, to evaluate the significance of differences between the mean values. P values 0.05 were considered as significant.

Results and their discussion. All DNA samples from the 127 cases and 389 controls were genotyped successfully. Genotypes distribution was consistent with Hardy-Weinberg equilibrium among controls.

Genotype frequencies and estimates for the association between *CCR6* polymorphism and psoriasis occurrence are shown in Table 1. There were no differences in the genotype frequencies of the polymorphism between psoriasis cases and healthy controls. When patients with arthropathic psoriasis were excluded from the analysis (Table 2), logistic regression showed that allele A was likely to reduce the risk of developing psoriasis in a dominant model (OR 0.58; 95% C.I. 0.35-0.95; p= 0.031).

Distribution of genotype frequencies for the *CCR6* polymorphism in the patients' series, stratified according to clinical characteristics, including age at onset gender, family history of psoriasis, type of psoriasis, severity, BMI, Smoking history and alcohol consumption was evaluated. As shown in Table 6, none of these clinical features was associated with the genotype frequencies of the tested *CCR6* polymorphism.

Table 1. Distribution of 127 psoriatic patients and 389 controls according to the genotype frequencies and the psoriasis risk estimates (adjusted for age and gender) for CCR6 rs3093024 polymorphism

Genotype/Allele	Cases (N=127)	Controls (N=389)	Odds Ratio	95% C.I.	P-Value
	N (%)	N (%)			
GG	43 (33.9)	106 (27.2)	ref		
GA	54 (42.5)	178 (45.8)	0.67	(0.41-1.08)	0.103
AA	30 (23.6)	105 (27)	0.80	(0.61-1.06)	0.125
Codominant			0.79	(0.60-1.06)	0.123
Dominant (GGvsAG+AA)			0.64	(0.41-1.01)	*0.054*
Recessive (GG+AGvsAA)			0.86	(0.53-1.39)	0.539

Table 2. Distribution of 90 patients with non arthropatic psoriasis and 389 controls according to the genotype frequencies and the psoriasis risk estimates (adjusted for age and gender) for CCR6 rs3093024 polymorphism

Genotype/Allele	Cases (N=90)	Controls (N=389)	Odds Ratio	95% C.I.	P-Value
	N (%)	N (%)			
GG	33 (36.7)	106 (27.2)	Ref		
GA	36 (40)	178 (45.8)	0.60	(0.35-1.03)	0.062
AA	21 (23.3)	105 (27)	0.59	(0.31-1.11)	0.103
Codominant			0.76	(0.55-1.05)	0.091
Dominant (GGvsAG+AA)			**0.58**	**(0.35-0.95)**	**0.031**
Recessive (GG+AGvsAA)			0.84	(0.48-1.45)	0.527

Table 3. Distribution of 37 patients with arthropatic psoriasis and 389 controls according to the genotype frequencies and the psoriasis risk estimates (adjusted for age and gender) for CCR6 rs3093024 polymorphism

Genotype/Allele	Cases (N=37)	Controls (N=389)	Odds Ratio	95% C.I.	P-Value
	N (%)	N (%)			
GG	10 (27)	106 (27.2)	Ref		
GA	18 (48.6)	178 (45.8)	0.94	(0.39-2.24)	0.890
AA	9 (24.4)	105 (27)	0.95	(0.35-2.61)	0.928
Codominant			0.99	(0.60-1.63)	0.961
Dominant (GGvsAG+AA)			0.91	(0.41-2.05)	0.829
Recessive (GG+AGvsAA)			1.06	(0.47-2.41)	0.890

Table 4. Distribution of 75 male psoriatic patients and 197 male controls according to the genotype frequencies and the psoriasis risk estimates (age adjusted) for CCR6 rs3093024 polymorphism

Genotype/Allele	Cases (N=75)	Controls (N=197)	Odds Ratio	95% C.I.	P-Value
	N (%)	N (%)			
GG	23 (30.7)	49 (24.9)	ref		
GA	31 (41.3)	98 (49.7)	**0.43**	**(0.21-0.91)**	**0.026**
AA	21 (28)	50 (25.4)	0.71	(0.30-1.66)	0.43
Codominant			0.87	(0.57-1.32)	0.51
Dominant (GGvsAG+AA)			0.51	(0.26-1.01)	0.054
Recessive (GG+AGvsAA)			1.33	(0.68-2.61)	0.403

Table 5. Distribution of 52 female psoriatic patients and 192 controls according to the genotype frequencies and the psoriasis risk estimates (age adjusted) for CCR6 rs3093024 polymorphism

Genotype/Allele	Cases (N=52)	Controls (N=192)	Odds Ratio	95% C.I.	P-Value
	N (%)	N (%)			
GG	20 (38.5)	57 (29.7)	ref		
GA	23 (44.2)	80 (41.7)	0.84	(0.42-1.68)	0.617
AA	9 (17.3)	55 (28.6)	0.47	(0.20-1.13)	0.091
Codominant			0.70	(0.46-1.07)	0.098
Dominant (GGvsAG+AA)			0.68	(0.36-1.30)	0.245
Recessive (GG+AGvsAA)			0.52	(0.24-1.15)	0.106

Table 6. Associations between CCR6 rs3093024 genotypes and clinical-pathologic features of psoriasis patients

Characteristic	A/A	A/G	G/G	p-value*
Mean age	35.9 (±14.7)	36.4 (±19.8)	40 (±18.6)	0.54
Male (N=75)	21 (28)	31 (41.3)	23 (30.7)	
Female (N=52)	9 (17.3)	23 (44.2)	20 (38.5)	0.35
Family history: negative (N=93)	21 (22.6)	39 (41.9)	33 (35.5)	
positive (N=34)	9 (26.5)	15 (44.1)	10 (29.4)	0.80
Type of psoriasis: arthropathic (N=37)	9 (24.3)	18 (48.6)	10 (27.1)	
not arthropathic (N=90)	21 (23.3)	36 (40)	33 (36.7)	0.55
Severity: mild (N=30)	7 (23.3)	11 (36.7)	12 (40)	
moderate/severe (N=97)	23 (23.7)	43 (44.3)	31 (32)	0.69
BMI: normal (N=69)	16 (23.2)	27 (39.1)	27 (37.7)	
overweight (N=14)	5 (35.7)	6 (42.9)	3 (21.4)	
obesity (N=35)	6 (17.2)	18 (51.4)	11 (31.4)	0.51
Smoking history: no (N=67)	14 (20.9)	30 (44.8)	23 (34.3)	
yes (N=46)	13 (28.2)	17 (37)	16 (34.8)	
ex smokers (N=10)	3 (30)	4 (40)	3 (30)	0.88
Alcohol consumption: no (N=99)	24 (21.7)	50 (45)	37 (33.3)	
yes (N=12)	5 (41.7)	2 (16.6)	5 (41.7)	0.13

** - for age p-value is from ANOVA test, for other characteristics p-value is from Chi-square test*

In blood samples of patients with psoriasis EPR signals of lipoperoxide (LOO·) free radicals were detected (these signals were not detected in blood samples of healthy volunteers) (Table 7). Activity of blood SOD, involved in O_2^- detoxification, was significantly decreased in psoriatic patients compared to healthy controls, and this might be related to epidermal hyperproliferation induced by excess amount of ROS. One of the antioxidant enzymes capable of reducing hydrogen peroxide is CAT. Activity of catalase was statistically significant increase in psoriatic patient, reflecting a high concentration of peroxide radicals (Table 8).

*Table 7. The lipoperoxide (*LOO·*)EPR signal intensity of the blood of patients with and without psoriasis*

PASI	LOO·
10 >	1,3±0,2*
3-10	0,8±0,1*
3 <	0,4±0,2*
control	0

** - statistically significant difference compared with the control (p<0.01)*

Table 8. Activity of catalase and SOD in blood of patients with and without psoriasis

PASI	Catalase	SOD
10 >	4,4±0,4* **	11,9±2,0***
3-10	2,7±0,3* **	12,8±2,2
3 <	2,1±0,2*	16,8±2,7
control	2,2±0,1	14,2±2.9

** - statistically significant difference compared with the control (p<0.01);*

Impairment of antioxidant system activity leads to the lipid peroxidation chain reactions and accumulation of LOO· It should be noted that alteration of blood redox-balance correlates with the severity of psoriasis.

In blood samples of psoriatic patients decrease of free spin-trapped NO content were detected, that may be explained by biological transformation of NO into other reactive nitrogen species (proxy nitrite or nitrosylated hemoglobin) (Table 9).

Table 9. EPR signal intensity of spin-trapped NO in the blood of patients with and without psoriasis

PASI	NO
10 >	6.8±0.6 P*<0.001 P**<0.001
3-10	10.9±0.2 P*<0.001
3 <	12.3±0.5 P*<0.001
Control	16.0±0.50

** - statistical significant difference in comparison to control;*
*** - statistical significant difference between the groups*

Decrease of free NO content in blood samples, in response to the increase concentration of reactive nitrogen species, correlated with the severity of psoriasis. Selective suppressor effect of NO on Th1 population leads to the prevalence of Th2 activity. Therefore, the alteration of the NO content during skin tissues inflammation plays a key role in the pathogenesis of psoriasis, promoting alterations of the immune response and vasomotor activity of subcutaneous capillaries.

In psoriasis, the initial activation of T-lymphocytes requires three steps: binding, signal 1 and signal 2. Binding occurs between surface adhesion molecules of both T-lymphocytes and antigen presenting cells (APCs), then additional interactions occur at “signal 1” between T-cell receptor (TCR) on T-cell and major histocompatibility complex (MHC) I or II on APC, a process that is followed by additional interactions “signal 2” which, in their absence, T-cells undergo apoptosis. The trafficking of T-cells into the skin involves three steps: rolling, binding and diapedesis. Activated T-cells produce Th-1 cytokines (IL-2, TNF-α, IFN-γ) which are along with chemokines (CXCR3, CCR4, CCR6, CC10) and their ligands (CCL9, CCL17, CCL10, CCL22, CCL20, CCL8, CCL27) and growth factors (VEGF, TGF-α, IGF-1, KGF, NGF, amphiregulin and IL-20) are responsible for the inflammatory infiltrate, vascular changes and the epidermal hyperproliferation present in psoriasis.

ROS are a family of oxygen-based free radicals that contain, or are capable of producing, an unpaired electron. They include $.O_2^-$, ·OH, H_2O_2 and singlet oxygen. In psoriasis, ROS are released from neutrophils, eosinophils and macrophages. They are induced by cytokines such as IL-1, TNF-α, IFN-γ (primary cytokines) and angiotensin II.

Increased ROS in psoriasis may result in increased lipid peroxidation products that is able to impair several biological mechanisms of the human body through its ability to react with molecules such as DNA and proteins. O_2^- can react with nitric oxide (NO) to form peroxynitrite (ONOO-) Nitric oxide is an important physiologic regulator of various intracutaneous processes; reduce of nitric oxide (NO) content may further decrease in cGMP activity and induce inbalanceof cAMP/cGMP ratio, hallmark of psoriatic process [9]. Excess production of peroxynitrite may causes tissue injury in part via the activation of poly(ADP-ribose) polymerase independent of oxidative stress by . regulation of proliferation, cell cycle, and transcription. O_2^- can react with nitric oxide to form peroxynitrite [11].

In our study, patients presented elevated levels of catalase and decrease SOD activity with an inverse relationship between them. Oxidative stress was associated with an increase of lipid peroxidation and accumulation of lipoperoxide (LOO.) free radicals and free NO content.We concluded that psoriasis might be an oxidative stress condition. In our study, oxidative stress was associated with an increase in lipid peroxidation s and free NO content.

Conclusion: Thus, the alterations of redox-balance and NO degradation leads to development impairment of skin perfusion disorder of proliferation and transcription cell cycle, initiation T-cell mediated immune responses, formation of chemokine receptor 6 (CCR6) related with intensification of cellular infiltration in the psoriatic plaques. Further more correction of redox-balance is responsible for inhibiting CCR6 formation resulted in suppressed cellular infiltration with concomitant decrease in oxidative stress. The data reviewed suggest the necessity of evaluation of other blood redox-balance and nitric oxide in psoriasis should with additional investigations to targeting CCR6 rs3093024 in the genetic susceptibility of psoriasis.

REFERENCES

1. Briganti S, Picardo M. Antioxidant activity, lipid peroxidation and skin diseases - what's new? JEADV 2003;17:663-669.
2. Dimon-Gadal S, Gerbaud P, Therond P, Guibourdenche J, Anderson WB, Evain-Brion D, et al. Increased oxidative damage to fibroblasts in skin with and without lesions in psoriasis. J Investig. Dermatol. 2000;114:984–989.
3. Kadam DP, Suryakar AN, Ankush RD, Kadam CY, Kishor H. Role of oxidative stress in various stages of psoriasis. J Clin Biochem. 2010; 25(4): 388–392.
4. Kim T-G. et al. CCL20/CCR6 in the Dermal Clusters of Chronic Psoriasis. Journal of Investigative Dermatology 2014; 134: 1462–1465.
5. Kochi Y, Okada Y et al. A regulatory variant in CCR6 is associated with rheumatoid arthritis susceptibility. Nat Genet. 2010;42(6):515-9.
6. Mabuchi T., Chang T.W. et al. Chemokine receptors in the pathogenesis and therapy of psoriasis. Journal of Dermatological Science 2012; 65; 4–11.
7. Nograles KE, Davidovici B, Krueger JG. New insights in the immunologic basis of psoriasis. SeminCutan Med Surg 2010;29:3–9.
8. Preedy V.R. Diabetes.Oxidative stress and dietary abtioxidants. 2014; 9.
9. Royer E.,Chaintreul J.,Meynadier J, Michel B., Guilhou J.J., de Paulet A. Cyclic AMP and Cyclic GMP Production in Normal and Psoriatic Epidermis. Dermatologica 1982;165:533–5430.
10. Sabat R, Philipp S, Höflich C et al. Immunopathogenesis of psoriasis. Experimental Dermatology 2007; 16: 779-798.
11. Virág L., Szabó É., Bakondi É., Bai P., Gergely P., Hunyadi J., Szabó C. Nitric oxide-peroxynitrite-poly(ADP-ribose) polymerase pathway in the Skin. Experimental Dermatology 2002; 11(3): 189–202.
12. Yang D, Chertox O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, et al. b-Defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 1999;286:525–8.
13. Zaba LC, Cardinale I, Gilleaudeau P et al. Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. J Exp Med 2007; 204:3183-94.

SUMMARY

EVALUATION OF BLOOD REDOX-BALANCE, NITRIC OXIDE CONTENT AND CCR6 RS3093024 IN THE GENETIC SUSCEPTIBILITY DURING PSORIASIS

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The aim of our study was to evaluate whether this polymorphism of *CCR6* gene and oxidative stress are associated with psoriasis risk in Caucasian population.

The association of the CCR6 polymorphism in the genetic susceptibility of psoriasis was performed at the Department of Dermatology and Venereology, Policlinico Umberto I of Rome (Italy). 516 participants were enrolled including 127 patients affected with psoriasis and 389 healthy controls. Cases and controls were genotyped, using a commercially available assay (Life Technologies, Carlsbad, California, USA) for CCR6 rs3093024 polymorphism. To verify the relations between genotypes and psoriasis risk we evaluated genotype frequencies for each individual DNA polymorphism in both case and control series.

There were no differences in the genotype frequencies of the polymorphism between psoriasis cases and healthy controls. When patients with arthropathic psoriasis were excluded from the analysis, logistic regression showed that allele A was likely to reduce the risk of developing psoriasis in a dominant model. Logistic regression showed that male patients harboring the heterozygous genotype GA presented a reduced risk of developing psoriasis, compared with the reference GG genotype. None of the clinical features as age at onset, gender, family history of psoriasis, type of psoriasis, severity, BMI, smoking history or alcohol consumption, were associated with the genotype frequencies of the tested *CCR6* polymorphism.

In blood samples of patients with psoriasis intensive EPR signals of lipoperoxide ($LOO^{\cdot}$) free radicals were detected. Activity of blood SOD was significantly decreased in psoriatic patients compared to healthy controls. Activity of catalase was significantly increased in psoriatic patients, reflecting a high concentration of peroxide radicals.

In blood samples of psoriatic patients decrease of free spin-trapped NO content were detected, that may be explained by biological transformation of NO into other reactive nitrogen species (proxy nitrite or nitrosylated hemoglobin).

Thus, the alterations of redox-balance and NO degradation leads to development of skin perfusion impairments, disorder of proliferation and transcription of cell cycle, initiation of T-cell mediated immune responses, formation of chemokine receptor 6 (CCR6) related with intensification of cellular infiltration in the psoriatic plaques. Furthermore, correction of redox-balance is responsible for inhibiting CCR6 formation resulted in suppressed cellular infiltration with concomitant decrease in oxidative stress. The data reviewed suggest the necessity of evaluation of other blood redox-balance and nitric oxide in psoriasis should with additional investigations to targeting CCR6 rs3093024 in the genetic susceptibility of psoriasis.

Keywords: blood redox-balance, *CCR6* gene polymorphism, oxidative stress, psoriasis risk.

РЕЗЮМЕ

ОЦЕНКА РЕДОКС-БАЛАНСА КРОВИ, СОДЕРЖАНИЯ ОКСИДОВ АЗОТА И CCR6 В ГЕНЕТИЧЕСКОЙ ПРЕДРАСПОЛОЖЕННОСТИ ВО ВРЕМЯ ПСОРИАЗА

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Тбилисский государственный медицинский университет, департамент дерматологии и венерологии, департамент медицинской физики и биофизики, департамент нормальной анатомии человека, Грузия; Римский университет «Сапиенца», департамент дерматологии и венерологии, Италия

Целью исследования явилась оценка связи полиморфизма CCR6 гена и оксидативного стресса с риском развития псориаза в Кавказской популяции.

Ассоциация CCR6 полиморфизма к генетической предрасположенности к псориазу проводилась на кафедре дерматологии и венерологии, в поликлинике Умберто I в Риме (Италия). Исследованы 516 лиц, из них 127 - больные псориазом и 389 - здоровые. Генотипирование проводилось коммерчески доступным анализом (Лайф технологис, Карлсбад, Калифорния, США). С целью выявления наличия связи между генотипами и риском развития псориаза оценивали частоту генотипов для каждого отдельного полиморфизма ДНК. Изучение возможной роли активных форм кислорода в патогенезе псориаза проведено в Тбилисском государственном медицинском университете, департаменте дерматологии и венерологии. В исследование были включены 187 лиц (84 - больные псориазом и 103 - здоровые лица). Редокс-статус крови - свободный оксид азота (NO) и свободные радикалы липопероксидов (LOO.) изучали методом электронного парамагнитного резонанса (ЭПР). Активность антиоксидантной системы крови (каталазы и супероксиддисмутазы) определяли посредством спектрофотометрического метода. В результате проведенных исследований различий в генотипной частоте полиморфизма в случаях псориаза и здоровых лиц не выявлено. Когда из анализа были исключены пациенты с артропатическим псориазом, логистическая регрессия показала, что вероятно аллель А снижает риск развития псориаза. Логистическая регрессия выявила, что у пациентов мужского пола с гетерозиготным генотипом GA риск развития псориаза ниже в сравнении с носителями генотипа GG. Ни один клинический признак не был связан с генотипной частотой CCR6 полиморфизма. В образцах крови больных псориазом обнаружены интенсивные ЭПР сигналы свободных радикалов $LOO^{\cdot}$; активность к SOD значительно понижена; активность каталазы - увеличена, отражая высокую концентрацию перекисных радикалов; обнаружено снижение уровня свободных NO радикалов, что, по всей вероятности, обусловлено биологической трансформацией NO в другие реактивные виды азота (пероксинитрит или нитрозилированный гемоглобин).

Таким образом, изменения редокс-баланса и деградация NO приводят к нарушениям перфузии кожи, пролиферации и транскрипции клеточного цикла, инициации Т-клеточного иммунного ответа и формированию рецептора хемокина 6 (CCR6), который связан с интенсификацией клеточной инфильтрации в псориатических бляшках. Кроме того, коррекция редокс-баланса несет ответственность за ингибирование образования CCR6 в результате подавления клеточной инфильтрации с сопутствующим снижением окислительного стресса. Рассмотренные данные свидетельствуют о необходимости дальнейшей оценки редокс-баланса и оксида азота при псориазе, а также о необходимости проведения дополнительных исследований для изучения роли CCR6 rs3093024 в генетической предрасположенности к псориазу.

რეზიუმე

რედოქს-ბალანსის, ჟანგბადის ოქსიდის შემცველობის და ქემოკინ რეცეპტორ 6-ის გენეტიკური წინასწარგანწყობის შეფასება ფსორიაზის დროს

მ. მათოშვილი, ა. კაციტაძე, თ. სანიკიძე,
დ. თოფურია, ს. დ'ეპირო, ა.ჯ. რიკეტა

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი,დერმატოლოგიისა და ვენეროლოგიის დეპარტამენტი, სამედიცინო ფიზიკისა და ბიოფიზიკის დეპარტამენტი,ადამიანის ნორმალური ანატომიის დეპარტამენტი, საქართველო; რომის უნივერსიტეტი "საპიენცა",დერმატოლოგიისა და ვენეროლოგიის დეპარტამენტი, იტალია

CCR6 პოლიმორფიზმის ასოციაციის შესწავლა ფსორიაზის გენეტიკურ მიდრეკილებასთან შეს-

რულდა კანისა და ვენერიულ სნეულებათა დეპარტამენტში, კლინიკა უმბერტო I, რომი (იტალია). კვლევაში მონაწილეობდა 516 ადამიანი, 127 ფსორიაზის დიაგნოზით, 389 ჯანმრთელი პირი. გენოტიპირება ჩატარდა კომერციულად ხელმისაწვდომი ანალიზით (ლაიფ ტექნოლოჯის, კარლსბადი, კალიფორნია, აშშ). გენოტიპებსა და ფსორიაზის განვითარების რისკს შორის კავშირის დასადგენად ფასდებოდა გენოტიპის სიხშირეები თითოეული დნმ-ის პოლიმორფიზმისათვის. ROS-ის შესაძლო როლის შესწავლა ფსორიაზის პათოგენეზში განხორციელდა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტის კანისა და ვენერიულ სნეულებათა დეპარტამენტში. კვლევაში მონაწილეობდა 187 ადამიანი (84 ფსორიაზის დიაგნოზით, 103 ჯანმრთელი პირი). სისხლის რედოქს-სტატუსი- (NO) და LOO.) - განისაზღვრა ელექტრონული პარამაგნიტური რეზონანსის (ეპრ) მეთოდით. სისხლის ანტიოქსიდანტური სისტემის (CAT და SOD) აქტიურობა განისაზღვრა სპექტროფოტომეტრული მეთოდით.

დადგენილია, რომ რედოქს-ბალანსის ცვლილებები და NO-ს დეგრადაცია იწვევს კანის პერფუზიის სისუსტის განვითარებას, უჯრედების პროლიფერაციის და ტრანსკრიფციის ცვლილებებს,T-ლიმფოციტებით განპირობებული იმუნური პასუხის დაწყებას,ქემოკინ-რეცეპტორ 6-ის (CCR6) ფორმირებას,რაც დაკავშირებულია ფსორიაზულ ბალთებში უჯრედული ინფილტრაციის გააქტიურებასთან. გარდა ამისა, უჯრედული ინფილტრაციის კლებისა და ოქსიდაციური სტრესის დაქვეითების დროს ვითარდება რედოქს-ბალანსის კორექცია,რაც,თავის მხრივ,განაპირობებს CCR6-ის წარმოქმნის ინჰიბირებას. მიღებული შედეგები ცხადყოფს დამატებითი კვლევების ჩატარების აუცილებლობას რედოქს-ბალანსის, აზოტის ოქსიდისა და CCR6-ის როლის ღრმად შესასწავლად ფსორიაზის დროს.

MECHANISMS OF RESISTANCE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TYROSINE KINASE INHIBITORS

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The mechanisms of CML progression were postulated based on in vitro modelling even before they were detected in the clinical practice [15]. CML progression can be driven by different mechanisms on tissue, cell, chromosomal and molecular level. Although, additional mutations can cause CML progression in 50-80% of cases, there are a lot of experimental evidence suggesting, that several other factors can lead to disease progression and therapy resistance in CML, such as quiescence of hematopoietic stem cells (HSCs) [1,7,15,16], evolution of hematopoietic progenitor cells (HPCs), overexpression of Bcr-Abl [12], changes in HSCs microenvironment, auto-secretion of growth factors [6], activation of additional signalling pathways, changes in adhesion properties and changes in level of transmembrane transporters expression in HSCs and HPCs. Those changes can be driven by Bcr-Abl dependent, so as Bcr-Abl independent mechanisms [4,5,13].

It was described previously, that even when targeted CML therapy is used - tyrosine kinase inhibitors (TKI) - disease relapse is observed in 59 % of patients with molecular remission after treatment discontinuation [20]. It was suggested, that such high level of relapses occurs because of persistence of quiescent $CD34^+$ HSCs that are residing in the G_0 phase, can't be eliminated by TKIs (probably due to low predisposition to TKI-triggered apoptosis) and are not susceptible endogenous growth factors. The quiescent status of such cells is also identified by $cdc25^-$, $Ki67^-$, $p21^+$ markers [18]. Some data also suggest that such quiescence may be driven by BCR-ABL independent mechanisms [11], such as alterations in HSCs microenvironment, HSCs adhesion properties and level of Pgp expression [14].

Recent in vitro studies of CML bone marrow cells also suggest that disease progression can be caused by functional changes in pool of more matured hematopoietic progenitor cells rather than in population of HSCs [6]. It was shown that HPCs, which possess normally do not have self-renewal capacities, can acquire stem cell properties during disease progression [3]. Such evolution of HPCs during CML progression can be identified by changes in their clonogenic potential during cultivation in semi-solid agar.

The aim of the study was to identify specific features of functional activity, proliferation rates and differentiation potential of CML hematopoietic progenitor cells of patients treated with tyrosine kinase inhibitors (TKI) by identifying number of Ki-67, Bcl-2 and CD34 positive cells in bone marrow, as well as in vitro colony-forming unit assay in patients with different response to the TKI therapy.

Materials and methods. *Samples:* Totally 51 samples of bone marrow were analyzed from patients with CML in chronic phase, who were treated with TKI, Imatinib. An informed consent was obtained before analysis. All samples were devised as to the response to TKI treatment to groups with optimal (26 patients), suboptimal (8 patients) response and treatment failure (26 patients) to TKI treatment, according to European LeukemiaNet recommendations for the management of chronic myeloid leukemia [2]. Bone marrow samples of 13 more patients were analyzed before they have started TKI treatment.

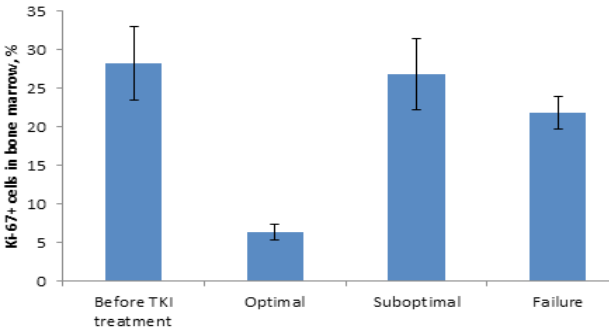
Surface markers expression: Apart of cytogenetic analysis, bone marrow cells were analysed for expression of biomarkers: CD34, CD95, Bcl-2, Pgp-170, Ki67 and co-expression of some of them. To assess the phenotypic characteristics of hematopoietic cells of patients the method of direct cytometry was used. For this purpose, bone marrow mononuclear cells were stained with monoclonal antibodies (Becton Dickinson, USA) using the method provided by the manufacturer. Cytometry study was performed using a flow cytometer laser FACScan (Becton Dickinson, USA) with an argon laser which has a wavelength of 488 nm. Data collection was performed by flow cytometry using the LYSYS-II Ver. 1.1 (Becton Dickinson) software. The program WinMDI 2.8 (Joseph Trotter, Scripps Institute, La Jolla, CA) was used to analyze the results.

CFU-assay: Mononuclear fraction was separated from bone marrow aspirates by centrifugation over Ficoll-Hypaque gradient (density, 1.077 g/ml). Median viability of the cells after separation was 92 % as indicated by trypan blue exclusion. Mononuclear cells in final concentration of $2x10^5$ cells/ml were cultivated for 14 days in full RPMI medium with addition of 0.33 % Bacto agar (Difco laboratories Ltd) and 50 ng/ml GM-CSF (Peprotech, Germany). All results were expressed as the mean number obtained from quadruplicate cultures. Clones of > 40 cells were counted as colony forming units granulocyte monocyte (CFU-GM) and those with 5 - 40 cells as cluster forming units (ClFU). The colony to cluster ratio (CCR) was calculated as number of colonies divided by the number of clusters.

All cells aggregates, formed during cultivation in semisolid agar were individually picked up from the culture and samples were prepared using centrifugation on cytocentrifuge. All samples were stained with Pappenheim method and cell's morphology was analyzed. Index of leukocyte's maturation (MI) was calculated for cells, comprising aggregates after cultivation in semisolid agar as a number of less differentiated myeloid cells (blast cells, promyelocytes, myelocytes), divided to the total number of more differentiated cells (banded and segmented granulocytes).

Colony and cluster numbers, so as CCR index were logarithmically distributed. For each group of data several parameters were calculated: the arithmetic mean value, mean standard error, standard deviation. Comparisons of colony or cluster numbers were made by the Mann-Whitney test. The difference was considered accurate at the level of significance of 5% ($p \leq 0.05$). For correlation analysis was performed by applying Spearman rank correlation coefficient.

Results and their discussion. All samples of bone marrow cells were divided according to response to TKI therapy into 4 groups, as described previously. Our results indicated that there was a significant decline ($p > 0.05$) in proliferation activity of HSCs and HPCs in group of patients with optimal response to the TKI therapy – mean level of Ki-67+ cells of bone marrow for this group of patients was 6.33±1.06%. As to other groups of patients, no difference was found in the number of Ki-67+ cells of patients before TKI treatment, patients with suboptimal response to the TKI therapy, so as for patients with treatment failure - mean values of $Ki67^+$ cells were 28.27±4.82%, and 26.83±4.58 % and 21.89±2.1 % respectively (Fig. 1).



** - p < 0,05 when compared to all other groups*

Fig. 1. Percentage of Ki67+ cells in bone marrow of CML patients with different response to the TKI therapy

There was also significant difference ($p < 0.05$) found when comparing mean numbers of $CD34^+$ cells in group of patients with optimal response to TKI therapy (mean number of CD34+ cells in bone marrow was 8.13±2.52%) with all other groups of patients (Fig. 2). Expression of CD34+ in bone marrow of patients of three other groups was significantly higher. Mean numbers of CD34+ cells in bone marrow of patients before TKI treatment was 35.10±13.2%, for group of patients with suboptimal response to TKI therapy - 37.10±13.80% and for group of patients with treatment failure - 28.27±8.80%.

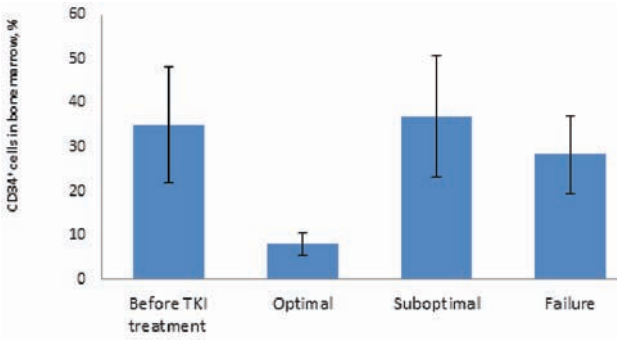
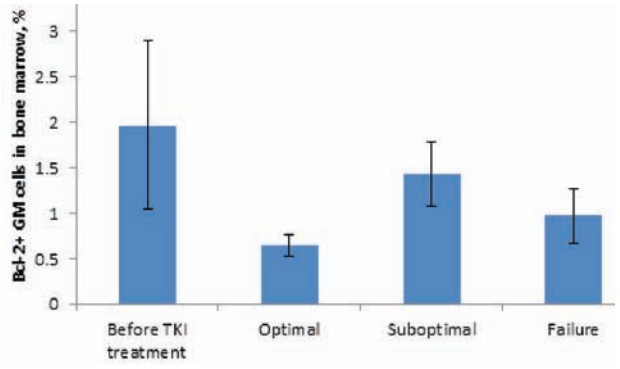


Fig. 2. Percentage of CD34+ cells in bone marrow of CML patients with different response to the TKI therapy

As to the level of apoptosis marker Bcl-2 on granulocyte and monocyte cells in bone marrow of CML patients (Fig. 3), the mean number of Bcl-2$^+$ cells was increased ($p<0.05$) in group of patients before TKI treatment (1.97±0.93%) comparing to level of Bcl-2$^+$ cells in bone marrow of patients with optimal response to TKI treatment (0.65±0.12%), patients with suboptimal response to TKI treatment (1.43±0.35%) and patients with TKI treatment failure (0.97±0.30%).



** - p < 0,05 when compared to all other groups*
Fig. 3. Bcl-2$^+$ granulocyte and monocyte cells in bone marrow of CML patients with different response to the TKI therapy

At the same time, correlation analysis, performed on individual basis for patients independently of response to the TKI therapy demonstrated that there was a negative correlation ($\rho = 0.7648$) between the number of Ki67$^+$ and CD34$^+$ cells (Fig. 4).

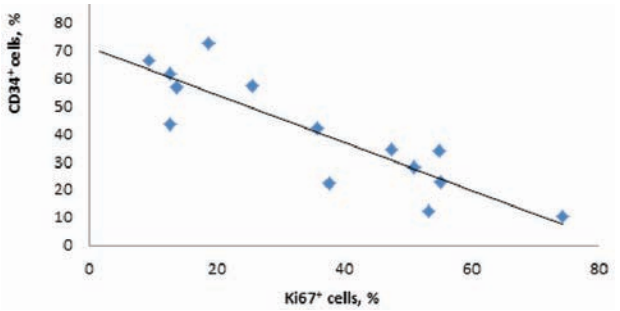
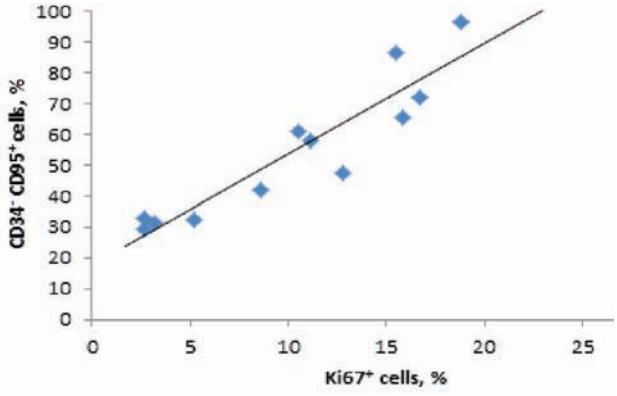
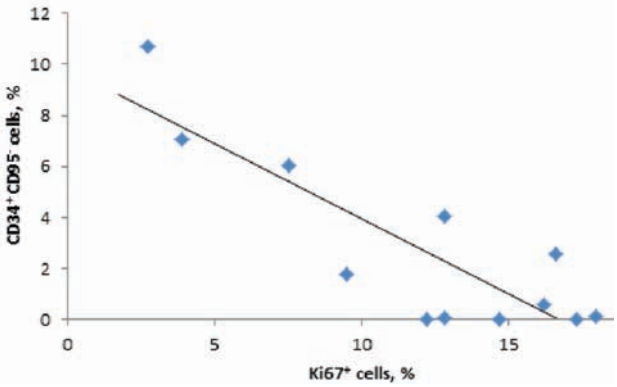


Fig. 4. Correlation between Ki67$^+$ and Cd34$^+$ cells in bone marrow of patients with CML

Furthermore, negative correlation was also estimated for co-distribution of Ki67$^+$ and CD34$^+$CD95$^-$ cells ($\rho=0.8556$; Fig. 5a), but positive correlation was found for Ki67$^+$ and CD34$^-$CD95$^+$ cells ($\rho=0.7363$; Fig. 5b). No correlation was found between percentages of Ki67$^+$ and CD34$^+$CD95$^+$ cells in bone marrow of CML patients ($\rho=0.0424$).



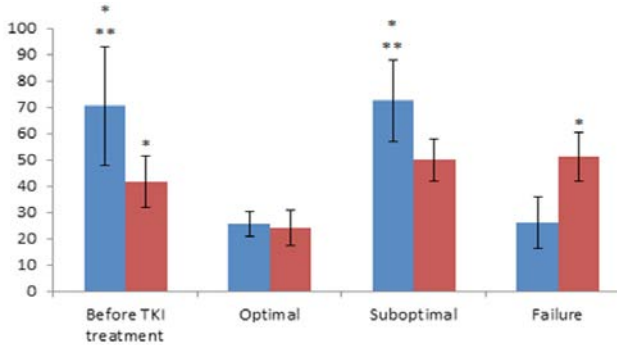
a



b

Fig. 5. Correlation between percentage of bone marrow cells of CML patients expressing different markers:
a) Ki67+ cells and CD34-CD95+ cells;
b) Ki67+ cells and CD34+CD95- cells

CFU assay indicated that there was a significant ($p<0.05$) difference in clonogenic potential (number of CFU-GM and ClFU) between groups of patients with different response to the therapy (Fig. 6). CFU numbers were lower in group of patients with optimal response to the TKI therapy (mean value of 25.54±4.6) and for patients with TKI treatment failure (26.00±9.70) when compared to CFU numbers for groups of patients before TKI treatment (70.40±22.51) and patients with suboptimal response to TKI treatment (72.37±15.32). Significant decline ($p<0.05$) was also indicated for mean numbers of ClFU for group of patients with optimal response for TKI (24.05±6.75) when compared to groups of patients with suboptimal response to TKI (49.94±7.89), but also for group of patients with TKI treatment failure (51.17±9.22). The mean number of ClFU for patients before TKI treatment was 41.80±9.78 and was not significantly different from other groups ($p>0.05$).



** - p<0.05 when compared to group of patients with optimal response to TKI treatment;*
*** - p<0.05 when compared to group of patients with treatment failure*
Fig. 6. Colony forming properties of granulocyte and monocyte bone marrow cells of CML patients with different response to the TKI therapy

As to colony to cluster ratio our results showed, that there is a correlation (ρ=0.6783) between CCR index and number of bone marrow cells with Philadelphia chromosome (Fig. 7).

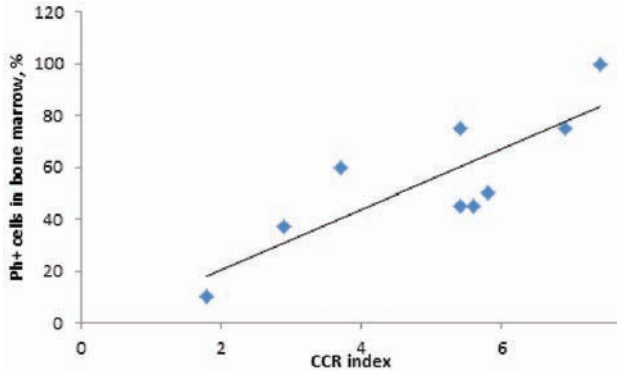
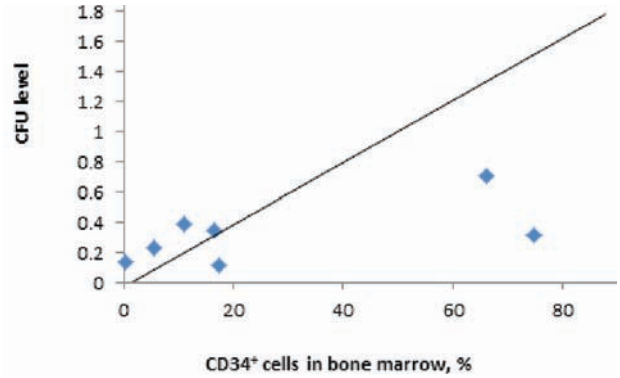
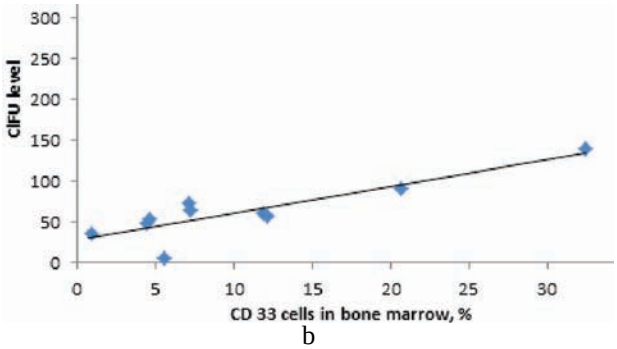


Fig. 7. Correlation between CCR and level bone marrow cells with Philadelphia chromosome

To have a possibility of identification of cells, composing aggregates obtained in CFU assay, a positive correlation was also found between numbers of colonies, formed during cultivation in CFU assay and level of CD34+cells (Fig. 8a) in bone marrow (ρ=0.4658) and between number of clusters and CD33+ cells (Fig. 8b) of bone marrow (ρ=0.7612).



a



b

Fig. 8. Identification of cells composing cell aggregates in CFU assay: a) CFU assay and CD34+ cells in bone marrow; b) ClFU number and percentage of CD33+ cells in bone marrow

To evaluate changes in the differentiation potential of HSCs and HPCs that can be related to pathologic process of CML, we picked-up individual cells aggregates, formed during in vitro CFU assay of bone marrow mononuclear cells of CML patients. Cells, composing those aggregates were analyzed with calculation of maturation index (MI) for each individual sample. It was indicated, that MI correlates with level of bone marrow cells, containing Philadelphia chromosome (Fig. 9).

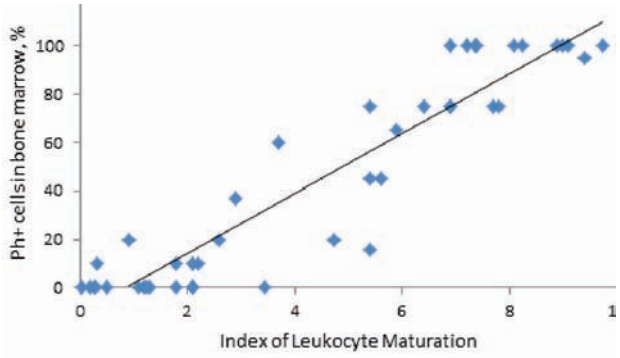


Fig. 9. Correlation between MI and level of Ph+ cells in bone marrow of CML patients with different response to TKI therapy

After additional correlation analysis a positive correlation was determined between MI and percentage of $CD34^+$ cells in bone marrow of CML patients (Fig. 10a). MI also appeared variable with number of Bcl-2 positive myeloid cells in bone marrow (Fig. 10b), so as with number of Pgp-170 positive cells (Fig. 10c). At the same time, there was a negative correlation between MI and number of $Ki67^+$ cells in bone marrow (Fig. 10d).

CML was the first hematological oncological malignancy with addiction to the specific chromosomal aberration was indicated and though the first one for which the targeted therapy was developed. But still the exact mechanisms of CML progression remain contradictory. In vitro culture of primitive CML cells in the presence of imatinib leads to accumulation of quiescent cells. As a phenotypically

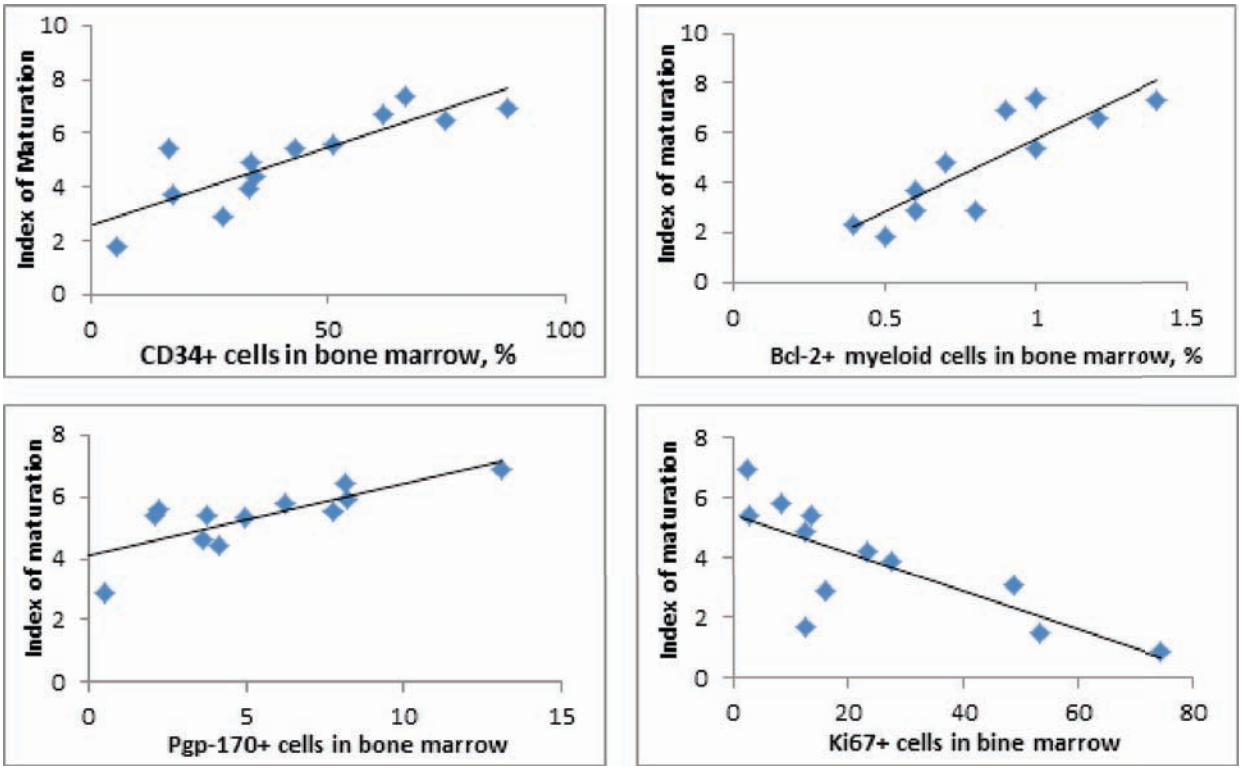


Fig. 10. Correlation between MI and level of a) CD34+ cells; b) Bcl-2+ cells; c) Pgp-170+ cells; d) Ki67+ cells in bone marrow of CML patients with different response to TKI therapy

similar population of cells from newly diagnosed patients can serially engraft immunodeficient mice, these cells may represent persistent leukemia cells in imatinib-treated patients; however, this is still contradictory [9]. It is also unclear whether the property of quiescence directs resistance. Finally, BCR-ABL independent mechanisms and bone marrow microenvironment–derived signals may also protect leukemic stem cells from the effects of imatinib [11]. In our study individual analysis was performed to evaluate potential markers of CML progression based on changes in functional properties of HSCs and HPCs, as in proliferation and differentiation potential and indication of quiescent HSCs.

Overlapping of resistance of quiescent HSCs is one of the biggest challenges for CML therapies. It was postulated previously, that imatinib-treated quiescent CML cells can be indicated by using Ki-67 negativity as a marker of quiescence [22]. It is absent during the G_0 phase - the stage at which neoplastic cells are totally unresponsive to chemotherapy The Ki-67 antigen is also one of the most sensitive markers of undifferentiated cell proliferation [19]. When analyzed the level of Ki-67 expression in monocyte and myelocyte cells of bone marrow of CML patient with different response to the TKI therapy, there was only statistically relevant increase in percentage of such cells for patients with optimal response to the TKI therapy. The same was true for the percentage of CD34+ cells in bone marrow. Anyhow, when correlation analysis was performed, the strong negative correlation was indicated between percentage of CD34+ and Ki-67+ cells in bone marrow, meaning, that with increase of total number of CD34+ cells, higher proportion of them are Ki-67 negative and can be indicated as quiescent. Even more, those cells were CD95 negative at the same time.

CFU assay is one of the standard methods used to identify functional properties of HSCs and HPCs of bone marrow. Our data indicated alteration of clonogenic activity of bone marrow hemopoietic cells for patients with different response to TKI treatment. Thus, CFU and ClFU numbers were increased in case of examination of bone marrow of patients before TKI treatment, so as for patients with suboptimal response to TKI treatment ant TKI treatment failure.

Modification of colony to cluster ratio (CCR), which was also identified in terms of cultivation of bone marrow mononuclear cells in CFU assay, was previously showed to be a prognostic factor for several hematological malignancies [21]. We have indicated that CCR index is interdependent with number of cells in bone marrow, containing Philadelphia chromosome, thus can be used as prognostic marker for CML patients.

Despite of long history of CFU assay usage, there is no clear understanding of phenotype characteristics of cells, composing colonies and clusters in CFU assay. Our data suggest, that colonies, formed in CFU assay after cultiva-

tion of bone marrow cells of patients with CML, may be composed primarily of CD34+ calls. At the same time, colonies are presumably formed mostly from CD33+ cells of bone marrow. This suggestion supports the idea of using CCR index as an individual prognostic factor, which potentially indicates increase of share of low differentiated and potentially quiescent CD34+ cells in bone marrow of CML patient during disease progression.

One more functional property of HSCs and HPCs that can be altered during CML progression is a differentiation potential. To identify changes in differentiation capacity of HSCs and HPCs of bone marrow of CML patients, in our study index of leucocyte maturation was used, defined as ratio between number of less and more differentiated cells of myeloid lineage in cell aggregates formed during cultivation in CFU assay. It was indicated, that MI correlates with percentage of bone marrow cells with Philadelphia chromosome, and thus can be potentially considered as a prognostic factor regarding disease progression. At the same time, positive correlation was also found between MI ratio and percentage of CD34+ cells of bone marrow, suggesting that accumulation of less differentiated cells may be driven by differentiation arrest of quiescent CD34+ HSCs. This observation can also be supported by the fact that negative correlation was found between MI ratio and percentage of Ki-67 positive cells, indicating decrease in proliferation capacity and potential quietness of cells CD34+ with differentiation arrest.

Imatinib affects cells by enhancing their sensitivity to apoptosis through mitochondrial pathway of apoptosis. Thus, increase of level of anti-apoptotic protein Bcl-2 during TKI therapy may indicate persistence of cells that are not susceptible to apoptosis. Such insusceptibility most probably can be driven through BCR-ABL independent pathways [8]. The results of our study have shown that the level of Bcl-2 protein was significantly lower for the group of patients with optimal response to the TKI therapy when comparing to three other groups of patients. At the same time, percentage of Bcl-2 positive cells was positively correlating with MI ratio, suggesting existence of BCR-ABL independent mechanisms involvement in maintenance of CD34+ quiescent cells.

Even more, it was previously shown, that enhanced expression of the drug efflux pump protein P-glycoprotein in CML stem cells may reduce sensitivity to TKI by lowering intracellular drug concentrations [10]. In our study we have also defined positive correlation between MI ratio and percentage of Pgp positive cells. This finding also supports our assumption regarding involvement of both BCR-ABL dependent and independent mechanisms in process of CML progression.

Conclusions. In summary, obtained results suggest that different mechanisms (BCR-ABL dependent and independent) may be involved in CML progression process at the same time. Disease prognosis should be preferably carried out on an individual basis.

REFERENCES

1. Куцев С.И., Оксенко О.С., Кравченко Е.Г. Лекарственный мониторинг хронического миелолейкоза иматинибом. Онкогематология 2010; 3: 1–9.
2. Baccarani M., Deininger M.W., Rosti G. et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia. Blood. 2013; 122 (6): 872-84.
3. Bhatia R., Holtz M., Niu N. et al. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. Blood 2003; 101: 4701-7.
4. Bolton-Gillespie E., Schemionek M., Klein H.U. et al. Genomic instability may originate from imatinib-refractory chronic myeloid leukemia stem cells. Blood 2013; 121 (20): 4175-83.
5. Cortes J.E., Talpaz M., O'Brien S. et al. Staging of chronic myeloid leukemia in the imatinib era: an evaluation of the World Health Organization proposal. Cancer 2006; 106: 1306-15.
6. Diaz-Blanco E., Bruns I., Neumann F. et al. Molecular signature of CD34 (+) hematopoietic stem and progenitor cells of patients with CML in chronic phase. Leukemia 2007; 21: 494-504.
7. Dube I.D., Kalousek D.K., Coulombel L. et al. Cytogenetic studies of early myeloid progenitor compartments in Ph1- positive chronic myeloid leukemia. II. Long-term culture reveals the persistence of Ph1-negative progenitors in treated as well as newly diagnosed patients. Blood 1984; 63 (5): 1172-7.
8. Gambacorti-Passerini C., le Coutre P., Mologni L. et al. Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL+ leukemic cells and induces apoptosis. Blood Cells Mol Dis. 1997; 23: 380-94.
9. Graham S.M. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 2002; 99 (1): 319-25.
10. Hiwase D.K., Saunders V., Hewett D. et al. Dasatinib cellular uptake and efflux in chronic myeloid leukemia cells: therapeutic implications. Clin Cancer Res. 2008; 14: 3881-8.
11. Ichim C.V. Kinase-independent mechanisms of resistance of leukemia stem cells to tyrosine kinase inhibitors. Stem Cells Transl Med. 2014; 3 (4): 405-15.
12. Ito T. Stem cell maintenance and disease progression in chronic myeloid leukemia. Int J Hematol. 2013; 98 (6): 641-7.
13. Jamieson C.H. Chronic Myeloid Leukemia Stem Cells. Hematology Am Soc Hematol Educ Program. 2008: 436-42.
14. Krause D.S., Fulzele K., Catic A., et al. Differential regulation of myeloid leukemias by the bone marrow microenvironment. Nat Med. 2013; 19 (11): 1513-17.
15. Lo Celso C., Wu J.W., Lin C.P. In vivo imaging of hematopoietic stem cells and their microenvironment. J Biophotonics. 2009; 2: 619-31.

16. Lowenberg B. Minimal residual disease in chronic myeloid leukemia. N Engl J Med. 2003; 349: 1399-1401.
17. Lugo T.G., Pendergast A.M., Muller A.J., Witte O.N. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science. 1990; 247: 1079-82.
18. Morotti A., Panuzzo C., Fava C., Saglio G. Kinase-inhibitor-insensitive cancer stem cells in chronic myeloid leukemia. Expert Opin Biol Ther. 2014; 14 (3): 287-99.
19. Nowicki M., Ostalska-Nowicka D., Mis´kowiak B. Prognostic significance of Ki67-negative blast cell clone in the high risk group of children treated for acute myeloid leukaemia. Folia histochemica et cytobiologica 2006; 44 (1): 49-52.
20. Rousselot P., Huguet F., Rea D., et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. Blood 2007; 109: 58 – 60.
21. Schunck H., Schütt M, Langen P. The colony-to-cluster ratio in agar cultures of bone marrow. II. Influence of a GM-CFC inhibiting factor. Biomed Biochim Acta. 1987; 46 (7): 587-93.
22. Tsurusawa M., Ito M., Zha Z., et al. Cell-cycle-associated expression of proliferating cell nuclear antigen and Ki67 reactive antigen of bone marrow blast cells in childhood acute leukemia. Leukemia 1992; 6: 669-74.

SUMMARY

MECHANISMS OF RESISTANCE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TYROSINE KINASE INHIBITORS

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Up to date, two major mechanisms have been proposed as an explanation for myeloid cells expansion in chronic myeloid leukemia (CML). One is a reduced susceptibility of hematopoietic stem or progenitor cells to apoptosis, while the other one is an increased activity within the hematopoietic progenitor cell population.

The aim of the study was to identify specific features of functional activity, proliferation rates and differentiation potential of CML hematopoietic progenitor cells of patients treated with tyrosine kinase inhibitors (TKI) by identifying number of Ki-67, Bcl-2 and CD34 positive cells in bone marrow, as well as in vitro colony-forming unit assay in patients with different response to the TKI therapy.

Our results indicated that there was a significant decline in proliferation activity of HSCs and HPCs in group of patients with optimal response to the TKI therapy. Correlation analysis, performed on individual basis for patients independently of response to the TKI therapy demonstrated that there was a negative correlation (ρ=0.7648) between the number of Ki67+ and CD34+ cells. As to colony to cluster ratio our results showed, that there is a correlation (ρ=0.6783) between CCR index and number of bone marrow cells with Philadelphia chromosome. It was indicated, that index of maturation correlates with level of bone marrow cells, containing Philadelphia chromosome, so as with percentage of CD34+, Bcl-2+, Pgp-170+ and Ki67+ cells in bone marrow of CML patients.

In summary, obtained results suggest that different mechanisms (bcr-abl dependent and independent) may be involved in CML progression process in the same time. Disease prognosis should be preferably carried out on an individual basis

Keywords: chronic myeloid leukemia, bone marrow progenitor cells, functional activity, disease progression in CML.

РЕЗЮМЕ

МЕХАНИЗМЫ РЕЗИСТЕНТНОСТИ ПРИ ЛЕЧЕНИИ ПАЦИЕНТОВ С ХРОНИЧЕСКОЙ МИЕЛОИДНОЙ ЛЕЙКЕМИЕЙ ИНГИБИТОРАМИ ТИРОЗИНКИНАЗЫ

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Для объяснения миелоидной экспансии клеток лейкемического клона при хронической миелоидной лейкемии (ХМЛ) на сегодня предложено два основных механизма: 1) уменьшение восприимчивости гемопоэтических стволовых клеток к апоптозу; 2) повышенная активность популяции непосредственно гемопоэтических клеток-предшественников.

Целью исследования явилось определить особенности морфофункциональной активности гемопоэтических клеток-предшественников, экспрессию Ki-67+, Bcl-2+ и CD34+ клеток в костном мозге и колония-образующую активность in vitro у пациентов при хронической миелоидной лейкемии с различным характером ответа на терапию ингибиторами тирокзинкиназы.

Результаты исследования показали, что существует значительное снижение пролиферативной активности гемопоэтических клеток-предшественников

в группе пациентов с оптимальным ответом в сравнении с субоптимальным и неэффективностью терапии ингибиторами тирозинкиназы (ИТК). Корреляционный анализ, выполненный на индивидуальной основе, независимо от ответа на терапию ИТК, показал, что существует отрицательная корреляция (ρ=0,7648) между количеством Ki67+ и CD34+ клеток. Что касается соотношения колоний и кластеров (пролиферативный потенциал), то результаты исследования свидетельствуют о существовании корреляции между пролиферативным потенциалом и количеством клеток костного мозга с Ph-хромосомой. Кроме того, индекс созревания коррелирует с числом клеток костного мозга, содержащих Ph-хромосому, а также с CD34+, Bcl-2+, Pgp-170 + и Ki67+ клеток в костном мозге у пациентов с ХМЛ.

Таким образом, полученные результаты свидетельствуют о том, что в процесс прогрессирования ХМЛ в одно и то же время могут быть вовлечены различные механизмы (BCR-ABL-зависимые и BCR-ABL-независимые). Прогноз ответа на терапию должен осуществляться на индивидуальной основе.

რეზიუმე

რეზისტენტობის მექანიზმები ქრონიკული მიელოიდური ლეიკემიით დაავადებული პაციენტების მკურნალობისას თიროზინკინაზას ინჰიბიტორებით

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კვლევის მიზანს წარმოადგენდა ჰემოპოეზური წინამორბედი უჯრედების მორფოფუნქციური აქტიურობის თავისებურებების შეფასება ძვლის ტვინში Ki-67+, Bcl-2+ და CD34+ უჯრედების ექსპრესიის განსაზღვრით, და, აგრეთვე, in vitro კოლონიაწარმომქმნელ აქტიურობის განსაზღვრით ქრონიკული მიელოიდური ლეიკემიით დაავადებულ პაციენტებში, რომელთაც ჰქონდათ განსხვავებული პასუხი თიროზინკინაზას ინჰიბიტორებით მკურნალობისას.

კვლევის შედეგად აღმოჩნდა, რომ ჰემოპოეზური წინამორბედი უჯრედების პროლიფერაციული აქტივობა მნიშვნელოვნადაა შემცირებული იმ პაციენტებში, რომელთაც აღენიშნათ ოპტიმალური პასუხი შედარებით თერაპიაზე, განსხვავებით იმ პაციენტებისაგან, რომალთაც აღენიშნებათ სუბოპტიმალური ან არაეფექტური თერაპია თიროზინკინაზას ინჰიბიტორებით. ინდივიდუალურ საფუძველზე თერაპიაზე პასუხის მიუხედავად, ჩატარებულმა კორელაციურმა ანალიზმა აჩვენა, რომ არსებობს უარყოფითი კორელაცია (ρ=0,7648) Ki67+ და CD34+ უჯრედების რაოდენობას შორის. კოლონიების და კლასტერების თანაფარდობის (პროლიფერაციული პოტენციალი) კვლევის შედეგები მოწმობენ, რომ არსებობს კორელაცია პროლიფერაციულ აქტივობასა და ძვლის ტვინის Ph-ქრომოსომას შემცველი უჯრედების რაოდენობას, აგრეთვე, CD34+, Bcl-2+, Pgp-170 + და Ki67+ უჯრედების რაოდენობას შორის.

ამრიგად, მიღებული შედგების საფუძველზე გამოტანილია დასკვნა, რომ ქრონიკული მიელოიდური ლეიკემიის პროგრესირების პროცესში ერთდროულად შეიძლება იყოს ჩართული სხვადასხვა მექანიზმები (BCR-ABL-დამოკიდებული და BCR-ABL-არადამოკიდებული). თერაპიაზე პასუხის პროგნოზი უნდა წარიმართოს ინდივიდუალურ საფუძველზე.

BCL1 POLYMORPHISM OF GLUCOCORTICOIDS RECEPTOR GENE AND BRONCHIAL ASTHMA

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Bcl1 polymorphism of glucocorticoids receptor gene (GR, h-GR/NR3C1) was first described by Murray et al. in 1987 [5]. The investigation of GR polymorphism frequency in different general populations has found that Asians have a high C allele frequency - 32,8%, Caucasians have 28,9%, and South Americans have 15,2% [7]. Bcl1 polymorphism of GR gene is associated with increased body mass index and body fat centralization ratio, dyslipidemia, insulin resistance, cardiovascular and autoimmune diseases, sensitivity to glucocorticosteroids (GCS), endothelial dysfunction and inflammation activity [1,3,6,10,12-14]. As for respiratory diseases, there are some publications on the association between BclI polymorphism and bronchial asthma (BA) [8,9], chronic obstructive pulmonary disease (COPD) [11], and cystic fibrosis [2].

In the opinion of Pietras T. et al. [8], GR gene polymorphism can play an important role in BA development and severity, and can influence response to corticosteroids. The study carried out in Polish population among individuals that have no history of BA and atopy [9], and in patients with BA [8] demonstrated heterogeneous distribution of genotypes among patients with BA and in the general population. Patients with BA were more likely to have G allele of the studied Bcl1 polymorphism. G allele frequency, as compared to C allele, correlated with a high prevalence of BA in the study population. BA developed more frequently in the carriers of allele G, both homozygotes and heterozygotes, as compared to the individuals with C/C genotype. C allele was associated with a lesser risk of BA, since C allele carriers (C/C+C/G) had BA less often than G allele carriers [8]. Thus, this study despite the small size of the study groups confirmed that the substitution of G allele for C allele contributed to the development of BA among the Polish population.

Some scientists conducted research of BclI polymorphism in the GR gene and found out that distribution of alleles and genotypes did not statistically differ between a control group and groups of boys and girls with BA of varying severity [15]. So far in Ukraine, there has been no research on the study of GR gene polymorphisms (h-GR/NR3C1), including Bcl1, in patients with BA.

Aim of our study was to investigate frequencies of alleles and genotypes of Bcl1 GR gene polymorphism and their correlation with prevalence of BA.

Materials and methods. The study has been approved by the Bioethics Committee of Medical Institute of Sumy State University. Prior to the study, all patients provided written informed consent to participate. 188 patients with BA have been examined. BA was diagnosed in accordance with the GINA guidelines. The control group consisted of 95 healthy adult individuals. Bcl1 (rs41423247) polymorphism in exon 2 was determined by means of polymerase chain reaction with subsequent RFLP analysis (restriction fragment length polymorphism) by Fleury I. et al. with modifications [4].

Statistical analysis of the results was performed using SPSS-17 program. To evaluate the influence of polymorphism genotype frequencies, the odds ratio (OR) and 95% confidence interval (CI) were calculated. Values of $p<0,05$ were considered statistically significant.

Results and their discussion. The frequency of the three possible genotypes for Bcl1 polymorphism of GR gene and compliance of main and minor alleles distribution with Hardy-Weinberg equilibrium were identified (Table 1).

Compliance test for Bcl1 polymorphism genotypes distribution and the Hardy-Weinberg equilibrium showed that deviations from the equilibrium were not statistically significant neither in the control group, nor in the main one. It was found out that alleles spreading in both groups didn't significantly differ from the predicted ($p>0,05$).

The control group had the following genotypes frequency for Bcl1 polymorphism of GR gene: C/C, C/G, G/G – 0,421/0,453/0,126, respectively. In patients with BA the frequency of studied genotypes were: 0,228/0,426/0,346, respectively. Thus, the analysis of genotype frequencies for Bcl1 polymorphism of GR gene asserted that there is a statistically significant difference in the distribution of allelic variants of the gene between patients with BA and healthy individuals: homozygous for the minor allele had a higher risk of the disease than the major allele carriers (C/C і C/G) ($\chi^2=19,234$; $p=0,001$).

The distribution of allele frequencies for Bcl1 polymorphism of GR gene in the general population and in patients with BA are shown in Table 2.

Table 1. The frequency of allelic variants and alleles for Bcl1 polymorphism of glucocorticoid receptor gene

Genotype/ allele	Control group, n (%)	Patients with bronchial BA, n (%)
C/C	40 (42,1)	43 (22,8)
C/G	43 (45,3)	80 (42,6)
G/G	12 (12,6)	65 (34,6)
C allele	0,65	0,44
G allele	0,35	0,56
	χ^2=0,01; p>0,05	χ^2=3,53; p>0,05

n – number of patients; χ^2 and p denote deviations from Hardy-Weinberg equilibrium in each group

Table 2. Allele frequencies for Bcl1 polymorphism of GR gene in the general population and in patients with bronchial asthma

Allele		Control group, n=95		Patients with bronchial asthma, n=188	
		n	%	n	%
C	present	83	87,4	123	65,4
	none	12	12,6	65	34,6
χ^2 =15,343; p=0,001					
G	present	55	57,9	145	77,1
	none	40	42,1	43	22,9
χ^2 =11,263; p=0,001					

n – number of patients; p – statistical significance of difference (according to Pearson's chi-squared test (χ^2))

Table 3. Distribution of various genotypes of Bcl1 GR gene polymorphism according to sex in the control group and in patients with bronchial asthma

Genotype	Women (n, %)		Men (n, %)	
	Control group	Bronchial asthma	Control group	Bronchial asthma
C/C	19 (38%)	30 (24,2%)	21 (46,7 %)	13 (20,3 %)
C/G	22 (44%)	50 (40,3%)	21 (46,7 %)	30 (46,9 %)
G/G	9 (18%)	44 (35,5%)	3 (6,7 %)	21 (32,8 %)
Total	50 (100%)	124 (100 %)	45 (100 %)	64 (100 %)
Women	χ^2 =6,1; p_1 =0,047		χ^2 =14,1; p_2 = 0,001	

n – number of patients; p_1 – statistical significance of difference in genotype distribution between female control group and female patients with BA; p_2 – statistical significance of difference in genotype distribution between male control group and male patients with BA

Using Pearson's chi-squared test revealed association between the G allele of Bcl1 polymorphism of GR gene and development of BA. Distribution of alleles between patients with BA and the control group demonstrated a statistically significant difference (p=0,001).

Distribution of allele frequencies for Bcl1 polymorphism of GR gene in male and female comparison groups is presented in Table 3.

Gender-stratified analysis showed a statistically significant difference in the distribution of genotypes for Bcl1 GR gene polymorphism among women in the control group and women with BA (χ^2=6,1; p=0,047). Males demonstrated comparatively higher statistically significant difference in the distribution of genotype frequencies for C647G polymorphism of GR gene (χ^2 = 14,1; p = 0,001) in the control group and in patients with BA.

By studying the prevalence of individuals in groups, formed according to different variants of polymorphism, it was ascertained that homozygotes for C/C allele comprised 47,5% of women and 52,5% of men in the control group, and 69,8% and 30,2%, respectively, – in the group of patients with BA. Heterozygotes were constituted by 51,2% of women and 48,8% of men in the control group and 62,5% and 37,5%, respectively, – among patients with BA. 75% of women and 25% of men were homozygous

for the minor allele in the control group, and 67,7% and 32,3%, respectively, – among patients with BA.

We have studied BA risk depending on the genotype of Bcl1 GR gene polymorphism in patients with BA as a whole and stratified by gender. Taking C/C genotype as a reference one, we demonstrated that G/G homozygous type of GR gene is likely to cause a fivefold increase of BA risk in patients regardless of gender (OR=5,039, CI – 95% 2,377-10,682, p<0,001). The results are given in Table 4.

Female carriers of G/G genotype showed a much lesser risk of BA (OR=3,096, CI 95% 1,235-7,761, p=0,016). As is evident from the data, OR is more than 1 that indicates the significance of G-allele of Bcl1 GR gene polymorphism with regard to the BA risk in women. Odds ratio calculated for men with BA showed the association between GR gene polymorphism (C647G) and predisposition to BA in G/G genotype carriers (OR=11,308, CI 95% 2,807-45,558, p<0,001).

Identification of modifier genes that may influence the development and progression of the disease is an important issue for patients with respiratory diseases. It helps not only for understanding the pathophysiology of progression, but also for identifying patients who may benefit from new therapeutic strategies and adaptation of treatment to their genetic profile. Among the candidate genes of interest there are genes that may influence the inflammatory cascade and response to anti-inflammatory drugs, in particular, genes that influence the effect of exogenous and endogenous GCS. The key factor of GCS action are steroid hormone receptors. According to the U.S. National Institute of Health, 2571 SNPs of GR gene are known up to now, of which only 161 has the minor allele frequency more than 10%, and 127 - more than 1%. The most common and well-studied polymorphism is BclI. Depending on population, G-allele frequency of the polymorphism constitutes more than 30%.

The mechanisms due to which the BclI polymorphism affects disease occurrence and progression, and lung function remain unclear. The results of several studies demonstrating the effect of this polymorphism on sensitivity to GCS may serve as an explanation [7, 14]. BclI GR gene polymorphism may cause changes in the receptor expression level and, respectively, affect sensitivity to GCS (either increase or decrease it, by tissue-specific manner in particular). This is ascertained by the studies of Panarelli et al. (1998) in healthy humans [7].

The data from the studies, carried out in patients with chronic respiratory diseases, mainly focused on BA. The study carried out in Polish individuals with no history of BA and atopy showed that the genotype frequency of BclI GR gene polymorphism was as follows: CC/CG/GG – 0,400/0,471/0,129 [8]. The following study by Pietras T. (2011) investigated the relationship between this polymorphism and the prevalence of BA; the following genotypes frequency for BclI GR gene polymorphism was found: CC/CG/GG–0,128/0,462/0,410, respectively. BA developed significantly more often in allele G carriers (GG + GC) as compared to CC carriers (OR = 5,44, CI: 95%, CI = 2,05–14,41, χ^2 = 13,16, p=0,00029) [9]. Our results are congruent with the data of Polish scientists. However Pietras T. et al. (2011) found no gender-dependent statistical difference in genotypes distribution of BclI polymorphism, which was found in our study.

At the same time, the researches of BclI GR gene polymorphism, conducted children with BA, found out that distribution of alleles and genotypes did not statistically differ between a control group and groups of children with BA of varying severity; gender-dependent statistical difference was not also noticed. However, when comparing the results of clinical and instrumental examination and molecular genetic testing, it was asserted that children with G/G genotype had BA earlier than C/C and C/G genotype carriers; they were also more likely to demonstrate bronchial hyperreactivity due to physical examina-

Table 4. Analysis of risk of BA depending on the genotype of Bcl1 GR gene polymorphism

Genotype	CR	SE	WS	P	OR	95% CI for OR lower	95% CI for OR upper
Patients with bronchial asthma							
C/G	0,549	0,290	3,581	0,058	1,731	0,981	3,054
G/G	1,617	0,383	17,794	0,001	5,039	2,377	10,682
Females							
C/G	0,364	0,389	0,876	0,349	1,439	0,671	3,086
G/G	1,130	0,469	5,811	0,016	3,096	1,235	7,761
Males							
C/G	0,836	0,453	3,403	0,065	2,308	0,949	5,611
G/G	2,425	0,711	11,638	0,001	11,308	2,807	45,558

the comparison was performed for the major allele (C/C) homozygous subjects; CR – regression coefficient; SE – standard error; WS – Wald statistic; P – statistical significance; OR – odds ratio; CI – confidence interval

tion and histamine challenge tests. It was also reported that children with C/C genotype had milder disease, less severe exacerbations and adequate control of the disease, as compared with children who had C/G and G/G genotypes [15]. Thus, the distribution of alleles and genotypes of BclI GR gene polymorphism in children with BA did not differ according to sex or severity, and was identical to that in the control group. Despite this fact, however, clinical and functional data, laboratory examination and assessment of the control level in children with different genotypes of BclI polymorphism make it clear that increasing number of G-alleles in genotype combinations in children with BA is associated with more severe course, acute exacerbations and decreased control level.

Thus, genotyping of BclI GR gene polymorphism together with clinical, laboratory and instrumental methods of examination can be used to assess the risk of BA, peculiarities of its clinical course and, in the future, for the selection of individual therapy and predicting the effectiveness of treatment in these patients.

Conclusions:
- statistically significant differences was revealed in genotypes distribution of BclI polymorphism of GR gene in the control group and in patients with BA, homozygous for the minor allele had a higher risk of the disease than the major allele carriers;
- G/G-homozygotes have a fivefold higher risk of BA, than those homozygous for C/C regardless of gender;
- BA risk in females, homozygous for the minor allele, is three times higher and in males – 11,3 times higher, as compared with C/C-homozygotes. That is, men with G/G genotype of Bcl1 GR gene polymorphism have the highest risk of BA.

REFERENCES

1. Buemann B., Vohl M.C., Chagnon M. et al. Abdominal visceral fat is associated with a BclI restriction fragment length polymorphism at the glucocorticoid receptor gene locus. Obes. Res. 1997; 5(3): 186–192.
2. Corvol H., Nathan N., Charlier C. et al. Glucocorticoid receptor gene polymorphisms associated with progression of lung disease in young patients with cystic fibrosis. Respir. Research. 2007; 8: 88.
3. Cuzzoni E., De Iudicibus S., Bartoli F. et al. Association between BclI polymorphism in the NR3C1 gene and in vitro individual variations in lymphocyte responses to methylprednisolone. Br. J. Clin. Pharmacol. 2012; 73(4): 651–655.
4. Fleury I., Beaulieu P., Primeau M. et al. Characterization of the BclI polymorphism in the glucocorticoid receptor gene. Clin. Chemistry. 2003; 49(9): 1528–1531.
5. Murray J.C., Smith R.F., Ardinger H.A. et al. RFLP for the glucocorticoid receptor (GRL) located at 5q11–5q13. Nucleic Acids Res. 1987; 15: 6765.
6. Otte C., Wüst S., Zhao S. et al. Glucocorticoid receptor gene and depression in patients with coronary heart disease: the Heart and Soul Study-2009 Curt Richter Award Winner. Psychoneuroendocrinology. 2009; 34(10): 1574–1581.
7. Panarelli M., Holloway C.D., Fraser R. et al. Glucocorticoid receptor polymorphism, skin vasoconstriction, and other metabolic intermediate phenotypes in normal human subjects. J. Clin. Endocrinol. Metab. 1998; 83(6): 1846–1852.
8. Pietras T., Panek M., Kuprys-Lipinska I. et al. Frequencies of Bcl I, E22E, and N363S of h-GR/NR3C1 restriction fragment length polymorphisms of glucocorticoid receptor gene in Polish adult population. Med. Sci Monit. 2010; 16(10): 475–479.
9. Pietras T., Panek M., Tworek D. et al. The BclI single nucleotide polymorphism of the human glucocorticoid receptor gene h-GR/NR3C1 promoter in patients with bronchial BA: pilot study. Mol. Biol. Rep. 2011; 38(6): 3953–3958.
10. Rosmond R. The glucocorticoid receptor gene and its association to metabolic syndrome. Obes. Res. 2002; 10: 1078–1086.
11. Schwabe K., Vacca G., Dück R., Gillissen A. Glucocorticoid receptor gene polymorphisms and potential association to chronic obstructive pulmonary disease susceptibility and severity. Eur. J. Med. Res. 2009; 14: 210–215.
12. Stamatelopoulos K., Saltiki K., Mantzou E. et al. Subjects homozygous for the BCL1 polymorphism of glucocorticoid receptor gene may have an increased risk for impaired endothelial function. Endocr. Abstracts. 2010; 22: 127.
13. Ukkola O., Rosmond R., Tremblay A., Bouchard C. Glucocorticoid receptor BclI variant is associated with an increased atherogenic profile in response to long-term overfeeding. Atherosclerosis. 2001; 157: 221–224.
14. Van Rossum E., Koper J., van den Beld A. et al. Identification of the BclI polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. Clin. Endocrinol. 2003; 59: 585–592.
15. Zhdanova M.V., Voitovich A.N., Bogdanova M.A. et al. Features of bronchial BA in children with various genotypes of BCL1 - polymorphism of the glucocorticoid receptor. Pediatria.2007; 86(4): 19–24.

SUMMARY

BCL1 POLYMORPHISM OF GLUCOCORTICOIDS RECEPTOR GENE AND BRONCHIAL ASTHMA

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The aim of our study was to investigate frequencies of alleles and genotypes of Bcl1 GR gene polymorphism and their correlation with prevalence of BA. Study involved 188 patients with BA and 95 healthy individuals. Bcl1 (rs41423247) polymorphism in exon 2 was determined

by means of polymerase chain reaction with subsequent RFLP analysis (restriction fragment length polymorphism) by Fleury I. et al. with modifications. Statistical analysis of the results was performed using SPSS-17 program.

The results showed statistically significant differences in genotypes distribution of BclI polymorphism of GR gene in the control group and in patients with BA. The frequency of genotypes distribution of Bcl1 polymorphism of GR gene in controls: C/C, C/G, G/G - 0,421/ 0,453/0,126; in patients with bronchial asthma: 0.228/0.426/0.346, respectively (p=0.001). It was found out that G/G-homozygotes have a fivefold higher risk of BA than those homozygous for C/C regardless of gender.

We demonstrated that BA risk in females, homozygous for the minor allele, is higher (p=0.016) and men with G/G genotype of Bcl1 GR gene polymorphism have the highest risk of BA. Thus, G/G genotype of Bcl1 polymorphism of the glucocorticoid receptor gene is associated with the development of asthma.

Keywords: Bcl1 polymorphism, glucocorticoid receptor gene, bronchial asthma.

РЕЗЮМЕ

BCL1 ПОЛИМОРФИЗМ ГЕНА РЕЦЕПТОРА ГЛЮКОКОРТИКОИДОВ И БРОНХИАЛЬНАЯ АСТМА

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Целью исследования явилось изучение частоты аллелей и генотипов Bcl1 полиморфизма гена глюкокортикоидного рецептора (ГР) и их связь с распространенностью бронхиальной астмы. Обследовано 188 больных бронхиальной астмой и 95 практически здоровых лиц. Bcl1 (rs41423247) полиморфизм 2-го экзона определяли методом полимеразной цепной реакции с последующим анализом длины рестрикционных фрагментов по Fleury I. Статистическую обработку результатов проводили с использованием программы SPSS-17. Результаты исследования показали, что существует разница в распределении аллельных вариантов гена между больными с бронхиальной астмой и практически здоровыми лицами. Частота распространения генотипов Bcl1 полиморфизма гена ГР в контроле составила: C/C, C/G, G/G – 0,421/0,453/0,126, а у больных бронхиальной астмой – 0,228/0,426/0,346 (p=0,001). Установлено, что у гомозигот по минорному аллелю G/G риск возникновения бронхиальной астмы в 5 раз выше по сравнению с гомозиготами по основному аллелю. Выявлено, что у женщин существует тенденция к увеличению риска возникновения бронхиальной астмы при наличии G/G генотипа (p=0,016), а самый высокий риск развития существует у мужчин с G/G генотипом. Таким образом, G/G генотип Bcl1 полиморфизма гена глюкокортикоидного рецептора ассоциирован с развитием бронхиальной астмы.

რეზიუმე

გლუკოპროტეიდული გენ-რეცეპტორ BCL1-ის პოლიმორფიზმი და ბრონქული ასთმა

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კვლევის მიზანს წარმოადგენს გლუკოპროტეიდული გენ-რეცეპტორ BCL1-ის პოლიმორფიზმის, ალელებისა და გენოტიპების შესწავლა ბრონქული ასთმის გავრცელებასთან კორელაციაში. გამოკვლეულია ბრონქული ასთმით 188 ავადმყოფი და 95 პრაქტიკულად ჯანმრთელი პირი. BCL1 (rs41423247) პოლიმორფიზმი ექსონი 2 განისაზღვრა პოლიმერაზული ჯაჭვური რეაქციის მომდევნო RFLP ანალიზით (რეს-ტრიქციის ფრაგმენტის სიგრძის პოლიმორფიზმი) I. Fleury მეთოდის მოდიფიკაციით. შედეგების სტატისტიკური ანალიზი ჩატარდა SPSS-17 პროგრამის გამოყენებით.

გამოვლინდა სტატისტიკურად სარწმუნო განსხვავება GR გენის Bcl1 პოლიმორფიზმის გენოტიპების გენოტიპური გავრცელებაში საკონტროლო ჯგუფსა და ბრონქული ასთმით დაავადებულ პაციენტებში. გენოტიპების გავრცელების სიხშირე საკონტროლო ჯგუფში იყო C/C, C/G, G/G – 0,421/0,453/0,126, ხოლო ბრონქული ასთმით დაავადებულ პაციენტებში - 0,228/0,426/0,346 (p=0,001). დადგენილ იქნა, რომ ჰომოზიგოტებში G/G მინორული ალელის ბრონქული ასთმის წარმოშობის რისკი 5-ჯერ უფრო მაღალია, ვიდრე ძირითადი ალელის მქონე ჰომოზიგოტებში. ქალებში აღინიშნა ბრონქული ასთმის განვითარების მაღალი რისკი G/G გენოტიპის არსებობის შემთხვევაში (p=0,016), ხოლო ყველაზე მაღალი რისკი აღენიშნებათ მამაკაცებს G/G გენოტიპით. ამრიგად, გლუკოპროტეიდული გენ-რეცეპტორ BCL1-ის პოლიმორფიზმის G/G გენოტიპი ასოცირდება ბრონქული ასთმის განვითარებასთან.

CORRELATION OF THE EXPRESSION OF CD32 AND CD180 RECEPTORS ON CLL CELLS AND MEC1 CELL LINE

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Chronic Lymphocytic Leukemia (CLL) presents with clonal expansion and accumulation of CD5+CD19+CD23+ cells in peripheral lymphoid organs and tissues and in bone marrow [1,2]. CLL is supposedly driven by exogenous or endogenous (auto)antigen(s) and there is increasing evidence that CLL cells receive microenvironmental signals which support their growth, survival and expansion *in vivo*. Identification of putative antigens and antigen-binding receptortors is a question of great importance that might help to reveal the mechanisms of CLL immunopathogenesis, and, subsequently, lead to the identification of optimal therapeutic approaches for this currently incurable disease [3].

We have previously shown [4-6] that apart from B cell receptor (BCR) some of the powerful signals are received by CLL through CD180 orphan receptor that belongs to the toll-like receptor (TLR) family and is predominantly expressed on antigen-presenting cells [7]. Some other accessory signals could be generated through FcγRII (CD32) that is also expressed on CLL cells as well as on control B cells [8]. According to our recent data [6], ligation of CD180 on CLL cells by monoclonal antibody (mAb) can trigger pro-survival as well as pro-apoptotic intracellular signaling events. CD32 is considered to be a negative regulator of BCR signaling pathway [9], as is CD5 receptor, uniformly expressed by all CLL cells [10,11].

In this paper we are presenting a data on the correlation of expression of these receptors on CLL cells separated from CLL patients as well as on MEC1 cell line. MEC1 cell line, which was established from EBV-seropositive patient's CLL cells is widely used as a model for CLL pathogenesis [12].

Materials and methods. Peripheral blood mononuclear cells (PBMC) from 15 CLL patients and 14 age-matched healthy volunteers were separated on Ficoll-Hypaque gradient (Sigma). Anti-CD180, anti-CD5, anti-CD32 and anti-CD19 mAbs conjugated to fluorochromes (BD Pharmingen) were used for immunophenotyping following the standard procedure described previously by us [4,5] and analysed by FACScan flow cytometer (Becton&Dickinson). MEC1 cell line has been kindly donated by the University of Westminster, UK. We have studied CD32 and CD180 expression levels at different time-points of actively cycling MEC1 cells: 24h, 48h, 72h and 96h of cell culture. Only viable cells have been assessed.

The data was statistically analysed using Mann-Whitney non-parametrical test. The values represent averages (M) with standard deviation (SD).

Results and their discussion. Our data indicates that expression of CD32 is significantly increased on CLL cells compared to control B cells as shown in Fig. 1 (p=0.0027).

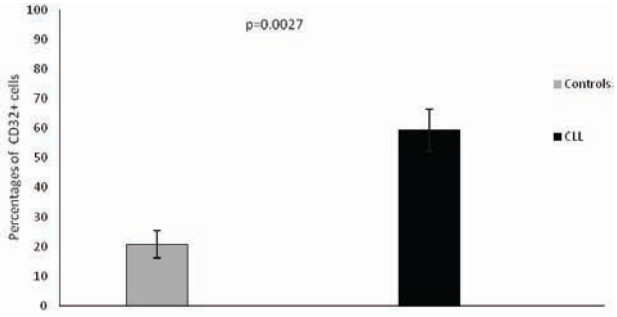

Fig. 1. Percentages of CD32+CD19+ CLL cells and CD32+CD19+ control B cells

Interestingly a strong positive correlation between the expression of CD32 and CD5 has been detected on CLL cells (corr.coef=0.78). Given that both receptors are involved in the negative regulation of BCR signaling [9] our results indicate heterogeneity of the expression of such receptors on CLL cells, ranging from very low to very high.

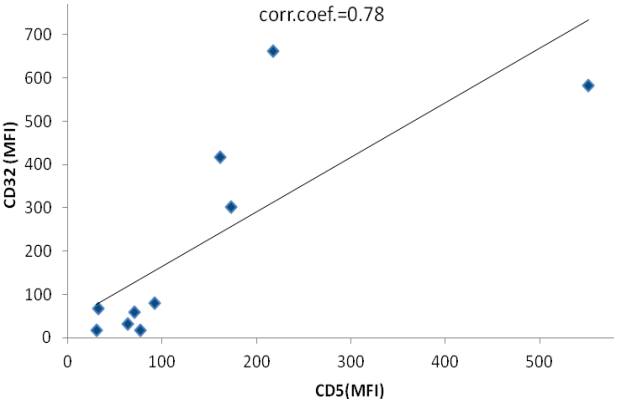

Fig. 2. Correlation between the expression of CD32 and CD5 on CLL cells (MFI)

We have previously shown that CD180 is also heterogeneously expressed on CLL cells. Therefore we next planned to assess the expression of CD32 and CD180 on CLL cells and its effect on CLL cell proliferation and expansion. Peripheral blood CLL cells do not proliferate, their division and expansion are limited to "proliferative centers"of bone marrow and lymph nodes. We therefore used MEC1 cell line in order to study dynamics of the expression of CD32 and CD180 on dividing CLL cells and hence reproduce events in proliferative centres. CD32 expression was

found to be stable on MEC1 cells during the first 48h of culture, but was surprisingly increased in long-term MEC1 cultures: from 11±3.8% at 24h after re-seeding and reaching 36±1.3% at 96h, p=0.0001 (Fig. 3). This is contrary to what has been expected given that CD32 is a negative regulator of BCR signaling. Perhaps CD32 is involved in the negative regulation of pro-apoptotic signaling pathways in MEC1 cells.

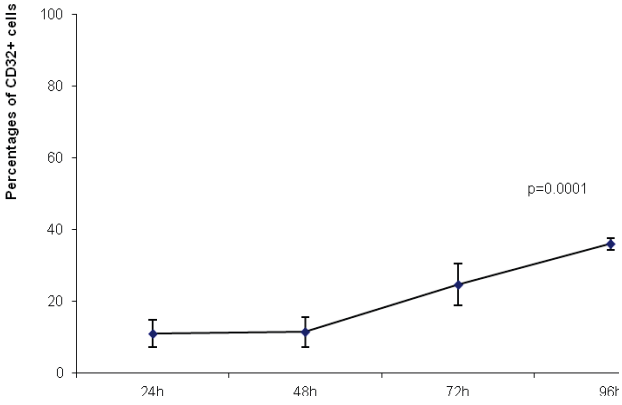

Fig. 3. CD32 expression at different time-points of actively cycling MEC1 cells

In contrast CD180 expression on MEC1 cells was fluctuating, and generally showed a significant tendency for decrease from 38.1±8.5 (CD180+ cells %: 24 h) to 10.2±2.9 (CD180+ cells %: 96 h) p=0.008 (Fig. 4). Interestingly the dynamics of the expression of CD180 and CD32 on a cycling CLL-like MEC1 cell line seems to be opposite.

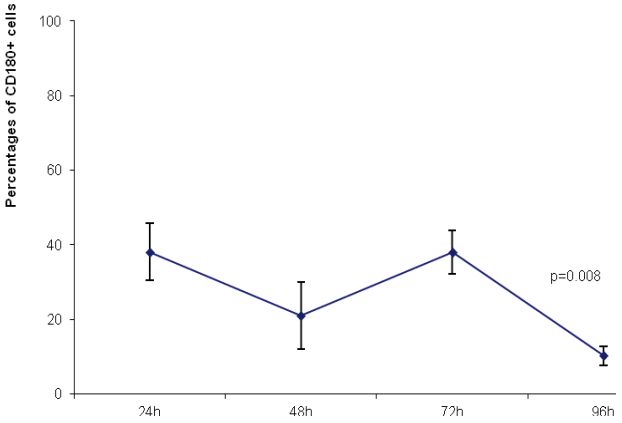

Fig. 4. CD180 expression at different time-points of actively cycling MEC1 cells

We have recently shown that CD180 ligation can redirect sIgM-mediated signaling from pro-survival to pro-apoptotic [6]. This data indicates that a drop in the expression of CD180 on cycling CLL cells might lead to a weakening of this effect and enhance further survival and expansion of CLL cells in proliferative centres of lymphoid tissues. Positive correlation of the expression of CD32 with MEC1 cell culture is puzzling. It has to be taken into account that MEC1 cells are derived from a CLL patient with mutated *IGVH* genes [13] (M-CLL), which is a good prognostic marker, as compared to CLL cases with unmutaded *IGVH* genes (UM-CLL). We have previously shown that CD180 is preferably expressed by M-CLL cells [4]. The observed negative correlation between CD180 and CD32 in MEC1 cell cultures could be limited to M-CLL.

Functional studies are underway to study the effect of ligation of CD180 and CD32 separately or in combination on cellular cycle and apoptosis of MEC1 cells and CLL cells.

REFERENCES

1. Ghia P, Ferreri AM, Caligaris-Cappio F. Chronic Lymphocytic Leukemia. Crit Rev OncolHemato. 2007; 64(3):234–246.
2. Ghia P, Chiorazzi N, Stamatopoulos K.Microenvironmental influences in chronic lymphocytic leukaemia: the role of antigen stimulation. J Int Med. 2008;264(6):549–562.
3. Clifford R, Schuh A. State-of-the-Art Management of Patients Suffering from Chronic Lymphocytic Leukemia. Clin Med InsightsOncol. 2012;6:165-178.
4. Porakishvili N,Kulikova N, Jewell AP,et al. Differential expression of CD180 and IgM by B-cell chronic lymphocytic leukaemia cells using mutated and unmutated immunoglobulin VH genes. Br J Haematol. 2005;131(3):313-319.
5. Porakishvili N, Memon A, Vispute K, et al. CD180 functions in activation, survival and cycling of B chronic lymphocytic leukaemia cells .Br J Haematol. 2011;153:486–498.
6. Porakishvili N, Vispute K, Rajakaruna N, et al. Re-wiring of sIgM-mediated signaling by CD180-toll-like receptor in CLL. Molecular Medicine 2015; Accepted.
7. Chiron D, Bekeredjian-Ding I, Pellat-Deceunynck C, Bataille R, Jego G.Toll-like receptors: lessons to learn from normal and malignant human B cells. Blood 2008;112(6): 2205–2213.
8. Younou P, Jamin C, Pers JO, Bertyhou C, Saraux A, Renaudineau Y. B Lymphocytes Are Required for Development and Treatment of Autoimmune Diseases. N.Y. Acad. Sci. 2005;1050:19–33.
9. Li DH, Tung JW, Tarner IH, et al. CD72 Down-Modulates BCR-Induced Signal Transduction and Diminishes Survival in Primary Mature B Lymphocytes1. The Journal of Immunology 2006; 176: 5321–5328.
10. Gary-Gouy HL, Harriague J, Dalloul A, Donnadieu E, Bismuth G. CD5-Negative Regulation of B Cell Receptor Signaling Pathways Originates from Tyrosine Residue Y429 Outside an Immunoreceptor Tyrosine-Based Inhibitory Motif. J. Immunol. 2002; 168: 232–239.
11. Sindhava VJ, Bondada S. Multiple regulatory mechanisms control B-1 B cell activation. Front. Immunol. 2012;3:372.
12. Hofbauer SW, Piñón JD, Brachtl G, et al. Modifying Akt Signaling in B-Cell Chronic Lymphocytic Leukemia Cells. Cancer Res. 2010;70:7336-7344.

13. Muzio M, Apollonio B, Scielzo C, et al. Constitutive activation of distinct BCR-signaling pathways in a subset of CLL patients: a molecular signature of anergy. Blood. 2008; 112(1):188–195.

SUMMARY

CORRELATION OF THE EXPRESSION OF CD32 AND CD180 RECEPTORS ON CLL CELLS AND MEC1 CELL LINE

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Chronic Lymphocytic Leukemia (CLL) presents with clonal expansion and accumulation of CD5+CD19+CD23+ cells in peripheral lymphoid organs and tissues and in bone marrow. CLL is supposedly driven by exogenous and/or endogenous (auto)antigen(s) and there is increasing evidence that CLL cells receive microenvironmental signals which support their growth, survival and expansion *in vivo*. We have previously shown that powerful signals are received by CLL cells through CD180 orphan toll-like receptor. Additional accessory signals could be generated through FcγRII (CD32), since both are expressed on CLL cells as well as on control B cells. Here we studied correlation of the expression of CD32 and CD180 on CLL cells as well as on MEC1 cell line.

Peripheral blood mononuclear cells (PBMC) from CLL patients and age-matched healthy volunteers were separated, stained with appropriate antibodies to CD19, CD32 and CD180 and analysed by flow cytometry. CD32 and CD180 expression on MEC1 cells was studied at different time-points. The data was statistically analysed using the Mann-Whitney non-parametrical test.

Our data indicates that expression of CD32 is significantly increased on CLL cells compared to control B cells as well as in long-term MEC1 cell culture. In contrast, CD180 expression on MEC1 cells significantly decreased throughout 0-96h of MEC1 cell culture. We have recently shown that CD180 ligation can redirect sIgM-mediated signaling from pro-survival to pro-apoptotic. This data indicates that a drop in the expression of CD180 on cycling CLL cells might lead to a weakening of this effect and enhance further survival and expansion of CLL cells in proliferative centres of lymphoid tissues. Since MEC1 cells are derived from a CLL patient with mutated *IGVH* genes (M-CLL) negative correlation between CD180 and CD32 expression on cycling MEC1 cells could be limited to M-CLL.

Keywords: chronic lymphocytic leukaemia (CLL), CD180, CD32. MEC1.

РЕЗЮМЕ

КОРРЕЛЯЦИЯ ЭКСПРЕССИИ CD32 И CD180 РЕЦЕПТОРОВ В ХЛЛ КЛЕТКАХ И ЛИНИИ КЛЕТОК MEC1

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При хронической лимфоцитарной лейкемии (ХЛЛ) происходит клональное распространение CD5+CD19+CD23+ клеток и их накопление в периферических лимфатических органах и тканях, а также в костном мозге. Развитие ХЛЛ, предположительно, обусловлено стимуляцией эндогенными и/или экзогенными (ауто)антигенами, в том числе исходящими из микроокружения, которые поддерживают рост, выживание и распространение *in vivo* лейкемических клеток. Ранее нами показано, что стимулирование ХЛЛ клеток опосредовано CD180 – рецептором-сиротой, принадлежащим семейству Toll-подобных рецепторов. Кроме этого вторичные сигналы могут исходить из от FcγRII (CD32) рецептора, так как оба рецептора экспрессированы как на клетках ХЛЛ, так и на контрольных В лимфоцитах. В данной работе изучена корреляция рецепторов CD32 и CD180 на клетках ХЛЛ, и в линии клеток MEC1.

Мононуклеарные клетки периферической крови пациентов с ХЛЛ и, подобранных в соответствии с возрастом здоровых доноров, обрабатывали соответствующими антителами к CD19, CD32 и CD180 рецепторам, конъюгированными с флюорохромами, и исследовали с использованием проточной цитометрии. Экспрессию CD32 и CD180 рецепторов на MEC1 клетках изучали в разных временных точках. Статистическую обработку полученных данных проводили с использованием метода Манн-Уитни.

Данные проведенного исследования показывают, что экспрессия CD32 рецептора на клетках ХЛЛ, в сравнении с контрольными В клетками, значительно увеличилась, также как и в длительной культуре MEC1 клеток. Экспрессия CD180 рецептора на MEC1 клетках значительно сократилась в 96-часовой культуре клеток MEC1. В ранее проведеных нами исследованиях показано, что с помощью CD180 рецептора возможно переключить sIgM-опосредованный сигнал с анти-апоптозного на про-апоптозный. Наши новые данные показывают, что снижение экспрессии CD180 рецептора в пролиферирующих ХЛЛ клетках может вызвать ослабление этого эффекта и дальнейшее выживание и экспансию ХЛЛ клеток в пролиферативных

центрах лимфоидных тканей. Так как MEC1 клетки были получены от ХЛЛ пациента с мутированными *IGVH* генами (М-ХЛЛ), отрицательная корреляция между экспрессией CD180 и CD32 рецепторов на пролиферирующих MEC1 клетках может быть ограничена М-ХЛЛ случаями.

რეზიუმე

CD32 და CD180 რეცეპტორების ექსპრესიის კორელაცია ქლლ უჯრედებსა და MEC1 უჯრედულ ხაზში

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ქრონიკული ლიმფოციტური ლეიკემია (ქლლ) ხასიათდება $CD5^{+}CD19^{+}CD23^{+}$ უჯრედების კლონური ექსპანსიით და აკუმულირებით პერიფერიულ ლიმფურ ორგანოებში, ქსოვილებსა და ძვლის ტვინში. ნაჩვენებია, რომ ქლლ უჯრედები მიკროგარემოსგან ღებულობენ სიგნალებს, რომლებიც ხელს უწყობენ მათ დაყოფას, გადარჩენასა და ექსპანსიას *in vivo*. სავარაუდოდ, ქლლ-ის განვითარებას ბიძგს აძლევს ჰიპოთეტური ენდოგენური ან ეგზოგენური (აუტო)ანტიგენ(ებ)ი. ნაჩვენებია, რომ ქლლ უჯრედები ძლიერ სიგნალებს იღებს CD180 თოლლმსგავსი რეცეპტორების საშუალებით. დამატებითი მეორადი სიგნალი შეიძლება გამომუშავებული იყოს FcγRII (CD32) რეცეპტორების მეშვეობით, რადგანაც ორივე რეცეპტორი ექსპრესირებულია, როგორც ქლლ უჯრედებზე, ასევე საკონტროლო B ლიმფოციტებზე. მოცემულ ნაშრომში შესწავლილია CD32 და CD180 რეცეპტორების ექსპრესიის კორელაცია როგორც ქლლ უჯრედებში, ასევე MEC1 უჯრედულ ხაზში.

ქლლ პაციენტებისა და შესაბამისი ასაკობრივი ჯგუფის ჯანმრთელი პირების პერიფერიული სისხლის მონონუკლეარულ უჯრედებს ვღებავდით CD19, CD32 და CD180 ანტისხეულებით, კონიუგირებულ ფლუოროქრომებით და შედეგებს ვსაზღვრავდით გამდინარე ციტომეტრის გამოყენებით. CD32 და CD180 რეცეპტორების ექსპრესიას MEC1 უჯრედებზე ვსწავლობდით დროის სხვადასხვა წერტილებში. მიღებული მონაცემების სტატისტიკურ დამუშავებას ვაწარმოებდით მან-უიტნის არაპარამეტრული კრიტერიუმის გამოყენებით.

მიღებული შედეგების თანახმად, CD32 რეცეპტორის ექსპრესია ქლლ უჯრედებზე მნიშვნელოვნად არის გაზრდილი საკონტროლო B უჯრედებთან შედარებით, ისევე როგორც MEC1 უჯრედების გრძელვადიან კულტურაში. ამისგან განსხვავებით, CD180 რეცეპტორის ექსპანსია MEC1 უჯრედებში მნიშვნელოვნად შემცირდა 0-96-საათიანი ზრდის პირობებში. ახლახანს ჩვენს მიერ ნაჩვენებია, რომ CD180 რეცეპტორის შებოჭვა იწვევს sIgM-გაშუალებული სიგნალის გადართვას გადასარჩენიდან აპოპტოზურზე. ახალი მონაცემები აჩვენებს, რომ CD180 რეცეპტორის ექსპრესიის შემცირებამ ქლლ უჯრედებში, შესაძლოა, გამოიწვიოს ამ ეფექტის შესუსტება და გააძლიეროს ქლლ უჯრედების შემდგომი გადარჩენა და ექსპანსია ლიმფოიდური ქსოვილების პროლიფერატორულ ცენტრებში. რადგანაც MEC1 უჯრედები მიღებულია ქლლ პაციენტებისგან მუტირებული IGVH გენებით (М-ქლლ), უარყოფითი კორელაცია CD180 და CD32 რეცეპტორების ექსპრესიებს შორის MEC1 უჯრედებში, შესაძლოა, შემოფარგლულია М-ქლლ-ით.

SELECTIVE LESION OF GABA-ERGIC NEURONS IN THE MEDIAL SEPTUM BY GAT1-SAPORIN IMPAIRS SPATIAL LEARNING IN A WATER-MAZE

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Given the central role the hippocampus plays in declarative memory formation [1-3] and the strong input to the hippocampus from the medial septum (MS), it is tempting to hypothesize that this input is critical for memory processes [8,12]. The septum and the hippocampus are heavily interconnected through the fimbria-fornix and are functionally coupled [5], often referred to collectively as the septohippocampal (SH) system [7]. The SH projection includes cholinergic and GABAergic components [5,15]. More recent studies have shown that a subpopulation of septal glutamatergic neurons projects to the hippocampus[13,17,24]. Extensive evidence indicates that

the SH system is involved in memory processes [16]. It is well established that hippocampal lesions causes spatial learning deficits in tasks such as the Morris water maze [22,23]. Cognitive dysfunctions after MS lesions are mainly considered to be due to hippocampal deafferentiation and therefore may result in behavioral effects similar to those of hippocampal lesions [14,18].

Much attention has been paid to the medial septal modulation of the hippocampus through cholinergic projections, However when spatial learning deficits are observed, the impairments with selective cholinergic lesions are generally smaller than those observed with nonselective MS lesions, suggesting a role for noncholinergic MS neurons in spatial memory. Many studies using the cholinergic immunotoxin 192 IgG-saporin have demonstrated that selective removal of cholinergic neurons in the basal forebrain does not disrupt simple place learning [6,9,19]. The most important SH noncholinergic neurons are the GABAergic neurons. GABAergic SH projections preferentially synapse on intrahippocampall GABAergic interneurons, an ideal target to enhance activity of hippocampal pyramidal neurons through disinhibition [11]. The involvement of GABAergic SH projections in hippocampal-based spatial learning remains unspecified. However, a new more selective toxin for GABAergic neurons would facilitate research. Pang et al [20], characterized the effects of GAT1-saporin on the MS neurons and showed that intraseptal GAT1-SAP preferentially reduced GABAergic neurons as compared to ChAT-ir neurons in the MS.

The aim of this study was to investigate the modulation of the cognitive function by the GABAergic cells of the MS and was designed to investigate the role of the MS GABAergic cells in hippocampal dependent spatial learning using the immunotoxin GAT1-SAP to produce selective lesions of GABAergic MS neurons. In current study rats were trained in a visible platform version of the Morris water maze in which either a place or cue strategy could be used to escape successfully.

Materials and methods. A total of 18 male outbred white rats weighing between 200 and 250 gm at the beginning of the experiment were used in the present study. The rats were housed in standard cages at a natural light/dark cycle and were tested during the light period. All animals were given access to food and water *ad libitum*. All experiments were approved by the Animal Care and Use Committee of the Institute and were in accordance with the principles of laboratory animal care.

Surgery. Rats were anaesthetized with i.p. injection of 4**%** chloral hydrate (9 ml/kg) and placed in a stereotaxic apparatus. All injections of GAT1-SAP (325ng/μl) for immunolesion surgeries or mouse saporin (this product serves as a control for the immunotoxin), for control surgeries (Advanced Targetting System, San Diego, USA) were performed stereotaxically according to Paxinos and Watson [21] sterotaxic atlas at two positions: AP – 0.7; ML – 0; DV - 7.8mm (0,3 μl; 0,05 μl/min) and DV - 6.2mm (0,2 μl; 0,05 μl/min). The needle was left in place for an additional 9 min and 6 min, respectively, after completion of the injection, to allow the toxin to diffuse from the injection site. All injections were made with a 1-μl Hamilton syringe with a microinjection pump **(**CMA 402 Syringe Pump, Sweden**).** For analgesia the rat was given a 0.1 mg/kg injection of buprenorfin after the surgery. The rats were allowed to recover from the surgery for two weeks before starting the behavioral experiments.

Morris water-maze. Animals were tested in a standard Morris water-maze, consisting of a circular tank (1.5-m in diameter and 0.5 m height) filled with opaque (white-colored) water. Escape platform (10 cm in diameter) was located 2 cm beneath the surface on hidden platform training days and raised 2 cm above the water surface on visible platform training days. The room, in which the tank was stationed, had sufficient number of the cues (door, window, furniture, posters on the walls, *etc*.) in order to provide spatial cues. The task was adapted from Bizon, at all. [4]. On days 1–9, rats received four trials per day, one from each of four equidistantly located start locations (N, S, E, W). On both visible- and hidden platform days, the rats were placed into the water facing the wall of the maze. The trial ended when the rat climbed on the available platform or until 60 s had elapsed. If a rat could not found the platform after 60 s, it was placed on the platform by the experimenter. Rats were left on the platform for 15 s and were then moved to a holding cage for a 2-min intertrial interval. On days 1 and 2, rats were trained to locate a visible platform in the southeast quadrant of the pool, followed by a third day in which the platform was submerged at the same location. This 3-day sequence was repeated twice on days 4-60 and 7-9 for a total of 36 trials (24 visible and 12 hidden). On day 10, a competition test was given in which the visible platform was moved to the northwest quadrant (opposite to its placement on the training days). Two trials were given with start points equidistant from the two platform locations (SE and NW). Video recordings were analyzed to determine whether rats swam within southeast quadrant before escaping to the visible platform in the northwest quadrant. Tracking the animal movements in water-maze, also collection of other numeric data (time in zone, latency to enter zone, and so on) were made with an aid of video tracking system.

Histology. At the end of behavioral testing a random sample of rats from each group (six control and six GAT1-SAP medial septal lesioned) were killed and brains collected in order to verify lesion effects. The immunotoxic GAT1-SAP lesions of MS were verified by observing decreased Acetylcholintransferase (ChAT) and parvalbumine (PV) staining of the MS. The 20 μ thick coronal sections using freezing microtome were stained with ChAT and PV

primary antibody and ABC Staining System. All necessary reagents and buffers were received from Santa Cruz Biotechnology, Inc. (USA). Totally 30 fields per animal were analyzed and average of immunostained cells per field was used to assess the effect of MS lesion on ChAT and PV-stained neurons. The sections were analyzed with a microscope Leica MM AF.

Differences of the escape latency obtained in various groups of animals were evaluated with the Student's *t*-test. Two-sample *t*-test was used to compare immunohistological data between control and lesioned groups. All data are presented as mean±standard error of the mean. Differences were considered significant when $p<0.05$.

Results and their discussion. Of the 10 rats that received GAT1-SAP lesions targeted at the MS two animals died before the end of the experiment and were excluded from the analysis. The number of animals in each group was as follows: MS immunotoxic - GAT1-SAP lesioned (n=8) and vehicle-injected -control rats (n=8).

Immunohistochemical studies showed that intraseptal injection of GAT1-SAP preferentially reduced GABAergic neurons as compared to cholinergic neurons in the MS. GAT1-SAP reduced the number of PV-ir neurons, representing GABAergic septohippocampal neurons by 78%. Counts of ChAT-ir neurons made in the same rats used to assess PV-ir neurons demonstrated a mild reduction following GAT1-SAP. The reduction of cholinergic neurons represented a loss of only 26%. Thus, GAT1-SAP when infused into the MS extensively damaged GABAergic MS neurons and spared most cholinergic neurons. The results of histological studies are presented in Table 1.

Table 1. Number of positive ChAT-ir and PV-ir neurons in MS of rats administered GAT1-SAP or mouse saporin (Control). The data represent means ± SEM

Group	MS	
	ChAT	PV
Control	720±80	560±60
MS (GAT1-sap)	377±60	75±35

The escape latencies for the training trials are shown in Fig.1. The control rats rapidly learned to escape to the visible platform and reached the 6 s asymptote on day 2. The MS-lesioned rats were significantly impaired on the first 4 days and improved in their ability to escape to the platform at slower rate. However, they also approached the 5 s asymptote on the fifth day. Thus, the learning was slower in the MS-lesioned group than in the control group, but differences in the platform reaching latency between the animals of different groups, at the 7th-8th days were not found. This fact certifies, that there were no obvious differences between the groups in perception, motivation, or motor abilities that could differentially influence acquisition of task.

Although in conditions of vissible platform testing, differences in the platform reaching latency between the animals of different groups, at the 7th-8th days were not found, at the day 9, when testing was performed in conditions of submerged platform, the latency of the platform finding was significantly increased ($P<0.05$). This fact certifies for obvious deficit of the place learning performance strategy in the MS-lesioned rats.

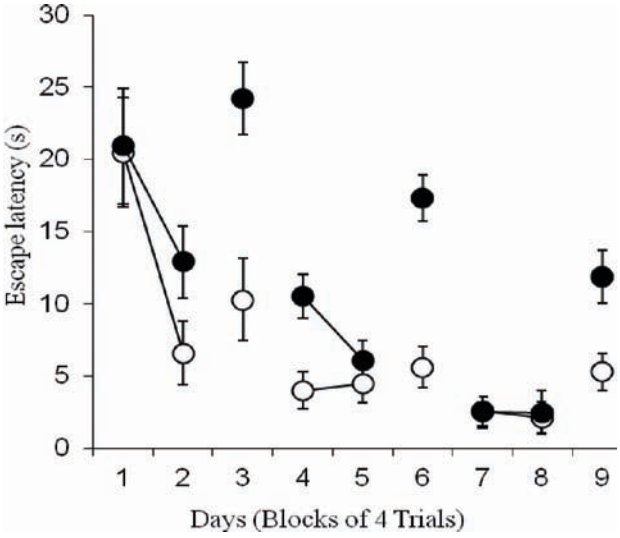

Fig. 1. Water maze acquisition. Mean±SEM escape latency for the visible (1,2,4,5,7,8 day) and hidden (3,6,9 day) platform tasks (○ - Control; ● - GAT1-SAP)

The rats' responses in the competition test were classified either as cue or as place, based on the swimming path for those trials. on the first competition trial, all of the control rats used place strutegy. Contrary to the control rats majority of the MS-lesioned rats, used a cue strategy: MS lesioned: n=2 place, n=6 cue; control: n=8 place, n=0 cue. On the second trial, all MS lesioned rats used a cue strategy: n=0 place, n=8 cue, while majority of the sham-operated rats used a place strategy: n=6 place, n=2 cue. Interestingly, although more rats from the control group used a place strategy on the second trial, two of those that on the first trial of the competition test used a place strategy, exhibited a cue strategy (Table 2). These data indicate that although the first trial might have influenced overall performance on the second competition trial, information regarding old location of the platform was still being accessed by a subset of rats on the second trial.

An overview of the data from both competition trials for each group show that the control rats in 14 trials out of 16 competition test trial used place strategy, while MS-lesioned ones used this strategy in 2 trials only. Decreased place-bias in MS-lesioned rats compared to the contrrol rats was significant ($P<0.01$).

The information obtained from a discrete trial such as the competition test, is limited; therefore, we also analyzed the rats' performance by combining data across the two compe-

Table 2. Number of rats (and % of Group) exhibiting place or cue strategies on two competition trials

Strategy in firs trial	Strategy in second trial	Control (n=8)	GAT1-SAP (n=8)
Place	Place	6 (75%)	
Place	Cue	2 (25%)	2 (25%)
Cue	Place		
Cue	Cue		6 (75%)

Table 3. Number of rats' classified on the basis of their performance across both trials of the competition test using established criteria

	Comtrol	MS lesioned
Place responder	6	--
Cue responder	--	6
Cue/place responder	2	2

tition trials. Rats were designated as 'place responder', if they swam within 10 cm of the previous platform location on two competition trial or as 'cue responders', if they swam toward visible platform location across both trials of the competition test. Rats were designated as 'cue/place responder', if they exhibited different strategy in two competition trials. Table 3 summarizes the rats' performance across both trials of the competition test, using established criteria.

Using these criteria, majority (75%) of the control rats were classified as 'place responders' and majority of the MS-lesioned rats were classified as 'cue responders'. As expected, escape latency averaged across both competition trials, on day 10 was significantly greater for place responders as compared with cue responders, confirming the more indirect path taken by the place responders.

Notably, the control and the MS-lesioned rats, designated as place- and cue responders on the competition test, exhibited corresponding differences in performance during training trials. The control rats, identified as place responders, had significantly more accurate searches on hidden platform days, providing an additional evidence of their effective use of a place learning strategy rather than the MS-lesioned rats exhibiting a cue strategy.

The data obtained in the control and MS electrolytic lesioned animals in the previous study [10] demonstrate that decreased place-bias in electrolytic MS-lesioned rats compared to the control rats was significant. The MS electrolytic lesioned rats acquired the visible platform version of the water maze task but failed to learn the platform location in space. When the visible platform was moved to a new location they often swam directly to it. The MS selective ACh lesioned rats, as well as control, acquired the platform location in space [9]. These findings suggest that the septo-hippocampal system is essential for accurate spatial learning and suggest its role in processing information about the spatial environment, but deficits observed after septal electrolytic lesions cannot be accounted solely to the loss of hippocampal ACh.

The data obtained in the control and GAT1-SAP lesioned animals in the present study, demonstrate that lesioned rats were impaired in hidden platform trials during training, and displayed a pronounced cue-bias in competition tests. Therefore, above data suggest involvement of the MS GABAergic neurons in organization of the spatial map-driven behavior and this structure, along with the hippocampus, should be viewed as a constituent of the functional system responsible for the cognitive types of spatial memory.

REFERENCES

1. Balderas I., Morin JP., Rodriguez-ortiz CJ., Bermudez-Rattoni f. Muscarinic receptors activity in the perirhinal cortex and hippocampus has differential involvement in the formation of recognition memory. Neurobiol Learn Mem. 2012; 97: 418-24.
2. Barbosa FF., de Oliveira Pontes IM., Ribeiro AM., Silva RH. Differential roles of the dorsal hippocampal regions in the acquisition of spatial and temporal aspects of episodic-like memory. Behav Brain Res. 2012; 232: 269-277.
3. Barker GR., Warburton EC. When Is the Hippocampus Involved in Recognition Memory? The Journal of Neuroscience 2011; 31: 10721–1073.
4. Bizon J.L., Han J.-S., Hudon C., Gallagher M. Effects of hippocampal cholinergic deafferentation on learning strategy selection in a visible platform version of the water maze. Hippocampus 2003; 13:676 –684.
5. Bland BH, Colom LV. Extrinsic and intrinsic properties underlying oscillation and synchrony in limbic cortex. Prog. Neurobiol. 1993; 341, 157–208.
6. Chang Q, Gold PE. Impaired and spared cholinergic functions in the hippocampus after lesions of the medial septum/vertical limb of the diagonal band with 192 IgG-saporin. Hippocampus 2004;14:170–179.
7. Colom LV, Castaneda MT, Reyna T, Hernandez S, Garrido-Sanabria E. Characterization of medial septal glutamatergic neurons and their projection to the hippocampus. Synapse 2005; 558: 151–164.
8. Drever BD, Riedel G, Platt B. The cholinergic system

and hippocampal plasticity. Behavioral Brain Research. 2011; 221(2):505–514.
9. Dashniani M, Beselia G, Maglakelidze G, Burjanadze M, Chkhvishvili N. Effects of the selective lesions of cholinergic septohippocampal neurons on different forms of memory and learning process. Georgian Medical News 2009; 166: 81-85.
10. Dashniani M., Beselia G., Maglakelidze G., Burjanadze M., Naneishvili T. Effects of Electrolytic Lesion of Medial Septal Nucleus on Learning Strategy Selection in a Visible Platform Version of the Water Maze. Georgian Med News. 2007; 11(152): 52-6.
11. Freund TF. GABAergic septohippocampal neurons contain parvalbumin. Brain Res. 1989;478:375–381.
12. Hasselmo M.E. The role of acetylcholine in learning and memory. Current Opinion in Neurobiology 2006; 16(6):710–715.
13. Hajszan T, MacLusky NJ, Leranth C. Dehydroepiandrosterone increases hippocampal spine synapse density in ovariectomized female rats. Endocrinology. 2004;145:1042–1045.
14. Hung MC, Shibasaki K, Nishizono S, Sato M, Ikeda I, Masuda Y, et al. Ibotenic acid-induced lesions of the medial septum increase hippocampal membrane associated protein kinase C activity and reduce acetylcholine systhesis: prevention by a phophatidylcholine /vitamin B12 diet. J Nurt Biochem 2000;11:159–64.
15. Kohler C, Chan-Palay V, Wu JY. Septal neurons containing glutamatic acid decarboxylase immunoreactivity project to the hippocampal region in the rat brain. Anat Embryol. 1984; 169:41-44.
16. Lecourtier L, de Vasconcelos AP, Leroux E, Cosquer B, Geiger K, Lithfous S, Cassel JC. Septohippocampal pathways contribute to system consolidation of a spatial memory: sequential implication of GABAergic and cholinergic neurons. Hippocampus 2011; 21: 1277-89.
17. Manseau F, Goutagny R, Danik M, WilliamsS. The hippocamposeptal pathway generates rhythmic firing of GABAergic neurons in the medial septum and diagonal bands: an investigation using acomplete septo-hippocampal preparation in vitro. J. Neurosci. 2008; 28: 4096–4107.
18. Morris R. Development of a water-maze procedure for studying spatial learning in the rat. J Neurosci Meth. 1984;11:47–60.
19. Pang KC, Nocera R Interactions between 192-IgG and intraseptal cholinergic and GABAergic drugs: role of cholinergic medial septal neurons in spatial working memory. Behav Neurosci. 1999; 113:265–275.
20. Pang ZP, Yang N, Vierbuchen T, Ostermeier A, Fuentes DR, Yang TQ, Citri A, Sebastiano V, Marro S, Südhof TC, Wernig M. Induction of human neuronal cells by defined transcription factors. Nature 2011; 476(7359):220-3.
21. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th ed. Academic, San Diego: CA, 1998.
22. Redish AD, Touretzky DS The role of the hippocampus in solving the Morris water maze. Neural Comput. 1998; 10: 73–111.
23. Silva AJ, Giese KP, Fedorov NB, Frankland PW, Kogan JH. Molecular, cellular, and neuroanatomical substrates of place learning. Neurobiol Learn Mem. 1998; 70:44–61.
24. Sotty F, Danik M, Manseau F, Laplante F, Quirion R, Williams S. Distinct electrophysiological properties of glutamatergic, cholinergic and GABAergic rat septohippocampal neurons: novel implications for hippocampal rhythmicity. J Physiol. 2003; 551:927–943.

SUMMARY

SELECTIVE LESION OF GABA-ERGIC NEURONS IN THE MEDIAL SEPTUM BY GAT1-SAPORIN IMPAIRS SPATIAL LEARNING IN A WATER-MAZE

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The aim of this study was to investigate the role of the medial septal (MS) GABAergic cells in hippocampal dependent spatial learning using the immunotoxin GAT1-SAP to produce selective lesions of GABAergic MS neurons. In current study rats were trained in a visible platform version of the Morris water maze in which either a place or cue strategy could be used to escape successfully. Immunohistochemical studies showed that intraseptal injection of GAT1-SAP extensively damaged GABAergic MS neurons and spared most cholinergic neurons. The rats' responses on the competition test were classified as either cue or place, based on the swim path for those trials. An overview of the data from both competition trials for each group show that the control rats in 14 trials out of 16 competition test trial used place strategy, while MS-lesioned ones used this strategy in 2 trials only. Decreased place-bias in MS-lesioned rats compared to the contrrol rats was significant ($P<0.01$). The data obtained in the control and GAT1-SAP lesioned animals in the present study, demonstrate that lesioned rats were impaired in hidden platform trials during training, and displayed a pronounced cue-bias in competition tests. Therefore, above data suggest involvement of the MS GABAergic neurons in organization of the spatial map-driven behavior and this structure, along with the hippocampus, should be viewed as a constituent of the functional system responsible for the cognitive types of spatial memory.

Keywords: medial septum, learning strategy, water maze, rats.

РЕЗЮМЕ

СЕЛЕКТИВНОЕ ПОВРЕЖДЕНИЕ GABA-ЕРГИЧЕСКИХ НЕЙРОНОВ МЕДИАЛЬНОГО ЯДРА ПРОЗРАЧНОЙ ПЕРЕГОРОДКИ УХУДШАЕТ ПРОСТРАНСТВЕННОЕ ОБУЧЕНИЕ В БАССЕЙНЕ МОРРИСА

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В представленной статье у крыс с иммунотоксическим (GAT1-SAP) повреждением GABA-ергических нейронов медиального ядра прозрачной перегородки (*medial septal neclei* - MS) и у контрольных животных исследовалась способность обучения местонахождения видимой, а также погруженной в непрозрачную воду платформы в условиях бассейна Морриса. Иммуногистохимическое исследование выявило значительное уменьшение количества GABA-ергических нейронов у животных с микроинъекцией GAT1-SAP по сравнению с контрольными животными. В поведенческих экспериментах выявлено, что в тестовых пробах (n=16), когда предоставлялся выбор ответа между приобретенной ранее пространственной локализацией платформы и видимой платформы, представленной на противоположной стороне бассейна, контрольные животные направлялись в сторону старого местонахождения платформы. Животные с повреждением MS в тестовых пробах чаще направлялись к видимой платформе. Оказалось, что во время тренировочных проб контрольные животные более аккуратны в поисках невидимой платформы, чем животные с повреждением MS, что служит дополнительным доказательством более эффективного применения ими стратегии обучения места (Place learning). Полученные данные подтверждают, что при повреждении GABA-ергических нейронов MS зависимость поведения от пространственной информации уменьшается и увеличивается реагирование на подкрепленный сигнал. Следовательно, полученные данные свидетельствуют об участии GABA-ергических нейронов MS в процессах пространственного обучения и данную структуру, наряду с гиппокампом, следует включить в функциональную систему, обслуживающую когнитивные формы пространственной памяти.

რეზიუმე

GAT1-საპორინით მედიალური სეპტუმის GABA-ერგული ნეირონების სელექტიური დაზიანება აუარესებს სივრცით დასწავლას მორისის წყლის აუზში

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შესწავლილია მედიალური სეპტუმის (MS) GABA-ერგული ნეირონების როლი ჰიპოკამპდამოკიდებულ სივრცით დასწავლაში. MS-ის GABA-ერგული ნეირონების სელექციურად დაზიანებისათვის გამოიყენებოდა იმუნოტოქსინი GAT1-საპორინი. ვირთაგვების ტრენირება ტარდებოდა მორისის წყლის აუზის ხილულბაქნიანი ვერსიით,რომლის დროს ამოცანის წარმატებით განხორციელება შესაძლებელია როგორც ადგილის, ასევე, სიგნალის დასწავლის სტრატეგიით. იმუნოჰისტოქიმიურმა კვლევამ აჩვენა, რომ GAT1-საპორინი, ძირითადად, აზიანებს GABA-ერგულ ნეირონებს და დაუზიანებელს ტოვებს ქოლინერგული ნეირონების უმრავლესობას. სატესტო სინჯებში გაცურვის ტრაექტორიის მიხედვით ცხოველები ფასდებოდნენ როგორც ადგილის, ან სიგნალის დამსწავლელები. ცხოველთა ყველა ჯგუფში ორივე სატესტო სინჯის შეფასების შედეგად აღმოჩნდა, რომ საკონტროლო ჯგუფის ვირთაგვები 16 სატესტო სინჯიდან 14-ს ასრულებდნენ ადგილის დასწავლის სტრატეგიით, ხოლო MS-ის დაზიანების მქონე ცხოველები ამ სტრატეგიას იყენებდნენ მხოლოდ 2 სინჯში. საკონტროლო ჯგუფთან შედარებით, MS-ის დაზიანების მქონე ცხოველებში ადგილის დასწავლა სარწმუნოდ იყო შემცირებული (P<0.01). მიღებული მონაცემების მიხედვით, ცხოველები MS-ის დაზიანებით წარუმატებელი არიან ფარული ბაქნის პირობებში ტრენირებისას, ხოლო სატესტო სინჯებში იყენებენ სიგნალის დასწავლის სტრატეგიას. აღნიშნული მონაცემები ადასტურებენ MS-ის GABA-ერგული ნეირონების ჩართულობას სივრცითი რუკით წარმართული ქცევის ორგანიზებაში და MS, ჰიპოკამპთან ერთად, შეიძლება მიეკუთნოს სივრცითი მეხსიერების კოგნიტიური ფორმების რეალიზაციაზე პასუხისმგებელ ფუნქციურ სისტემას.

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BIOLOGICAL CHARACTERIZATION OF *V. CHOLERAE*-SPECIFIC BACTERIOPHAGES ISOLATED FROM WATER SOURCES IN GEORGIA

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Cholera is one of the most serious enteric infections with the potential to appear in an explosive outbreak, epidemic or even worldwide pandemic. Disease is transmitted via food and water contaminated with *Vibrio cholerae*, a naturally occurring bacterium in marine, fresh- and brackish waters. Only toxigenic strains belonging to the *V. cholerae* serogroups O1 (both classical and El Tor biotypes) and O139 can cause disease cholera [3] while those belonging to the other serogroups known as *V. cholerae* non-O1/non-O139 usually lead to a sporadic diarrhea and also rarely cause extraintestinal infections [16]. Cholera outbreaks are among major public health problems in developing countries with poor social-economic conditions in Asia, Africa, and Latin America [7]. The most recent (2010-2014) serious cholera outbreaks occurred in Haiti, Democratic Republic of Congo, Sierra Leone, Mexico and South Sudan [22].

Understanding the ecology and pathogenicity of *V. cholerae*, as well as origin of various isolates is very important for prevention and control of cholera. In this regard, phage typing of *V. cholerae* was shown to be useful both- for differentiating classical and El Tor strains and for epidemiological purposes [6]. In recent years, there has been a renewed interest in the role of *Vibrio*-specific bacteriophages as indicators of host bacteria. The works of Faruque et.al [7] demonstrated that detection of *V. cholerae* specific bacteriophages in water environment may indicate future or past recent outbreaks of cholera. Studies of *V. cholerae*-specific phages as infection control agents have been of historical interest. Felix D'Herelle used phage preparations to treat thousands of people with cholera in India [18]. Later phages have been used also for the identification of toxigenic *V. cholerae* strains and correspondingly for the diagnosis of the disease cholera.

The goal of this study was to reveal, isolate and characterize the naturally occurring *V. cholerae*- specific bacteriophages in aquatic environments of Georgia, in the Black Sea coastal zone and inland reservoirs near the capital city Tbilisi and to estimate their potential role for detection, diagnosing, and biocontrol for *V. cholerae*.

Materials and methods. *Microbiological media and buffers:* Luria-Bertani broth (LBB) and agar (LBA); LBB+MgSO4; LBB+2% NaCl; LBB +$CaCl_2$; Tryptone salt broth (T_1N_6 and T_1N_8); Saline; Phosphate buffered saline (PBS) pH7.4; Buffered peptone water (BPW) pH7.8; SM-Buffer (100mMNaCl, 8mM $MgSO_4 \cdot 7H_2O$, 50mMTris-Cl (1 M, pH 7.5), 0.002% (w/v) Gelatine) [11,17].

Bacterial strains. Two standard strains obtained from the collection of NCDC (Tbilisi, Georgia): *V. cholerae* O1 El Tor (V.ch890-M-878), *V. cholerae* O1 classical (V.ch 145-P-1); 4 strains from collection of Institute Pasteur (CIP): *V. cholerae* O1 classical (V.chCIP 55.91, V.chCIP 62.13), *V. cholerae* O1 El Tor (V.chCIP 106855), *V. cholerae* O139 (V.chCIP104151); 846 environmental *V. cholerae* isolates collected from the aquatic environments of Georgia (Eliava IBMV collection).

Bacteriophages: 82 primary phage isolates specific to *V. cholerae* collected during the presented study in 2006-2009.

Sampling sites and time. Regular monitoring and sampling was performed in 2006-2009 at the 4 sites in the Black Sea coastal zone and in the three lakes in the vicinity of Tbilisi as described earlier [13,14].

Isolation of phages from water samples was done by standard enrichment technique [2,15] using selected *V. cholerae* strains. The primary lysates revealing lytic activity against sensitive bacteria were processed for obtaining phage pure lines, their propagation and concentration.

Isolation of phages from bacterial cultures was done according to the Fisk's method [1,2].

Phage titre was estimated using double layer method [1,2] by number of developed phage negative plaques (NP).

Host range of phages was determined by spot test technique [19] against the set of bacterial strains. Phage suspensions with $1x10^8$ plaque forming units per millilitre (pfu/ml) were spotted onto the lines or lawns of test cultures on solid media and different lytic reactions were recorded after 18-24 hours incubation at 37^0 C.

Stability of phages was studied in different media and solutions (see above), also in the autoclaved Lisi lake water (LLW) and the Black Sea water (BSW). Sensitivity to chloroform was tested in the mixture LBB+chloroform. The phages with the initial titre $1x10^7$ pfu/ml were inoculated in the test solutions and kept at the temperature 4^0C in the dark with periodic (24 hours, 1 week, 2 weeks, 1 month, 3 months, and 6 months) check of phage titre.

Influence of temperature on phage viability was studied according to [2]. Phage suspensions in the saline with the final concentration of $1x10^8$ pfu/ml were incubated at

selected temperatures (55^{0}C, 60^{0}C, 65^{0}C, 70^{0}C) with different exposure time (10, 20, 30 min) and the samples were studied for number of viable phage particles by double layer method. For each temperature and phage velocity constant was determined.

Influence of concentration of Hydrogen ions (pH) on phages was studied through incubation in the buffer SM adjusted to different pH values (pH=1,4,7,9,12). Phages with initial concentration 1x10^8 pfu/ml were exposed to test solutions for 30 and 60 minutes at 37°C with following determination of phage titer.

Influence of UV-irradiation on phage was studied according to [2]. Phage suspension with a final concentration of 1x10^8 pfu/ml in saline was exposed to UV short waves (254nm) in Petri dish with a distance of 30-35cm in a dark condition. Phage survivability was studied after different exposure times (30, 60, 90 seconds).

Influence of ionic strength and osmotic shock on phages was studied by a Anderson's standard method [1,2]. Phages with the concentration of 1x10^8 pfu/ml were added to a 3,5 M sodium chloride (NaCl) solution, equilibrated for 15 minutes at 37°C and number of viable phage particles was determined. 0,1 ml of phage suspension in 3,5M NaCl was quickly inoculated into a 10 ml of distilled water with following immediate measuring of viable phage particles.

Influence of Sodium citrate (SC- $C_6H_5Na_3O_7$) on phage viability was studied to determine the requirement of phages in Ca^{++} ions [2]. Test phages were diluted in 0.1-2% SC solutions, incubated at 37°C for 15 min and the amount of developed phage NP's was determined.

Data analysis. All measurements were carried out in triplicate for each sample. The numbers were averaged to calculate a mean value and a standard error using Windows Excel descriptive statistics program. Phylogenetic trees were constructed using FreeTree software [12] based on a Distance-matrix method such as Unweighted Pair Group Method with Arithmetic Mean (UPGMA)-based approach and phylogenetic tree visualization software (TreeView) [21].

Results and their discussion. In total, 71 primary phage isolates specific to *V. cholerae* were obtained through enrichment of 882 samples collected in 2006-2009 at the 4 sites (Chorokhi estuary, Green Cape, Supsa estuary, Batumi Boulevard) of Black Sea coastal zone of Georgia and in the inland reservoirs around Tbilisi (Kumisi lake, Lisi lake and Tbilisi Sea, also a few samples of river Mtkvari). The average rate for *V. cholerae* phage isolation comprised 8% to 15%, calculated as ratio of samples positive for phage content to total number of samples. Like many other autochthonous bacteria in the aquatic environment, *V. cholerae* was supposed to have considerably low phage potential. For example, out of twenty-four lake water samples enriched for *V. cholerae* in July 2009 only five samples appeared to be the positive for the phage isolation. The intensity of *V. cholerae* phage isolation was varying by season. The majority of *V. cholerae* -specific phages (64.6%) were isolated from the fresh and brackish water lakes around Tbilisi that is not surprising since the host bacterium- *V. cholerae* was shown to be abundant in these water bodies [13]. *V. cholerae* phages were less frequently isolated from the Black Sea water: marine isolates comprised 22% of all phage isolates. Several *V. cholerae* phages were obtained from filtrates of *V. cholerae* strains. The most of *V. cholerae* O1-specific phages were obtained from brackish water lake Kumisi (Fig. 1). Interestingly, phages specific to *V. cholerae* non-O1 were most often isolated in the late spring- early summer months (from April to June), while the isolation rate for *V. cholerae* O1-specific phages was higher in the period of July-September (Fig. 2).

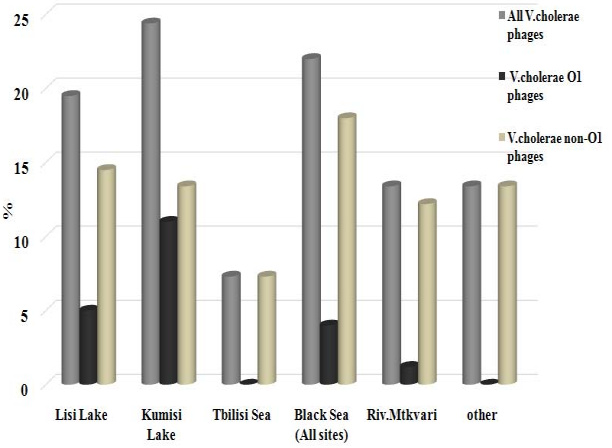

Fig.1. Distribution of V. cholerae phages by isolation sites

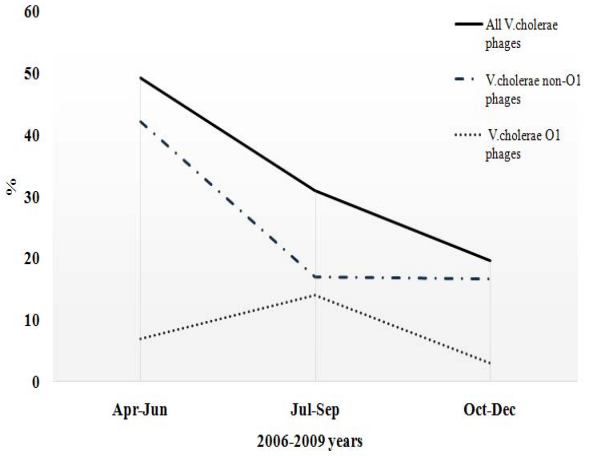

Fig.2. Isolation dynamics of V. cholerae phages by season

Host range of phages

All primary phage isolates (82) were processed for further cloning and series of screenings for antibacterial activity.

For the next step of investigations 23 different *V. cholerae* –specific phages were selected, among them all 19 phages specific to *V. cholerae* O1 and 4 phages specific to *V. cholerae* non-O1. The collected phages were investigated for specificity to the host bacteria and lytic spectrum within the constructed set of isolates of the same species. Spot test was done on large set of *V. cholerae* strains from EIBMV collection (60 strains of *V. cholerae* O1 and 75 - *V. cholerae* non-O1/ non O139). In total, 58% of *V. cholerae* strains appeared to be susceptible to phages. Tested 85% of *V. cholerae* O1 strains and 40% of *V. cholerae* non-O1 strains were lysed by at least one of the tested phages. Nine phages showed a narrow host range with activity against only their host strains. The rest (14 phages) *V. cholerae* O1-specific phages showed considerably wide spectrum of lytic activity lysing more than 2 strains simultaneously. Some of the *V. cholerae* O1-specific phages showed similar lytic profiles.

The relatedness between phages was estimated based on the results of lytic activity using UPGMA-based approach (Fig. 3). On the phylogenetic tree phages specific to *V. cholerae* non-O1 (Vch105, Vch105S, VchBS3) have been placed within one small cluster while another *V. cholerae* non-O1 specific phage- Vch10CH was most clearly separated. As to *V. cholerae* O1- specific phages, two groups have obtained: i) the larger cluster consisting of 14 *V. cholerae* O1-specific phages and ii) the small group of nearly identical 5 phages specific to *V. cholerae* O1 (VchK1, VchK3, Vch801, Vch802, and Vch803).

Based on the obtained results for the phage lytic spectrum, as well as virion morphology and DNA restriction profiles (detailed data not shown in this paper), we can conclude that *V. cholerae* - specific phages express significant diversity.

Here we present results of the studies on characterization of selected 3 phages of *V. cholerae* non-O1 and 6 phages of *V. cholerae* O1. The summarized basic properties of these phages (based partly on previous results) are present in the Table 1. The selected phages have been further studied for a number of biological features such as stability in different solutions and at different temperatures, pH- and UV- sensitivity, influence of high ionic strength and sodium citrate.

Stability of phages

Five different *V. cholerae* phages were selected for stability testing. *V. cholerae* O1-specific phages demonstrated high stability observed over six months of storage in majority of media and solutions, including natural water- LLW and BSW. One log decrease in viable phage counts was registered for *V. cholerae* O1-specific phages in 6 months, while in case of *V. cholerae* non-O1 phages 1 log reduction was observed after one month and 2 logs -after 6 months. Some *V. cholerae* O1-specific phages (Vch16-O1, Vch20-4805) revealed less stability in saline, decrease of viability up to 2 logs occurred in 6 months. In general, *V. cholerae* O1 -specific phages maintained their stability in tested solutions longer than those specific to non-O1 *V. cholerae* (Table 2).

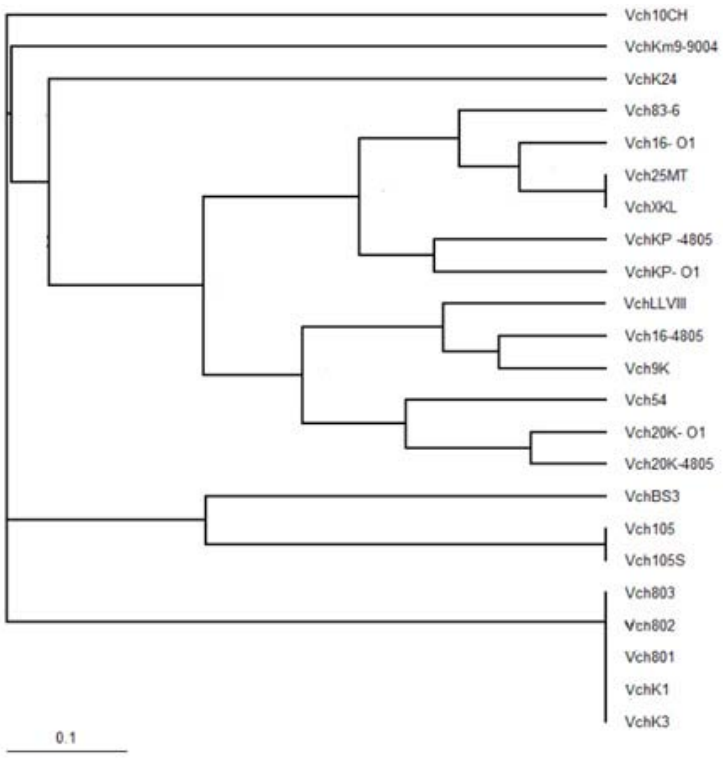

Fig. 3.UPGMA Nei-Li Distance bootstrap analysis map based on lytic activity of V. cholerae –specific phages

Table 1. Basic properties of selected phages specific to V. cholerae O1 and non-O1

Phages		Host Strain	Isolation Site/Date	Morphology	Sencitivity of phage DNA for restriction endonucleases
V.cholerae non-O1	Vch 105	V.ch L105	Lisi Lake/Oct2006	Myoviridae	EcoR I, EcoR V, BamH I, Not I
	VchBS3	V.ch L89	Chorokhi estuary/Jul2008	Siphoviridae	BamH I
	Vch10CH	V.ch L469	Kumisi Lake/Sept2007	Myoviridae	EcoR I
V.cholerae O1	Vch16K-4805	V.chCIP 62.13	Chorokhi estuary/Sept2007	Podoviridae	EcoR I, EcoR V
	Vch20K-4805	V.chCIP 62.13	Chorokhi estuary/Sept2007	Podoviridae	EcoR I, EcoR V
	Vch54	V.ch 145-P-1	Kumisi Lake/Jun2007	Myoviridae	EcoR I, EcoR V
	Vch9K	V.ch 145-P-1	Kumisi Lake/Aug2008	Podoviridae	EcoR I, EcoR V, BamH I
	Vch16K-O1	V.ch 145-P-1	Chorokhi estuary/Sept2007	Podoviridae	EcoR I, EcoR V
	Vch20K-O1	V.ch 145-P-1	Chorokhi estuary/Sept2007	Podoviridae	EcoR I, EcoR V

Table 2. Viability of V. cholerae –specific phages in different media and solutions

Phages	Storage time	LB Broth	LB+MgSO4	LB + 2%NaCl	PBS pH7.4	BPW pH7	PW pH7.8	Saline	SM-Buffer	LLW	BSW	LB+Cacl2	TiN6	TiN8	LB+Chloroform
Vch54	24 Hours	$2,7x10^7$	$1,7x10^7$	$2,1x10^7$	$1,4x10^7$	$1,9x10^7$	$2x10^7$	$1,7x10^7$	$2,1x10^7$	$2,2x10^7$	$2,5x10^7$	$1,8x10^7$	$1,6x10^7$	$1,8x10^7$	$1,6x10^7$
Vch16K-O1		$8x10^7$	$8x10^7$	$7,5x10^7$	$7x10^7$	$6,5x10^7$	$6x10^7$	$6x10^7$	$4x10^7$	$6x10^7$	$6x10^7$	$7x10^7$	$8x10^7$	$7,5x10^7$	$6x10^7$
Vch20K-4805		$8x10^7$	$7x10^7$	$6x10^7$	$6x10^7$	$5x10^7$	$5x10^7$	$6x10^7$	$7x10^7$	$7x10^7$	$7,5x10^7$	$8x10^7$	$7x10^7$	$7x10^7$	$7,5x10^7$
Vch105		$5x10^7$	$4x10^7$	$3x10^7$	$2x10^7$	$4x10^7$	$3x10^7$	$6x10^7$	$5x10^7$	$2x10^7$	$2x10^7$	$1x10^7$	$1x10^7$	$1x10^7$	$2x10^7$
VchBS3		$6x10^7$	$5x10^7$	$8,6x10^7$	$3,2x10^7$	$9x10^7$	$6x10^7$	$7x10^7$	$5,5x10^7$	$5,5x10^7$	$8,2x10^7$	$8,3x10^7$	$8x10^7$	$9x10^7$	$7x10^7$
Vch54	1 week	$2,2x10^7$	$1,4x10^7$	$1,8x10^7$	$1,7x10^7$	$1,8x10^7$	$1,8x10^7$	$1,8x10^7$	$2x10^7$	$1,7x10^7$	$1,9x10^7$	$1,9x10^7$	$1,9x10^7$	$1,8x10^7$	$1,9x10^7$
Vch16K-O1		$5,6x10^7$	$4x10^7$	$6,8x10^7$	$5x10^7$	$6,4x10^7$	$6x10^7$	$6x10^7$	$4x10^7$	$6x10^7$	$6x10^7$	$6x10^7$	$7x10^7$	$7x10^7$	$6x10^7$
Vch20K-4805		$8x10^7$	$8x10^7$	$7x10^7$	$7x10^7$	$6x10^7$	$6x10^7$	$6x10^7$	$6,8x10^7$	$7x10^7$	$7x10^7$	$7,5x10^7$	$7x10^7$	$7x10^7$	$7x10^7$
Vch105		$5x10^7$	$3x10^7$	$3x10^7$	$2x10^7$	$2x10^7$	$3x10^7$	$6x10^7$	$5x10^7$	$2x10^7$	$1x10^7$	$1x10^7$	$1x10^7$	$1x10^7$	$2x10^7$
VchBS3		$6x10^7$	$4,5x10^7$	$7,5x10^7$	$3x10^7$	$8x10^7$	$6x10^7$	$6x10^7$	$5x10^7$	$5x10^7$	$8x10^7$	$8x10^7$	$7x10^7$	$9x10^7$	$7x10^7$
Vch54	2 weeks	$2,1x10^7$	$1,5x10^7$	$2x10^7$	$1,3x10^7$	$1,6x10^7$	$1,6x10^7$	$1,7x10^7$	$2x10^7$	$1,6x10^7$	$1,9x10^7$	$1,7x10^7$	$1,5x10^7$	$1,7x10^7$	$1,9x10^7$
Vch16K-O1		$4x10^7$	$3,5x10^7$	$4x10^7$	$4x10^7$	$3,8x10^7$	$4,3x10^7$	$4x10^7$	$3,7x10^7$	$4x10^7$	$4,2x10^7$	$4,5x10^7$	$5x10^7$	$4,7x10^7$	$4x10^7$
Vch20K-4805		$6x10^7$	$6x10^7$	$5x10^7$	$5,5x10^7$	$5x10^7$	$5x10^7$	$4x10^7$	$5x10^7$	$5x10^7$	$5x10^7$	$6x10^7$	$5,5x10^7$	$5,5x10^7$	$5x10^7$
Vch105		$5x10^7$	$3x10^7$	$3x10^7$	$2x10^7$	$1x10^7$	$3x10^7$	$6x10^7$	$5x10^7$	$1x10^7$	$1x10^7$	$1x10^7$	$1x10^7$	$1x10^7$	$1x10^7$
VchBS3		$6x10^7$	$4x10^7$	$7x10^7$	$2x10^7$	$5x10^7$	$5x10^7$	$4,5x10^7$	$4,5x10^7$	$5x10^7$	$8x10^7$	$7,5x10^7$	$7x10^7$	$4x10^7$	$4x10^7$
Vch54	1 month	$2x10^7$	$1,2x10^7$	$2x10^7$	$1,1x10^7$	$1,5x10^7$	$1,5x10^7$	$1,7x10^7$	$2x10^7$	$1,6x10^7$	$1,8x10^7$	$1,7x10^7$	$1,4x10^7$	$1,7x10^7$	$1,8x10^7$
Vch16K-O1		$4x10^7$	$3x10^7$	$4x10^7$	$4x10^7$	$3x10^7$	$4x10^7$	$3,4x10^7$	$3x10^7$	$4x10^7$	$4x10^7$	$4x10^7$	$4x10^7$	$4x10^7$	$4x10^7$
Vch20K-4805		$2x10^7$	$2,5x10^7$	$2x10^7$	$2,2x10^7$	$2,3x10^7$	$4x10^7$	$4x10^7$	$5x10^7$	$5x10^7$	$5x10^7$	$5,5x10^7$	$5x10^7$	$5x10^7$	$4x10^7$
Vch105		$2x10^7$	$1x10^7$	$2x10^6$	$9x10^6$	$1x10^7$	$1x10^7$	$4x10^7$	$1x10^7$	$6x10^6$	$4x10^6$	$8x10^6$	$4x10^6$	$2x10^6$	$1x10^7$
VchBS3		$3,2x10^7$	$3,2x10^7$	$4x10^7$	$2x10^7$	$4x10^7$	$3x10^7$	$3x10^7$	$3,5x10^7$	$5x10^7$	$5x10^7$	$3,2x10^7$	$2x10^7$	$2x10^7$	$1,5x10^7$
Vch54	3 months	$1x10^7$	$1x10^7$	$1,5x10^7$	$1x10^7$	$1x10^7$	$1,3x10^7$	$1,5x10^7$	$1,5x10^7$	$1,3x10^7$	$1,3x10^7$	$1,2x10^7$	$1,1x10^7$	$1,2x10^7$	$1,5x10^7$
Vch16K-O1		$3x10^7$	$2,8x10^7$	$2x10^7$	$2,2x10^7$	$2x10^7$	$3x10^7$	$3x10^6$	$2,2x10^7$	$2x10^7$	$3x10^7$	$4x10^7$	$4x10^7$	$4x10^7$	$4x10^7$
Vch20K-4805		$1x10^7$	$1x10^7$	$1x10^7$	$1,5x10^7$	$1,6x10^7$	$2,5x10^7$	$3x10^6$	$4x10^7$	$5x10^7$	$5x10^7$	$5,5x10^7$	$5,5x10^7$	$4,5x10^7$	$6x10^7$
Vch105		$9x10^6$	$8x10^6$	$1x10^6$	$4x10^6$	$6x10^6$	$7.4x10^6$	$8x10^5$	$7x10^6$	$2x10^6$	$2x10^6$	$4x10^6$	$3x10^6$	$1x10^6$	$8x10^6$
VchBS3		$6x10^6$	$9x10^6$	$8x10^6$	$2x10^6$	$3x10^6$	$4x10^6$	$3x10^6$	$4x10^6$	$5x10^6$	$5x10^6$	$2x10^6$	$6x10^6$	$2x10^6$	$2x10^6$
Vch54	6 months	$2,4x10^6$	$2,1x10^6$	$2,2x10^6$	$1,8x10^6$	$1x10^6$	$2x10^6$	$1,5x10^5$	$1,9x10^5$	$2x10^5$	$2x10^5$	$1,8x10^6$	$1,7x10^6$	$1,8x10^6$	$2x10^6$
Vch16K-O1		$7x10^6$	$6,8x10^6$	$5,8x10^6$	$6x10^6$	$5,4x10^6$	$5,2x10^5$	$8x10^5$	$7x10^6$	$7,2x10^5$	$7x10^6$	$6,8x10^6$	$6x10^6$	$5,4x10^6$	$6,2x10^6$
Vch20K-4805		$5,6x10^6$	$5x10^6$	$5x10^6$	$4,8x10^6$	$5,4x10^6$	$4x10^6$	$4,8x10^5$	$5,8x10^6$	$4,8x10^6$	$5,2x10^6$	$5x10^6$	$5x10^6$	$4x10^6$	$4,5x10^6$
Vch105		$2x10^5$	$1x10^5$	$6x10^5$	$1x10^6$	$1x10^6$	$8x10^5$	$3x10^5$	$1x10^5$	$2x10^5$	$1x10^5$	$1x10^5$	$1x10^5$	$1x10^5$	$1x10^6$
VchBS3		$1x10^5$	$1x10^5$	$5x10^5$	$1x10^5$	$5x10^5$	$1x10^5$	$1x10^5$	$1x10^5$	$1x10^5$	$3x10^5$	$4x10^5$	$1x10^5$	$1x10^5$	$1x10^5$

** - The highlighted sections indicate decrease of viable counts by 1 log (light gray) and 2 logs (dark gray). The presented numbers are mean values of the parallel measurements*

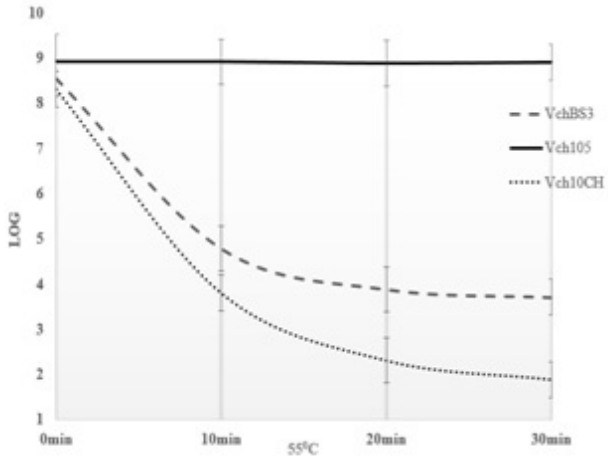

Fig. 4. Survival rate of V. cholerae non-O1-specific phages at 55^{0}C

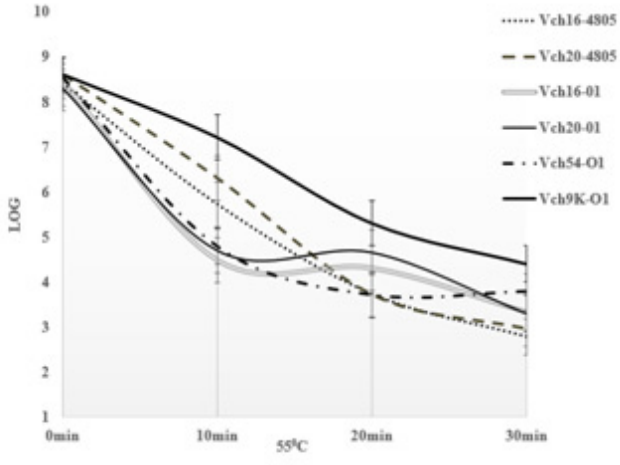

Fig.5. Survival rate of V. cholerae O1-specific phages at 55^{0}C

Influence of temperature on phage viability

Selected phages specific to *V. cholerae* non-O1 and *V. cholerae* O1 strains were subjected to changing temperatures (from 55^{0}C to 70^{0}C). The velocity constant (K) values for the heat inactivation of these phages were varying according to changing temperature and exposure time. For great majority of phages (except Vch105) a high susceptibility to temperature increase was revealed, expressed in the decrease of viable phage counts by >90% at 55^{0}C during 10 minutes (Fig.4,5). Experiments indicated that exposure in <30 minutes at 60^{0}C resulted in almost total inactivation of *V. cholerae* non-O1-specific phage Vch10CH. The same was observed for phages specific to *V. cholerae* O1 (Vch20K-4805, Vch20K-O1, and Vch16K-O1) at 65^{0}C, and for phage specific to *V. cholerae* non-O1 (VchBS3) at 70^0, correspondingly. The phage Vch105 specific to non-O1 *V. cholerae* appeared to be the most thermotolerant, for which significant decrease of viable particles by 90% appeared only at 70^{0}C during10 minutes of exposure.

Influence of concentration of Hydrogen ions (pH) on phages was studied in the wide range of pH values (1 to 12) with estimation of phage infectivity after 30 and 60 minutes of exposure. *V. cholerae* non-O1-specific phages showed higher resistance in the pH range 4 -9. For *V. cholerae* O1-specific phages up to 2 logs decrease in viable counts was registered after 10 minutes of exposure at pH values 4, 9 and 12. The full inactivation (survival rate <1%) was observed for all phages at pH1. Only two of *V. cholerae* non-O1-specific phages- Vch105 and Vch10CH retained their activity after 30 and 60 minutes exposure at pH values ranging from 4-12. Thus, *V. cholerae* O1 phages appeared to be less resistant to both alkaline and acidic pH than *V. cholerae* non-O1- specific phages (Table 3).

Influence of UV-irradiation on phage viability was estimated as viable phage counts for each UV length and exposure time compared to those of the control group. It was shown that lytic activity was decreasing with increased exposure to UV. Especially clear downward of lytic activity trend was seen in groups exposed for 60 and 90 seconds trials. Surprisingly *V. cholerae* non-O1-specific phages were more sensitive to UV than *V. cholerae* O1 specific ones. The percentage of survived phage counts were taken and plotted as a line graph in order to show the trend of the data as the phage was exposed for different times to the UV (Fig. 6,7).

Table 3. Viability of V. cholerae –specific phages at different pH values

Phages		pH1		pH4		pH7 (Control group)		pH9		pH12	
		30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min
V.cholerae non-O1	VchBS3	2±0.1	2±0.1	8.6±0.6	8.3±0.4	8.6±0.6	8.6±0.5	8.5±0.5	8±0.4	7.8±0.4	6±0.3
	Vch105	2±0.1	2±0.1	8.6±0.5	8.3±0.5	8.8±0.5	8.8±0.6	8.7±0.4	8.3±0.5	8.6±0.5	8.5±0.4
	Vch10CH	2±0.1	2±0.1	8.5±0.6	8±0.4	8.9±0.6	8.9±0.5	8.7±0.4	8.6±0.4	8.6±0.5	8.5±0.4
V.cholerae O1	Vch16-4805	4.5±0.2	3±0.1	7.6±0.5	7.3±0.5	8.3±0.6	8.3±0.4	7.7±0.3	7.3±0.4	7.6±0.3	7.1±0.3
	Vch20-4805	2.8±0.2	2.7±0.2	7.9±0.5	7.7±0.5	8.7±0.5	8.7±0.4	7.5±0.4	7.5±0.4	7.5±0.4	7.3±0.3
	Vch16-O1	2.3±0.1	2±0.1	7.8±0.5	7.3±0.5	8.3±0.6	8.3±0.5	7.5±0.4	7.1±0.4	7.6±0.3	6.9±0.4
	Vch20-O1	3.6±0.2	3±0.2	7.6±0.5	7.2±0.5	8.3±0.6	8.3±0.4	7.7±0.4	6.8±0.3	7.6±0.4	7.8±0.4
	Vch54	3±0.2	2±0.1	6.9±0.4	6.7±0.4	8.5±0.5	8.5±0.6	7.3±0.4	7.1±0.3	7.3±0.4	7±0.4
	Vch9K	5±0.3	3.5±0.2	7.7±0.4	7±0.5	8.8±0.6	8.8±0.5	7.9±0.4	7.7±0.4	7.6±0.4	7.5±0.4

** - The viable numbers are given as logs of PFU*

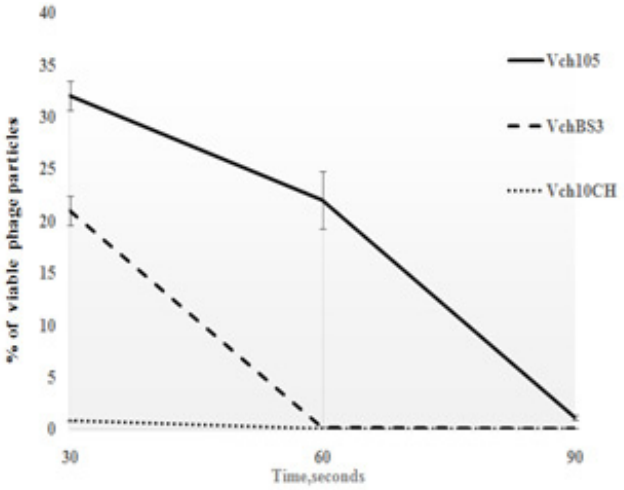

Fig. 6. Survival rate of V. cholerae non-O1-specific phages after UV-irradiation

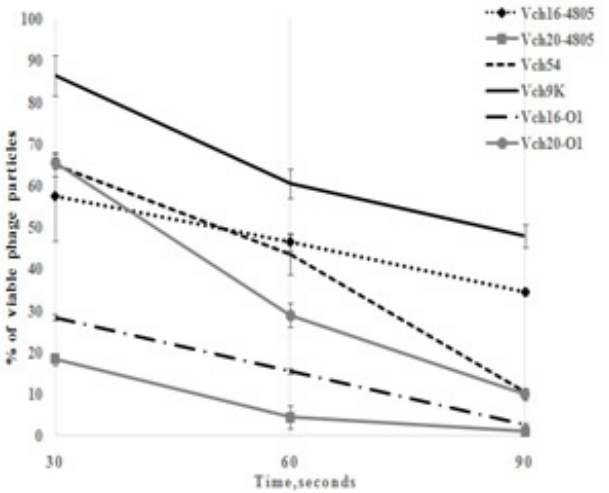

Fig. 7. Survival rate of V. cholerae O1-specific phages after UV-irradiation

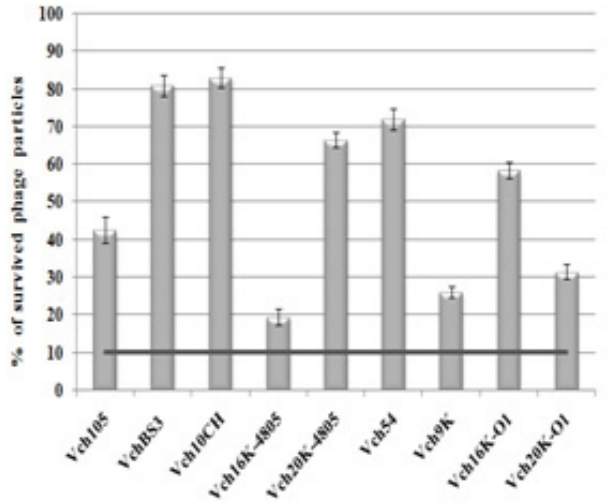

Fig. 8.Effect of osmotic shock on V. cholerae-specific phages

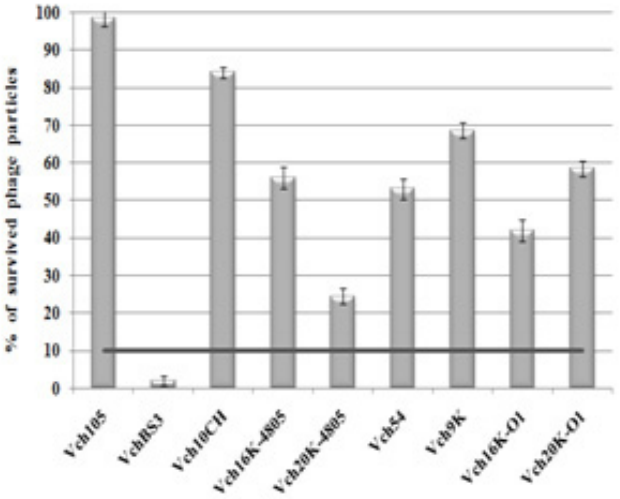

Fig. 9. Effect of SC on V. cholerae-specific phages

Susceptibility of phages to high ionic strength and osmotic shock. Tested phages specific to *V. cholerae* non-O1 and *V. cholerae* O1 revealed low to moderate resistance to a deactivating factor such as osmotic shock. One log decrease of phage count was lined as a threshold (Fig.8). It can be said that *V. cholerae* non-O1-specific phages were capable to maintain their resistance more than those of *V. cholerae* O1. In particular, decreased survival rates- 18, 25, 30% were registered for *V. cholerae* O1 phages: Vch16-4805, Vch9K, Vch20-O1, while 79, 40, 85% survived in case of non-O1-specific phages:VchBS3, Vch105, Vch10CH, correspondingly.

Susceptibility of phages to Sodium Citrate (SC). Effect of 2% SC was observed only in case of the phage specific to *V. cholerae* non-O1 -VchBS3 with the survival rate equal to a 1%. This may indicate that there is a high demand for Ca^{++} ions as an adsorption cofactor for inactivated phage. As to other *V. cholerae* non-O1-specific phages no decrease in viable phage count was registered, for Vch105, and only a minor titer change for phage Vch10CH (survival rate 83%). Phages specific to *V. cholerae* O1 showed low to moderate resistance to SC. The obtained results are given in the Fig.9. Percentage rates of vibriophages are plotted as a bar graphs and one log decrease of phage counts is lined as a threshold.

Summarizing above presented data, we can conclude that *V. cholerae*- specific phages collected in Georgian aquatic environments express significant diversity by basic biological features with obvious difference between phages specific to epidemic and non-epidemic serotypes of *V. cholerae***.**

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REFERENCES

1. Адамс М. Бактериофаги. М.: изд-во «Иностранная Литература»;1961.
2. Габрилович И.М. Основы бактериофагии. Минск: «Вишейшая школа»; 1973.
3. Barua D. History of cholera, In: D. Barua, and W. B.Greenough (ed.), Cholera. Plenum Publishing Co. New York: 1992; 1-36.

4. Baudoux A.-C., Hendrix R. W., Lander G. C., Podell S., Paillard C., Johnson J. E., et al. Genomic and functional analysis of Vibrio phage SIO-2 reveals novel insights into ecology and evolution of marine siphoviruses. Environ. Microbiol. 2012; 14(8):2071–2086.
5. Chakrabarti A.K., Ghosh A.N, Balakrish Nair G., Niyogi S.K., Bhattacharya S.K., and Sarkar B.L.. Development and Evaluation of a Phage Typing Scheme for Vibrio cholerae O139. J. Clin. MicroBiol. 2000; 1(38):44–49.
6. Chattopadhyay D. J., Sarkar B. L., Ansari M. Q., ChakrabartiB. K., Roy M. K., Ghosh A. N., and. Pal S. C. New Phage Typing Scheme for Vibrio cholerae O1 Biotype El Tor Strains. J. Clin. Microb. 1993;6 (31): 1579-1585.
7. Faruque Shah M., Iftekhar Naser Bin, Islam M. Johirul, Faruque A.S.G., Ghosh A.N., Nair G. Balakrish, Sack David A., and Mekalanos John J. Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. PNAS 2005; 5(102):1702–1707.
8. Faruque Shah M., Mekalanos John J. Phage-bacterial interactions in the evolution of toxigenic Vibrio cholerae. Virulence. 2012; 3(7): 556–565.
9. Fazil MH, Singh DV. Vibrio cholerae infection, novel drug targets and phage therapy. Future Microbiol. 2011; 6(10):1199-208.
10. Grim C., Jaiani E., Whitehouse C., Janelidze N., Kokashvili T., Tediashvili M., Colwell R.R., Huq A.. Detection of toxgenic vibrio cholerae 01 from freshwater environments in the Former Soviet Republic of Georgia: a potential source of disease. Environ. Microbiol. Reports 2010; 2(1):2-6.
11. Huq, A., Ch. Grim., R. R. Colwell., Nair G. Balakrish. Detection, Isolation and Identification of Vibrio cholerae from the Environment. Current protocols in microbiology. 2006. chapter 6.Unit 6 A.5.
12. IJSEM. International Journal of Systematic and Evolutionary Microbiology. FreeTree application program (Freetree.exe). http://ijs.sgmjournals.org/content/51/3/731/suppl/DC1. Page last updated 2001.
13. Jaiani E., Kokashvili T., Mitaishvili N., Elbakidze T., Janelidze N., Lashkhi N., Kalandadze R., Mikashavidze E., Natroshvili G., Whitehouse C.A., Huq A., Tediashvili M.. "Microbial water quality of recreational lakes near Tbilisi, Georgia J. Water and Health 2013: 2(11):333-345.
14. Janelidze N., Jaiani E., Lashkhi N., Tskhvediani A., Kokashvili T., Gvarishvili T., Jgenti D.,Mikashavidze E., Diasamidze R., Narodny S., Obiso R.,Whitehouse C.A., Huq A., Tediashvili M. Water microbial quality of the Georgian coastal zone of the Black Sea. Mar. Poll. Bull. 2011; 62(3):573-80.
15. Martha R.J. Clokie, Andrew M. Kropinski. Bacteriophages Methods and Protocols, Volume1: Isolation, Characterization and Interactions.Methods in Molecular Biology 2009; 501: 2009.
16. Onishenko G.G., Ganin V.C., Golubinskiy E.P. (ed). Non-O1 Vibrios and their importance in humane pathogeneses. Moscow: 2001; 384.
17. Sambrook Joseph, Fritsch E.F., Maniatis Tom. Molecular cloning: A Laboratory manual. Second Edition. New York: Cold spring harbor laboratory press: 1989 (7.58).
18. Sulakvelide A., Alavidze Z., Morris J. G, JR. Bacteriophage Therapy. Antimicrobial Agents and Chemotherapy 2001; 3(45): 649–659.
19. Tediashvili M.I., Nikolskaia I.I., Chanishvili T.G., Debov S.S.. Search for host specificity systems in Shigella using DD-series phages. Zhurnal Mikrobiologii, Epidemiologii, I Immunobiologii 1979; (5):78-83.
20. Telesphore Sime-Ngando. Environmental bacteriophages: viruses of microbes in aquatic ecosystems. Front Microbiol. 2014; (5):355.
21. TreeView X. http://darwin.zoology.gla.ac.uk/~rpage/treeviewx/history.html. Page last updated 2005.
22. World Health Organization. Global Alert and Response (GAR). Cholera. http://www.who.int/csr/don/archive/disease/cholera/en/. Page last updated 2014.
23. Zahid M. Shamim Hasan, Udden S. M. Nashir, Faruque A. S. G., Calderwood Stephen B., Mekalanos John J., Faruque Shah M. Effect of Phage on the Infectivity of *Vibrio cholerae* and Emergence of Genetic Variants. Infect Immun. 2008; 76 (11):5266–5273.

SUMMARY

BIOLOGICAL CHARACTERIZATION OF *V. CHOLERAE*-SPECIFIC BACTERIOPHAGES ISOLATED FROM WATER SOURCES IN GEORGIA

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Vibrio cholerae, a widely spread bacterium in various marine, fresh, and brackish water environments, can cause a devastating diarrheal disease - cholera and also mild forms of gastroenteritis. Bacterial viruses are natural controllers of bacterial population density in water systems. The goal of this study was to isolate and characterize *V. cholerae*-specific bacteriophages occurring in the Georgian coastal zone of the Black Sea and inland water reservoirs in the eastern part of Georgia. During 2006-2009, 71 phages lytic to *V. cholerae* were collected from these aquatic environments. The phage isolation rate was varying from 8% to 15%, depending on the sampling season and site, and the abundance of host bacteria. The majority of phages specific to *V. cholerae* were collected from freshwater sources. The phage isolation showed seasonal character covering warm period –from April to September. Based on basic characteristics of primary phage isolates (lytic spectrum, virion morphology and DNA restriction profiles) 23 *V. cholerae* –specific phages were selected for series of consecutive screenings. Comparatively wide spectrum of lytic activity was revealed in case of 14 phages specific to *V. cholerae* O1, and one phage - VchBS3, active against non-O1 *V. cholerae*. Three phages active against *V. cholerae* non-O1 and six *V. cholerae* O1 -specific phages have been

studied in detail for a number of biological features (stability in different solutions, temperature-, pH- and UV- sensitivity, influence of high ionic strength etc.), considered to be additional important characteristics for selection of phages with therapeutic potential.

Keywords: Cholera, *V. cholerae,* Bacteriophages, Biological properties.

РЕЗЮМЕ

БИОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА *V. cholerae*-СПЕЦИФИЧЕСКИХ БАКТЕРИОФАГОВ, ВЫДЕЛЕННЫХ ИЗ ВОДНОЙ СРЕДЫ ГРУЗИИ

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Бактерии вида *Vibrio cholerae* – возбудителя тяжелого диарейного заболевания холеры и легких форм гастроэнтерита широко распространены в различных морских, пресных и солоноватых водоемах. Бактериальные вирусы контролируют плотность бактериальных популяций в естественных условиях. Целью данного исследования явилось выделение и характеристика *V. cholerae*-специфических бактериофагов, распространенных в грузинской прибрежной зоне Черного моря и внутренних водоемах восточной части Грузии. В 2006-2009 гг. фаги (n=71), активные в отношении *V. Cholerae,* выделены путем обогащения 882 проб воды из водных систем Грузии. Частота выделения холерных фагов колебалась в пределах от 8 % до 15%, в зависимости от сезона и типа водоема. Большинство *V. Cholerae*-специфичных фагов были получены из пресноводных водоемов. Выделение фагов носило сезонный характер, охватывающий теплый период – начиная с апреля по сентябрь включительно. На основании первичной оценки основных фаговых характеристик (литический спектр, морфология вириона и профили рестрикции ДНК) 23 *V. cholerae* -специфических фагов были отобраны для последующих серий скрининга. Сравнительно широкий спектр литической активности выявлен в случае 14 фагов, специфичных для *V. cholerae* O1, и одного фага -VchBS3, активного в отношении не-O1 *V. cholerae.*

Три фага, специфичных к *V. cholerae* не-O1, и шесть фагов, специфичных к *V. cholerae* O1, отобраны и подробно характеризованы по ряду биологических свойств (стабильность в различных растворах, чувствительность к температуре, pH- и УФ-облучению, влияние высокой ионной силы), которые имеют определенное значение для отбора фагов с терапевтическим потенциалом.

რეზიუმე

საქართველოს წყლიანი გარემოდან გამოყოფილი *V. cholerae*-ს მიმართ სპეციფიკური ბაქტერიოფაგების ბიოლოგიური თვისებების შესწავლა

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Vibrio cholerae ზღვის,მტკნარ და მომლაშო წყლიან გარემოში ფართოდ გავრცელებული ბაქტერიაა, რომელიც იწვევს მძიმე დიარეულ დაავადებაქოლერას და, ასევე, მსუბუქი ფორმის გასტროენტერიტებს. წარმოდგენილი კვლევის ძირითადი მიზანი იყო *V. cholerae*–ს მიმართ სპეციფიკური ბაქტერიოფაგების გამოყოფა შავი ზღვის სანაპირო ზოლიდან და აღმოსავლეთ საქართველოს შიდა წყალსატევებიდან და მათი დახასიათება. 2006-2009 წლებში საკვლევი წყალსატევების გამოკვლეული ნიმუშებიდან გამოყოფილი იყო *V. cholerae*–ს მიმართ სპეციფიკური ბაქტერიოფაგების 71 პირველადი იზოლატი. გამოყოფის სიხშირე მერყეობდა 8%-დან 15%-მდე სინჯის აღების დროსა და ადგილზე დამოკიდებულებით. *V. cholerae*–ს მიმართ აქტიური ფაგების უმეტესობა შეგროვებული იყო მტკნარი წყალსატევებიდან. ფაგების გამოყოფა ატარებდა სეზონურ ხასიათს, გამოხატული ინტენსივობით თბილ პერიოდში - აპრილიდან სექტემბრამდე. თავდაპირველ ძირითად მახასიათებლებზე დაყრნობით (ლიზისური სპექტრი, ვირიონის მორფოლოგია, დნმ-ის რესტრიქციული პროფილი) შემდგომი დახასიათების მიზნით შერჩეული იყო *V. cholerae*–ს მიმართ სპეციფიკური 23 ბაქტერიოფაგი. ლიზისური აქტივობის შედარებით ფართო სპექტრი აღინიშნა *V. cholerae* O1–ის მიმართ აქტიური 14 ფაგის და *V. cholerae* არა-O1–ის მიმართ აქტიური ერთი ფაგის (VchBS3) შემთხვევაში.

ჩატარდა შერჩეული ფაგების (*V. cholerae* არა-O1-ის მიმართ აქტიური 3 ფაგი და *V. cholerae* O1-ის მიმართ სპეციფიკური 6 ფაგი) ისეთი მახასიათებლების კვლევა (ფაგების სტაბილობა სხვადასხვა არეში,მგრძნობელობა სხვადასხვა ტემპერატურის, წყალბად-იონთა და ულტრაიისფერი სხივების მიმართ,მაღალი იონური ძალის ზემოქმედება და სხვ.),რომელთაც,როგორც დამატებით მახასიათებლებს, გარკვეული მნიშვნელობა ენიჭება თერაპიული პოტენციალის მქონე ბაქტერიოფაგების შერჩევისას.

MYCOPLASMA PNEUMONIAE AND *CHLAMYDOPHILA PNEUMONIAE* IN HOSPITALIZED CHILDREN WITH BRONCHIOLITIS

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Respiratory tract infections (RTIs) are a major cause of morbidity and mortality worldwide. Acute RTI is most common in children under five years of age, and represents 30-50% of the pediatric medical admissions, as well as 20-40% of hospitalizations in children [7].

Bronchiolitis is an acute lower respiratory tract infection in early childhood caused by different viruses, with coughing, wheeze and poor nutrition as the major symptoms.

Although the term "bronchiolitis" refers to inflammation of the bronchioles, these findings are rarely observed directly, but inferred in young children who present with respiratory distress in association with signs of a viral infection. The guideline of the American Academy of Pediatrics defines bronchiolitis as "a constellation of clinical symptoms and signs including a viral upper respiratory prodrome followed by increased respiratory effort and wheezing in children less than 2 years of age" [21].

A substantial proportion of children experience at least one episode with bronchiolitis, and as much as 2-3% of all children are hospitalized with bronchiolitis during their first year of life. Bronchiolitis is the most common reason for children hospitalization in many countries, challenging both economy, area and staffing in pediatric departments [15].

The causes of bronchiolitis have been studied in different environments and populations. *Respiratory syncytial virus(RSV), human Metapneumovirus (hMPV), human Bocavirus (hBoV), human Rhinoviruses (hRV)* have consistently been shown to predominate, with some displaying strong seasonal peaks and co-infection with more than one viral pathogen occurring in 4–33% of children. RSV is the most common virus involved in children with bronchiolitis. In most studies it accounts for 60–80% of bronchiolitis cases in children under 12 months of age. In this age group *Rhinovirus* is the second most common virus (14-30%), thereafter *human bocavirus* (14-15%), *human metapneumovirus* (3-12%), *entero-, adeno-, corona* and *influenza* viruses (1-8%) [2,12,19,20].

A few studies, however, have attempted to determine whether other, particularly atypical pathogens *Mycoplasma Pneumoniae (Mpn)* and *Chlamydophila pneumoniae (Cpn),* which are frequently detected in older children and adults with asthma exacerbation, are associated with bronchiolitis in children under 2 years of age [4,8,9].

The aim of this study was to determine the prevalence and clinical features of atypical pathogens in a cohort of well-characterized children under the age of 2 years presenting to the Iashvili Central Children Hospital in Tbilisi with various severities and clinical manifestations of bronchiolitis.

Materials and methods. The Ethics Committee at Tbilisi State Medical University approved the study and informed consent was obtained from the parents prior to enrolment.

This study was a prospective and descriptive clinical study. The etiology and clinical features were evaluated in children between one month and two years of age, who were admitted to Iashvili Central Children Hospital with acute bronchiolitis from 2011 to 2012.

Demographic features of the patients were registered on a questionnaire form. Patients included in the study were those hospitalized and who suffered from acute bronchiolitis during the previous week before admission and who did not develop bronchiolitis during the hospital stay due to other reasons.

Bronchiolitis were stated in patients with a constellation of clinical symptoms and signs including a viral upper respiratory prodrome followed by fever, nasal discharge, dry, frequent cough, increased respiratory effort and wheezing in children less than 2 years of age [11,21].

Baseline observations including temperature, oxygen saturation, respiratory and pulse rates were recorded at the time of admission and daily thereafter in all participants. Total leukocyte counts with differential and C-reactive protein (CRP) levels were studied in all patients. Chest radiographs of all cases were evaluated by a pediatric radiologist.

Daily dyspnea score (Table 1) was assessed in all patients by using symptom score on a scale from 0 to 10 based on a clinical scoring system according to Kristj'ansson et al [10]. Children with dyspnea score from 0 to 3 were considered as a mild bronchiolitis, with score 4-6 as a moderate and with score 7-10 as a severe bronchiolitis.

The following investigations were performed within 24 hours of admission: full blood count, chest x-ray and pulse-oximetry.

Table 1. Dyspnea Score

Respiratory rate	**0**	**1**	**2**
	normal < 40/min	slightly increased 40 - 60/min	clearly increased > 60/min
Oxygen saturation	≥ 95% in room air	92-94% in room air	< 92% in room air, or need for supplemental oxygen
Wheezing	none	audible with stethoscope	audible without stethoscope
Retractions	none	mild-moderate	severe
General condition	not affected: alert/quietly sleeping	moderately affected: Irritable or agitated	severely affected: lethargic, poor feeding

Acute and convalescent serum samples were tested by ELISA for IgM and IgG antibodies to *RSV, Cpn and Mpn*. Positive results were defined by a significant antibody response in specific IgM or a 4-fold increase in IgG titer in paired serum samples. A positive infection was defined either in a single test or in paired sera taken 2-3 weeks apart.

Children ≤2 years of age with impaired general state or fever ≥38.3°C, extensive crepitations, extended sibilant rhonchi and prolonged expiration on auscultation, apnea, and dyspnea or tachypnea (respiratory rate ≥50 for infants 1-11 months and ≥40 for infants 12-24 months) who were hospitalized were eligible.

Infants with extended crepitations on auscultation and bronchopneumonic infiltration in posteroanterior lung X-ray without sibilant rhonchi and prolonged expiration were defined as pneumonia and not included in the study.

The data assessed and recorded on the study form included: date of the study initiated for the patient, age andsex of the patient, duration of symptoms, risk factors such as prematurity, low birth weight, history of previous bronchiolitis, presence of asthma, allergy and /or atopic dermatitis in parents and cigarette smoking in the family.

Children included in the study were divided into age groups of 0-6 months, 7-11 months, and 12-24 months.

The results have been analyzed by the SPSS Statistics versions 16.0. $p<0.05$ has been considered as significant difference.

Results and their discussion. Thirty sevenchildren under two years of age were studied. Their median (range) age was 11.86 month (age distribution from 3 to 23 months). Distribution of the patients according to their age is shown on Fig. 1.

In 19 patients out of 37 (51.35%) etiological diagnosis was established and in 18 patients (48.65%) no pathogens were found (Fig. 2).

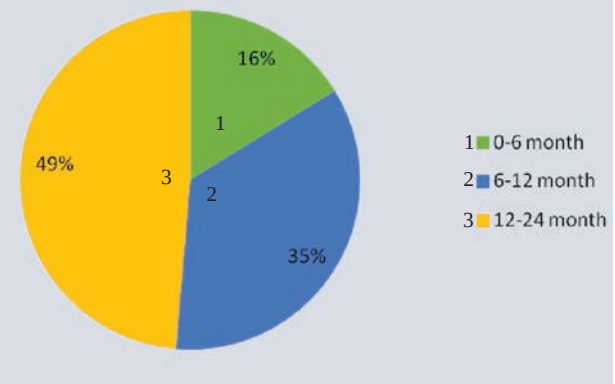

Fig. 1. Age distribution of the patients

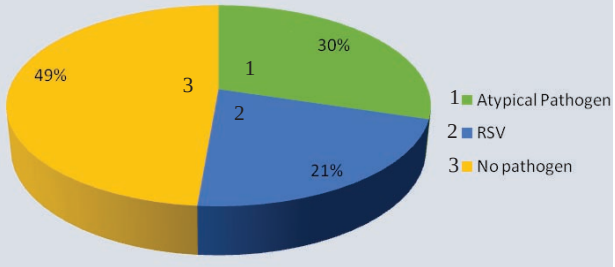

Fig. 2. Distribution of the pathogens

Patients were grouped according to pathogens in three groups: in group I was included patients with atypical pathogens - *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae;* in group II patients with *RSV*; in group III patients with mixed-infections with *Chlamydophila pneumoniae, Mycoplasma pneumoniae* and *Respiratory syncytial virus.*

Group I involved 11 patients, who had either *Chlamydophila pneumonia* or *Mycoplasma pneumonia* or their co infections. In this group the median age was 12.87 (age range from 6 to 23 months).

Group II involved 8 patients with *RSV,* their median age was 11.62 (age range from 5 to 19 months), who had either *RSV* or its coinfections with *Chlamydophila pneumoniae* or *Mycoplasma pneumoniae*.

Group III involved 7 patients, who had co infections of *Chlamydophila pneumoniae, Mycoplasma pneumoniae* and

RSV. Their median age was 10.28 (age range from 6 to 16 months). Co-infection with two or more pathogens was present in 7 samples (18.9%). Two pathogens were detected in 6 (16.2%) samples, three pathogens in 1 (2.7%) sample.

Median birth weight in the whole grope was 3147 grams (range from 2100 g to 4700 g), in atypical bacteria group 3136 (range from 2300 g to 3900 g), 3175g in RSV group (range from 2000 g to 4700 g) and 2900 g in mixed-infection group (range from 2600 g to 3600 g).

The median gestational age in the whole group was 38.21 weeks, in atypical pathogen group 38.0, in the RSV group 37.75 and in the mixed infection group 37.57.Twenty three (63%) of infants were male. Mother median age was 24.79 years. Sixty five percent of infants were exclusively breastfed for the first six months of life and 73.6% were ever exposed to household tobacco smoke. Thirty one percent of infants had parents with allergic disease (asthma, allergic rhinitis, atopic dermatitis).Sixty seven percent of infants had a history of prior wheezing (Table 2).

There was no significant difference in age between infants presenting with bronchiolitis associated with different pathogens, although in group II infants with RSV were youngest (11.62 vs. 12.87 in group I).

All infants in all groups had cough as the chief presenting complaint, followed closely by temperature, tachypnea and wheezing. Infants with RSV were more likely to have a prior history of wheezing than those with atypical pathogens (77.7% in RSV group vs. 54.5% in atypical pathogens group, p<0.05). Infants with RSV were more likely to be hospitalized in previous months (62.5% in RSV group vs. 36.4% in atypical pathogens group, p<0.05).

Overall, 57.9% (n = 11) of children had *mild* disease, 31.6% (n=6) *moderate* disease and 10.5% (n=2) *severe* disease. Children with RSV were more likely to have *moderate* and *severe* than *mild* disease (62.5% vs. 27.3%, p<0.05) compared to children without RSV infection, whilst children with *Chlamydophila pneumonia* and *Mycoplasma pneumonia* infection were more likely to have *mild* than *moderate* disease (72.7% vs. 37.3%, p=0.05).

Infants with RSV had higher bronchiolitis severity scores (Fig. 3) with a median of 4.89 vs. infants with atypical pathogens (median 3.37, p<0.05) and vs. infants with mixed-infections (median 3.57, p<0.05).

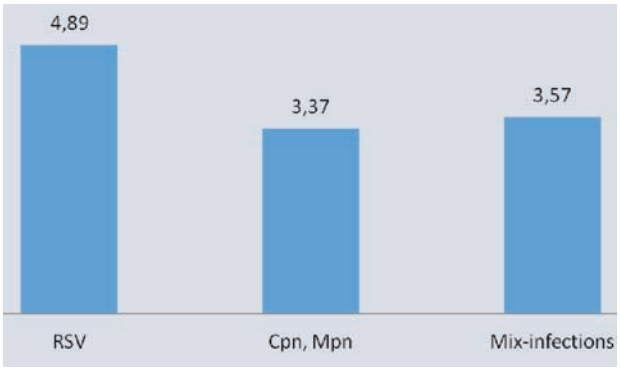

Fig. 3. Bronchiolitis severity score by pathogens

Mean duration of hospital stay for patients in atypical pathogen group was 6.90 days, in RSV group 6.5 days and in mix-infections group 5.33 days. The difference between the groups was not significant.

We were able to contact the parents of all patients after discharged from hospital for a telephone interview. Infants with RSV were most likely to have wheezing episodes after hospitalization to compare patients with atypical pathogens (average 2.65 in atypical pathogens group vs. 3.25 in RSV group, p<0.05).

Bronchiolitis is a leading cause of acute illness and hospitalization in young children. Bronchiolitis is an acute lower respiratory tract infection caused by different viruses, with coughing, wheezing and poor nutrition as the major symptoms. A substantial proportion of children will experience at least one episode with bronchiolitis, and as much as 2-3% of all children will be hospitalized with bronchiolitis dur-

Table 2. Baseline characteristics of the study population

Characteristics	Total n=37	Atypical bacteria n=11	RSV n=8	Not found n=18
Age (mo) Median	3-23 11.86	6-23 12.87	5-19 11.62	3-18 11.38
Birth weight Median	2000-4700 3147	2300-3900 3136	2000-4700 3175	2100-3650 3141
Gestational age Median	36-40 38.21	37-39 38	36-39 37.75	36-40 38.55
Breast feeding, N (%)	24 (65)	7 (64)	5 (63)	12 (66)
Atopic parent, N (%)	17 (44.7)	6 (54.5)	3 (37.5)	8 (44.4)
Self atopy, N (%)	12(32.4)	1 (9.1)	5 (62.5)	6 (33.3)
Passive smoker, N (%)	27 (72.9)	8 (72.7)	8 (100)	11 (61.1)

ing their first year of life [17, 18]. Bronchiolitis is the most common reason for hospitalization of children in many countries, challenging both economy, area and staffing in pediatric departments. *Respiratory syncytial virus* is the most common virus causing bronchiolitis, occurring in epidemics during winter months. Approximately, 75% of bronchiolitis cases occur in children younger than 1 year of age and 95% in children younger than 2 years of age, with the peak incidence at 2-8 months of age [1,12].

According to the data collected at our site, the most common pathogen in single infections was *RSV* (21.6%). This finding is consistent with several studies conducted in children younger than 2 years hospitalized due to acute bronchiolitis. The frequency of RSV positive LRTI was found as 17.5-66% in children hospitalized in developed countries and as 15.2-50% in developing countries [5,6,14].

Published literature states that *Chlamydophila pneumonia* and *Mycoplasma pneumonia* are well-recognized causes of community-acquired pneumonia in older children and adults, but less common causes in preschool children, although few studies have examined prevalence in this age group [3,8,17]. There are a few studies that investigated prevalence of atypical bacteria in children with bronchiolitis and in these studies the prevalence rate is 10-19.46% [3,10,16].Thus our findings that *Chlamydophila pneumoniae* and *Mycoplasma pneumonia* could be detected in approximately 29.7% of all children with bronchiolitis is important and has potential implications for the management, given that current treatment guidelines for bronchiolitis in this age group do not include macrolide antibiotics.

Our results showed that taking into account the various etiologies, the differences in clinical manifestations and severities were statistically significant. Children infected with *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* had less severe bronchiolitis than those infected with RSV. These findings are similar to the studies from Brazil [2] and USA [13], but contrast with one from Thailand [20], which showed that children infected with RSV had no more significantly severe disease to compare to other pathogens.

In accordance with previous studies [2] co-infection was not associated with the disease severity: mean severity score in atypical pathogen group was 3.37, in RSV group 4.87 and in the mixed infection group 3.57.

In summary, we have shown a high prevalence of *Chlamydophila pneumoniae* and *Mycoplasma pneumonia* in children under 2 years of age with bronchiolitis. Even without assessing the role played by 'typical' viruses, we have highlighted the complexity of viral/atypical bacterial epidemiology in bronchiolitis in this age group. Although detection of pathogens using more sophisticated methods (e.g. PCR) will provide much useful information, our data will also provide the bases for management challenges. Overcoming these challenges will be necessary to accurately direct resources,treatment and preventive strategies, particularly in the age group where bronchiolitis accounts for higher rate of morbidity and mortality.

REFERENCES

1. Ahmad SA, Mujawar Q, Othman MA, Salleh HB, Alsarfandi MA. Clinical profile of bronchiolitis in infants younger than 90 days in Saudi Arabia. J Emerg Trauma Shock. 2014; 7(1): 49–52.
2. Bezerra PGM, Britto MCA, Correia JB, Duarte MdC, Fonceca AM, Rose C, et al. Viral and Atypical Bacterial Detection in Acute Respiratory Infection in Children Under Five Years. 2011; PLoS ONE 6(4): e18928.
3. Brittain-Long R, Nord S, Olofsson S, Westin J, Anderson L, Lindh M. Multiplex real-time PCR for detection of respiratory tract infections. J ClinVirol. 2008; 41: 53–6.
4. Chen Q, Shi SY, Hu Z, Zhang QH, Cao X. Analysis of non-bacterial respiratory pathogen infection in children with asthmatic diseases. Zhongguo Dang Dai ErKeZaZhi. 2012; 14(11): 834-7.
5. Gupta S, Shamsundar R, Shet A, Chawan R, Srinivasa H. Prevalence of respiratory syncytial virus infection among hospitalized children presenting with acute lower respiratory tract infections. Indian J Pediatr 2011; 78: 1495-1497.
6. Hacımustafaoğlu M, Çelebi S, Bozdemir SE, Özgür T, Özcan I, Güray A, Çakır D. RSV frequency in children below 2 years hospitalized for lower respiratory tract infections. The Turkish Journal of Pediatrics 2013; 55: 130-9.
7. Hatipoğlu N, Somer A, Badur S, Unüvar E, Akçay-Ciblak M, Yekeler, et al. Viral etiology in hospitalized children with acute lower respiratory tract infection. Turk J Pediatr. 2011; 53(5): 508-16.
8. Ji W, Chen ZR, Zhou WF, Sun HM, Li BQ, Cai LH, et al. Detection of *Mycoplasma pneumoniae, Chlamydia trachomatis* and common respiratory viruses in children with acute respiratory infection in Nanjing. Zhongguo Dang Dai ErKeZaZhi. 2010; 12(6):450-4.
9. Ji W, Chen ZR, Zhou WF, Sun HM, Li BQ, Cai LH, et al. Etiology of acute respiratory tract infection in hospitalized children in Suzhou from 2005 to 2011.Zhonghua Yu Fang Yi Xue Za Zhi. 2013; 47(6):497-503.
10. Kristj'ansson S, Loudrup C, Wennergren G, Strannegard I-L, Carlsen K-H. Nebulised racemic adrenaline in the treatment of acute bronchiolitis in infants and toddlers. Archives of Disease in Childhood 1993; 69(6): 650-4.
11. Lakhanpaul M, MacFaul R, Werneke U, Armon K, Hemingway P, Stephenson T. An Evidence Based Guideline for the Management of Children Presenting With Acute Breathing Difficulty. Emerg Med J. 2009; 26(12):850-3.
12. Lucion MF, Juárez M, Viegas M, Castellano V, Romanin VS, Grobaporto M. et al. Respiratory syncytial virus. Clinical and epidemiological pattern in pediatric patients admitted to a children's hospital between 2000 and 2013. Arch Argent Pediatr 2014;112(5):397-404.
13. Miller KE, Gebretsadik T, Carroll KN, Dupont WD, Mohamed YA, Morin LL, et al. Viral Etiologies of Infant Bron-

chiolitis, Croup, and Upper Respiratory Illness during Four Consecutive Years. Pediatr Infect Dis J. 2013; 32(9): 950-5.
14. Nair H, Nokes DJ, Gessner BD. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010; 375: 1545-55.
15. Nagakumar P, Doull I. Current therapy for bronchiolitis. Arch Dis Child. 2012; 97 (9):827-830.
16. Pientong C, Ekalaksananan T, Teeratakulpisarn J, Tanuwattanachai S, Kongyingyoes B, Limwattananon CJ. Atypical bacterial pathogen infection in children ith acute bronchiolitis in northeast Thailand. Microbiol Immunol Infect. 2011; 44(2):95-100.
17. Qumar K, Skjerven HO, Mikalsen IB. Mikalsen IB. Acute bronchiolitis in infants, a review. Scand J Trauma Resusc Emerg Med. 2014; 3: 22-23.
18. Ralston S, Garber M, Naranq S, Shen M, Pate B, Pope J, et al. Decreasing unnecessary utilization in acute bronchiolitis care: results from the value in inpatient pediatrics network. J Hosp Med. 2013; 8:25–30.
19. Sung RY, Chan PK, Tsen T, Yeung AC, et al. Identification of viral and atypical bacterial pathogens in children hospitalized with acute respiratory infections in Hong Kong by multiplex PCR assays. J Med Virol 2009; 81: 153–159.
20. Teeratakulpisarn J, Pientong C, Ekalaksananan T, Ruangsiripiyakul H, Uppala R. Rhinovirus infection in children hospitalized with acute bronchiolitis and its impact on subsequent wheezing or asthma: a comparison of etiologies. Asian Pac J Allergy Immunol. 2014; 32(3):226-34.
21. Zorc J.J, Breese Hall C. Bronchiolitis: recent evidence on diagnosis and management. Pediatrics 2010; 125(2): 342-9.

SUMMARY

MYCOPLASMA PNEUMONIAE AND *CHLAMYDOPHILA PNEUMONIAE* IN HOSPITALIZED CHILDREN WITH BRONCHIOLITIS

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Bronchiolitis is an acute lower respiratory tract infection in early childhood caused mainly by different viruses. Etiology of bronchiolitis have been studied in different environments and populations. *Respiratory syncytial virus (RSV), human Metapneumovirus (hMPV), human Bocavirus (hBoV), human Rhinoviruses (hRV)* have consistently been shown to predominate. Few studies however have attempted to determine whether other pathogens, particularly *Mycoplasma Pneumoniae (MP)* and *Chlamydophila pneumoniae (CP),* are associated with bronchiolitis in children under 2 years of age.

The aim of this study was to determine the prevalence and clinical features of *MP* and *CP* in children under the age of 2 years presenting to the Iashvili Central Children Hospital in Tbilisi with various severities and clinical manifestations of bronchiolitis. Acute and convalescent serum samples were tested by ELISA for IgM and IgG antibodies to *RSV, CP and MP.*37 children under two years of age were studied. In 19 patients out of 37 (51.35%) etiological diagnosis were established and in 18 patients (48.65%) no pathogens were found. 11 patients (29.72%) had either *CP* or *MP* and 8 patients (21.62%) had *RSV*. Children infected with CP and MP had less severe bronchiolitis than those infected with RSV. Co-infection was not associated with disease severity. There were no statistically significant differences between groups with respect to length of hospital stay. Our study underlines the importance of atypical bacterial pathogens in acute bronchiolitis in children under 2 years and highlights the complex epidemiology and clinical features of these pathogens in this age group.

Keywords: Mycoplasma pneumoniae, Chlamydophila pneumoniae, Respiratory syncytial virus, Bronchiolitis.

РЕЗЮМЕ

MYCOPLASMA PNEUMONIAE И *CHLAMYDOPHILA PNEUMONIAE* У ГОСПИТАЛИЗИРОВАННЫХ ДЕТЕЙ С БРОНХИОЛИТОМ

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Целью данного исследования явилось определить распространенность и клинические особенности MP и CP у детей в возрасте до 2 лет с различной степенью тяжести и клинических проявлений бронхиолита.

Острые и конвалесцентные образцы сыворотки изучены методом ELISA для IgM и IgG антител к RSV, CP и MP. Исследовано 37 детей в возрасте до двух лет. У 19 (51,35%) пациентов из 37 этиологический диагноз был установлен, в 18 (48,65%) случаях возбудители не выявлены. У 11 (29,72%) пациентов из 19 обнаружены CP или MP, у 8 (21,62%) - RSV. У детей с CP и MP бронхиолитом заболевание протекало менее тяжело, чем у пациентов с RSV. Микст-инфекция не была связана с более тяжелым течением заболевания. Статистически значимых различий между группами в отношении продолжительности пребывания в стационаре не обнаружено.

Результаты исследования выявили значимую роль *Mycoplasma pneumoniae* и *Chlamydophila pneumoniae* при остром бронхиолите у детей в возрасте до 2 лет и неоднородность эпидемиологии и клинических особенностей указанных микроорганизмов в этой возрастной группе.

რეზიუმე

Mycoplasma pneumoniae და *Chlamydophila pneumoniae* ბრონქიოლიტის დიაგნოზით ჰოსპიტალიზებულ ბავშვებში

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ბრონქიოლიტი ადრეული ასაკის ბავშვთა ქვედა სასუნთქი გზების მწვავე ინფექციაა, რომელიც ძირითადად გამოწვეულია სხვადასხვა ვირუსით. ბრონქიოილიტის ეტიოლოგია შესწავლილია სხვადასხვა გარემოში და სხვადასხვა პოპულაციაში. ცნობილია, რომ მის ძირითად გამომწვევებს წარმოადგენენ რესპირაციულ-სინციტიური ვირუსი *(RSV)*, ადამიანის მეტაპნევმოვირუსი *(hMPV)*, ადამიანის ბოკავირუსი *(hBoV)*, ადამიანის რინოვირუსი *(hRV)*. ჩატარებულა ერთეული კვლევები ბრონქიოლიტის ეტიოლოგიაში სხვა პათოგენების, განსაკუთრებით მიკოპლაზმის (*Mycoplasma Pneumoniae)* და ქლამიდიის (*Chlamydophila pneumoniae)* როლის განსაზღვრისთვის 2 წლამდე ასაკის ბავშვებში. კვლევის მიზანს წარმოადგენდა მიკოპლაზმის და ქლამიდიის სიხშირის და კლინიკური თავისებურებების განსაზღვრა 2 წლამდე ასაკის ბავშვებში, რომლებიც ჰოსპიტალიზებულნი იყვნენ მ.იაშვილის ბავშვთა ცენტრალურ საავადმყოფოში სხვადასხვა სიმძიმის ბრონქიოლიტის დიაგნოზით. IgMდა IgG ანტისხეულები *RSV, CP* და *MP*-ს მიმართ შესწავლილია ELISA-ს მეთოდით მწვავე და კონვალესცენტურ შრატში. ჩვენი დაკვირვების ქვეშ იმყოფებოდა 2 წლამდე ასაკის 37 ბავშვი. მათ შორის 19 (51.35%) პაციენტში დადგინდა ეთიოლოგიური დიაგნოზი, ხოლო 18 (29.72%) პაციენტში გამომწვევი არ იყო დადგენილი. 11 (29.72%) პაციენტთან დადგინდა *CP* და *MP* ინფექცია, 8 (21.62%) - *RSV*. ბავშვები, რომელთაც ჰქონდათ *CP* და *MP* ინფექცია, ხასიათდებოდნენ ბრონქიოლიტის ნაკლებად მძიმე მიმდინარეობით, ვიდრე პაციენტები RSV ინფექციით. პაციენტებში მიქსტ-ინფექციებით ბრონქიოლიტი არ მიმდინარეობდა უფრო მძიმედ. კლინიკაში დაყოვნების ხანგრძლივობის მიხედვით ჯგუფებს შორის განსხვავება არ დაფიქსირებულა. ავტორები მიუთითებენ მიკოპლაზმის და ქლამიდიის როლზე 2 წლამდე ასაკის ბავშვებში ბროქიოლიტით და ხაზს უსვამენ ამ პათოგენების ეპიდემიოლოგიური და კლინიკური თავისებურებების გათვალისწინების აუცილებლობას 2 წლამდე ასაკის ბავშვებში.

ПОВЕДЕНЧЕСКИЕ ЭФФЕКТЫ ОКСИДАТИВНОГО СТРЕССА

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Устойчивость к стрессу является одним из важнейших показателей жизнеспособности организма и, естественно, что выяснение механизмов, формирующих эту устойчивость, имеет фундаментальное значение [12].

Известно, что оксидативный стресс вовлечен в развитие множества патологических процессов; вместе с тем он играет значительную роль и в процессах физиологической адаптации и регулировании внутриклеточной трансдукции сигналов.

Полагают, что наиболее приемлемой дефиницией оксидативного стресса является следующее: «состояние, когда оксидативные процессы довлеют над антиоксидатными системами, вторично по отношению к нарушению баланса между ними» [11].

Определение состояния оксидативного стресса имеет принципиальное значение не только с точки зрения исследования многих заболеваний, но и для повышения эффективности их лечения [11]. Согласно теории D. Harman [4,5], оксидативный стресс играет важнейшую роль в развитии процессов старения, однако дальнейшее развитие этой теории, как ни странно, привело к результатам, которые свидетельствуют, что свободные радикалы могут внести существенный вклад в поддержании метаболических процессов и способствовать увеличению продолжительности жизни [8]. Этот эффект известен под названием гормезиса (hormesis), который базируется на принципе стимулирования восстановительных процессов с использованием повторяющихся стрессовых воздействий низкого уровня. Одной из первых попыток такого подхода было использование

повторяющихся термических шоков, проводимых на культуре человеческих клеток, результаты которых показали, что использование горметического подхода в геронтологических исследованиях носит весьма многообещающий характер [7]. Фундаментальной базой для понимания феномена гормезиса является понятие «доза-ответ», который показывает стимулирование восстановительных процессов при низких дозах и их ингибирование при высоких. Имеется предположение, что оксидативный стресс может внести существенный вклад в нарушение процессов обучения и памяти [6].

Целью исследования явилось изучение эффекта оксидативного стресса низкого и высоко уровня на поведение белых крыс в многоходовом лабиринте.

Материал и методы. Эксперименты проводились на белых крысах (n=30) весом 250-300 г. В качестве экспериментальной модели оксидативного стресса, индуцированного пероксидом водорода (H_2O_2), использовали пероральное введение 0,1 и 0,2% водного раствора H_2O_2, который вместо питьевой воды давали двум группам животных в течение 25 дней до начала поведенческих экспериментов и в процессе экспериментов (7-8 дней) до их завершения. I группа (n=12) получала 0,1% раствор, II группа (n=12) – 0,2% раствор. Использовали перекись водорода пищевого качества (Wellness, 35% food grade H_2O_2). Контрольную группу (n=6) составили интактные животные.

С 26 дня животные I и II групп начинали обучаться в многоходовом лабиринте определению оптимальной траектории движения для попадания в ящик-гнездо (рис. 1).

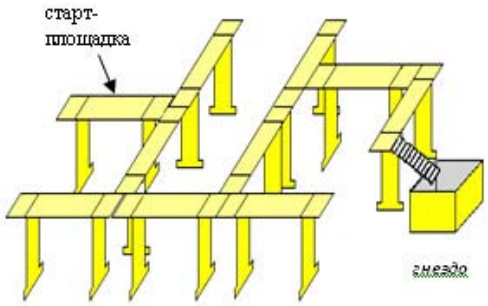

Рис. 1. Конструкция многоходового лабиринта

Общий вид использованного лабиринта представлен на рис. 1. Лабиринт состоит из отдельных мостков (высотой 30 см.), что дает возможность легко менять его конфигурацию, усложнять или упрощать решаемую задачу – попадание в ящик-гнездо. При первых пусках животных по лабиринту (первый день обучения) им обычно помогают в нахождении оптимальной траектории движения от старт-площадки до ящика-гнезда, а в дальнейшем они обучаются уже самостоятельно, методом проб и ошибок. Опыты проводятся без пищевого подкрепления, стимулом для поиска пути служит избавление от неэтологических условий. Условия в экспериментальной комнате (освещенность, расположение предметов и т.д.) до полного завершения экспериментов сохранялись строго неизменными. Оценка тестирования проводилась по двум показателям – число совершаемых ошибок (отклонений от оптимальной траектории движения) и времени, затрачиваемого на прохождение от старт-площадки до ящика гнезда. Регистрацию указанных показателей начинали со второго дня обучения, так как в первый день большинство из животных получало помощь от экспериментатора.

Достоверность разницы между полученными результатами оценивали по критерию Стьюдента.

Результаты и их обсуждение. Анализ полученных данных показал, что статистически достоверной разницы в процессе обучения прохождению многоходового лабиринта с точки зрения уменьшения количества совершаемых ошибок (отклонений от оптимальной траектории движения) между указанными выше группами не выявлено. На седьмой день обучения (по пять пусков в день) практически все животные проходили лабиринт без единой ошибки. Но принципиально другая картина наблюдалась с точки зрения времени, которое животные той или иной группы, в среднем, затрачивали на прохождение лабиринта.

График, представленный на рис. 2, показывает изменение времени прохождения лабиринта животными контрольной группы, начиная со второго дня обучения, которые в стадии «автоматизированного» поведения, на седьмой день тестирования проходят лабиринт в среднем за 20-22 секунды и тот же показатель для животных первой экспериментальной группы, которые подвергались оксидативному стрессу от введения 0,1% раствора пероксида водорода. Среднее время прохождения лабиринта в данной группе в «автоматизированном» режиме составляет 11-12 сек.

Разница между этими показателями по указанным двум группам статистически достоверна ($p<0,05$).

Оказалось, что животные, которые подверглись стрессогенному воздействию хроническим введением перекиси водорода, существенно повысили свою поведенческую активность. По сравнению с контрольными животными они вели себя на эстакадах лабиринта весьма живо и энергично и к концу седьмого дня лабиринтных сессий, безошибочно проходили весь путь до ящика-гнезда, практически, в два раза быстрее контрольных.

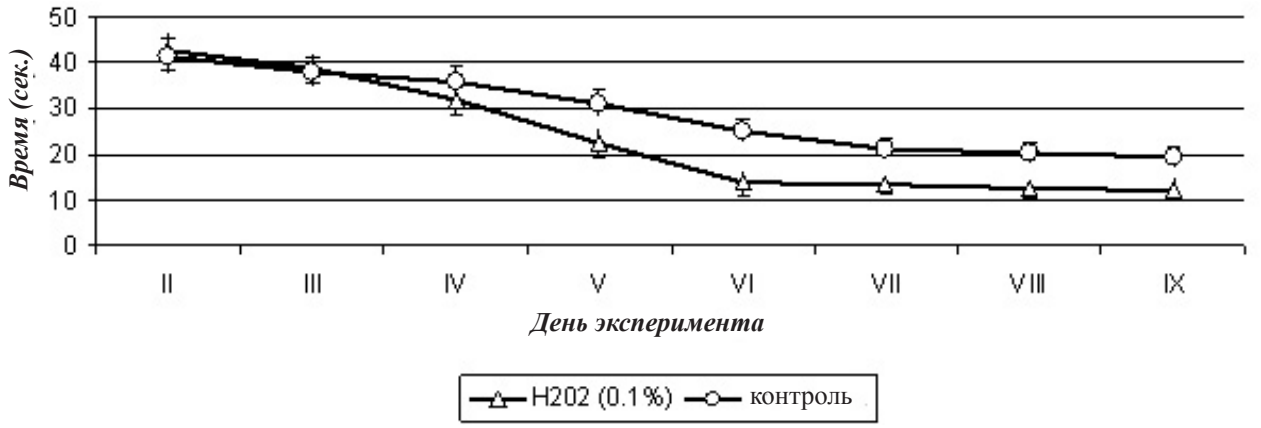

Рис. 2. Время прохождения многоходового лабиринта крысами контрольной группы, а также крысами, которые до лабиринтной сессии получили хроническое воздействие пероксидом водорода (H_2O_2, 0,1%)

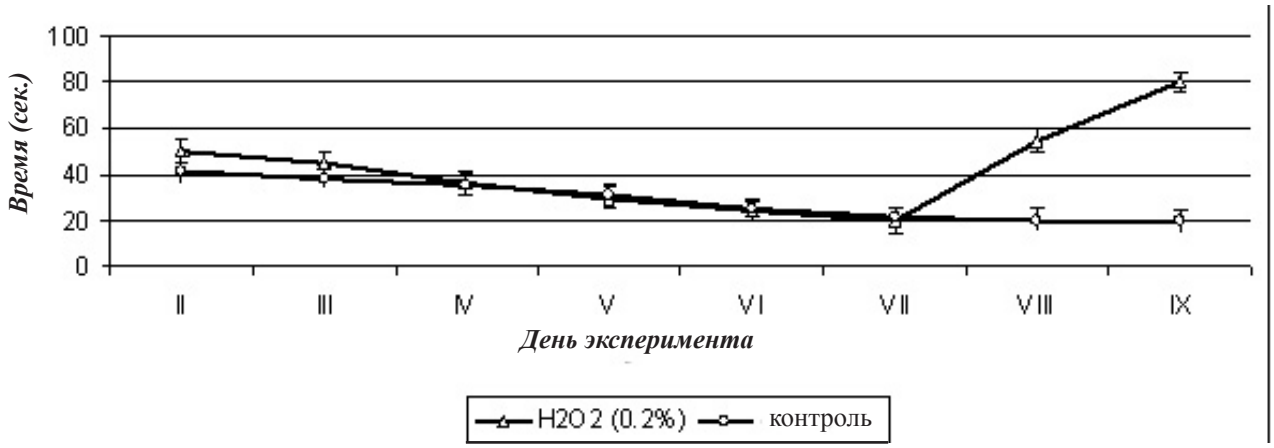

Рис. 3. Время прохождения многоходового лабиринта крысами контрольной группы, а также крысами, которые до лабиринтной сессии получили хроническое воздействие пероксидом водорода (H_2O_2, 0,2%)

Каковы же результаты, полученные во II экспериментальной группе, животные которой, по сравнению с первой группой, получали двойную дозу H_2O_2?

Как показано на рис.3, до седьмого дня тестирования поведение животных этой группы не отличалось от поведения контрольной, но начиная с восьмого дня, они резко уменьшили поведенческую активность и на прохождение лабиринта им потребовалось в среднем 55 сек, а на 9 день – 80 сек, что, примерно, в 8 раз превышает время, затрачиваемое на ту же задачу, I экспериментальной группой и в 4 раза – контрольной.

Анализируя механизмы индуцированного перекисью водорода оксидативного стресса в in-vitro моделях Coyle CH. [2] пришел к выводу, что в его развитии задействованы системы синтазы оксида азота (NOS) и NADPH-оксидазы, которые, по сути, служат источником увеличения реактивных форм кислорода.

Наличие цитопротекторного свойства ответов на стрессовые факторы вызвало широкий интерес к созданию фармако-агентов, способных индуцировать стресс-реакции, однако их уровень не должен выходить за пределы горметических реакций.

Анализ полученных результатов позволяет заключить, что в проведенных экспериментах наблюдается поведенческое проявление феномена гормезиса. Весьма существенное повышение поведенческой активности, направленной на избавление от неэтологического состояния животных после оксидативного стресса, вызванного введением перекиси водорода (первая группа), по нашему мнению, указывает на то, что доза нанесенного стрессового воздействия оказалась в пределах стимуляции горметических механизмов. Повышение же дозы воздействия (группа 2), видимо, вывело на уровень их ингибирования. Примерно аналогичные данные были получены ранее на дрозофилах, скорость полета которых после нанесения оксидативного стресса перекисью водорода, по данным авторов, была «драматично повышена» [3]. Еще ранее, опять же на дрозофилах, показано, что гипертермическое прекондиционирование (36^0C в течение одного часа) также повышает их локомоторную активность [10]. Помимо этого, оказалось, что стресс низкой дозы повысил и

продолжительность жизни дрозофил [1]. Полагают, что этот эффект достигается за счет активации белков теплового шока и их шаперонной функции, в результате чего происходит репарация не только нанесенных стрессом повреждений, но и имеющих место до нанесения стрессогенного воздействия [1]. Функционирование указанного механизма обычно ассоциируют с продуцированием оксида азота [9].

Исходя из вышеизложенного, следует заключить, что проведенные эксперименты на белых крысах показали возможность наблюдения поведенческой манифестации феномена гормезиса, что, в отличие от экспериментов на дрозофилах, предоставляет неизмеримо большие возможности для максимального раскрытия механизмов этого явления, теоретическое и практическое значение чего трудно переоценить.

ЛИТЕРАТУРА

1. Butov A, Johnson T, Cypser J, Sannikov I, Volkov M, Sehl M, Yashin A. Hormesis and debilitation effects in stress experiments using the nematode worm Caenorhabditis elegans: in model of balance between cell damage and HSP levels. Experimental gerontology 2001; 37: 57-66.
2. Coyle CH. Mechanisms of H2O2-induced oxidative stress in endothelial cells. Thesis and Dissertation. 2004, http://ir.uiowa.edu/etd/117.
3. Grover D, Ford D, Brown C, Hoe N, Erdem A, et al. Hydrogen Peroxide Stimulates Activity and Alters Behavior in Drosophila melanogaster. PloS ONE 2009; 4(10): 75-80.
4. Harman D. Aging: a theory based on free radical and radiation chemistry. Journal of Gerontology 1956; 11(3): 298–300.
5. Harman D. A biologic clock: the mitochondria? Journal of the American Geriatrics Society 1972; 20(4): 145–147.
6. Jeong YH, Park CH, Yoo J. et al. Chronic stress accelerates learning and memory impaiments and increases amyloid deposition in APP-CT100 transgenic mice, an Alzheimer's disease model. The FASEB Journal 2006; 10:1096/fj.05-4265fje (published online).
7. Rattan S.I.S. Principles and practice of hormetic treatment of aging anf ahe related diseases. BELLE Newsletter 2005; 13 (2): 1-5.
8. Ristow M, Schmeisser S. Extending life span by increasing oxidative stress. Free Radic Biol Med. 2011; 51(2): 327-336.
9. Romano G, Costantini M, Buttino I, Ianora A, Palumbo A. Nitric Oxide Mediates the Stress Response Induced by Diatom Aldehydes in the Sea Urchin Paracentrotus lividus. PLoS ONE 2011; 6(10): 25980.
10. Xiao C, Mileva-Seitz V, Seroude L, Robertson RM.Targeting HSP70 to motoneurons protects locomotor activity from hyperthermia in Drosophila. Dev Neurobiol. 2007; 67(4): 438-455.
11. Yoshikawa T, Naito Y. What is oxidative stress? JMAJ 2002; 45(7): 271-276.
12. Михальский А.И., Новосельцев В.Н. Количественный анализ и моделирование старения, заболеваемости и смертности. Успехи геронтол. 2005; 17: 117–129.

SUMMARY

BEHAVIORAL EFFECTS OF OXIDATIVE STRESS

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It is known that oxidative stress is involved in the development of many pathological processes, but at the same time it became clear that it can play a significant role in processes of physiological adaptation. There are suggestions that oxidative stress can make a significant contribution to the disorders of learning and memory processes. This assumption served as a basis of this study, in which we tried to reveal the effects of low and high-level oxidative stress on behavior of rats in a multi-way maze. As an experimental model of oxidative stress, induced by hydrogen peroxide (H_2O_2) we used oral administration of 0.1 and 0.2% aqueous solution of H_2O_2. It was found that animals treated with 0.1% hydrogen peroxide, significantly increased their behavioral activity and in the stage of "automatic behavior" they passed all the maze twice as fast as animals from the control group. But in case of 0.2% H_2O_2 locomotor activity of animals decreased sharply and for maze passing they spent in average 4-fold more time in comparison with the control group.

In our opinion, the first dose of stress was within the range of hormetic mechanism's stimulation, but increased dose, led to its inhibition, which is a classic manifestation of hormetic effects of oxidative stress.

Keywords: oxidative stress, physiological adaptation, hormetic effects.

РЕЗЮМЕ

ПОВЕДЕНЧЕСКИЕ ЭФФЕКТЫ ОКСИДАТИВНОГО СТРЕССА

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Известно, что оксидативный стресс вовлечен в развитие многих патологических процессов; вместе с тем он играет значительную роль в процессах физиологической адаптации. Имеется предположение, что оксида-

тивный стресс может внести существенный вклад в нарушение процессов обучения и памяти.

Целью исследования явилось изучение эффекта оксидативного стресса низкого и высоко уровня на поведение белых крыс в многоходовом лабиринте.

В качестве экспериментальной модели оксидативного стресса, индуцированного пероксидом водорода (H_2O_2), использовали пероральное введение 0,1 и 0,2% водного раствора H_2O_2. Оказалось, что животные, получавшие 0,1% перекиси водорода, существенно повысили свою поведенческую активность и в стадии «автоматизма» проходили весь лабиринт в два раза быстрее контрольных. В случае 0,2% H_2O_2 животные резко уменьшили поведенческую активность, и на прохождение лабиринта им требовалось в среднем в 4 раза больше времени в сравнении с контрольной группой.

Авторы предполагают, что первая доза стрессового воздействия оказалась в пределах стимуляции гормезисных механизмов, а ее повышение, привело к их ингибированию, что является классическим проявлением гормезисного эффекта оксидативного стресса.

რეზიუმე

ოქსიდაციური სტრესის ქცევითი ეფექტები

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კვლევაში თეთრ ლაბორატორიულ ვირთაგვებზე შესწავლილია დაბალი და მაღალი დონის ოქსიდაციური სტრესის ეფექტი მრავალსვლიან ლაბირინთში მათი ქცევის მიხედვით.

წყალბადის ზეჟანგით (H_2O_2) ინდუცირებული ოქსიდაციური სტრესის საექსპერიმენტო მოდელის სახით გამოიყენებოდა H_2O_2-ის 0.1 და 0.2%-იანი წყალხსნარის პერორალური შეყვანა. აღმოჩნდა, რომ ცხოველებს, რომლებმაც მიიღეს 0.1%-იანი წყალბადის ზეჟანგი, შესამჩნევად აუმაღლდათ ქცევითი აქტივობა და "ავტომატიზმის" სტადიაში 2-ჯერ სწრაფად გადიოდნენ მთლიან ლაბირინთს, ვიდრე საკონტროლო ცხოველიბი. ხოლო 0.2 %-იანი H_2O_2-ის მიღების შემთხვევაში ცხოველებმა მკვეთრად შეამცირეს ქცევითი აქტივობა და ლაბირინთზე გავლისათვის დასჭირდათ 4-ჯერ მეტი დრო, ვიდრე საკონტროლო ჯგუფის ცხოველებს.

ავტორების მიერ გამოტანილია დასკვნა, რომ სტრესული ზემოქმედების პირველი დოზა აღმოჩნდა ჰორმეზული მექანიზმების აღძვრის მასტიმულირებელ ფარგლებში, ხოლო დოზის მომატება (2-ჯერ გაზრდა) იწვევს ამ მექანიზმების ინჰიბირებას, რაც წარმოადგენს ოქსიდაციური სტრესის ჰორმეზული ეფექტის კლასიკურ გამოვლენას.

ТЕНДЕНЦИИ И РИСКИ САМОЛЕЧЕНИЯ В ГРУЗИИ

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Европейская ассоциация производителей безрецептурных препаратов (AESGP) термин «самолечение» преобразовала в «ответственное самолечение». Ключевой характеристикой самолечения является ответственность больного за свое здоровье. Ответственное самолечение, определяемое ВОЗ как «разумное применение пациентами лекарственных средств, находящихся в свободной продаже, с целью профилактики или лечения "легких расстройств здоровья", в настоящее время является одним из наиболее проблемных аспектов отечественного здравоохранения [3,6]. Ключевой характеристикой самолечения является ответственность больного за свое здоровье. Основой ответственного подхода к самолечению служит наличие соответствующей информации. Во-первых, необходимо четко разъ-

яснить, когда можно заниматься самолечением, а когда необходимо обратиться к врачу. Грань между этими случаями должна быть достаточно понятной для больного. Очевидно, что при наличии любых сомнений пациента лучше ориентировать на целесообразность визита к врачу. Во-вторых, больного необходимо информировать об эффективных и безопасных безрецептурных средствах, которые могут быть использованы для самолечения в тех или иных ситуациях [4,7,12].

Концепция ответственного самолечения допускает самостоятельное применение безрецептурных средств для лечения некоторых заболеваний, которые не требуют вмешательства врача или по которым больной уже получил консультации медицинских работников. Конечно, никакие советы друзей или знакомых не могут заменить рекомендаций врача. Желательно, чтобы даже безрецептурный препарат был впервые рекомендован специалистом, имеющим медицинское или фармацевтическое образование. Затем любой человек, внимательно прочитавший инструкцию, соблюдающий ее рекомендации и предписания врача, способен самостоятельно пользоваться безрецептурным лекарством без особой угрозы здоровью.

На практике понятие самолечения включает также лечение членов семьи и знакомых, особенно это касается лечения детей. Однако, в средствах массовой информации широко рекламируются лекарственные средства, на телевидении растет число передач, посвященных вопросам здоровья, что приводит к бесконтрольному применению лекарственных средств населением [1,12].

Европейское региональное бюро ВОЗ в своем сборнике статей «Национальная политика в области лекарственных средств» отмечает преимущества «самолечения» как для потребителей, экономящих время на посещение врача и приобретающих семейный опыт по лечению, так и для медицинских работников, которые получают возможность уделять большее время пациентам, действительно нуждающимся в рекомендациях врача. При таком подходе к лечению, естественно, необходимо обеспечить безопасность безрецептурных препаратов, повысить роль фармацевтов. как консультантов потребителей; информировать специалистов и население о применении таких препаратов.

В опубликованном в 1988 году исследовании ВОЗ «Самолечение в Европе» отмечалось, что медицинские работники, и в первую очередь фармацевты, нуждаются в расширении знаний о самолечении и безрецептурных препаратах. В реализации концепции «самолечения» аптечные работники стали одной из ключевых фигур, участвующих в лекарственном обеспечении населения. В 1994 году ВОЗ приняла резолюцию «Роль фармацевта в поддержку пересмотренной стратегии ВОЗ в области лекарственных средств», в которой и закрепила за аптечными работниками ключевую роль в лекарственном обеспечении и призвала их предоставлять « населению информированную и объективную консультативную помощь по лекарственным средствам и их использованию» [9,11].

Естественно, для предоставления такой помощи потребителю, фармацевт должен быть сам профессионально информирован, получая знания о лекарственных средствах и повышая свою квалификацию, чтобы быть способным дать адекватный совет и определить долю своей компетентности в том или ином случае. Грань между рекомендациями по выбору самолечения и рекомендациями посещения врача должна быть совершенно очевидна для профессионального фармацевта при изучении симптомов заболевания посетителя. Потенциально фармацевт имеет необходимую квалификацию для выполнения такой задачи [1,9,11]:
- предоставление лекарственных средств и изделий медицинского назначения требуемого качества;
- обучение пациентов правильному применению лекарственных средств (ЛС) и изделий медицинского назначения;
- сотрудничество с врачом при составлении плана фармакотерапии пациента, информирование врача о применяемых пациентом ЛС безрецептурного отпуска;
- контроль применения ЛС безрецептурного отпуска на основе постоянного сотрудничества с пациентом;
- введение фармацевтического досье в базу данных о пациенте и его фармакотерапии.

В задачи, поставленные перед фармацевтом, также входят систематическое повышение уровня знаний фармацевта в области фармакотерапии данного заболевания, информация о ЛС, психология общения, развитие рынка ЛС, соблюдение профессиональной этики и конфиденциальности, стандартизация и контроль [6,9].

Фармацевт в рамках своей компетенции в стационарах и поликлиниках должен выполнять следующие функции:
- оказывать помощь врачам в выборе лекарств, учитывая как безопасность, эффективность и качество лекарств, так и стоимость лечения;
- обеспечивать пациентов информацией о рецептурных препаратах. Наблюдать за их применением, особое внимание обращая на выявление и предотвращение побочных эффектов лекарств;
- следить за применением лекарств как в стационарных лечебных учреждениях, так и в амбулаторных условиях. Помогать врачам оценивать эффективность лечения, способствуя таким образом рациональному назначению и применению лекарств;

- консультировать клиентов аптек о применении безрецептурных препаратов и контролировать процесс самолечения;
- способствовать популяризации здорового образа жизни и профилактики заболеваний.

Выполняя вышеперечисленные функции, фармацевт осуществляет фармацевтическую опеку. Фармацевтическая опека — часть медицинского обеспечения, которую в рамках своей компетенции выполняют фармацевты, способствуя улучшению качества жизни пациентов [7,10,11]. Во всем мире в настоящее время особое значение уделяется самолечению. Развитие самолечения позволяет эффективно экономить общественные затраты на медицинское обслуживание

Чтобы пациенты аптеки, затратив минимальные средства, получили максимальную пользу, фармацевту необходимо участвовать в этом процессе как консультанту и одновременно контролировать самолечение, т.е. осуществлять фармацевтическую опеку [6,9].

Целью исследования явилось определение проблем самолечения в Грузии и поиск их решений, анализ спроса безрецептурных лекарств в аптеках и потребления лекарств различных фармакотерапевтических групп, приобретаемых в аптеках с целью самолечения.

Материал и методы. В процессе исследования были использованы следующие методы: системный и информационный; непосредственного наблюдения; сравнительного, документального анализа; социологические (опрос, интервьюирования).

Исследование проводилось в 2013-2014 гг. в сетевых аптеках «PSP» и других аптеках различных городов Грузии.

Результаты и их обсуждение. 2013-2014 гг. в сетевых аптеках «PSP» и других аптеках различных городов Грузии проводили видеомониторинг (видеонабюдение) визитов и потреблений пациентов. Обобщая данные наблюдений и опросов, на основании вычисленной частоты приема лекарств в течение недели среди мужчин и женщин, были уточнены причины применения различных лекарств в зависимости от возраста и пола клиента (рис. 1 и 2).

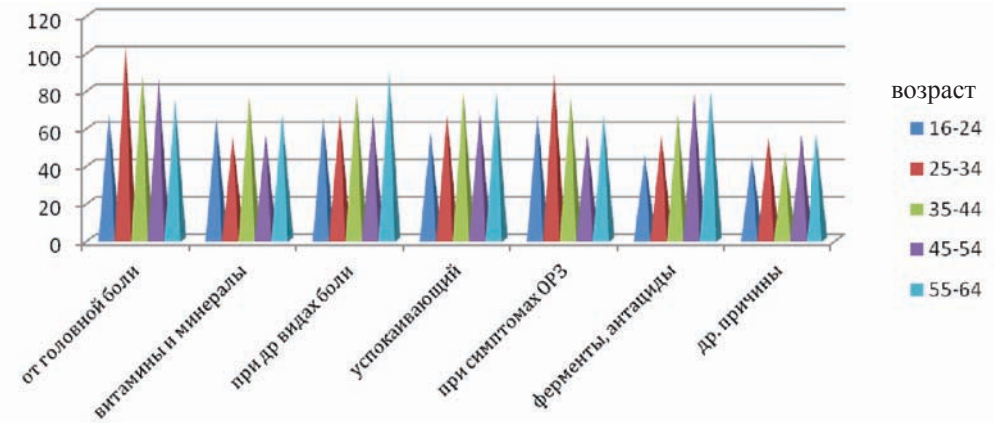

Рис. 1. Частота приема лекарственных препаратов в течение недели среди мужчин (число респондентов 1948)

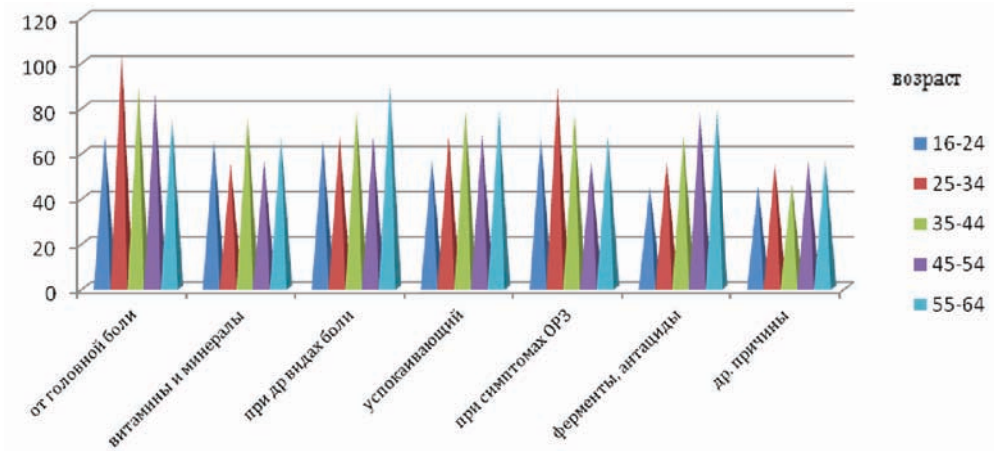

Рис. 2. Частота приема лекарственных препаратов в течение недели среди женщин (число респондентов 2171)

Данные, представленные на рисунках, показывают, что мужчины и женщины всех возрастных групп чаще пользуются препаратами от головной боли.

Следует отметить, что женщины лекарства применяют почти в 2 раза чаще, чем мужчины. Женщины употребляют преимущественно витамины и минеральные вещества, что указывает на то, что они больше заботятся о своем здоровье.

Показатель потребления лекарств с возрастом увеличивается как у женщин, так и у мужчин.

Анализ симптомов и синдромов, чаще всего вынуждающих пациентов обращаться в аптеку, показал, что они совпадают с причинами обращения пациентов в аптеку в государствах Европейского Союза [9,11]. Таковыми являются: простудные заболевания -35%; нервозность и бессонница – 26%; головная боль – 21%; нарушения пищеварения – 18%; усталость – 15%; незначительные кожные проблемы – 9%; запоры и т. д. -7%.

Соотношение частоты потребления наиболее популярных фармакотерапевтических групп, используемых в целях самолечения в Грузии, отображено на рис. 3. Самой потребляемой является группа лекарств для лечения острых респираторных заболеваний (ОРЗ). Пациенты чаще обращаются в аптеку именно с симптомами ОРЗ: кашель, насморк, боль в горле, повышенная температура, головная боль.

Анализ данных опроса (200 респондентов) выявил, что пациенты при первых симптомах заболевания предпочитают обращаться в аптеку. Считаем, что причинами являются: высокая стоимость амбулаторного лечения; экономия времени (длительный период записи на услуги врачей в страховых компаниях); в аптеке меньше негативной атмосферы, чем в больнице (длинные очереди и т.д.); фармацевт может точно перенаправить (терапевт, гинеколог, хирург).

На вопрос: «выявлены ли последствия самостоятельного применения лекарственных средств при симптомах ОРЗ», в интервью (250 респондентов) выявлено, что, в основном, в 81% случаев, при самолечении наблюдались аллергические реакции, из них 15% пациентов были госпитализированы. В остальных случаях наблюдалась аллергическая сыпь по типу крапивницы. Например, одна из пациенток, зная, что у нее в анамнезе была аллергическая реакция в виде крапивницы на пенициллин, начала самостоятельный прием амоксициллина, без назначения врача. С отеком Квинке больная была доставлена в стационар. Разные названия препаратов ввели ее в заблуждение. Больная не была проинформирована, что указанные препараты из одной группы, и, следовательно, наличие аллергии на пенициллин является противопоказанием для применения амоксициллина.

По результатам опроса, к самолечению прибегали чаще женщины - 89%, средний возраст которых составил 51 год. Самой молодой пациентке было 20 лет, а самой пожилой - 81 год. У большинства (56%) пациентов в анамнезе отмечались аллергические реакции. Причиной возникновения неблагоприятных побочных реакций (НПР) , как правило, были антибактериальные препараты (рис. 4).

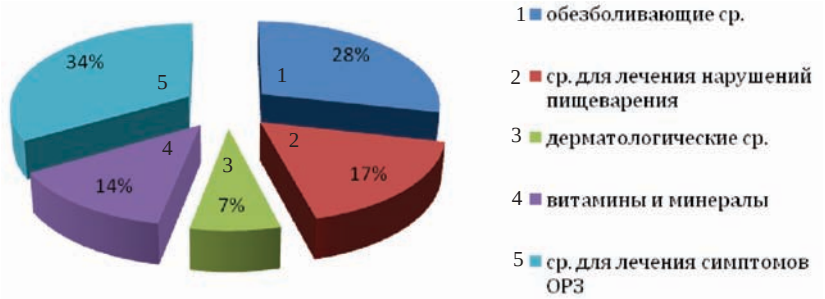

Рис. 3. Соотношение частоты потребления наиболее популярных фармакотерапевтических групп, используемых в целях самолечения в Грузии

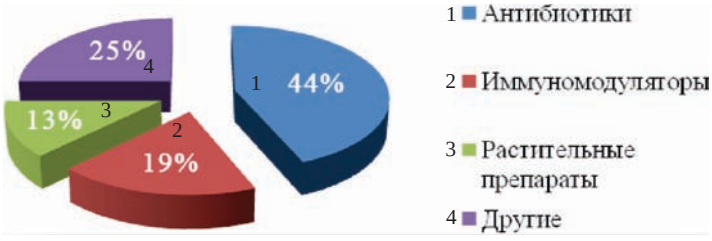

Рис. 4. Группы лекарственных средств, ставших причиной НПР при самолечении

Выводы

В результате провведенного исследования установлено, что в Грузии, как и в других государствах Европейского Союза, фармацевт является самым доступным специалистом системы медицинского обеспечения. Часто именно в компетенцию фармацевта входит принятие решения — в каком случае посетитель аптеки должен обращаться к врачу, а когда можно заняться самолечением.

Исследование показало, что основными принципами эффективной фармацевтической помощи для ответственного самолечения являются:
- более жесткая государственная регуляция отпуска лекарственных средств, включая контроль за выполнением инструкций по применению лекарственных препаратов.
- качество (законодательство, стандарты, образование, оценка соответствия стандартам и надзор, точно определенная ответственность фармацевта);
- доступность;
- обеспечение потребителей фармацеатической информацией.

ЛИТЕРАТУРА

1. American Society of Hospital Pharmacists. ASHPstatement on continuing education. Am J Hosp Pharm. 2009; 47:1855.
2.American Society of Health-System Pharmacists.ASHP Guidelines on Quality Assurance for Pharmacy-Prepared Sterile Products. Am J Health-Syst Pharm.2009; 57:1150–69.
3. Berta College of Pharmacists, Standards of Practice. The Pharmacist January 1 2009; http://www.altapharm.org/document_library/standards_of_practice.pdf
4. College of Pharmacists of British Columbia. Framework of Professional Practice. April 1 2009; http://www.bcpharmacists.org/standards/pdf/FPP.pdf
5. Compliance Policy Guide, Compliance Policy Guidance for FDA Staff and Industry, Chapter 4, Sub Chapter 460, Sec. 460.200 Pharmacy Compounding. 2009; http://www.fda.gov/ora/compliance_ref/cpg/cpgdrg/cpg460-200.html
6. Good Compounding Practices Applicable to State Licensed Pharmacies. 2011; http://www.nabp.net/law/modelact/download/appendixc.pdf
7. Guidelines for Bulk Compounding of Products in Hospitals, Canadian Society of Hospital Pharmacists 2012; http://www.cshp.ca/default.asp
8. Health Products and Food Branch Inspectorate: A Policy Framework, June 2009, Manufacturing and Compounding Drug Products in Canada. http://www.hc-sc.gc.ca/hpfb-dgpsa/inspectorate/pol_fram_man_comp_drug_prod_can_e.pdf
9. International Academy of Compounding Pharmacists Code of Ethics. 2012; http://www.iacprx.org/code_of_ethics/index.html
10. Kastango E.S. “The Cost of Quality in Pharmacy”. International Journal of Pharmacy Compounding 2013; 6(6) 404-408. http://www.ijpc.com/_pdf/quality.pdf
11. Royal College of Veterinary Surgeons Practice Standards Manual. 2013; http://www.bvha.org.uk/standard.pdf
12. U.S. Department of Health and Human Services Officeof Inspector General Exclusions Program. http://oig.hhs.gov/fraud/exclusions.asp (accessed 12 January2011).

SUMMARY

TRENDS AND RISKS OF SELF-MEDICATION IN GEORGIA

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The aim of this study was to identify the problems of self-medication in Georgia.

The study has once again shown that the basic principles of effective pharmaceutical software are: quality (legislation, standards, conformity assessment standards and supervision, well-defined responsibilities specialist pharmaceutical software and individual); accessibility; providing information.To date, Georgia has no specific guidelines defining the concept of the use of OTC self-medication. Physicians and pharmacists are not enough systematized and standardized non-commercial information about medicines. Specialists are often guided by commercial information provided by pharmaceutical companies, which is not always complete and objective.

Self-treatment of lung diseases with the use of non-prescription drugs - is becoming increasingly popular. The patient should be instructed in the proper use of drugs, and should receive adequate commercially independent information professionals medical care. Only in this case we can speak of a properly organized, evidence-based and cost-effective to society towards a system of primary medical care - to self-medicate.

Keywords: self-medication, pharmacy.

РЕЗЮМЕ

ТЕНДЕНЦИИ И РИСКИ САМОЛЕЧЕНИЯ В ГРУЗИИ

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Целью исследования явилось определение проблем самолечения в Грузии и поиск их решений, анализ спроса безрецептурных лекарств в аптеках и потребле-

ния лекарств различных фармакотерапевтических групп, приобретаемых в аптеках с целью самолечения.

Исследование показало, что основными принципами эффективного фармацевтического обеспечения являются: качество; доступность; обеспечение информацией.

В Грузии по сей день не разработаны специальные методические рекомендации, определяющие правила применения безрецептурных средств для самолечения.

Врачам и фармацевтам не доступна систематизированная и стандартизированная некоммерческая информация о лекарствах. Специалисты часто руководствуются коммерческой информацией, предоставляемой фармацевтическими фирмами, которая не всегда является полной и объективной.

Самолечение - лечение заболеваний с применением безрецептурных препаратов - приобретает все большую популярность. Исходя из этого, пациент должен быть информирован о правильном применении лекарственных средств путем предоставления адекватной, коммерчески независимой информации специалистами системы медицинского обеспечения. Только в этом случае можно судить о правильно организованной, научно обоснованной и экономически выгодной обществу деятельности системы первичного медицинского обеспечения - самолечении.

რეზიუმე

თვითმკურნალობის რისკები და ტენდენციები საქართველოში

ზ. ჭანტურია, თ. ჭურმბურიძე, ვ. ერიაშვილი, ნ. ნემსიწვერიძე, ნ. დუღაშვილი

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ფარმაცევტული ფაკულტეტი, საქართველო

კვლევის შედეგად დადასტურდა, რომ ფარმაცევტული დახმარების ეფექტური უზრუნველყოფის ძირითად პრინციპებს წარმოადგენენ:
- ხარისხი (საკანონმდებლო, სტანდარტები, სტანდარტებთან შესაბამისობის შეფასება, ზედამხედველობა, ფარმაცევტის პასუხისმგებლობების ზუსტი განსაზღვრა); ხელმისაწვდომობა; ინფორმაციით უზრუნველყოფა.

სადღეისოდ საქართველოში არ არის ჩამოყალიბებული თვითმკურნალობის კონცეფცია.

ექიმებსა და ფარმაცევტებს არ გააჩნიათ საკმარისი სისტემატიზებული და სტანდარტიზებული, არაკომერციული ინფორმაცია წამლებზე, ამიტომ ხშირ შემთხვევაში ხელმძღვანელობენ ფარმაცევტული ფირმების მიერ მიწოდებული კომერციული ინფორმაციით, რომელიც ხშირად არ არის ობიექტური.

თვითმკურნალობა თავისი არსით წარმოადგენს მსუბუქი დაავადების მკურნალობას ფარმაცევტის თანამონაწილეობით და ურეცეპტოდ გასაცემი მედიკამენტების საშუალებით, რაც სადღეისოდ დიდი პოპულარობით სარგებლობს. პაციენტი ინფორმირებული უნდა იყოს მედიკამენტების მართებული გამოყენების თაობაზე, ასევე უნდა ფლობდეს ადექვატურ, კომერციულად დამოუკიდებელ ინფომაციას სამედიცინო უზრუნველყოფის სისტემის სპეციალისტებისგან. მხოლოდ ამ შემთხვევაში შესაძლებელია სწორად ორგანიზებულ, მეცნიერულად დასაბუთებულ და ეკონომიკურად სარგებლიან თვითმკურნალობაზე მსჯელობა.

ИЗУЧЕНИЕ ПРОТИВОВОСПАЛИТЕЛЬНОЙ АКТИВНОСТИ ТИОСЕМИКАРБАЗИДОВ N-МОРФОЛИНИЛ УКСУСНОЙ КИСЛОТЫ

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В настоящее время в комплексной терапии острых и хронических заболеваний для купирования боли и уменьшения выраженности воспалительной реакции применяют множество лекарственных препаратов преимущественно из группы нестероидных противовоспалительных средств и глюкокортикостероидов. Однако наряду с благоприятным фармакологическим действием и достаточной степенью клинической эффективности, все они вызывают ряд нежелательных побочных реакций. В связи с этим остается актуальным поиск высокоэффективных лекарственных средств, подавляющих воспаление и обладающих минимальными побочными реакциями.

Известно, что в патогенезе воспаления значительную роль играют свободные радикалы, повреждающие клеточную мембрану и вызывающие развитие заболеваний. Для подавления свободнорадикального окисления используются антиоксиданты различной химической структуры, обладающие противовоспалительными свойствами как при внутреннем, так и при наружном применении (мексидол, димефосфон, витамин Е) [6,14]. Среди 1,3,4-тиадиазолов, являющихся циклическими аналогами гидразидов и тиосемикарбазидов, обнаружены вещества, обладающие наряду с антиконвульсантными свойствами [13], также антигипоксической [5], противосудорожной [4,11] и антимикробной [12] активностью. Многие соединения этого ряда в настоящее время нашли широкое применение в медицинской практике и сельском хозяйстве [3,11]. По мере изучения этих соединений постоянно выявляются новые фармакологические свойства, представляющие интерес для медицины.

Целью настоящего исследования явилось изучение синтеза и противовоспалительной активности ряда новых производных N-ацилзамещенных тиосемикарбазидов и продукта их гетероциклизации (тиадиазола).

Материал и методы. ИК-спектры сняты на спектрометре с Фурье-преобразователем «AVATAR-320» в таблетках с KBr, спектры ЯМР ^{1}H записаны на спектрометре Bruker DRX500 с частотой 500 МГц в растворе DMSO-d6 относительно внутреннего стандарта тетраметилсилан. Рентгеноструктурный анализ проведен на четырехкружном автоматическом дифрактометре “Xcalibur”. Масс-спектры сняты на приборе FINNIGAN MAT.INCOS 50 прямым вводом вещества с энергией ионизации 70 эВ. Тонкослойный хроматографический (ТСХ) анализ выполнен на пластинках «Sorbfil» в системе изопропиловый спирт-бензол-аммиак - 10:5:2, проявление парами йода. Температуры плавления определены на приборе «Boetius». Данные элементного анализа соединений (II-IV) и (VI) соответствуют вычисленным.Рентгеноструктурный анализ соединения (VI): параметры ячейки и интенсивности 2938 и 4063 независимых отражений для кристалла (VI) измерены на дифрактометре “Xcalibur” (CuK_α, графитовый монохроматор, θ/2θ-сканирование, $2\theta \leq 151^O$ и 169^O для VI). Кристаллы (VI) триклинные: a=8,837(6), b=9,818(5), c=12,126(13) Å, α=83,78(6), β=80,74(7), γ=80,40(5)^O, V=1020(2) Å^3, Z=4 ($C_7H_{11}N_3OS_2$), пространственная группа P1, $d_{выч}$=1,413 г/см^3. Структура расшифрована прямым методом. Позиции неводородных атомов уточнены в анизотропном приближении полноматричным методом наименьших квадратов. Атомы водорода помещены в геометрически рассчитанные положения и включены в уточнение в модели «наездника», которые выявлены из разностного синтеза электронной плотности и уточнены в изотропном приближении. В расчетах использовано 2306 и 3447 отражений с $I \geq 2\sigma(I)$. Окончательные факторы расходимости R_1=0,0731, $_wR_2$=0,2122. Структура расшифрована и уточнена по программам "SHELXS-97" и "SHELXL-97" [10]. Координаты атомов в виде CIF-файлов депонированы в Кембриджском центре кристаллоструктурных данных (CCCD 861096) для (VI).

N-Аллилтиосемикарбазид N-морфолинил уксусной кислоты (II). 1,59 г (0,01 моль) гидразида N-морфолинил уксусной кислоты растворяют в этаноле, затем приливают по каплям 0,9 г (0,011 моль) аллилизотиоцианата. Смесь перемешивают в течение 60 мин при температуре 50-60^{0}C. Завершение реакции контролируют по ТСХ. Раствор охлаждают, выпавший мелкокристаллический осадок отфильтровывают, промывают небольшим количеством холодного этанола. После перекристаллизации из бензола получают 1,91 г (74%) соединение (II) с т. пл. 137-138 ^{0}C. Масс-спектр, m/z, (Iотн): 258 $[M]^+$ (0,2), 115 (26,7), 100 (100), 56 (39,5), 41 (34,3).

N-(2-(2-морфолиноацетил)гидразинокарбонотиоил) бензамид (III). К раствору 1,55 г (0,011 моль) хлорангидрида бензойной кислоты в 15 мл ацетона при перемешивании на магнитной мешалке добавляют 1,07 г (0,011 моль) роданида калия. Перемешивают 2 часа при комнатной температуре и полчаса кипятят с обратным холодильником. Раствор отфильтровыва-

ют от выпавшей соли KCl через двойной бумажный фильтр, промывают несколько раз ацетоном и затем прикапывают к раствору 1,59 г (0,01 моль) гидразида морфолинил уксусной кислоты (I) в 10 мл абс. изопропилового спирта. Затем перемешивают при 60°C 3 ч. Отгоняют растворитель. Остаток кристаллизовывают при охлаждении. Получают 1,91 г (59,5%) белого кристаллического вещества с т. пл. 186-187°C (2-пропанол). ПМР-спектр (ДМСО-d6),δ, м.д.: 2.55 (т, 4H, $N(CH_2)_2$, J 4.3Гц), 3.32 (уш.с, 2H, $N-CH_2$), 3.63 (т, 4H, $O(CH_2)_2$, J 4.6 Гц), 7.66 (м, аром. 5H), 10.7 (уш.с, 1H, H-NHNC=O), 11.8 (с, 1H, H-NHNC=O), 12.7 (уш. с, 1H, C(O)H-N-C=S). Масс-спектр, m/z (Iотн): 322 $[M]^+$ (2), 105 (76), 100 (100), 77 (66.8), 56 (32.5), 51 (33.9), 42 (28.4).

4-Бром-(N-(2-(2-морфолиноацетил)гидразинокарбонотиоил)бензамид (IV) **с**интезирован аналогично соединению (III) из 2,41г (0,011 моль) хлорангидрида 4-бромбензойной кислоты, 1,07 г (0,011 моль) роданида калия и 1,59 г (0,01 моль) гидразида морфолинил уксусной кислоты (I). Получают 2,09 г (52,3%) белого кристаллического вещества с т.пл. 229-230°C (2-пропанол). ПМР-спектр (ДМСО-d6),δ, м.д.:2.55 (т, 4H, $N(CH_2)_2$, J4.2 Гц), 3.30 (уш.с, 2H, $N-CH_2$), 3.63 (т, 4H, $O(CH_2)_2$, J 4.5 Гц), 7.74, 7.89 (д., д, 4H, H-ArBr, J 8.55 Гц), 10.65 (уш.с, 1H, H-NHNC=O), 11.85 (с, 1H, H-NHNC=O), 12.60 (уш. с, 1H, C(O)H-N-C=S). Масс-спектр, m/z, (Iотн): 402 $[M]^+$ (4), 400$[M]^+$ (4), 100 (100), 56 (19), 42 (14.7).

Калиевая соль гидразинодитиоморфолинил уксусной кислоты (V). Смесь 1,59 г (0,01 моль) гидразида морфолинил уксусной кислоты (I), 0,84 г (0,015 моль) гидроксида калия растворяют в 15 мл безводного этанола и при охлаждении прикапывают 1,52 г (0,02 моль) сероуглерода. Реакционную смесь перемешивают в течение 2-3 часов. Выпавший порошкообразный продукт отфильтровывают, промывают несколько раз безводным диэтиловым эфиром. Получают 2,27 г (83%) калиевой соли гидразинодитиоморфолинил уксусной кислоты (V) с т.пл. 212-214°C.

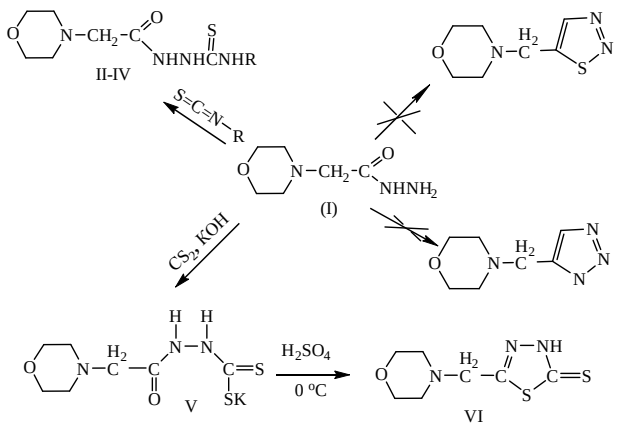
5-(Морфолинометил)-1,3,4-тиадиазол-2-тион (VI). 1,36 г (5 ммоль) калиевой соли гидразинодитиоморфолинил уксусной кислоты (V) растворяют порциями в 3 мл охлажденной до 0 ^{0}C концентрированной серной кислоты. Полученный раствор приливают к 50 мл ледяной воды и доводят до нейтральной среды. Продукт реакции экстрагируют этилацетатом. После сушки над прокаленным поташом и отгонки растворителя получают 0,97 г (45%) продукта (VI) с т. пл. 136-137°C (этанол). Для рентгеноструктурного исследования кристаллы соединения (VI) получают при естественном упаривании насыщенного спиртового раствора соединения. ПМР-спектр (ДМСО-d6)δ, м.д.:2.46 (т, 4H, $N(CH_2)_2$, J4.44 Гц), 3.32 (с, 2H,$N-CH_2$), 3.57 (т, 4H, $O(CH_2)_2$, J4.58 Гц), 14.37 (с, 1H, N-H). Масс-спектр, m/z,(Iотн): 217$[M]^+$ (50.3), 132 (20.1), 100 (100), 86 (65.5), 56 (28.8), 55 (24.6), 42 (22.3), 41 (15.7).

Экспериментальная биологическая часть. Подопытных животных содержали в условиях вивария (с естественным режимом освещения; при температуре 22–24°C; относительной влажности воздуха 40–50%) с использованием стандартной диеты (ГОСТ Р 50258-92). Исследования проводили в соответствии с правилами качественной лабораторной практики (GLP) при проведении доклинических исследований, а также правилами и Международными рекомендациями Европейской конвенции по защите позвоночных животных, используемых в экспериментальных исследованиях [1,9]. Перед постановкой эксперимента животные проходили карантин в течение 10–14 дней. Противовоспалительную активность образцов (II-IV, VI) изучали на модели острой экссудативной реакции (перитонит) на белых беспородных крысах [9]. Острую экссудативную реакцию (перитонит) вызывали внутрибрюшинным введением 1% раствора уксусной кислоты в объеме 1 мл на 100 г массы тела крыс. 3 часа спустя животных забивали под эфирным наркозом декапитацией, вскрывали брюшную полость, собирали экссудат и оценивали его объем [9]. Исследуемые вещества применяли в дозе 100 мг/кг при пероральном введении в виде крахмальной суспензии. Препарат сравнения «диклофенак натрия» применяли в дозе 25 мг/кг. Контрольные животные получали эквиобъемное количество крахмальной слизи. Исследуемые объекты вводили однократно за 1 час до введения 1% раствора уксусной кислоты. Статистическая обработка результатов проводилась с использованием пакета программ «Statistica 6,0». Полученные результаты представлены в виде «среднее значение, стандартная ошибка среднего значения». Межгрупповые отличия оценивали непараметрическим критерием Mann-Whitney U-test. Достоверными считались различия при достигнутом уровне значимости $p< 0,05$ [7].

Результаты и их обсуждение. Реакцию нуклеофильного присоединения гидразида N-морфолинил уксусной кислоты (I) к аллил-, бензоил-, 4-бромбензоилизотиоционатам осуществляли в спиртовой среде при эквимольных соотношениях реагентов. В результате реакции получены тиосемикарбазидные производные (II-IV), представляющие собой кристаллические вещества белого цвета, растворимые в полярных органических растворителях. Обычно подобные соединения (II-IV) в водно-щелочной среде способны к гетероцик-лизации с образованием производных тиадиазола [2,8]. В условиях реакций в спиртовой среде присутствие продуктов гетероциклизации не обнаружено, что, возможно, связано с наличием и характером электрононасыщенных заместителей (R) в тиосемикарбазидной группе, стабилизирующих молекулу продуктов (II-IV).

Известно, что используя разнообразные реагенты и меняя условия проведения реакции, возможно на-

править реакцию образования тиосемикарбазидных производных не только в сторону образования соединений, аналогичных строению (II-IV), но также и в 1,2,4-триазолов, 1,3,4-оксадиазов и 1,3,4-тиадиазолов [4,12]. С целью изучения противовоспалительных свойств других аналогов полученных соединений (II-IV) изучена возможность внутримолекулярной гетероциклизации тиосемикарбазидного производ-ного гидразида N-морфолинил уксусной кислоты (I) в кислой среде. Реакция осуществлялась в две стадии в условиях «*one pot*»: на первой стадии взаимодействием гидразида N-морфолинил уксусной кислоты (I) с сероуглеродом получили калиевую соль гидразинодитиоморфолинил уксусной кислоты (V). На следующей стадии калиевая соль гидразинодитиоморфолинил уксусной кислоты (V) под действием серной кислоты (конц.) при пониженной температуре подверглась циклизации в 5-(морфолинометил)-1,3,4-тиадиазол-2(3H)-тион (VII).



R = CH_2=CH-CH_2- (II); $C_6H_5C(O)$- (III); 4-Br-$C_6H_4C(O)$- (IV)

Состав, строение и индивидуальность синтезированных соединений (II-IV) подтверждены данными элементного анализа, ИК-, ЯМР ^{1}H- спектроскопией, масс – спектрометрией. В ИК-спектрах синтезированных соединений (II-IV) проявляется полоса поглощения в области 1140-1240 $см^{-1}$, характерная для - NH-CS группы тиосемикарбазидного фрагмента, полосы поглощения амидной группы C(O)NH появляются в области 1690-1675 $см^{-1}$ и NH-группы в области 3390-3360 $см^{-1}$ [2,8].

При анализе спектров ЯМР ^{1}H соединений (II-IV) наблюдаются характерные сигналы метиленовых протонов морфолинового и тиосемикарбазидных фрагментов. Так, например, в спектре соединения (III) сигналы метиленовых протонов прописываются в виде двух триплетов в области с центром 2.55 м.д. и 3.63 м.д. Метиленовые протоны NCH_2-фрагмента проявляются в области 3.18 м.д. узким синглетом. Протоны ароматического фенильного кольца резонируют соответственно в виде дуплета и 2 триплетов при 7.36 м.д., 7.53 м.д., и 7,65 м.д. Амидные и тиоамидные N-H протоны выписываются в области слабых полей в виде трех синглетов в области 10.65 м.д., 11.8 м.д. и 12.7 м.д.

5-(Морфолинометил)-1,3,4-тиадиазол-2(3H)-тион (VI) представляет собой бесцветное кристаллическое вещество, хорошо растворимое в полярных органических растворителях. Такого рода соединения способны к таутомерным тион-тиольным превращениям и, как правило, в кристаллическом состоянии представляют собой тионы, что подтверждается ИК-спектрами. В области 2700-2450 $см^{-1}$ не наблюдаются полосы, характерные для валентных колебаний SH-группы, но обнаруживаются отчетливые полосы для группы NH (в области 3300-3100 $см^{-1}$) и группы C=S (в области 1350 $см^{-1}$) [2,8].

Физико-химические константы и выходы соединений (II-IV,VI) приведены в таблице 1. Кристаллическое строение вещества (VI) подтверждено рентгеноструктурным анализом строения молекулы (рис.).

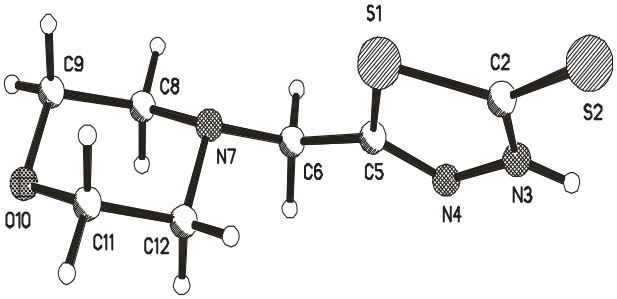


Рис. Кристаллическая структура молекулы (VI)

В независимой части элементарной ячейки (VI) находятся две молекулы VIa и VIb. Длины связей и валентные углы в обеих молекулах близки к обычным [11]. Морфолиновые циклы в VIa и VIb принимают конформацию почти идеального кресла. Тиадиазольные циклы в обеих молекулах плоские (атомы S1, C2, N3, N4 и C5 в VIIa и S1', C2', N3', N4 и C5' в VIb копланарны). Атом C6 ориентирован экваториально относительно морфолинового цикла.

Исследования показали, что у крыс в опытной группе через три часа после введения уксусной кислоты объем экссудата был существенно ниже (на 39,4%, 25,4%, 39,2%, 47%) по сравнению с показателем в контрольной группе и сопоставим по сравнению с показателем в группе животных, получавших «диклофенак натрия», т.е. соединения (II, IV, VI) проявляют выраженную противовоспалительную активность на модели острой экссудативной реакции.

Соединение (III) оказывает умеренное действие. Результаты исследований противовоспалительной активности образцов (II-IV) и (VI) приведены в таблице 2.

Таблица 1. Физико-химические константы соединений II-IV, VI

Соед.	Выход, %	Т.пл. ^{0}C	Брутто-формула
II	74	137	$C_{10}H_{18}N_4O_2S$
III	60	186-187	$C_{14}H_{18}N_4O_3S$
IV	59	229-230	$C_{14}H_{17}BrN_4O_3S$
VI	45	136-137	$C_7H_{11}N_3OS_2$

Таблица 2. Влияние образцов соединений (II-IV, VI) и диклофенака натрия на количество образовавшегося экссудата в брюшной полости крыс

№ соед.	Химическое название	Масса животных, гр.	Количество экссудата, мл
II, n=10	N-аллилтиосемикарбазид N-морфолинилуксусной кислоты	272±9	5,12±0,30*
III, n=10	N-(2-(2-морфолиноацетил) гидразинокарбонотиоил)бензамид	282±10	6,30±0,31*#
IV, n=10	4-бром-(N-(2-(2-морфолиноацетил) гидразинокарбонотиоил)бензамид	294±9	5,14±0,34*
VI, n=10	5-(морфолинометил) -1,3,4-тиадиазол-2-тион	276±9	4,47±0,88*
Контроль, n=10		289±7	8,45±0,89
Диклофенак натрия, n=10		284±8	4,05±0,67*

*примечание - * - p< 0,05 по сравнению с контролем;*
 # - p < 0,05 по сравнению с группой сравнения (диклофенак натрия)

Таким образом, результаты экспериментального исследования показали, что среди изученных производных N-морфолинил уксусной кислоты имеются соединения (II-IV и VI), обладающие противовоспалительной активностью. Возможно, что противовоспалительные свойства новых производных связаны с их антибактериальными свойствами, обусловленными наличием в их химической структуре тиосемикарбазидного и 1,3,4-тиадиазол-2(3H)-тионового фрагментов. Полученные результаты позволяют рекомендовать проведение в дальнейшем расширенных доклинических испытаний с целью изучения механизмов противовоспалительной активности и безопасности исследуемых соединений.

Выводы:

1. Реакция нуклеофильного присоединения гидразида N-морфолинил уксусной кислоты к аллил-, бензоил-, 4-бромбензоилизотиоционатам в спиртовой среде приводит к получению новых тиосемикарбазидов. В реакционной среде отсутствие продуктов гетероциклизации (тиадиазолов) объясняется наличием электрононасыщенных заместителей в тиосемикарбазидной группе и их стабилизирующим действием.
2. Взаимодействие гидразида N-морфолинил уксусной кислоты с сероуглеродом в щелочной среде приводит к образованию калиевой соли гидразинодитиоморфолинил уксусной кислоты, которая при подкислении серной кислотой (концентрированной) при пониженной температуре легко претерпевает циклизацию в 5-(морфолинометил)-1,3,4-тиадиазол-2(3H)-тион.
3. Выявлены соединения тиосемикарбазидов N-морфолинил уксусной кислоты, обладающие выраженной противовоспалительной активностью, которые представляют интерес для дальнейших более углубленных фармакологических исследований.

ЛИТЕРАТУРА

1. Европейская конвенция «О защите позвоночных животных, используемых для экспериментов или в иных научных целях». Страсбург, EST №123. Электронный ресурс. – URL: http://www.lawmix.ru/abro.php?id=11036. (по состоянию на 21.01.2013).
2. Ильиных Е.С., Ким Д.Г. Исследование реакций 2-амино-5-трифторметил-1,3,4-тиадиазола в основной среде. Вестник ЮУрГУ 2011; 12: 18-21.
3. Ингерлейб М.Б. Жизненно важные лекарственные средства: карманный справочник. М.: Феникс; 2011: 416.
4. Коньшин М.Е., Сыропятов Б.Я., Вахрин М.И., Мельникова Н.А., Ефремов А.Л. Синтез, Антикоагулянтная, антимикробная и противогрибковая активность бета-антранилоилгидразидов 2-ариламиноникотиновых кислот. Хим.-фарм. журнал 2011; 3: 25-26.
5. Марышева В.В., Шабанов П.Д. Способ повышения антигипоксической активности 3,5-диамино-1.2,4-тиадиазола. Патент РФ №2431483. Опубл. 20.10.2011. Бюлл. №29.
6. Машковский М.Д. Лекарственные средства. 16 издание. М.: Новая Волна; 2012: 1216.

7. Мятлев В.Д., Панченко Л.А., Ризниченко Г.Ю., Терехин А.Т. Теория вероятностей и математическая статистика, математические модели**.** Серия: Университетский учебник. Высшая математика и её приложения к биологии. М.: «Академия»; 2009: 320.
8. Нуркенов О.А., Фазылов С.Д., Сатпаева Ж.Б., Сейлханов Т.М. Синтез и строение новых 1,2,4-триазолов на основе гидразида *n*-гидроксибензойной кислоты. Журнал общ. химии 2015; 85 (Вып. 1): 62-67.
9. Хабриев Р.У. Руководство по экспериментальному (доклиническому) изучению новых фармакологических веществ. М.: Медицина; 2005: 832.
10. Chantler C.T., Islam M.T., Rae N. A., Tran C.Q., Glover J.L., Barnea Z. New consistency tests for high-accuracy measurements of X-ray mass attenuation coefficients by the X-ray extended-range technique. Acta Crystallographica. Section A 2012; 68 (2): 188-195.
11. Karakurt A., Ozalp M., Isik S., Stables J.P., Dalkara S. Synthesis, anticonvulsant and antimicrobial activities of some new 2-acetylnaphthalene derivatives. Bioorg. Med. Chem. 2010; 18(8): 2902-2911.
12. Rajasekaran A., Rajamanickam V., Darlinquine S. Synthesis of some new thioxoquinazolinone derivatives and a study on their anticonvulsant and antimicrobial activities. European Review for Medical and Pharmacological Sciences 2013; 17: 95-104.
13. Sharma B., Verma A., Prajapati S., Sharma U. K. Synthetic Methods, Chemistry, and the Anticonvulsant Activity of Thiadiazoles. International Journal of Medicinal Chemistry 2013; 16.
14. Schepetkin I.A., Kirpotina L.N., Khlebnikov A.I., Cheng Ni., Ye R.D., Quinn M.T. Antagonism of human formyl peptide receptor 1 (FPR1) by chromones and related isoflavones. Biochemical Pharmacology 2014; 92: 627–641.

SUMMARY

RESEARCH OF ANTI-INFLAMMATORY ACTIVITY OF THE THIOSEMICARBAZIDEN-MORPHOLINILACETIC ACID

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Studying of synthesis and anti-inflammatory activity of a number of new derivatives of N-acylsubstituted of thiosemicarbazide and product of their heterocyclization (thiadiazole).

In work the following reagents are used: hydrazide of N-morfolinilacetic acids, allil-, benzoil-, 4-brom-benzoil isothiocyanates. IR spectrums of compounds are removed on a spectrometer from Fourier converter by "AVATAR-320" in tablets with KBr, 1H NMR spectra were recorded on Bruker DRX500 spectrometer with a frequency of 500 MHz in DMSO-d6 solution relative to internal tetramethylsilane standard. X-ray diffraction analysis was carried out on four circuitous automatic diffractometer "Xcalibur". Mass spectra were recorded on a device FINNIGAN MAT.INCOS 50 direct input material with an ionization energy of 70 eV. Thin-layer chromatographic (TLC) analysis was performed on plates «Sorbfil» system benzene-isopropanol-ammonia 10:5:2, display iodine vapor. Melting point defined on the device «Boetius». Anti-inflammatory activity of compounds is studied on white not purebred rats. Statistical processing of results was carried out with use of the software package of "Statistica 6,0".

The experimental results showed that, among the received new hydrazide derivatives of N-morpholinilacetyc acids are compounds (II-IV and VI), which have anti-inflammatory activity. It is possible that novel anti-inflammatory properties associated with their antibacterial properties due to the presence in their chemical structure and thiosemicarbazides 1,3,4-thiadiazol-2 (3H)-thione fragments. The obtained results allow us to recommend the test compounds for advanced pre-clinical trials to study their properties.

Based on N-hydrazide morpholinil acetic acid, a number of new N-acyl-substituted derivatives of thiosemicarbazide is synthesized and described, composition and structure of which is proved by IR, 1H NMR spectroscopy and X-ray analysis. In an experimentation rats is founded anti-inflammatory activity of N-acyl-substituted thiosemicarbazide.

Keywords: hydrazide, thiosemicarbazide, 1,3,4-thiadiazole-2-tion, anti-inflammatory activity.

РЕЗЮМЕ

ИЗУЧЕНИЕ ПРОТИВОВОСПАЛИТЕЛЬНОЙ АКТИВНОСТИ ТИОСЕМИКАРБАЗИДОВ N-МОРФОЛИНИЛ УКСУСНОЙ КИСЛОТЫ

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Цель исследования - изучение синтеза и противовоспалительной активности ряда новых производных N-ацилзамещенных тиосемикарбазидов и продукта их гетероциклизации (тиадиазола).

В работе использованы следующие реагенты: гидразид N-морфолинил уксусной кислоты, ал-

лил-, бензоил-, 4-бромбензоилизотиоционаты. ИК-спектры соединений сняты на спектрометре с Фурье-преобразователем «AVATAR-320» в таблетках с KBr, спектры ЯМР ^{1}H записаны на спектрометре "Bruker DRX500" с частотой 500 МГц в растворе DMSO-d6 относительно внутреннего стандарта тетраметилсилан. Рентгеноструктурный анализ проведен на четырехкружном автоматическом дифрактометре“Xcalibur”. Масс-спектры сняты на приборе "FINNIGAN MAT.INCOS 50" прямым вводом вещества с энергией ионизации 70 эВ. Тонкослойный хроматографический анализ выполнен на пластинках «Sorbfil» в системе изопропиловый спирт-бензол-аммиак - 10:5:2, проявление парами йода. Температуры плавления определены на приборе «Boetius». Противовоспалительная активность соединений изучена на белых беспородных крысах. Статистическая обработка результатов проводилась с использованием пакета программ «Statistica 6,0».

Результаты экспериментального исследования показали, что среди полученных новых производных гидразида N-морфолинил уксусной кислоты имеются соединения (II-IV и VI), обладающие противовоспалительной активностью. Возможно, что противовоспалительные свойства новых производных связаны с их антибактериальными свойствами, обусловленными наличием в их химической структуре тиосемикарбазидного и 1,3,4-тиадиазол-2(3H)-тионового фрагментов. Полученные результаты позволяют рекомендовать проведение в дальнейшем расширенных доклинических испытаний с целью изучения механизмов противовоспалительной активности и безопасности исследуемых соединений.

На основе гидразида N-морфолинил уксусной кислоты синтезирован и описан ряд новых производных N-ацилзамещенных тиосемикарбазидов, состав и строение которых доказаны данными ИК-, ЯМР^{1}H-спектроскопии и рентгеноструктурного анализа. В эксперименте на крысах изучена противовоспалительная активность производных N-ацилзамещенных тиосемикарбазидов.

რეზიუმე

N-მორფოლინძმარმჟავას თიოსემიკარბაზიდის ანთების საწინააღმდეგო აქტივობის შესწავლა

[1]რ. ბაკიროვა, [2]ს.ფაზილოვი, [2]ო.ნურკენოვი, [1]ლ. მურავლევა, [3]ა. ჟაკუპოვა

[1]ყარაგანდის სახელმწიფო სამედიცინო უნივერსიტეტი, ყარაგანდა, ყაზახეთი; [2]ორგანული სინთეზის ინსტიტუტი, ყარაგანდა, ყაზახეთი; [3]ევრაზიული ინოვაციური უნივერსიტეტი, პავლოდარი, ყაზახეთი

კვლევის მიზანს შეადგენდა N-აცილჩანაცვლებული თიოსემიკარბაზიდების ახალი წარმოებულების და მათი ჰეტეროციკლიზაციის პროდუქტის (თიადიაზოლი) სინთეზისა და ანთების საწინააღმდეგო აქტივობის შესწავლა.

ექსპერიმენტული კვლევის შედეგებით გამოვლინდა,რომ N-მორფოლინძმარმჟავას ჰიდრაზიდის მიღებულ წარმოებულებს შორის არის ნაერთები (II – IV,VI) გამოხატული ანთების საწინააღმდეგო აქტივობით. შესაძლოა, ახალი წარმოებულების ანთების საწინააღმდეგო მოქმედება დაკავშირებულია მათ ანტიბაქტერიულ თვისებებთან, რაც განპირობებულია თიადიაზოლ-2(3H)-თიონური ფრაგმენტების არსებობით. მიღებული შედეგები იძლევა აღნიშნული ნაერთების თვისებების შესწავლის მიზნით მათი გაფართოებული წინაკლინიკური კვლევის რეკომენდების შესაძლებლობას.

N-მორფოლინძმარმჟავას ჰიდრაზიდის საფუძველზე სინთეზირებულია და აღწერილია N-მორფოლინძმარმჟავას თიოსემიკარბაზიდების ახალი წარმოებულები,რომელთა შემადგენლობა და შენება შესწავლილია ბირთვულ-მაგნიტური რეზონანსული^{1}H-სპექტროსკოპით და რენტგენოსტრუქტურული ანალიზით. ექსპერიმენტში ვირთაგვებზე შესწავლილია N-აცილჩანაცვლებული თიოსემიკარბაზიდების წარმოებულების ანთების საწინააღმდეგო აქტივობა.

ИННОВАЦИОННЫЕ ОБРАЗОВАТЕЛЬНЫЕ ТЕХНОЛОГИИ В ПРЕПОДАВАНИИ ПРОПЕДЕВТИКИ ВНУТРЕННИХ БОЛЕЗНЕЙ

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В эпоху роста научно-экономического и медицинского потенциала общества, правового сознания населения, информационно-технологической модернизации производства, повышаются требования к подготовке высококвалифицированных специалистов, обладающих широким кругозором, ориентированных в вопросах не только своей профессии, но и права, менеджмента, маркетинга [1,4]. В этой связи перед медицинскими вузами Казахстана встают вопросы совершенствования системы медицинского образования, разработки и внедрения инновационных образовательных программ, повышения качества образования. Решающую роль в этом должны сыграть реорганизация учебного процесса и контроля знаний студентов, учебно-методическое и материальное обеспечение высших учебных заведений. Современный подход к образовательным технологиям диктует необходимость реформирования системы подготовки медицинских кадров, внедрения как известных зарубежных, так и собственных эффективных форм и методов обучения [7].

Целью исследования явилось проведение анализа внедрения инновационных технологий обучения при преподавании пропедевтики внутренних болезней.

Материал и методы. Методами исследования являлись теоретические; педагогический эксперимент; наблюдение; тестирование; метод научного анализа.

Результаты и их обсуждение. Пропедевтика внутренних болезней является одной из первых клинических дисциплин, с которой студенты начинают познавать внутренние болезни и продолжают этот процесс до окончания медицинского вуза. Дисциплина в нашем университете преподается на третьем курсе. Основной задачей пропедевтики внутренних болезней является обучение студентов методике клинического обследования больных, основным лабораторным и инструментальным методам исследования, семиотике заболеваний внутренних органов, построению синдромного диагноза. Выявление ведущего синдрома, причин и обстоятельств его возникновения, характера развития патологического процесса основывается на клинической компетентности будущего врача. Поэтому обучающийся должен владеть не только теоретическими знаниями, но и грамотно, последовательно выполнять практические навыки обследования пациентов. Инновационные технологии, используемые в университете, приводят к видоизменению и совершенствованию методик и приемов обучения, способствующих трансформации содержания и качества образовательного процесса на более высокий уровень [7,9].

На кафедре пропедевтики внутренних болезней Карагандинского государственного медицинского университета учебный процесс проводится по кредитно-модульному принципу с применением интегрированного обучения. Интегрированное обучение – это форма организации образовательной деятельности с использованием системного подхода, междисциплинарной связи, способствующее расширению кругозора, творческой активности субъектов этого процесса, повышению их компетентности [8]. Междисциплинарная интеграция на 3 курсе содержит и объединяет материалы восьми дисциплин: нормальной анатомии и физиологии, патологической анатомии и физиологии, гистологии, визуальной диагностики, фармакологии и пропедевтики внутренних болезней. Кредитно-модульная система включает 8 модулей по следующим системам: дыхательная, сердечно-сосудистая, опорно-двигательная, эндокринная, мочеполовая, кроветворная, нервная, пищеварительная.

Учебный процесс на кафедре пропедевтики внутренних болезней проходил в форме аудиторной работы (лекции, практические занятия, самостоятельная работа студента под руководством преподавателя) с использованием интерактивных методов обучения: разбора клинических случаев (Problem-Based Learning - PBL), решения ситуационных задач (Team-Based Learning - TBL, Case-Based Learning - CBL), ролевых игр и др. По методике проведения занятий по данным технологиям обучения получены свидетельства о регистрации прав на объект авторского права [2,3]. Приоритетными направлениями данных интерактивных методов обучения являются приобретение клинических, коммуникативных и практических навыков у студентов, обучение навыкам работы в команде и уважительному отношению к мнениям коллег. При подготовке к занятиям студенты совершенствуют навыки научно-исследовательской работы. Так, наши студенты ежегодно занимают призовые места в международных студенческих научных форумах.

Главными действующими лицами на таких занятиях являются сами студенты. Преподаватели, выступая в роли тьютора, являются экспертами в решении обсуждаемой проблемы, не вмешиваясь в дискуссию, они наблюдают за творческой работой студентов. Участники дискуссии, выдвигают различные гипотезы, обосновывают

их, принимают решения, используя знания смежных и базовых дисциплин. При решении проблемных ситуаций активизируется клиническое мышление студента, улучшается познавательный процесс, качество освоения учебного материала, он ощущает свою интеллектуальную состоятельность.

План самостоятельной работы студентов был согласован со смежными дисциплинами и выполнялся в форме презентации проекта, написания фрагмента истории болезни тематического больного, решения ситуационных задач, ответов на вопросы самоконтроля.

Открытые занятия, проводимые на смежных кафедрах, позволяли преподавателям делиться опытом своего профессионального мастерства с коллегами, обучаться методологии и структуре проведения занятий и лекций, использованию новейших технических средств в образовательном процессе.

Сбор жалоб и анамнеза у больных осуществлялся в палатах профильных отделений. В процессе работы студент осваивал навыки психологического контакта с больным, обращал внимание на его поведенческие реакции. Проведенный расспрос и физикальное обследование пациента позволяло студенту формулировать предварительную диагностическую гипотезу. Обучающийся проводил сравнительный анализ клинической симптоматики пациента с классической картиной заболевания, изложенной в учебных пособиях. Вопросы, возникшие при обследовании пациента, требовали поиска дополнительной медицинской информации в библиотечном фонде и электронных базах университета. Информационная структура университета, оснащенная современной аппаратурой и средствами связи, обеспечивает широкий доступ обучающихся к Интернет-ресурсам, электронным учебно-образовательным комплексам и библиотекам, мультимедийным обучающе-тестирующим системам и др. В библиотеке имеется достаточная электронная картотека учебных материалов и научных изданий.

Применение компьютерных технологий в образовательном процессе и тренажеров стало возможным после создания в университете Центра практических навыков (ЦПН). Тренажерные системы позволяют подготовить обучающихся к принятию качественных и безотлагательных решений в различных клинических ситуациях. Результаты работы студентов автоматически обрабатывались, что позволяло проводить мониторинг их достижений. Оснащенность видеооборудованием позволяло наблюдать и оценивать качество выполнения навыков студентами и эффективность проведения практического занятия преподавателями.

Внедрение интегрированного обучения, современного технического оснащения (мультимедийные проекторы, видео-, кинофильмы и др.) внесло коррекцию в методику изложения лекционного материала. Так, в проблемной лекции освящался определенный раздел с обязательной демонстрацией больного, что стимулировало познавательный и мыслительный процесс студентов. Лекционный материал излагался не в форме монолога, а в виде диалога, дискуссии, что способствовало активизации студенческой аудитории к решению проблемных вопросов.

Проводились лекции-симпозиумы с участием лекторов смежных дисциплин (патологической анатомии, патофизиологии, визуальной диагностики, фармакологии и др.), на которых раскрывалась структура конкретного заболевания, например: пневмонии, гломерулонефрита, геморрагического инсульта, язвенной болезни.

Студенты младших курсов порой неспособны осознать значение фундаментальных дисциплин для будущей деятельности врача. Сущностный (или инвариантный) подход в обучении необходимо использовать не только в отдельных дисциплинах, но и при установлении межпредметных связей для достижения конечных целей подготовки специалиста [4,9]. В этой связи использование междисциплинарных ситуационных задач позволяет, с одной стороны, обобщить и интегрировать теоретические и практические знания смежных дисциплин, с другой стороны, оценить клиническое мышление студентов. Следует отметить, что в текущем учебном году с целью повышения познавательной мотивации студентов были выпущены преподавателями смежных дисциплин 3 курса интегрированные учебники по 8 модулям на государственном и русском языках, которые включали вопросы нормы и патологии.

Проверка и оценка знаний, умений и навыков студентов – важный структурный компонент всего процесса обучения. В соответствии с принципами систематичности, последовательности и прочности обучения она должна осуществляться в течение всего периода обучения [9]. Текущий контроль уровня знаний и умений студентов по пропедевтике внутренних болезней проводился в виде оценки текущей успеваемости и мини-клинического экзамена (МКЭ). МКЭ проводился после каждого пройденного модуля и включал оценку следующих критериев: сбор анамнеза, физикальный осмотр, коммуникативные навыки, профессиональная этика, клиническое мышление, организованность/эффективность, общая клиническая компетентность. Результаты выполнения этих критериев заносились в оценочные листы МКЭ. Известно, что профессиональные навыки, в частности контакт с больным, являются составными элементами успешной деятельности врача. Научить этому можно при непосредственном доступе к больному в учебном процессе. Преимущество мини-экзамена состоит в том, что он позволяет наблюдать и анализировать работу студентов с тематическим

больным непосредственно в палате. Обсуждение итогов проведения, формулирование синдромов, с выделением ведущего, проводилось в учебной комнате. Предоставление «обратной связи» экзаменатором заключалось в выяснении удовлетворенности студента выполненным заданием, указании достоинств и недостатков их выполнения, демонстрации навыков после их оценки. Мини-клинический экзамен требовал определенной подготовки экзаменаторов, которые должны иметь научную степень, либо не менее 5-летний опыт клинической практики и преподавательской деятельности. Преимуществами МКЭ являются возможность наблюдения за выполнением студентом заданий в реальной обстановке; оценка способностей студентов; минимальные административные расходы; увеличение достоверностии с ростом количества проводимых модулей. Анализ результатов МКЭ показал, что средний балл по 8 модулям в 2013-2014 учебном году был выше (77%) в сравнении с предыдущим годом (75%), за счет улучшения выполнения таких навыков, как сбор анамнеза, физикальное обследование, общая клиническая компетентность. На английском отделении средний балл в последние 2 года составил 70% и 69%, соответственно, что, по-видимому, связано с трудностями расспроса пациентов иностранными студентами.

Рубежный контроль знаний студентов в конце каждого модуля оценивался в форме тестовых заданий по восьми дисциплинам с ежегодным их обновлением на 40%. Качественный показатель по модулю указывает на клиническую компетентность обучающегося в данной области, т.е. трансформацию приобретенных знаний в профессиональные навыки.

Итоговый контроль знаний студентов проводился также с соблюдением интегрированного подхода, т.е. задания экзамена включали вопросы всех восьми дисциплин. Итоговый экзамен прохожил в два этапа: первый этап – объективный структурированный клинический экзамен (ОСКЭ), второй этап – комплексное тестирование. ОСКЭ проводился в центре практических навыков и включал в себя 9 станций с ситуационными задачами и заданиями смежных дисциплин 3 курса. Умение студентами проводить сбор жалоб и анамнеза больного анализировался на стандартизированном пациенте.

Результаты ОСКЭ прошлого учебного года у 953 студентов специальности «общая медицина» показали достаточно высокий средний балл по пропедевтике внутренних болезней - 85% (B+). Причем, на русском и английском отделениях он превышал общий показатель (89% и 86% соответственно), на казахском отделении был ниже (81%). Наиболее высокие результаты студенты показали на следующих станциях: «Сбор анамнеза и коммуникативные навыки», «Аускультация сердца в норме и патологии», «Аускультация легких в норме и патологии». Низкий балл (менее 60%) получили 11 (2,3%) студентов казахского отделения, 4 (0,9%) студента русского отделения и 1 (4,2%) студент английского отделения.

Средний балл второго этапа итогового контроля - письменного экзамена по дисциплине составил B+(86%), что подтвердил результат предыдущего года.

Таким образом, студенты 3 курса продемонстрировали достаточный уровень клинической компетентности по пропедевтике внутренних болезней. В целом, средний рейтинг успеваемости студентов русского и казахского отделения не изменился по сравнению с предыдущим годом и составил B (81%) и B-(79%) соответственно; у студентов иностранного отделения снизился с B- (76%) до C+ (72%) за счет снижения балла текущей успеваемости.

Значимой составляющей частью учебного процесса на кафедре является воспитательная работа, направленная на формирование у студентов патриотизма, гражданственности, интернационализма, высокой морали и нравственности. Следует отметить, что преподаватель клинической дисциплины воспринимается не только как врач, но и как воспитатель, оказывающий большое влияние на окружающих его людей. А это, в свою очередь, требует постоянного педагогического самосовершенствования и самообразования путем изучения вопросов педагогики, психологии, теории управления, методики учебной дисциплины, культурологии и других дисциплин. В связи с чем надо отметить, что профессорско-преподавательский состав кафедры прошел курсы повышения педагогической квалификации, с участием известных ученых и докторов университетов США, Великобритании, России, по формированию навыков использования инновационных технологий в учебном процессе.

Таким образом, кредитно-модульная технология обучения способствует повышению мотивации студентов к приобретению знаний, а преподавателей - к совершенствованию их педагогического мастерства. Повышение качества медицинского образования до уровня международных стандартов является главным условием успешной реализации программы подготовки высококомпетентного специалиста.

ЛИТЕРАТУРА

1. Арзыкулова Б.Ж., Арыстанбаева А.Т. Интерактивные методы обучения в образовательном процессе. Научный мир Казахстана 2009; 1: 139.
2. Бакирова Р.Е., Нурсултанова С.Д.,Турханова Ж.Ж., Мирзо Е.И., Ли В.В. The methodological approaches to organization and teaching of practical lessons by module “Respiratory system” with use innovative technology which include the clinical case (Case Based Learning - CBL). Свидетельство о регистрации прав на объект авторского права 2014; 632.

3. Бакирова Р.Е., Тусупбекова К.Т., Турханова Ж.Ж., Беков Е.К., Ли В.В. Методические подходы к организации и проведению занятий по модулям «Пищеварительная система», «Эндокринная система», «Кроветворная система» с применением инновационной технологии, основанной на проблемном обучении (Problem-Based Learning). Свидетельство о регистрации прав на объект авторского права № 722. 2014.
4. Денисов И.Н., Артамонов Р.Г., Улумбеков Э.Г., Улумбекова Г.Э. Модульный принцип- основа современного образования врачей. Методические рекомендации. М.: 2005: 29.
5. Досмагамбетова Р.С., Молотов-Лучанский В.Б., Муратова А.З., Калиева Ш.С. Инновационные технологии в обучении и оценке учебных достижений студентов Карагандинского государственного медицинского университета: Руководство для преподавателей медицинских вузов. Караганда: 2011; 127.
6. Денисов И.Н., Артамонов Р.Г., Улумбеков Э.Г.и др. Модульный принцип - основа современного образования врачей: Метод.-учеб. пособие. М.: изд-во ММИ им. И.М. Сеченова; 2002: 140.
7. Досмагамбетова Р.С., Калиева Ш.С., Кемелова Г.С. и др. Педагогический процесс в медицинском образовании: монография. КГМУ: 2012; 127.
8. Досмагамбетовой Р.С., Кемеловой Г.С., Мулдаевой Г.М. Сферы компетентности выпускника Карагандинского государственного медицинского университета специальности «Общая медицина». Учебно-методическое пособие. Караганда: 2012; 115.
9. Мухина С.А., Соловьева А.А. Современные инновационные технологии обучения. М.: ГЭОТАР - Мед.: 2008; 504.

SUMMARY

INNOVATIVE EDUCATIONAL TECHNOLOGY IN THE TEACHING OF PROPAEDEUTIC OF INTERNAL DISEASES

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This article presents analysis of the results of inculcation of innovative learning technologies in teaching on propaedeutic of internal diseases which is first clinical discipline faced by medical students of the University. Credit-modular training included integration of propaedeutic of internal diseases with basic disciplines of the third year (the normal anatomy, physiology, pathological anatomy, histology, pathophysiology, visual diagnostics and pharmacology). There are 8 models on following systems: respiratory, cardiovascular, musculoskeletal, endocrine, urogenital, hematopoietic, nervous, digestive. The innovative implementation of learning technologies (Problem-based learning, clinical cases, team-oriented teaching, lectures, symposium lectures, discussions, role plays, etc.) and knowledge control (mini-clinical examination, objective structured clinical exam, comprehensive testing) help students to acquire clinical skills, team working and skills of researching work.

Keywords: credit-modular training, interdisciplinary integration, Problem-based Learning, Team-Based Learning, Case-Based Learning, objective structured clinical examination.

РЕЗЮМЕ

ИННОВАЦИОННЫЕ ОБРАЗОВАТЕЛЬНЫЕ ТЕХНОЛОГИИ В ПРЕПОДАВАНИИ ПРОПЕДЕВТИКИ ВНУТРЕННИХ БОЛЕЗНЕЙ

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В статье проанализированы результаты внедрения инновационных технологий обучения при преподавании пропедевтики внутренних болезней. Кредитно-модульное обучение с интеграцией пропедевтики внутренних болезней с базовыми дисциплинами третьего курса (нормальная анатомия, физиология, патологическая анатомия, гистология, патофизиолгия, визуальная диагностика и фармакология) включает 8 модулей по следующим системам: дыхательная, сердечно-сосудистая, опорно-двигательная, эндокринная, мочеполовая, кроветворная, нервная, пищеварительная. Внедрение нновационных технологий обучения (проблемно-ориентированное обучение, обучение на клинических случаях, командно-ориентированное обучение, лекции-симпозиумы, лекции-дискуссии, ролевые игры и др.) и контроля знаний (мини-клинический экзамен, объективный структурированный клинический экзамен, комплексное тестирование) способствует приобретению студентами клинических навыков, навыков работы в команде, навыков научно-исследовательской работы.

რეზიუმე

ინოვაციური საგანმანათლებლო ტექნოლოგიები შინაგან სნეულებათა პროპედევტიკის სწავლებაში

კ. ტუსუპბეკოვა, რ. ბაკიროვა, ს. ნურსულტანოვა

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სტატიაში გაანალიზებულია შინაგან სნეულებათა პროპედევტიკის სწავლების პროცესში ინოვაციური ტექნოლოგიების დანერგვის შედე-

გები. კრედიტ-მოდულური სწავლება შინაგან სნეულებათა პროპედევტიკის ინტეგრაციით მესამე კურსის საბაზისო დისციპლინებთან (ნორმალური ანატომია,ფიზიოლოგია,პათოლოგიური ანატომია, გისტოლოგია, პათოფიზიოლოგია, ვიზუალური დიაგნოსტიკა და ფარმაკოლოგია) შეიცავს 8 მოდულს შემდეგ სისტემებში: სუნთქვითი, გულ-სისხლძარღვთა, საყრდენ-მამოძრავებელი, ენდოკრინული, საშარდო-სასქესო, ნერვული, მომნელებელი. სწავლების ინოვეციური ტექნოლოგიების (პრობლემაზე ორიენტირებული სწავლება, კლინიკურ შემთხვევებზე,დაფუძნებული სწავლება, გუნდობაზე ორიენტირებული სწავლება,ლექცია-სიმპოზიუმი,ლექცია-დისკუსიები,როლური თამაშები) და სწავლის კონტროლი (მინი-კლინიკური გამოცდა, ობიექტურად სტრუქტურირებული კლინიკური გამოცდა,კომპლექსური ტესტირება) ხელს უწყობს სტუდენტების მიერ კლინიკური უნარ-ჩვევების,ჯგუფში მუშაობის და სამეცნიერო კვლევითი მუშაობის ათვისებას.

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