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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

No 9 (258) 2016

This special issue of the journal is dedicated to the project
"Improving research and writing skills among young Georgian academics"
funded through the University Research Program by the U.S. Embassy in Georgia
Guest Editor – George Kamkamidze, MD, PhD, MS

Номер журнала посвящается проекту "Улучшение научно-исследовательских и писательских навыков среди молодых научных работников Грузии", который финансируется в рамках Университетской научно-исследовательской программы при поддержке посольства США в Грузии
Приглашенный редактор – доктор медицинских наук Георгий Камкамидзе

ჟურნალის ნომერი ეძღვნება პროექტს "სამეცნიერო და წერითი უნარ-ჩვევების გაუმჯობესება საქართველოს ახალგაზრდა აკადემიური პერსონალისთვის", რომელიც დაფინანსებულია საქართველოში აშშ-ის საელჩოს საუნივერსიტეტო კვლევითი პროგრამის ფარგლებში
მოწვეული რედაქტორი – მედიცინის მეცნიერებათა დოქტორი გიორგი კამკამიძე

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.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией и Международной академией наук, образования, искусств и естествознания (IASEIA) США с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, рецензии, научные сообщения, новости медицины и здравоохранения.

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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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В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Библиографическое описание литературы составляется на языке текста документа. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующему номеру данной работы в списке литературы.

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Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or computer-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

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).

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Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

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.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

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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.

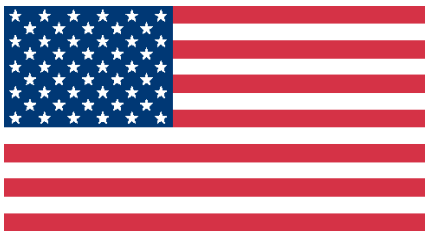
9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

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.



Government of the United States
THE U.S EMBASSY IN GEORGIA
აშშ-ის საელჩო საქართველოში

Health Research Union
ჯანმრთელობის კვლევის კავშირი

The scientific publications presented in this special issue of the "Georgian Medical News" are the products generated through the project "Improving research and writing skills among young Georgian academics" funded through the University Research Program by the U.S. Embassy in Georgia.

The objectives of the project were to create and implement educational and training program for young Georgian academics to introduce skills such as critical thinking, data analysis, literature review, research proposal writing, and publication preparation; promote and facilitate ongoing mentoring of project beneficiaries by the local and international experts; help young Georgian academics in establishing inter-institutional, personal and peer-mentoring networks to share strategies for pursuing their scientific career advancements and peer review of research proposals and manuscripts.

Several universities from different regions of Georgia participated in the project. Participants got familiar with different methodologies used in qualitative and quantitative research. Training provided young Georgian academics with detailed understanding of research process and necessary practical skills for conducting research projects and publishing in local and international peer-reviewed journals.

This project is funded by the U.S. Embassy to Georgia in Tbilisi. The contents of this publication are those of the Author(s) and do not necessarily represent the views of the Department of State.

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НАУКА

ROTAVIRUS VACCINE IMPLEMENTATION IN GEORGIA – IMPACT OF KNOWLEDGE, APPROACH, AND PRACTICES OF HEALTH CARE WORKERS

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Rotavirus (RV) is the most common cause of severe gastroenteritis in infants and young children worldwide. The virus causes approximately half a million deaths each year among children aged <5 years, and more than 80% of these deaths occur in developing countries [5,24]. By age 5, nearly every child will have experienced an episode of rotavirus gastroenteritis; 1 in 5 will visit a clinic, and 1 in 60 will be hospitalized [21]. Rotavirus-associated illness is the leading cause of dehydration and hospitalization due to gastroenteritis in children [6,29]. One study in Canada reported that the cost per person of rotavirus infection varies from $350 for mild cases to $2663 for hospitalized cases [13]. Several other studies in both developing and developed countries have also shown that rotavirus-associated diarrhea incurs significant costs [19,27].

Rotavirus gastroenteritis is a significant public health problem in Georgia. According to the World Health Organization (WHO) estimate for 2008, there were approximately 10 to 50 deaths of young children due to rotavirus diarrhea in Georgia [30]. The rotavirus sentinel surveillance was established in Georgia in 2006 with WHO support. A high proportion (36%) of severe diarrhea events in children below 5 years of age was caused by RV [31].

Two main types of rotavirus vaccines that are approved worldwide are the monovalent vaccine and the pentavalent bovine-derived vaccine. Different studies have shown that these vaccines have good safety profiles and no significant adverse effects [3,12]. Additionally, these rotavirus vaccines appear to exhibit high cost-effectiveness in developing countries. Studies have shown that vaccination will prevent 2.4 million rotavirus deaths and >82 million disability-adjusted life-years (DALYs) in 64 of the 72 GAVI-eligible countries that have or will introduce vaccine between 2007 and 2025. The introduction of rotavirus vaccines is very cost-effective and is projected to substantially reduce child mortality [1,8,17,23].

Given the high burden of the disease, the availability of safe and effective vaccines, and the high cost-effectiveness of new interventions, the Ministry of Labour, Health and Social Affairs (MoLHSA) of Georgia decided to introduce the rotavirus vaccine into the routine immunization program in 2013.

International experience with the introduction of new vaccines has shown that existing concerns and resistances to immunization in the population might lead to refusal of the RV vaccine. Moreover, the introduction of any new vaccine, including the RV vaccine, can cause anti-vaccine movements and decrease the overall immunization coverage in the country. Studies conducted in the United States and in developing countries have indicated that the main barriers to the introduction of the rotavirus vaccine are the cost of purchasing the vaccine and concerns about vaccine safety [14,15].

There is a scarcity of evidence-based data that provide insight about the main barriers to vaccination and the existing vaccine-resistant groups in the Georgian population. Research studies of health care workers (HCWs) that were performed in 2007 revealed that the most common barriers for hepatitis B vaccine uptake by HCWs were concerns about safety and adverse events and negative media reporting[4,28]. Another study performed by the National Center for Disease Control and Public Health with the assistance of UNICEF in 2010 revealed that HCWs' and parents' mistrust toward vaccination, particularly multi-component Hib vaccine, was due to the low competence of the HCWs in the area and the perception of low risk of the disease among the parents [9]. According to national immunization reports and research performed by UNICEF, inadequate interpretation of the contraindications also represents an important barrier against vaccine uptake [2]. These data indicate that the opinions of HCWs are of great importance for achieving high vaccination coverage.

The study aim was to identify factors influencing Georgian HCWs' attitude and practices regarding introduction of RV vaccine in Georgia.

Material and methods. In May and June of 2012, the National Center for Disease Control and Public Health (NCDC) conducted a cross-sectional survey of 462 primary health care workers (HCWs) who were involved in the expanded program of immunization (EPI) using self-administered anonymous questionnaires in three regions of Georgia. The study sites were selected to include a range of population densities, ethnic diversity and a range of vaccine coverage according to the official immunization coverage estimates for the year 2010 (Penta3 [DPT+Hib+HepB] was used as the indicator). The following regions were included:

1) the capital city Tbilisi in which 1/3 of country's population resides; 2) Kvemo Kartli, which is a rural region with high ethnical diversity; and 3) Shida Kartli, which is one of the regions with the lowest vaccine coverage. The vaccination coverage were 93%, 88% and 86%, respectively. For Shida Kartli and Kvemo Kartli, which each include districts with disparate coverage statistics, two districts were included for each region; i.e., those with the highest and lowest coverage statistics. These districts were Tetritskaro and Marneuli in Kvemo Kartli (95.4% and 77.5%, respectively) and Kaspi and Kareli in Shida Kartli (98.3% and 81.2%, respectively).

The sample size was calculated using a formula for finite populations (based on a total number of 578 physicians involved in immunization in the selected regions) using the standard parameters of alpha=0.05 and power=0.80. Assuming that 50% of the health care workers had sufficient knowledge of rotavirus infections, the minimum required sample size for the proposed survey was 420.

The eligibility criteria included being a medical doctor who was employed full-time at a primary health care unit (PHCU) participating in the EPI. The HCWs were selected by simple random sampling using employee lists provided by the medical facilities of the selected regions. The potential participants who met the eligibility criteria were invited to participate in the survey until the designated sample size was met for each PHCU. The response rate for each institution was calculated.

The questionnaires were developed in collaboration with local experts in pediatrics, infectious diseases, preventive medicine and public health. The self-administered anonymous questionnaires were pilot-tested on a convenience sample of 10 HCWs from PHCUs located in Tbilisi.

The questionnaires were hand-delivered to HCWs during working hours and collected after completion. Brief oral and written descriptions of the purpose and objectives of the study were provided, and verbal consent was obtained from each prospective participant. Prior to administration, the HCWs arranged to complete the self-administered standardized questionnaires at pre-arranged times.

Data quality assessments were conducted prior to the analyses. The relationship between the HCWs' willingness to provide recommendations for rotavirus vaccine inclusion in the National Schedule of Immunization (i.e., favorable versus undecided or opposed) and potential predictive factors were studied using chi-square or Fisher's exact tests. A stepwise backward logistic regression was used to determine the most suitable model for multivariate analysis. Variables with p values ≤ 0.20 in the bivariate analysis were entered into the model, and p values ≤ 0.05 were considered statistically significant. The data analyses were performed using EpiInfo 7 software.

Results and their discussion. *Demographic and professional characteristics*

In May 2012, 475 health care workers (HCWs) were invited to participate in the study, and 462 agreed and completed the self-administered questionnaire (97.3% response rate). Three hundred sixty-four HCWs (78.8%) were employed in primary health care facilities located in Tbilisi, 38 (8.2%) were from the Shida Kartli region (22 from Kaspi and 16 from Kareli), and 60 (13.0%) were from the Kvemo Kartli region (20 from Tetritskaro and 41 from Marneuli).

Table 1. Perceived Burdens of Rotavirus Infection and Diarrheal Diseases in Georgia

Statement	Absolutely agree, N(%)	Somewhat agree, N(%)	Somewhat disagree, N(%)	Absolutely disagree, N(%)
Diarrhea is common in children under 2 years	105 (22.7)	96 (20.8)	216 (46.8)	45 (9.7)
Diarrhea is a serious health problem in children under 2 years	176 (38.1)	110 (23.8)	157(34.0)	19 (4.1)
Rotavirus is the most common cause of infectious diarrhea in children <2 y old in Georgia	72 (15.6)	95 (20.6)	249 (53.9)	46 (10.0)
Rotavirus infection is the most frequent cause of severe diarrhea disease in <2-y-olds in Georgia	120 (26.0)	127 (27.5)	195 (42.2)	20 (4.3)
There is need for a safe and effective rotavirus vaccine in Georgia	142 (30.7)	123 (26.6)	168 (36.4)	29 (6.3)
Because rotavirus infections are common and potentially severe in developing countries, there is a need for a safe and effective rotavirus vaccine in Georgia	168 (36.4)	122 (26.4)	144 (31.2)	28 (6.1)

Table 2. Perceived Barriers to the Implementation of the Rotavirus Vaccine

Barrier	Definite barrier, N(%)	Somewhat a barrier, N(%)	Minor/not a barrier at all, N(%)
Lack of state financial resources for immunization	214 (46.3)	198 (42.9)	50 (10.8)
Parental refusal	180 (39.0)	234 (50.6)	48 (10.4)
Parental concerns about the safety of the rotavirus vaccine	146 (31.6)	245 (53)	71 (15.4)
Physicians'concerns about the safety of the rotavirus vaccine	79 (17.1)	184 (39.8)	199 (43.1)
Parental concerns about the vaccine safety in GENERAL	114 (24.7)	268 (58.0)	80 (17.3)
Parents not thinking that a rotavirus vaccine is necessary	125 (27.1)	239 (51.7)	98 (21.2)
Physician's concern about adding another vaccine to an already overloaded vaccine schedule	49 (10.6)	134 (29.0)	279 (60.4)
Physician's belief that rotavirus is not a severe disease that requires vaccination	59 (12.8)	160 (34.6)	243 (52.6)
The time it will take for physicians to discuss rotavirus vaccine safety with parents	54 (11.7)	160 (34.6)	248 (53.7)
Parents not thinking that infectious diarrhea is risk for their children	100 (21.6)	211 (45.7)	151 (32.7)

The study participants included pediatricians (164, 35.5%), family doctors (137, 29.7%), HCWs with joint specialties (pediatricians with family doctor licenses; 155, 33.5%) and other specialists in the field (6,1.3%; i.e., immunologists and infectious disease and internal medicine specialists). The vast majority of the recruited HCWs were females (91.3%), had 10 or more years of work experience (91.6%) and worked in the primary health care facilities located in Tbilisi (78.8%). Less than half of the HCWs (43.9%) reported having moderate vaccination-related workloads (between 11 to 30 children vaccinated per month) and having taken continuous medical education courses during the previous 1 or 3 years (35.3% and 31.0%, respectively).

Perceptions of Diarrheal Diseases and Rotavirus Infection
The majority of the health care workers (67.9%) recognized diarrhea as a frequent health-related problem in children below the age of 2 years. However, only 7.8% of the HCWs reported diarrhea as the most commonly encounter disease in their clinical practice. Only 28.4% of the respondents named rotavirus infection as the leading cause of diarrhea in this age group.

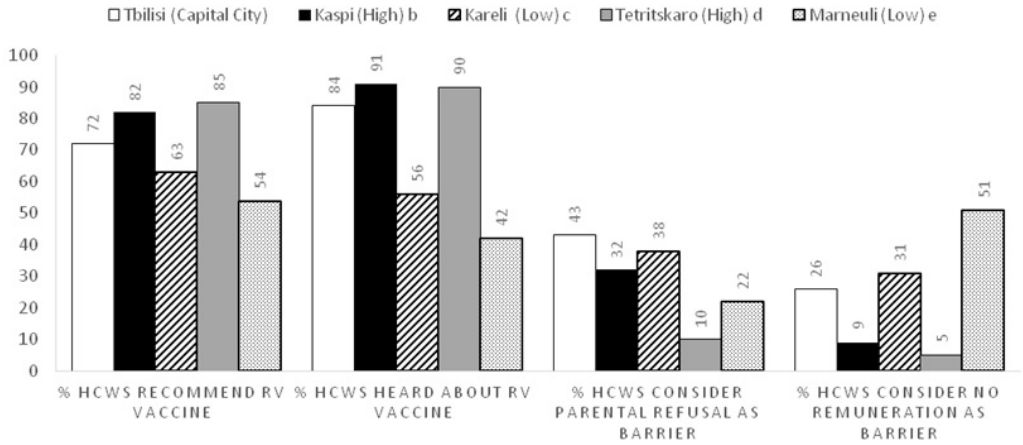
The participants were asked to provide their level of agreement with 6 statements concerning diarrhea and rotavirus infection burden in Georgia on a scale of one to four in which one indicated "absolutely agree" and four indicated "absolutely disagree" (Table 1).

Perceptions about rotavirus vaccine
Overall, the participants recognized the need for a safe and effective rotavirus vaccine in Georgia, and 36.4% absolutely agreed that the high burden of rotavirus disease in developing countries is a sufficient argument to justify the need for a rotavirus vaccine in Georgia.

The majority of HCWs had already heard about the rotavirus vaccine (79.7%). Regarding the question of whether they would recommend the inclusion of the rotavirus vaccine in the National Schedule of Immunization, less than half recommended this inclusion with confidence (44.2%).

The top three perceived "definite" barriers to rotavirus vaccine implementation in Georgia were the scarcity of state financial resources to cover all costs related to immunization (a definite barrier among 46.3%), the expectancy of parents' refusal (definite barrier - 39.0%), and concern about the safety of the rotavirus vaccine (definite barrier - 31.6%; Table 2).

The other most common perceived barriers to vaccine implementation were primarily associated with the parents' concerns regarding immunization, including the parents' general concerns about vaccine safety (combined responses of definite and somewhat of a barrier: 82.7%), parents not thinking that the rotavirus vaccine is necessary (78.8%) and the belief that rotavirus infection is not a severe disease that requires vaccination (68.4%).



a - Pearson Chi-Square ≤0.002;
b - High vaccine coverage settlement in the Kvemo Kartli region for the year 2010 (Indicator: Penta3 [DPT+Hib+HepB]);
c - Low vaccine coverage settlement in the Kvemo Kartli region;
d - High vaccine coverage settlement in the Shida Kartli region;
e - Low vaccine coverage settlement in the Shida Kartli region
Fig. RV infection- and vaccine-related perceptions among health care workers from different settlements

RV infection and vaccine-related perceptions: Regional differences

Perceptions regarding diarrheal diseases, RV infection and vaccination significantly differed in the low vaccine coverage settings. Specifically, RV vaccine awareness and willingness to recommend the inclusion of the vaccine in the National Schedule of Immunization were considerably lower in both low vaccine coverage rural settings (Kareli and Marneuli) than in their high-coverage neighboring settlements. Moreover, the perception that the parents' refusals would be the main barrier to RV vaccine implementation in the country was more commonly named in low vaccine coverage areas (38% vs. 32% in Shida Kartli Reg. and 22% vs. 10% in Kvemo Kartli; Fig. 1). Similarly, the HCWs from these areas were more prone to report the lack of remuneration of the HCWs for each immunization visit as an important barrier, which distinguished the HCWs from low-coverage settings from those of high-coverage neighborhoods (31% vs. 9% in Shida Kartli Reg. and 51% vs. 5% in Kvemo Kartli; Fig.).

Factors Associated With Rotavirus Vaccine Recommendation

The bivariate analyses revealed that the willingness to provide a recommendation for the inclusion of the RV vaccine in the national immunization schedule were significantly lower among the HCWs with low vaccine awareness (OR=2.1395%CI: 1.33-3.41), low perception of RV disease burden (OR=1.91 95%CI: 1.26-2.91), low perception of the severity (OR=1.9795%CI: 1.31-2.96), low perception of the need for RV vaccine in routine immunization in the country (OR= 4.99 95%CI: 3.23-7.70) and among HCWs with concerns about vaccine safety (OR= 2.8895%CI: 1.85-4.50), parental refusal due to safety concerns (OR=2.2595%CI: 1.17-4.35) and the additional time required for discussions about vaccine safety issues with parents (OR=1.55 95%CI: 1.04-2.32) (Table 3).

After adjustment for the other variables in the multivariate regression analyses, only three factors remained statistically significant and were identified to be independent predictors of vaccine recommendation and acceptance (Table 3). Specifically, the HCWs were 2.54 times less likely to recommend the inclusion of the rotavirus vaccine in the national immunization schedule if they did not believe that there is a need for the safe and effective rotavirus vaccine in the country in general (OR=2.54, CI: 1.46-4.42), and they were 2.75 times less likely to provide this recommendation if they did not see the need for the vaccine because rotavirus infection is common and potentially severe in developing countries (OR=2.75, CI: 1.59-4.75). Moreover, the HCWs who were concerned about vaccine safety were 2.51 times less likely to provide this recommendation than were the others (OR=2.51, CI: 1.56-4.02).

Numerous studies performed in different countries have indicated that the knowledge and attitudes of HCWs have strong influences on vaccination coverage [18,25]. Therefore, the opinion of HCWs should be considered before any change in the vaccination calendar. These types of surveys also help to identify potential barriers to the uptake of a new vaccine.

Past experiences in Georgia indicate that concerns about vaccine safety might detrimentally affect vaccination coverage. In 2008, a supplementary measles-rubella immunization campaign exhibited low coverage (50.3%) due to safety concerns among the population [16]. Other studies have also shown that the primary barriers to vaccination are concerns about vaccine safety among HCWs and the general population [4,28].

Table 3. Factors associated with RV vaccination acceptance among primary health care workers (N=462) according to bivariate and multivariate analyses

Factors	Not Recommend RV vaccine (N=135)	Bivariate Analysis			Multivariate Final Model		
		Crude OR	95% CI	p value	Adjusted OR	95% CI	p value
Have ever heard about rotavirus vaccine							
Yes	95 (25.8)	1					
No	40 (42.6)	2.13	1.33-3.41	0.001			
Diarrhea is common in children under 2 years							
Yes	44 (21.9)	1					
No	91(34.9)	1.910	1.26-2.91	0.002			
There is need for a safe and effective rotavirus vaccine in Georgia							
Yes	41 (15.5)	1					
No	94 (47.7)	4.99	3.23-7.70	0.000	2.54	1.46-4.42	0.001
Because rotavirus infections are common and potentially severe in developing countries, there is a need for a safe and effective rotavirus vaccine in Georgia							
Yes	48 (16.6)	1					
No	87 (50.6)	5.16	3.36-7.94	0.000	2.75	1.59-4.75	0.000
Parents' concerns about the safety of rotavirus vaccine is a barrier							
No	12 (16.9)	1					
Yes	123 (31.5)	2.25	1.17-4.35	0.013			
Physicians'concerns about the safety of rotavirus vaccine is a barrier							
No	35 (17.6)	1					
Yes	100 (38.0)	2.88	1.85-4.50	0.000	2.51	1.56-4.02	0.000
Physician's belief that rotavirus is not a severe disease that requires vaccination is a barrier							
Yes	80(36.5)	1.97	1.31-2.96	0.001			
No	55(22.6)	1					
The time it will take for a physicians to discuss rotavirus vaccine safety with parents is a barrier							
Yes	73(34.1)	1.553	1.04-2.32	0.032			
No	62(25.0)	1					

The bivariate analyses of our data also indicated that the physicians' intentions to recommend rotavirus vaccine inclusion in the National Schedule of Immunization were associated with vaccine safety concerns and other factors including the following: (1) RV vaccine awareness, (2) the HCWs' perceptions of the burden and severity of diarrheal diseases, (3) the perceived need for rotavirus vaccination, and (4) attitudes about the potential barriers to rotavirus immunization (i.e., RV vaccine safety concerns among parents, the extra time required for discussions about the RV vaccine with parents and low rotavirus disease severity perceptions among physicians). However, the multivariate analysis revealed that the independent predictors of the willingness of HCWs to provide RV vaccine recommendations were their concerns related to vaccine safety and perceptions of low existing need for the introduction of a new vaccine in the country. This list of barriers is similar but not identical to those identified in studies performed in other countries [10,11,26].

Importantly, the main three barriers to the introduction of a new rotavirus vaccine as perceived by the HCWs did not include the health providers' concerns about vaccine safety. The top three "definite" barriers for rotavirus vaccine implementation in Georgia as perceived by the physicians were the following factors: (1) the perception of the scarcity of state financial resources to cover all costs related to remuneration of the HCWs for immunization, (2) the expectancy of parental refusal, and (3) parental safety concerns. The HCWs' perceptions about the parents' vaccine safety concerns were more commonly named as a barrier specifically for the RV vaccine than for vaccination in general. This finding partially corresponds to findings from similar studies conducted in developed countries. For example, among Canadian clinicians, the main perceived barriers are the risk of adverse effects, the cost of the vaccine and the fact that it is a new vaccine. The expectancy of parental refusal of new vaccines has been named as a barrier by HCWs including those from developed countries [7,22]. The results of previous studies suggest that the identification of parental safety concerns as the main barrier by health providers might be related to the following two main factors: (1) greater resistance against immunization among the general population in Georgia compared to the populations of developed countries; and (2) the HCWs' lower levels of knowledge, self-confidence and interpersonal communication skills to reduce parental refusal [4,9,28].

Despite the fact that diarrhea was considered by primary health care workers involved in National Immunization Program to be a serious health problem in children below the age of 2 years (61.9%), rotavirus was not perceived to be the most common cause of infectious diarrhea in children <2 years old in Georgia (36.2%) and thus the introduction of the rotavirus vaccine was not perceived to be important for the country (42.7%). Similar to other developing countries [26], in Georgia, this finding might be attributable to the fact that the majority of diarrhea cases are not diagnosed in the laboratory, and the etiology of the disease remains unknown. However, severe cases of diarrhea that require the detection of the etiologic agent are typically treated in specialized infectious disease clinics. Therefore, primary health care workers might have insufficient awareness about the magnitude of the problem, which has been identified as a major barrier to the prioritization of the RV disease and vaccine in other countries [20,26].

Despite obvious variation in the perceptions related to public RV vaccine acceptance and barriers, financial motivations and greater concerns about negative parental attitudes toward vaccination (presumably due to the low communication skills for handling parental refusal) were still the main factors that distinguished HCWs from low coverage settlements from their counterparts in high-coverage neighborhoods.

More than half of the health providers agreed that better preventive interventions were needed to decrease the number of cases of acute diarrhea and expressed willingness to learn more about the vaccine against rotavirus (62.1% and 62.3 absolutely agreed, respectively). These findings suggest that educational interventions will be well accepted by HCWs.

Conclusion. The findings from our study suggest that the HCWs' concerns about vaccine safety, low levels of awareness about the RV disease burden and vaccine in combination with a lack of communication skills represent the main predictors of RV vaccine uptake by health care providers. These revealed factors underline the need for intensive vaccine promotion interventions that focus on the education of HCW, particularly in low vaccine coverage settlements, and provide an opportunity for the development of comprehensive and effective vaccine communication campaigns.

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SUMMARY

ROTAVIRUS VACCINE IMPLEMENTATION IN GEORGIA – IMPACT OF KNOWLEDGE, APPROACH, AND PRACTICES OF HEALTH CARE WORKERS

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Rotavirus (RV) is the most common cause of severe gastroenteritis in infants and young children worldwide. RV causes approximately half a million deaths each year among children aged <5 years. According to WHO estimates for 2008, there were approximately 10 to 50 deaths annually in young children due to rotavirus diarrhea in Georgia. The purpose of this study was to assess the knowledge, attitudes, and practices related to rotavirus diarrhea and the rotavirus vaccine among health care workers (HCWs). The National Center for Disease Control and Public Health (NCDC) conducted a cross-sectional survey of HCWs involved in the expanded program of immunization (EPI). The HCWs were selected by simple random sampling using employee lists, and questionnaires were hand-delivered to selected HCWs during working hours. The majority of HCWs (67.9%) recognized diarrhea as a frequent health-related problem in children under 2 years of age. However, 53.9% partially disagreed with the statement that rotavirus is the most common cause of all forms of diarrhea. Multivariable analysis revealed that the following perceptions among HCWs more than doubled the likelihood that they would not support adoption of the RV vaccine: no perception of need for this vaccine in Georgia specifically (OR=2.54, CI: 1.46-4.42), no perception of need to address burden of disease in developing countries (OR=2.75, CI: 1.59-4.75), and concerns about the vaccine's safety (OR=2.51, CI: 1.56-4.02). Concerns about vaccine safety, low awareness about the RV disease burden and the effectiveness of the RV vaccine, combined with a lack of communication skills represented the main predictors of RV vaccine uptake among HCWs. Intensive vaccine promotion interventions that focus on the epidemiology of disease and vaccine effectiveness are urgently needed.

Keywords: rotavirus, vaccine, diarrhea.

РЕЗЮМЕ

ВНЕДРЕНИЕ РОТАВИРУСНОЙ ВАКЦИНЫ В НАЦИОНАЛЬНЫЙ КАЛЕНДАРЬ ВАКЦИНАЦИЙ ГРУЗИИ - ВЛИЯНИЕ ЗНАНИЙ, ОТНОШЕНИИ И ПРАКТИКИ МЕДПЕРСОНАЛА

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Ротавирус (RV) является наиболее частым возбудителем острого гастроэнтерита в мире среди грудных и малолетних детей, ежегодно являясь причиной смертности полмиллиона детей в возрасте до пяти лет. По данным Всемирной Организации Здравохранения за 2008 год, каждый год в Грузии от ротавирусной диареи погибают от 10 до 50 детей.

Целью данного исследования явилась оценка информированности медперсонала о ротавирусной инфекции и их отношения к проведению вакцинации.

С этой целью Национальный центр контроля болезней и общественного здравоохранения провел перекрестно-секционное исследование среди медперсонала, участвовавшего в программе обширной иммунизации (n=462). Медперсонал отобран методом простой случайной выборки. Опрос проведен посредством вопросника, разработанного в сотрудничестве авторов с местными специалистами в области педиатрии, инфекционных болезней, профилактической медицины и общественного здравоохранения. Анализ заполненных медперсоналом вопросников выявил, что большая часть респондентов - 313 (67,9%) считает диарею самой частой причиной заболеваемости детей в возрасте до 2 лет. 250 (53.9%) опрошенных не согласны с тем, что самой частой причиной всех форм диареи является ротавирус. Мультивариационный анализ показал, что вероятность негативной рекомендации, т.е. против включения анти-ротавирусной вакцины в национальный календарь вакцинаций, доминирует среди респондентов, считающих, что в Грузии нет необходимости включения ротавирусной вакцины в календарь вакцинаций (OR=2.54, CI: 1.46-4.42); не считают нужным включить эту вакцину в календарь вакцинаций в развивающихся странах (OR=2.75, CI: 1.59-4.75); медперсоналом высказано сомнение в безопасности самой вакцины (OR=2.51, CI: 1.56-4.02). Результаты проведенного исследования позволяют заключить, что определяющими факторами задержки внедрения ротавирусной вакцины являются недостаточная ин-

формированность медперсонала о последствиях ротавирусных болезней, о сути ротавирусной вакцины и ее безопасности. Исходя из вышеизложенного, авторы статьи считают целесообразным проведение интенсивных информационных мероприятий среди медперсонала о безопасности и необходимости вакцинации.

რეზიუმე

როტავირუსის საწინააღმდეგო ვაქცინის დანერგვა საქართველოში - სამედიცინო პერსონალის ცოდნის, დამოკიდებულების და პრაქტიკის გავლენა

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დაავადებათა კონტროლისა და საზოგადოებრივი ჯანმრთელობის ეროვნული ცენტრი; ჯანმრთელობის კვლევის კავშირი,თბილისი,საქართველო

მსოფლიოში ჩვილებსა და მცირეწლოვან ბავშვებში მწვავე გასტროენტერიტის ყველაზე ხშირი გამომწვევი არის როტავირუსი (RV), რომელიც ყოველწლიურად ნახევარი მილიონი 5 წლამდე ასაკის ბავშვის სიკვდილობის მიზეზს წარმოადგენს. ჯანმრთელობის მსოფლიო ორგანიზაციის 2008 წლის მონაცემებით საქართველოში ყოველწლიურად როტავირუსული დიარეით მცირეწლოვანი ბავშვების გარდაცვალების 10-დან 50 შემთხვევამდე ფიქსირდება.

კვლევის მიზანია სამედიცინო პერსონალის როტავირუსულ დიარეასთან და ვაქცინასთან დაკავშირებული ცოდნის, დამოკიდებულების და პრაქტიკის შეფასება. დაავადებათა კონტროლის და საზოგადოებრივი ჯანმრთელობის ეროვნული ცენტრის მიერ ჩატარდა იმუნიზაციის გაფართოებული პროგრამის განხორციელებაში მონაწილე სამედიცინო პერსონალის ჯვარედინ-სექციური კვლევა. სამედიცინო პერსონალი შეირჩა მარტივი შემთხვევითი შერჩევის მეთოდით, პერსონალის (n=462) სიების გამოყენებით,ხოლო შერჩეულ პერსონალს კითხვარები გადაეცათ პირადად სამუშაო საათებში. კითხვარი შემუშავებულია ავტორების მიერ პედიატრებთან, ინფექციონისტებთან, პროფილაქტიკური მედიცინის და საზოგადოებრივი ჯანდაცვის სპეციალისტებთან ერთად. სამედიცინო პერსონალის დიდი ნაწილი 313 (67.9%) 2 წლამდე ბავშვების ავადობის ხშირ მიზეზად დიარეას ასახელებს, 250 (53.9%) ნაწილობრივ არ ეთანხმება მოსაზრებას, რომ როტავირუსი არის ყველა ფორმის დიარეის ყველაზე ხშირი გამომწვევი. მულტივარიაციულმა ანალიზმა აჩვენა,რომ უარყოფითი რეკომენდაციის გაცემის ალბათობა მაღალია რესპოდენტებში,რომლებიც: ვერ ხედავენ საქართველოში ვაქცინის დანერგვის საჭიროებას (OR=2.54, CI: 1.46-4.42); არ თვლიან საჭიროდ ვაქცინის დანერგვას განვითარებად ქვეყნებში (OR=2.75, CI: 1.59-4.75); ვაქცინის დანერგვის მთავარი ბარიერი ექიმების ვაქცინის უსაფრთხოებასთან დაკავშირებული ეჭვია (OR=2.51, CI: 1.56-4.02). ამდენად, სამედიცინო პერსონალის მიერ როტავირუსული ვაქცინის დანერგვის შეფერხების ძირითად განმსაზღვრელ ფაქტორებს წარმოადგენს კომუნიკაციის უნარების ნაკლებობა და სამედიცინო პერსონალის ეჭვები ვაქცინის უსაფრთხოებაზე, მათი დაბალი ინფორმირებულობა როტავირუსული დაავადების ტვირთისა და როტავირუსის საწინააღმდეგო ვაქცინის შესახებ. ყოველივე ზემოაღნიშნულიდან გამომდინარე, ავტორებს მიზანშეწონილად მიაჩნიათ ჩატარდეს სამედიცინო პერსონალისთვის ინტენსიური საინფორმაციო ღონისძიებები ვაქცინის უსაფრთხოებისა და ვაქცინაციის აუცილებლობის შესახებ.

CHANGES OF ENDOTHELIN-1 CONTENT AND BLOOD RHEOLOGICAL PROPERTIES IN CRUSH SYNDROME

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Our previous studies have already revealed that crush syndrome (CS) together with other important consequences, causes microcirculation and arterioles adrenergic regulation disorders. In particular, in relation with compression and decompression period increase, the intensity of the local blood flow is reduced, both vascular α- and β- adrenergic receptors' sensitivity is violated [5].

In the above mentioned vascular dysfunction pathogenesis, with other important factors, the active role player should be changes of one of the most potent vasoconstrictor – endothelin-1 (ET-1) content in blood. On the other hand, occurred significant hemocirculation changes should be resulted by blood rheological properties disorders. According to researched data, information about endothelin-1 and blood rheology changes in CS is scarcely available.

Accordingly, the present study aimed to determine hematocrit values along with the quantitative changes investigation of endothelin-1 content in blood in different regimens of crush syndrome compression and decompression periods.

Material and methods. The experiments were carried out on randomly selected 200-250 gr mass 50 Wistar rats. Crush syndrome modeling was conducted by tightening of femoral muscles in the middle third of both hips during 3 or 6 hours in narcotized rats (by Ether). Investigations were conducted at various stages of compression and post-compression periods – immediately after decompression, 1 or 6 hours from decompression. After the experiments the rats were euthanized with ether.

The content of Endothelin-1 in blood was determined by immunoenzime method [11,12,14], using ELISA REDEAR - URIT 660. The method is based on the so-called "Sandwich" technique. We used a special Enzyme-linked Immune Sorbent Assay (ELISA) kit – (Enzo life Sciences, Lausen, Switzerland). Spectrophotometric analysis of the samples was performed on 450 nm. After constructing the standard curve, endothelin concentration was measured according to color intensity. The sample color intensity is directly proportional to the content of endothelin in specimen. The data is presented in picograms per milliliter (pg/ml).

Hematocrit was determined with the use of standard method. Rat blood was collected in anticoagulant containing (EDTA) test tubes, blood samples were spun in centrifuge on 1500 revolutions per second (rev/sec) during 10 minutes, after which plasma and blood formed elements were separated from each other. Hematocrit - height of RBC column from the whole sample height was measured according to tube column divisions and was expressed in liter per liter (L/L) units.

The obtained data was processed statistically with the use of Student t-test (statistical program IBM SPSS Statistics for Windows, Version 19.0). The data is statistically significant, for all tables $p<0.05$.

Results and their discussion. Our data show that with the increase of compression and especially decompression period there is observed a rise of endothelin-1 in blood. The results are presented on Table 1.

Table 1. Content of Endothelin-1 in different regimens of CS compression and decompression periods

Crush Syndrome Regimen	Endothelin concentration (pg/ml)
Control Group	5,2±0,08
3 hours compression	7,24±1,04
6 hours compression	8,75±1,27
3 hours compression +1 hour decompression	9,25±0,95
6 hours compression +6 hours decompression	9,87±1,09

Table 2. Changes of Hematocrit during different regimens of Crush Syndrome

Control Group	Hematocrit (L/L)
3 hours compression	0,44±0,02
6 hours compression	0,51±0,01
3 hours compression +1 hour decompression	0,67±0,02
6 hours compression +6 hours decompression	0,58±0,01
Control Group	0,71±0,02

Our data shows that the content of endothelin in 3 hours compression is 7.24 pg/ml, which is 39% higher compared with the control (5.2 pg/ml). In 6 hours compression blood ET-1 is increased by 68% (8.75pg /ml) compared to the control.

After 1 hour from 3 hours compression ET-1 is increased by 77% (9.25pg/ml), whereas after 6 hours decompression from 6 hours compression ET-1 has the maximum increase – by 89% (9.87pg/ml).

According to the experiment results, (Table 2), it is clear that hematocrit progressively increases in relation with the severity of the crush syndrome. In particular, in 3 hour crush syndrome modeling hematocrit blood test value (0.51 L/L) indicates 15% increase compared to control (0.44 L/L), while 6 hours compression causes 52% increase of heamtocrit level (0.67 L/L).

As for 1 hour decompression period after 3 hours of compression, this time hematocrit slightly increases by 13% (0.58 L/L) compared with the same regimen of compression period, while it is 31% higher than the control value. After 6 hours following the 6 hours compression period hematocrit gets 0.71 L/L, which is 61% higher than the control value.

Our study revealed that in CS circulatory disorders number of factors play important role, including also local disturbances of vascular tone. Concretely, along with crush syndrome severity and especially decompression period increase ET-1 concentration was elevated statistically significantly to some extent, but it did not probably activated surface endothelial B (ET-B) receptors located in the vessel endothelium, having a vasodilative effect due to nitrogen oxide (NO) and prostacyclin synthesis induction [2,3,9,15]. As it is known, NO paracrinically enhances smooth muscle cells soluble guanilatcyclase leading to Cyclic Guanosine Monophosphate (CGMP) generation, which in turn activates CGMP dependent proteinkinases; as a result cell CA^{2+} concentration decreases, myosin light chains phosphorylation is disturbed and actin-myosin complexes are not created, finally resulting in blood vessel smooth muscle relaxation and vessel dilation [10].

As for hematocrit values, their rise in crush syndrome should be related to plasmorrhagia induced by traumatic and toxic (rhabdomyolysis) shock [6-8]. It is known that during skeletal muscle necrosis (rhabdomyolysis) nephrotoxic compounds are produced and released into the bloodstream after reperfusion. Specifically, in addition to myoglobinemia, creatine phosphokinase, uric acid, lactate dehydrogenase is elevated in the blood; metabolic acidosis develops [1,7]. Acidosis with other injury mediators (histamine, bradikynin) promotes vasodilation, increases vascular permeability, intravascular fluid moves into the interstitial space ("third space" shift), and due to hemoconcentration there develops polycytemic hypovelemia with elevated hematocrit values [4,13,16].

Thus, in CS, elevation of ET-1 concentration and hematocrit values leads to serious microcirculation disturbances in parallel with compression and decompression severity and duration increase.

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SUMMARY

CHANGES OF ENDOTHELIN-1 CONTENT AND BLOOD RHEOLOGICAL PROPERTIES IN CRUSH SYNDROME

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This study describes hematocrit values and quantitative changes in plasma endothelin-1 (ET-1) levels according to the severity of crush syndrome (CS) compression and decompression periods. The experiments were carried out on 50 randomly selected 200-250 gr mass Wistar rats with the use of the standard crush syndrome modeling method. The plasma level of ET-1 was determined by the immuneenzyme method with the use of ELISA REDEAR URIT 660. Hematocrit was determined using the standard method and measured according to tube column divisions. Our data show that ET-1 and hematocrit values rise commensurate with an increased duration of compression, and especially decompression periods. In CS, elevation of ET-1 concentrations and hematocrit values leads to significant microcirculation disturbances in parallel with longer and more severe compression and decompression periods. Specifically, the ET-1 concentration was significantly elevated, possibly in response to activation of surface endothelial B (ET-B) receptors located in the vessel endothelium. These receptors, in turn, have a vasodilative effect due to nitrogen oxide synthesis induction and vascular smooth muscle relaxation. The rise in hematocrit values during crush syndrome is associated with plasmorrhagia induced by trauma and toxic (rhabdomyolysis) shock.

Keywords: crush syndrome, microcirculation, endothelin, hematocrit.

РЕЗЮМЕ

ИЗМЕНЕНИЯ СОДЕРЖАНИЯ ЭНДОТЕЛИНА-1 И РЕОЛОГИЧЕСКИХ СВОЙСТВ КРОВИ ПРИ КРАШ-СИНДРОМЕ

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Целью исследования явилась оценка изменений показателей гематокрита и эндотелина-1 (эн-1) в периодах компрессии и декомпрессии краш-синдрома различной тяжести. Эксперименты проведены на 50 половозрелых крысах линии Вистар массой тела 200-250 г. Моделирование краш-синдрома осуществлялось классическим методом. Содержание эндотелина в крови определяли иммунноферментным методом с помощью анализатора ELISA REDEAR URIT 660; показатели гематокрита - стандартным методом с использованием градуированных пробирок. Согласно результатам исследования, при краш-синдроме, с развитием периодов компрессии и, особенно, декомпрессии концентрация эн-1 в крови и гематокрит увеличивались, что является причиной тяжелых нарушений микроциркуляции. Повышенная концентрация эндотелина, по всей вероятности, обусловливает активацию расположенных на поверхности эндотелия эн-В рецепторов, усиление синтеза оксида азота и расширение сосудов. Увеличение гематокрита, очевидно, вызвано выходом плазмы из сосудистого русла в результате травматического и токсического (рабдомиолиз) шока при краш-синдроме.

რეზიუმე

ენდოთელინის შემცველობის და სისხლის რეოლოგიური თვისებების ცვლილებები კრაშ-სინდრომის დროს

ნ. გამყრელიძე, ნ. პავლიაშვილი, რ. ოთარაშვილი

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, პათოფიზიოლოგიის დეპარტამენტი, საქართველო

კვლევის მიზანს წარმოადგენდა ენდოთელინ-1 (ეთ-1) შემცველობის და ჰემატოკრიტის მაჩვენებლების ცვლილებების შესწავლა სისხლში განსხვავებული სიმძიმის კრაშ-სინდრომის (კს) კომპრესიის და დეკომპრესიის პერიოდებში. ექსპერიმენტი ჩატარდა რანდომულად შერჩეულ ზრდასრული ასაკის 200-250 გრ მასის ვისტარის ჯიშის ვირთაგებზე (n=50). კრაშ-სინდრომი მოდელირდებოდა სტანდარტული მეთოდით. სისხლში ენდოთელინის შემცველობა ისაზღვრებოდა იმუნოფერმენტული მეთოდით, ELISA REDEAR URIT 660-ის გამოყენებით, ჰემატოკრიტის მაჩვენებელი - სტანდარტული მეთოდით, სინჯარის სვეტში დანაყოფების მიხედვით. კვლევის შედეგები მიუთითებს კს-ის დროს კომპრესიის და, განსაკუთრებით, დეკომპრესიის პერიოდის განვითარებასთან ერთად სისხლში ეთ-1-ის კონცენტრაციაზე და ჰემატოკრიტის მაჩვენებლის პროგრესულად მატებაზე. აღნიშნული დინამიკა, კომპრესიის და დეკომპრესიის ხანგრძლივობის და სიმძიმის პარალელურად, იწვევს მიკროცირკულაციის მძიმე დარღვევებს; ეთ-1-ის კონცენტრაცია აღწევს დონეს, რომელიც, სავარაუდოდ, ააქტიურებს ენდოთელიუმზე ზედაპირულად

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TONIC INFLUENCE OF NEOCORTEX ON HIPPOCAMPAL SEIZURES

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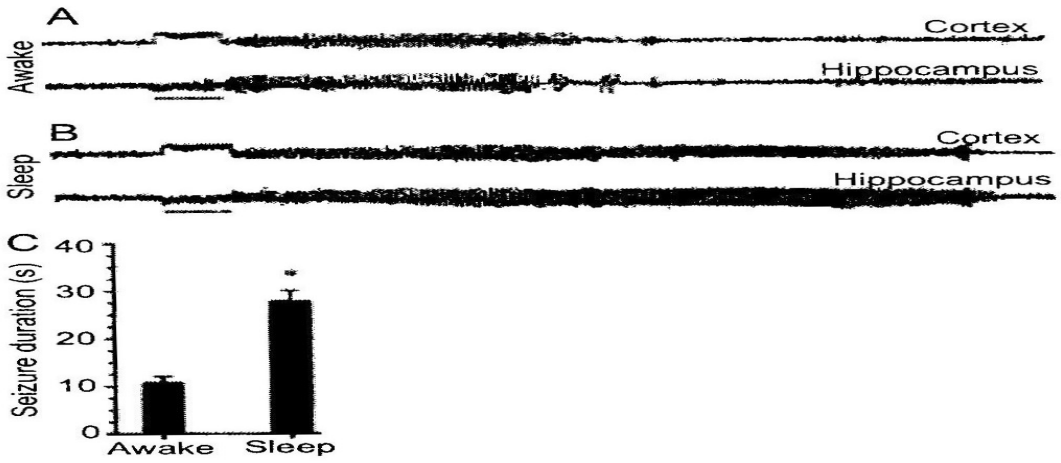
Epilepsy is a complex disease of central nervous system with diverse clinical characteristics. Despite our advances in understanding of molecular, cellular and structural mechanisms of seizure development, the mechanisms of seizure initiation, spread and control still remain unclear. It is well established that seizures are triggered when there is a misbalance between excitation and inhibition in defined brain structures [7,8]. Increased excitation and/or decreased inhibition lead to enhanced susceptibility of the brain to the seizures. In contrary, decreased excitation and/or increased inhibition will lead to decreased seizure readiness and diminished probability of spontaneous epileptic discharges [7]. Based on this concept, it is obvious that in many patients epileptic seizures occur during the period of awakens when the excitability of neurons is high and their seizure readiness is increased [1-4]. However, it is striking that in many patients the spontaneous epileptic seizures occur more often during the sleep when the excitability of neurons should be low. Recent research has elucidated the complex relationship between sleep and epilepsy. It is well known that sleep can affect seizure occurrence, threshold and spread. Interictal electroencephalography (EEG) abnormalities are often potentiated during sleep, suggesting a change in seizure threshold [3]. However, the sleep–epilepsy interaction varies according to the epilepsy syndrome. In some, such as the frontal lobe epilepsies, sleep may facilitate seizures [4], whereas in others, sleep may protect against epilepsy. In frontal lobe seizures have a greater chance of occurring during sleep whereas temporal lobe seizures are more likely to occur during wakefulness though secondarily generalization is more likely during sleep than wakefulness. These observations imply that sleep has distinct effects on seizure threshold in different brain regions [4]. Furthermore, sleep can influence the extent of seizure spread, such that seizures in temporal lobe epilepsy are more likely to secondarily generalize during sleep than during wakefulness [1,4]. These observations suggest that sleep may influence the pattern and extent of seizure spread, and therefore the EEG and clinical characteristics of the seizures [5].

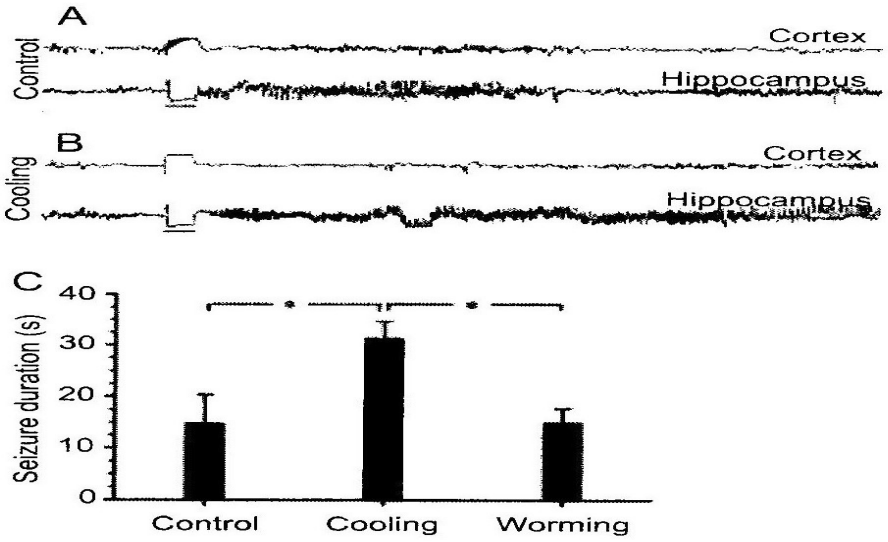
The main objective of current study was to induce limbic seizures by electrical stimulation of the hippocampus during sleep-awakens cycles of the animals and assess how the different phases of the sleep affect seizure characteristics and to elucidate the possible role of cortex in controlling hippocampal seizures.

Material and methods. The experiments were carried out on 15 male Wistar rats weighing 300 gr. Animals were housed in 12:12 h light/dark conditions with ad libidum access to food and water. All precautious were taken to minimize the pain or discomfort of the animals. All experiments were carried out according to NIH guide and permission was obtained from local ethical committee. Rats were anaesthetized with ketamine (100 mg/kg) and the stimulation and recording constantan electrodes (o. d. 0.15 mm) were implanted in the dorsal hippocampus and neocortex according at following coordinates: (toothbar at 3.3 mm):Hippocampus: 2.8 mm caudal to bregma; 2 mm lateral to midline; and 3 mm ventral to dura for stimulation and 3.8 mm caudal to bregma; 2.5 mm lateral to midline; and 3.5 mm ventral to dura for recording; Cortex: 2.3 mm caudal to bregma; 3 mm lateral to midline; and 1.5 mm ventral to dura for recording according to the rat atlas. Epileptic seizures were induced by hippocampal stimulations (3-5 V, pulse duration 0.2-0.3 ms at 30-50 Hz) for 5 s and the EEG was monitored continuously with electroencephalographer (Medicor EEG 8S, Hungary). In order to decrease electrical activity, the neocortex was either cooled down by topical application of chlorethyl directly to the scull or cortical spreading depression (CSD) (Leao, 1944) SD was induced by a small drop (about 1µl) of 4% KCl solution applied to the pial surface on the right side through a burr hole. Both treatments were carried out ipsilaterally to the implanted electrodes.

Results and their discussion. For each individual animal, the super threshold stimulation intensity was determined which would cause seizure activity in three consecutive time when applied with 30 min intervals. Initially, the sei-



A - EEG from cortex and hippocampus during wakefulness;
B - EEG from cortex and hippocampus during slow wave sleep. The seizures are evoked by stimulation of right hippocampus;
C – Duration of hippocampal seizures evoked by electrical stimulation of hippocampus during wakefulness and slow wave sleep.
Means±SD, p<0.001 Student's t-test.*
Fig. 1. Hippocampal seizures evoked during wakefulness and sleep



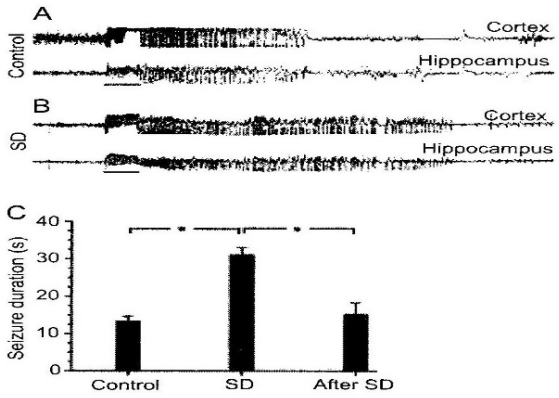
A – EEG from cortex and hippocampus under normal conditions;
B -EEG from cortex and hippocampus 30 min after cooling the cortex with chlorethyl. T he seizures are evoked by stimulation of right hippocampus;
C – Duration of hippocampal seizures evoked by electrical stimulation of hippocampus under normal conditions, after cooling the brain surface and after restring the brain temperature.
Means±SD, p<0.001 One-Way ANOVA followed by Bonferroni post hoc test*
Fig. 2. Hippocampal seizures evoked under normal conditions and after cooling of the brain surface

zures were induced when animal was awake and then when they were in slow sleep phase as determined by EEG activity. As illustrated by Fig.1A, hippocampal stimulation with super-threshold stimulation led to appearance of high-frequency seizure discharges of 10.7±1.4 s duration (Fig.1C).

The seizure activity of about same duration was also observed in the cortex. When the same stimulation was applied to the hippocampus at a time when animal was in slow sleep phase as determined by its cortical EEG, the duration of hippocampal as well as cortical seizures when stimulated with the same parameters was significantly increased (Fig. 1B and C). In order to test whether the cortical input influence the duration of hippocampal seizures, we decreased cortical activity by cooling the scull with chlorethil at least 30 min after the induction of seizures. Cooling of the brain

surface lead to the decrease of frequency and the amplitude of cortical EEG. When under these conditions we stimulated the hippocampus, the evoked hippocampal seizures where strongly increased in duration (Fig. 2B and C).

In contrast to normal conditions, hippocampal stimulation at a time when brain surface was cooled down, did not lead to induction of cortical seizures. Almost immediately after the induction of seizures the cold brain surface was wormed up by application of worm (+ 37-38°C) 0.9 % NaCl solution. When the brain temperature was restored, repeated hippocampal stimulation exhibited the seizures with the duration similar to that under control conditions (Fig. 2C). To confirm that cortical activity can control the duration of hippocampal seizures, we also tested another way of decreasing of cortical EEG activity. It is well known that CSD induced by topical application of KCl leads to a transient depression of the cortical EEG activity. When hippocampus was stmulated during the episodes of CSD, the duration of hippocampal seizures was significantly increased (Fig. 3B and C), and after disappearance of CSD the duration was decreased to initial control values.



A – EEG from cortex and hippocampus under normal conditions;
B - EEG from cortex and hippocampus after induction of SD by application of KCl. The seizures are evoked by stimulation of right hippocampus;
C - Duration of hippocampal seizures evoked by electrical stimulation of hippocampus under normal conditions, after induction of SD and after termination of SD.
Means±SD, p<0.001 One-Way ANOVA followed by Bonferroni post hoc test*

Fig. 3. Hippocampal seizures evoked under normal conditions and after induction of spreading depression (SD)

Our data clearly indicate that the hippocampal seizures are under certain control from the neocortex: the decrease of cortical the electrical activity either during slow-phase sleep or by cooling of the cortical surface and induction of CSD causes increase of the hippocampal seizure duration.

Since the major efferent input to the hippocampus goes through entorhinal cortex, it is conceivable that afferent inputs from motor, somatosensory and the frontal parts of the neocortex [2] represent anatomical substrate to exhibit that interaction between neocortex and hippocampus. It has been demonstrated also that neocortical activity could also influence epileptic discharges in the amygdala. Induction of CSD in the frontal cortex ipsilateral to amygdala kindling stimulations significantly decreased the duration of EEG seizures in amygdala and suggested strong interaction of amygdala and cortex in severity of the seizures.

Our data indicate that neocortex probably exerts tonic inhibitory influence on hippocampal seizures and cortico-hippocampal interaction could be important component in exhibition and secondary generalization of limbic seizures.

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SUMMARY

TONIC INFLUENCE OF NEOCORTEX ON HIPPOCAMPAL SEIZURES

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The interaction between different brain structures could be crucial to predicting seizure occurrence, threshold and spread. Moreover, the sleep-wake cycle and electrical activity of brain structures in different phases of sleep could significantly affect the pattern and extent of seizure spread, and therefore the characteristics of epileptic activity. In this animal model using 15 Wistar rats, we show that the duration of hippocampal seizures, induced by electrical stimulation of the hippocampus, is significantly increased during slow sleep. Moreover, decreasing the electrical activity of the neocortex by cooling of the cortical surface or induction of cortical spreading depression also caused an increase in hippocampal seizure duration. Conversely, warming the cortical surface triggered a remission in spreading depression, in turn restoring the duration of epileptic episodes. Our data suggest that the neocortex probably exerts a tonic inhibitory influence on hippocampal seizures. Thus, cortico-hippocampal interaction could be an important component in the manifestation and generalization of limbic seizures.

Keywords: sleep, epilepsy, neocortex, hippocampus.

РЕЗЮМЕ

ТОНИЧЕСКОЕ ВЛИЯНИЕ НЕОКОРТЕКСА НА СУДОРОГИ ГИППОКАМПАЛЬНОГО ПРОИСХОЖДЕНИЯ

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Взаимодействие между различными структурами головного мозга, очевидно, является решающим фактором, влияющим на идентификацию судорог, определение их порога и распространения. Эксперименты проводились на 15 мышах линии Вистар. Показано, что длительность гиппокампальных судорог, вызванных электрическим раздражением, значительно увеличивается в период медленного сна. Электрическая активность неокортекса уменьшается при охлаждении поверхности коры головного мозга или во время распространения в ней депрессии, вызывающей увеличение длительности гиппокампальных судорог. Согревание поверхности коры и исчезновение распространяющейся депрессии вызывает восстановление эпилептической активности. Полученные данные свидетельствуют, что неокортекс имеет тонически-воздерживающее влияние на гиппокампальные судороги. Кортикогипокампальное взаимоотношение, очевидно, является значительным показателем в выявлении повторных генерализированных лимбических судорог.

რეზიუმე

ნეოკორტექსის ტონური გავლენა ჰიპოკამპური წარმოშობის კრუნჩხვებზე

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[1]ბათუმის შოთა რუსთაველის სახ. სახელმწიფო უნივერსიტეტი; [2]ძილის მედიცინის ცენტრი,საქართველოს ძილის მედიცინისა და კლინიკური ელექტროფიზიოლოგიის ასოციაცია; [3]ი. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი. საქართველო

თავის ტვინის სხვადასხვა სტრუქტურებს შორის ურთიერთქმედება შეიძლება იყოს კრუნჩხვების იდენტიფიცირების, მათი ზღურბლის და გავრცელების განმსაზღვრელი. ექსპერიმენტები ჩატარდა ვისტარის 15 თაგვებზე. ნაჩვენებია, რომ ჰიპოკამპური კრუნჩხვების ხანგრძლივობა, გამოწვეული ჰიპოკამპის ელექტრული გაღიზიანებით, მნიშვნელოვნად იზრდება ნელი ძილის დროს. ქერქის ზედაპირის გაცივებით, ან ქერქში გავრცელებული დეპრესიის პირობებში,რომელიც ჰიპოკამპური კრუნჩხვების ხანგრძლივობის ზრდას იწვევს,ნეოკორტექსის ელექტრული აქტივობა მცირდება. ქერქის ზედაპირის გათბობა და გავრცელებადი დეპრესიის გაქრობა განაპირობებს ეპილეფსიური აქტივობის აღდგენას. მიღებული მონაცემები მიუთითებს,რომ ნეოკორტექსი ტონურ შემაკავებელ გავლენას ახდენს ჰიპოკამპურ კრუნჩხვებზე. სავარაუდოა,რომ კორტიკოჰიპოკამპური ურთიერთობა განმეორებითი გენერალიზებული ლიმბური კრუნჩხვების გამოვლინის მნიშვნელოვანი მაჩვენებელია.

FACTORS ASSOCIATED WITH DEPRESSIVE EPISODE IN PATIENTS HOSPITALIZED WITH ACUTE CORONARY EVENTS

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Depression is a common condition associated with acute coronary syndrome. There is a growing evidence suggesting that the majority of patients with acute coronary syndrome (acute myocardial infarction, unstable angina) have a subclinical depression, and almost 20% of them are diagnosed with a major depression [5]. A meta-analyses [6] showed that in depressed post-MI patients an average risk of adverse cardiac outcomes was 2.0-2.6 greater compared to those without depression. One study [3] suggests that detection of depression during hospitalization is very important in order to provide an optimal care and reduce an increased risk. Furthermore, Enrich study [7] revealed, that initial major depressive episodes are more strongly predictive, than recurrent ones. After reviewing 53 studies in their meta-analyses [5], American Heart Association suggested, that depression should be elevated to the status of risk factor of adverse medical outcomes in patients with Acute Coronary Syndrome (ACS).

Since severe infarction or its consequences are considered as triggering factors of incidental depression [9], it is very important to reveal factors, associated with a depressive episode. The DepreMi study [9] found that myocardial infarction disease severity markers, such as decreased ejection fraction, cardiac arrhythmias and revascularization were associated with the first depressive episode in MI patients. However, no significant differences were found between initial and recurrent depression in Left Ventricular ejection fraction (LVEF), Killip class heart failure in Enreach study [7]. Thus the controversy between trials still exists.

The aim of our study was to reveal factors associated with depressive episode in patients hospitalized with acute coronary syndrome (acute myocardial infarction, unstable angina).

Material and methods. The Beck Depression inventory (BDI) was used for assessement of depressive symptoms in patients with coronary disease in Emergency Cardiology Clinic Tbilisi, Georgia. The initial study sample included patients with acute coronary events – ST-segment elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction, unstable angina and stable angina. The total number of participants was 123. Unstable angina was considered, if patient had angina after myocardial infarction, new onset angina or crescendo-type angina during last 2 months, as well as prolonged chest pain [1]. We exluded of patients with STEMI and stable angina, and conducted all analyses in 84 participants.

The clinical Information was collected from hospital recordings. All subjects signed an informed consent at the admission to the hospital. Depression screening was approved by the ethical committee of the Tbilisi State Medical University.

Coronary disease and risk factors: Coronary obstruction was defined as ≥50% stenosis in the left main coronary artery or ≥70% stenosis in major coronary arteries (6). History of arterial hypertension was categorized into two groups, negative/ positive. *Body mass index* (BMI) was calculated and then categorized into BMI ≤25 and >25 respectively. *Tobacco consumption:* All participants were classified as non-smokers (which included past smokers) and current smokers.

After assessment of depressive symptoms, the BDI score was categorized into two categories: >16 and 16. Our decision was based on the BDI interpreting manual, which concerns scores <10 as normal, 11-16 – mild mood disturbance, 17-20 as borderline clinical depression, 21-30 – moderate depression, 31-40 – severe depression and >40 – extreme depression.

Ejection fraction was assessed by echocardiography at admission. The variable for ejection fraction was provided by two categories – ejection fraction <40% and >40%. In 89% of patients ejection fraction was given as a continuous variable, therefore we used it as a continuous score in our regression analyses [9].

Descriptive statistical tests were used for the calculation of frequencies, means and standard deviations. Chi-square test was used for assessment the difference between groups. Independent t-test was used to compare means for numerical variable "age". P value was set at 0.05. A binary logistic regression was applied in order to assess a relationship between disease severity factors (ejection fraction, coronary events and revascularization) and depressive episode. 95% Confidence interval was used for statistical analysis. All statistical tests were performed using IBM SPSS 20.0.

Results and their discussion. Study sample included 79% of men and 21% of women with a mean age 59 years. Coronary obstruction as well as cardiac risk factors (arterial hypertension, obesity) was revealed in majority of participants. The mean depression score was 13.0, while BDI score >16 was revealed in 28.8% of patients (Table 1).

Table 1. General characteristics of participants

N84	All
Sex % Men Women	 78.6 21.4
Age (years), Mean and SD	58.2 (10.2)
Body mass index, % >25 <25	 72.6 21.4
Smoking status, % Smokers Non-smokers	 38.1 61.9
History of arterial hypertension, % Yes No	 90.5 8.3
BDI score, Mean and SD	13.3 (7.7)
Depression score, % >16 ≤16	 29.8 70.2
Coronary artery disease, % Unstable angina Myocardial infarction	 75.0 25.0
Diabetes Yes No	 25.0 73.8
Obstructive coronary artery disease, % Yes No	 86.9 13.1
Ejection fraction (EF), Mean and SD	50.3 (9.6)
Ejection fraction, % <40% >40%	 11.9 75.0

We found, that women rather than men had a greater number of depressive symptoms (BDI >16). 70% of patients with decreased ejection fraction (EF <40%) had depressive score > 16 (Table 2).

In a binary regression model ejection fraction was associated inversely with depressive episode even after adjustment with age and sex and coronary risk factors (Table 3). Every one unit increase of ejection fraction was related with almost 10% reduced number of depressive symptoms in a fully adjusted model.

No statistically significant association was found between myocardial infarction, revascularization and depressive episode.

When disease severity markers (ejection fraction, revascularization) together with classic risk factors were included into the model, they explained only 42% variance of outcome variable (depressive episode).

In current study we tried to revealed factors, associated with a depressive episode in patients with coronary disease (myocardial infarction, unstable angina).

Decreased ejection fraction is associated positively with a depressive mood. This could be explained by the fact that patients with decreased ejection fractions frequently report fatigue and disability, which may contribute to depressive mood. In other words, the disease-related processes can trigger somatic symptoms of depression [8].

However, our results showed that disease severity markers together with traditional Cardio-Vascular Disease risk factors explained only 40% of depressive symptoms. Therefore further research is needed to establish behavioral factors contributing to depression in patients with acute coronary events.

Table 2. Differences between groups for depressive scores

N 84	Depression score >16	Depression score ≤16	p value
Age, mean, SD	62.0 (11.8)	58.2 (9.3)	0.169
Sex % Men Women	 24.2 50.0	 75.6 50.0	0.034
Obstructive CAD, % Yes No	 31.1 27.3	 69.9 72.7	0.846
EF, % <40% >40%	 70.0 25.4	 30.0 74.6	0.005
Revascularization, % Yes No	 35.7 26.8	 64.3 73.2	0.399
CAD, % Unstable angina Myocardial infarction	 33.3 19.0	 66.7 81.0	0.215
History of arterial hypertension% Yes No	 31.6 14.3	 68.4 85.7	0.340
Diabetes, % Yes No	 42.9 24.2	 57.1 75.8	0.103
BMI, % >25 <25	 56.5 43.5	 85.7 14.3	0.005
Tobacco consumption, % Yes No	 15.6 38.5	 84.4 61.5	0.026

Chi-squared tests for contingency tables were applied. p value was set at 0.005

Table 3. Multivariate logistic regression analyses for association disease severity markers and depressive episode

N=84	Depression score > 16 / Odds Ratio	95% Confidence Intervals	p- value
Model 1 crude			
EF	0.93	0.88 – 0.98	0.010
Revascularization	1.91	0.63 – 5.7	0.252
Myocardial infarction	0.40	0.10 – 1.5	0.184
Model 2. Adjusted with age and sex			
EF	0.91	0.85 – 0.97	0.006
Revascularization	2.28	0.69 – 7.51	0.174
Myocardial infarction	0.48	0.12 – 1.9	0.301
Model 3. Additional adjustment with CVD risk-factors			
EF	0.91	0.83 – 0.98	0.021
Revascularization	2.94	0.70 - 11.8	0.128
Myocardial infarction	1.22	0.23 – 6.48	0.808

No statistically significant association was found with other disease severity markers such as myocardial infarction, revascularization.

Our results are lined in with the DepriMi study, which suggested the positive relationship of LVEF during hospitalization with the incident depression [9]. Furthermore, the association between angiographycally determined CAD severity and incident depression was shown in the Copes study [2]. Meanwhile, the evidence regarding the role of inflammatory markers suggests that not all ACS patients with increased levels of inflammatory markers develop depression [8]. Therefore, along with these trends, the role of behavioral and psychosocial stressors and coping mechanisms should be taken into account [8].

Anyway, since ACS associated depression is related with poor prognosis [8], incorporation of psychiatric and mental health care specialists is needed in order to provide optimal care.

There are some limitations in our study. Particularly, in some cases information for cholesterol was not available therefore we did not include cholesterol as a potential confounder in our analyses.

Furthermore, we did not assessed depressive symptoms in follow-up period, during a year after hospitalization. However, we were focused only on a depressive episode during hospitalization rather than on a severity of depression.

It may be concluded, that disease severity markers together with classic cardiac risk factors explain only partially a depressive episode in patients, hospitalized for acute coronary events. A multidisciplinary approach is needed in order to provide optimal care and improve prognosis of patients with ACS.

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SUMMARY

FACTORS ASSOCIATED WITH DEPRESSIVE EPISODE IN PATIENTS HOSPITALIZED WITH ACUTE CORONARY EVENTS

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Severe infarction or its consequences are considered as triggering factors of incidental depression. The aim of our study was to reveal factors associated with depressive episode in patients hospitalized with acute coronary syndrome (acute myocardial infarction, unstable angina). The Beck Depression inventory (BDI) was used for assessment of depressive symptoms in patients with coronary disease in Emergency Cardiology Clinic Tbilisi, Georgia. The study sample included 84 patients. The clinical Information was collected from hospital recordings. The chi-square test was used for assessment the difference between groups. Independent t-test was used to compare means for numerical variable “age”. A binary logistic regression was applied in order to assess a relationship between disease severity fac-

tors (ejection fraction and revascularization) and depressive episode. Study sample included 79% of men and 21% of women with a mean age 59 years. Coronary obstruction as well as cardiac risk factors was revealed in majority of participants. The mean depression score was 13.0, while BDI score > 16 was revealed in 28.6% of patients. In the binary regression model ejection fraction was inversely associated with depressive episode even after adjustment to the age, gender and coronary risk factors. When disease severity markers (ejection fraction, revascularization) together with classic risk factors were included into the model, they explained only 42% of depressive episodes. It may be concluded that disease severity markers together with classic cardiac risk factors explain only partially depressive episode in patients, hospitalized for acute coronary events. A multidisciplinary approach is needed in order to provide optimal care and improve prognosis of patients with acute coronary syndrome (ACS).

Keywords: cardio-vascular disease, acute coronary syndrome, Beck depression inventory, ejection fraction.

РЕЗЮМЕ

ФАКТОРЫ, СВЯЗАННЫЕ С ЭПИЗОДОМ ДЕПРЕССИИ, У ПАЦИЕНТОВ С ОСТРЫМ КОРОНАРНЫМ СИНДРОМОМ

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Инфаркт миокарда и его осложнения в большинстве случаев связаны с эпизодом депрессии**.** Целью данного исследования явилось выявление факторов, связанных с эпизодом депрессии при остром коронарном синдроме. Больным острым коронарным синдромом скрининг депрессии проведен в Центре неотложной кардиологии им. Г. Чапидзе. Исследовано 84 больных, средний возраст которых составил 59 лет. Использованы параметрические и непараметрические методы статистики. Данные клинических и лабораторных результатов заимствованы из историй болезни пациентов. У большинства пациентов выявлены факторы риска ишемической болезни сердца и обструкция коронарных артерий. Снижение фракции выброса связано с эпизодом депрессии. Снижение фракции выброса, пониженный процент реваскуляризации и классические факторы риска частично объясняют развитие эпизода депрессии при остром коронарном синдроме.

რეზიუმე

დეპრესიის ეპიზოდთან დაკავშირებული რისკ-ფაქტორები მწვავე კორონარული სინდრომით პაციენტებში

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თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, შინაგანი მედიცინის №3 დეპარტამენტი; აკად. გ. ჩაფიძის სახ. გადაუდებელი კარდიოლოგიის ცენტრი, თბილისი, საქართველო

მიოკარდიუმის მწვავე ინფარქტი და მისი გართულებები ხშირად დაკავშირებულია დეპრესიის ეპიზოდებთან.

კვლევის მიზანს წარმოადგენდა მწვავე კორონარული სინდრომის დროს დეპრესიის ეპიზოდთან დაკავშირებული ფაქტორების გამოვლენა. დეპრესიის ეპიზოდი შეფასებულია გადაუდებელი კარდიოლოგიის ცენტრში. კვლევაში ჩართვის კრიტერიუმს წარმოადგენდა მწვავე კორონარული სინდრომის არსებობა. კვლევაში ჩართული იყო 84 პაციენტი, საშუალო ასაკი - 59.2±10.2 წელი. მონაცემები პაციენტების კლინიკური და ლაბორატორიული გამოკვლევების შესახებ მოძიებული იყო პაციენტების ისტორიებში. კვლევისთვის გამოყენებულია სტატისტიკის პარამეტრული და არაპარამეტრული ტესტები და რეგრესიის მეთოდი. პაციენტთა უმრავლესობას გამოუვლინდა კორონარული არტერიების ობსტრუქცია და კორონარული რისკის ფაქტორები. დაქვეითებული განდევნის ფრაქცია ასოცირებული იყო დეპრესიის ეპიზოდთან. კვლევის შედეგად ავტორებს გამოტანილი აქვთ დასკვნა, რომ დაქვეითებული განდევნის ფრაქციის არსებობა,რევასკულარიზაციის ნაკლები სიხშირე და კლასიკური რისკის ფაქტორები ნაწილობრივ ხსნიან დეპრესიის ეპიზოდის განვითარებას მწვავე კორონარული სინდრომის დროს.

POSSIBLE MECHANISM OF DEVELOPMENT OF SALT SENSITIVE ESSENTIAL HYPERTENSION

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Arterial hypertension remains one of the major health problems in the world. According to the WHO epidemiological data 2014, 30% of adult population diagnosed with arterial hypertension, or every third adult suffers from high blood pressure. Antihypertensive treatment is effective in 52% of all Hypertension cases, but in 48% blood pressure is not controlled. Since 1994 to 2004 arterial hypertension caused mortality increased in 26.6% [4].

The most cases of Hypertension are Essential Hypertension, also referred as Primary Hypertension. Numbers of studies has been reported to evaluate of its different types. In the individuals with decreased sodium excretion by the kidneys low salt diet leads to lowering blood pressure, but high salt intake causes arterial hypertension. This is referred as salt-sensitivity and is defined genetically as well as can be developed secondary to either decreased renal function or by influence of other environmental factors [1,3,10,11]. The RAAS activation plays an important role in so called salt-resistant type of hypertension but has found to be suppressed in volume expanded salt-sensitive hypertension. The high levels of cardiotonic steroids such as Endogenous Marinobufagenin and Ouabain has been found in the individuals with salt-sensitive hypertension. These compounds are synthesized in human adrenal glands, hypothalamus and heart muscle and represented as Na+K+ATP-ase endogenous inhibitors in the kidneys and blood vessels [9]. Although a lot of studies suggest their role in the development of salt-sensitive hypertension, the approved mechanism has not been defined yet [1,7].

The aim of the study was to evaluate the possible mechanism for the development of salt-sensitive essential hypertension in Georgian population.

Material and methods. Case-Control study was performed. 185 subjects, 94 cases with Essential Hypertension stage I (JNC7) without prior antihypertensive treatment, and 91 controls - normotensives were involved into the study. Accordingly, two groups were defined: Group 0 – Normotensives and Group 1 – patients with Essential Hypertension. From 72 males 29 were normotensives and 43 with hypertension. From 113 females 62 were normotensives and 51 with hypertension.

Using the salt-sensitivity test the total number of subjects were divided into salt-sensitive and salt-resistant subgroups. Salt-sensitivity was found in 60.5% of total population investigated. 63.8% of case group were salt-sensitive and 36.2% salt-resistant. 57.1% were salt-sensitive and 42.9% salt-resistant in normotensive controls. As for salt-sensitivity distribution by sex it was found in 60.7% females and 39.3% males (Table 1).

Before enrollment into the study all individuals have signed the informed concept statement approved by the ethics committee of the Institute of Medical Biotechnology. The patients' selection and their clinical investigations were performed at the Department of Internal Medicine of the Tbilisi State Medical University as well as outpatient primary health care centers in Tbilisi. The inclusion criteria for the case group were defined as followings: 1. Essential Hypertension diagnosed at the first visit. 2. Essential Hypertension Stage I JNC 7 3. Age 19-72 years. 4. No other co morbidities. 5. No use of GFR modifying medications. 6. No alcohol and tobacco use during the study period. 7. BMI <35. All individuals who agreed to participate and signed the informed concept statement were involved in the case group. Subjects for the control group were selected from the population coming to the outpatient centers for the prophylactic checkup. As mentioned above, salt-sensitivity test was used to divide both case and control groups into salt-sensitive and salt-resistant subgroups. The salt-sensitivity of blood pressure was defined as the difference of Mean Arterial Pressure (MAP) between the averages of 6 readings (3 readings in sitting and 3 readings in standing positions)

Table 1. Distribution of salt-senstivity according to Blood Pressure stages

		Salt-sensitivity		Total
		Salt-resistant	Salt-sensitive	
BP Stage	0	39	52	91
		42.9%	57.1%	100.0%
	1	34	60	94
		36.2%	63.8%	100.0%
Total		73	112	185
		39.5%	60.5%	100.0%

Table 2. Difference between Mean Arterial Pressure at the different dietary sodium conditions in salt-sensitive and salt-resistant subjects

Salt-sensitivity	N	Min.	Max.	Mean
0	73	-11.4400	21.7900	0.06
1	112	3.0300	28.8700	8.97

Table 3. Paired statistics of Fractional excretion of sodium on high and low dietary salt intake

Sltsens			Mean	N	Std. Deviation
1	Pair 1	FENaBs	0.124	73	0.042
		FENaLs	0.030	73	0.011
0	Pair 1	FENaBs	0.157	112	0.060
		FENaLs	0.032	112	0.012

at the end of the high (200 mmlo) and low (40 mmol) salt periods. Salt sensitivity was considered when difference between MAP exceeded 3 mm Hg. MAP was calculated as DBP plus one-third of the difference between DBP and SBP. BMI was calculated to find its correlation with the salt-sensitivity as other risk factor of essential hypertension.

GFR was calculated for the assessment of renal function during different sodium intake in all groups.

Assessment of Renal Function by Creatinine clearance (CrC) and Fractional excretion of sodium (FENa) were calculated using the following equations: CrC=uCr×uVol×1.73/(pCr×T×BSA), where uCr is urine creatinine concentration (mg/ml), uVol is urine volume (ml), pCr is plasma creatinine concentration (mg/ml), T is time (min). BSA (body surface area) = W0.425×H0.725×0.007184, where W is weight (kg), H is height (cm). BSA is expressed as m2. CrC is expressed as ml/min/1.73m2. FENa=uNa×pCr×100/(pNa×uCr), where uNa and pNa are urine and plasma sodium concentra- tions (mmol/L), uCr and pCr are urine and plasma creatinine concentrations (mg/ml). FENa is expressed as %.

Blood samples were collected in 10 ml EDTA and 10 ml plain vacutainers in the morning after an overnight fast, before starting and after low salt diet. Also, 20 ml sample from 24 hour urine indicating the 24 hour urine amount were obtained at the same time. All samples were centrifuged at 1500 x g for 15 min, aliquoted at 2 ml portions and stored at -20 °C until using. For the assessment of high-salt condition as well as low-salt diet adequacy, sodium in 24 hour urine was quantitated. Determinations of circulating levels of endogenous sodium pump inhibitors were carried out using the ELISA and RIA methods. In these assays, a monoclonal antibodies specific for ouabain and marinebufinogen, respectively, were used. PRA was assessed by Radioimmunoassay [8].

Descriptive statistics were used to describe the basic features of the data. Differences in variables between dietary sodium conditions were assessed using paired t tests.

Results and their discussion. At the baseline high salt condition Mean Arterial Pressure in normotensive controls was 101.5 and decreased to 97.1 after law salt diet. In hypertensive probands these measurements were 117.39 and 110.77 respectively. The MAP difference was very noticeable between salt-sensitive and salt-resistant subgroups (Table 2).

The mean 24 hour urine sodium level in salt-sensitive group was 374.55 and 295.43 in salt-resistant group. After low salt diet these measurements decreased to 79.01 and 74.36 respectively. Statistically significant positive correlation was found between 24 hour urine sodium concentration changes and salt-sensitivity, $r=0.334$, $p<0.01$.

Salt-sensitivity positively correlated with age, $r=0.117$ in males, $r=0.262$ in females, also, only the last correlation was statistically significant $p<0.01$ and prevalence of salt-sensitivity was higher in females, compared to males. The mean Body mass index in males was 28 and 26 in females; in salt-sensitive subjects 27.6 and 26.4 in salt-resistant. Since no significant correlations were found between BMI and salt-sensitivity, we assume that BMI and salt-sensitivity should be discussed as different independent risk factors for the development of Essential Hypertension.

For better and in-depth assessment of renal function we used Creatinine clearance (CrC) and Fractional excretion of sodium (FENa) during different dietary sodium conditions in both groups. Both salt-sensitive and salt-resistant subjects revealed decrease in Glomerular Filtration Rate (GFR) after low salt diet, but significant correlation was found between changes in GFR in salt-sensitive cases and controls $p<0.01$. This can be explained with comparable hyperfiltration of the kidneys at high sodium load in salt-sensitive individuals and discussed as early sign of hypertensive nephropathy. As far as we know, fractional excretion of sodium is the percent of filtered sodium which is excreted with the urine. We have found higher baseline FENa in salt-resistant group compared to salt-sensitive. This can be explained by the hypothesis that salt-sensitive kidneys are characterized by comparable decreased sodium excretion during salt loading (Table 3).

The interesting data were obtained in the changes of concentrations of endogenous cardiotonic steroids during high and low salt diets. After low salt diet Marinobufagenin concentration in blood and 24 hour urine samples decreased three folds both in probands and controls in salt-sensitive subjects, but remained at the same level in salt-resistant hypertensive cases and normotensive controls.

The lowest Plasma Renin Activity (PRA) was administered in salt-sensitive subjects at the high sodium condition, which markedly increased after low salt diet. PRA is elevated in salt-resistant subjects at the both sodium conditions (Fig.).

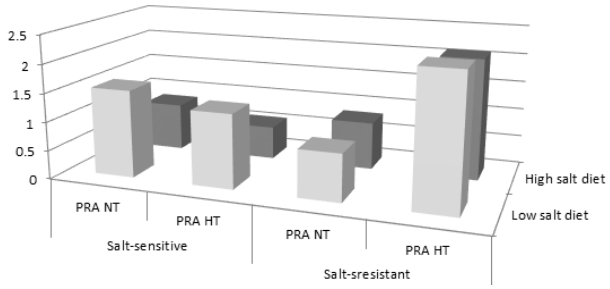

Fig. PRA in salt-sensitive and salt-resistant subjects at the high and low sodium conditions

PRA on high salt condition was higher in salt-resistant group compared to salt-sensitive. Statistically significant negative correlation was found between PRA and salt-sensitivity. r=0.853 (PRABs), r=0.887 (PRALs) $p<0.01$ in salt-resistant subgroup. r= -0.720 (PRABs), r=-0.498 (PRALs), $p<0.01$. In salt sensitive subgroup. Sodium handling is associated with volume expansion and leads to renin suppression in salt-sensitive subjects. High PRA is administered in salt-resistant subjects as RAAS plays key role in this type of Hypertension.

On the basis of our results we can assume that the certain populations in Georgia consume excessive amount of salt. The high prevalence of Salt-sensitivity is found in hypertensive cases as well as in normotensive controls. Our results support the idea that chronic high sodium loading (>200 mmol) which is typical in Georgian as well as other diets switch such humoral and pathophysiological mechanisms that can lead to the development of certain type of hypertension in salt-sensitive individuals. We have demonstrated that chronic salt loading is accompanied by volume expansion and decreased Plasma Renin Activity in salt-sensitive group compared to salt-resistant, where the PRA is increased. Also plasma MBG and OU levels are elevated in salt-sensitive subjects, but remain within the lower level in salt-resistant individuals [2,5,6]. Despite numerous studies performed regarding the salt-sensitivity there are still many questions to explain the mechanism of its development. We have tried to evaluate the issue of its direct role in the pathogenesis of essential hypertension. There is evidence that reduction of salt intake doesn't always decrease the risk of hypertension, the reason of what should be in salt-sensitivity. And salt restriction can become preventive measure for salt-sensitive individuals only, because in salt-resistant individuals the high concentration of sodium will not switch the above mentioned mechanisms for the development of hypertension. Thus salt intake reduction can prevent development of hypertension in salt-sensitive subjects, although hypertension develops in salt-resistant individuals but with other mechanism such as Rein Angiotensin Aldosterone System (RAAS).

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SUMMARY

POSSIBLE MECHANISM OF DEVELOPMENT OF SALT SENSITIVE ESSENTIAL HYPERTENSION

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It has been known that salt-sensitivity of blood pressure is defined genetically as well as can be developed secondary to either decreased renal function or by influence of other environmental factors. The aim of the study was to evaluate the possible mechanism for the development of salt-sensitive essential hypertension in the population of Georgia.

The Case-Control study included 185 subjects, 94 cases with Essential Hypertension stage I (JNC7) without prior antihypertensive treatment, and 91 controls. Salt-sensitivity test was used to divide both case and control groups into salt-sensitive (n=112) and salt-resistant (n=73) subgroups. Endogenous cardiotonic steroids, sodium and PRA were measured in blood and urine samples at the different sodium conditions. Determinations of circulating levels of endogenous sodium pump inhibitors and PRA were carried out using the ELISA and RIA methods. Descriptive statistics were used to analyze the data. Differences in variables between sodium conditions were assessed using paired t-tests.

Salt-sensitivity was found in 60.5% of total population investigated, with higher frequency in females. Salt-sensitivity positively correlated with age in females ($r=0.262$, $p<0.01$). Statistically significant positive correlation was found between 24 hour urine sodium concentration changes and salt-sensitivity $r=0.334$, $p<0.01$. Significant negative correlation was found between salt-sensitivity and PRA. Since no significant correlations were found between BMI and salt-sensitivity, we assume that BMI and salt-sensitivity should be discussed as different independent risk factors for the development of Essential Hypertension. Significant correlation was found between changes in GFR in salt-sensitive cases and controls $p<0.01$. This can be explained with comparable hyperfiltration of the kidneys at high sodium load and discussed as early sign of hypertensive nephropathy in salt-sensitive individuals. At the high sodium condition Endogenous MBG and OU were high in salt-sensitive subjects compared to salt-resistant. These compounds decreased after low salt diet in salt-sensitive cases as well as controls but remained within the same level in salt-resistant individuals. MBG and OU levels positively correlated with SBP in salt-sensitive individuals but salt-resistant subjects didn't show any changes.

Our results support the idea that chronic high sodium loading (>200 mmol) which is typical in traditional Georgian as well as other diets switch those humoral and pathophysiological mechanisms that can lead to the development of certain type of hypertension in salt-sensitive individuals. Salt intake reduction can prevent development of hypertension in salt-sensitive subjects, although hypertension develops in the salt-resistant individuals but by other mechanism such as RAAS.

Keywords: salt-sensitivity, essential hypertension, marinobufagenin, ouabain, renin.

РЕЗЮМЕ

ВОЗМОЖНЫЙ МЕХАНИЗМ РАЗВИТИЯ СОЛЬЧУВСТВИТЕЛЬНОЙ АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИИ

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Известно, что соль-чувствительность артериальной гипертензии определяется генетически, а также может развиваться вследствие снижения почечной функции или под воздействием других факторов окружающей среды.

Целью исследования явилось определение возможного механизма развития соль-чувствительной артериальной гипертензии в грузинской популяции.

Методом случай-контроль исследовано 185 лиц, из них 94 - с артериальной гипертензией I стадии (JNC7) без предварительного антигипертензивного лечения и 91 здоровый волонтер. Согласно результатам теста на соль-чувствительность, обследуемые разделены на 2 группы: соль-чувствительные (n=112) и соль-резистентные (n=73). Образцы крови и мочи получены в период диеты с высоким и низким содержанием соли. Эндогенные кардиотонические стероиды, такие как маринобуфагенин (МБГ) и уабаин (УА), а также натрий и ренин измеряли в образцах обеих групп на фоне двух солевых диет. Для анализа данных использованы описательные статистические методы. Различия показателей между диетами с высоким и низким содержанием соли оценивали с использованием парных t-тестов.

Соль-чувствительность выявлена у 60,5% от общей численности исследуемых, с более высокой частотой у женщин. Соль-чувствительность положительно коррелирует с возрастом у женщин (r=0,262, p<0,01). Выявлена статистически значимая положительная корреляция между концентрацией натрия в 24-часовой моче и соль-чувствительностью (r=0,334, p<0,01). Значимая отрицательная корреляция обнаружена между соль-чувствительностью и ренином. Поскольку никакой достоверной корреляции между индексом массы тела и соль-чувствительностью не выявлено, их следует считать независимыми риск-факторами развития гипертензии. Значимые различия показателей скорости клубочковой фильтрации у соль-чувствительных лиц объясняются относительной гиперфильтрацией почек при высокосолевой диете и обсуждаются как ранний признак гипертонической нефропатии. При диете с высоким содержанием соли показатели МБГ и УА были выше у соль-чувствительных лиц в сравнении с соль-резистентными. При низкосолевой диете уровень этих веществ понижался у соль-чувствительных лиц, однако оставался в пределах того же уровня у соль-резистентных. Уровни МБГ и УА положительно коррелировали с систолическим давлением только у соль-чувствительных лиц.

Результаты проведенного исследования выявили, что хроническое употребление соли с высокой концентрацией натрия (>200 ммоль), которое весьма распространено в Грузии, активирует гуморальные и патофизиологические механизмы, приводящие к развитию определенного типа гипертензии у соль-чувствительных лиц. Сокращение потребления соли является превентивной мерой для развития гипертензии у соль-чувствительных лиц. Развитие гипертензии у соль-резистентных лиц происходит по другим механизмам, таким как система ренинангиотензиналдостерона.

რეზიუმე

მარილმგრძნობიარე ესენციური ჰიპერტენზიის განვითარების შესაძლო მექანიზმი

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თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, შინაგანი სნეულებათა დეპარტამენტი,საქართველო

კვლევის მიზანს წარმოადგენდა მარილმგრძნობიარე ესენციური ჰიპერტენზიის განვითარების შესაძლო მექანიზმის შესწავლა ქართულ პოპულაციაში.

ჩატარდა შემთხვევა-კონტროლის კვლევა, სადაც ჩართული იყო 185 პირი, 94 ესენციური ჰიპერტენზიით (I სტადია JNC7), რომელთაც მანამდე მკურნალობა არ ჩატარებიათ და 91 ჯანმრთელი პირი. ყველა მათგანს ჩაუტარდა მარილმგრძნობელობის ტესტი, რის შედეგადაც პაციენტები გაიყო მარილმგრძნობიარე (n=112) და მარილრეზისტენტულ (n=73) ქვეჯგუფებად. სისხლისა და შარდის ნიმუშების აღება და ენდოგენური კარდიოტონული სტეროიდების - მარინობუფაგენინის (მბგ), უაბაინის (უა), ნატრიუმის და რენინის კონცენტრაციათა განსაზღვრა ჩატარდა, როგორც მაღალ-, ასევე დაბალმარილოვანი სტატუსის დროს. რენინი, მბგ და უა განისაზღვრა ELISA და RIA მეთოდებით. მასალის ანალიზისათვის გამოყენებული იყო აღწერილობითი სტატისტიკა, მაჩვენებლებს შორის კორელაციის დადგენა განხორციელდა წყვილი t ტესტების საშუალებით.

მარილმგრძნობელობა გამოვლინდა გამოკვლეულთა 60.5%-ში. მისი სიხშირე უფრო მაღალი იყო ქალებში და დადებითად კორელირებდა ასაკთან - r=0.262, p<0.01. სტატისტიკურად სარწმუნო დადებითი კორელაცია გამოვლინდა 24-საათიან შარდში ნატრიუმის კონცენტრაციათა სხვაობასა და მარილმგრძნობელობას შორის - r=0.334, p<0.01. პლაზმის რენინსა და მარილმგრძნობელობას შორის დაფიქსირდა უარყოფითი სარწმუნო კორელაცია. სხეულის მასის ინდექსსა (სმი) და მარილმგრძნობელბას შორის სარწმუნო კორელაცია არ გამოვლინდა,რაც მიგვითითებს იმაზე,რომ მარილმგრძნობელობა და სმი ესენციური ჰიპერტენზიის განვითარების დამოუკიდებელი რისკფაქტორებია. სარწმუნო კორელაცია აღმოჩნდა გლომერულური ფილტრაციის სიჩქარეებს შორის სხვადასხვა დიეტაზე,მარილმგრძნობიარე ჯგუფში, რაც მაღალმარილვან დიეტაზე თირკმლების შედარებით ჰიპერფილტრაციაზე მიუთითებს და შესაძლოა განვიხილოთ, როგორც ჰიპერტენზიული ნეფროპათიის ადრეული ნიშანი. მაღალმარილოვან სტატუსზე მბგ-ს და უა-ს დონე მაღალი იყო მარილმგრძნობიარე ქვეჯგუფებში, ხოლო დაბალმარილოვანი დიეტის შემდეგ დაიკლო. ასევე, გამოვლინდა მათი დადებითი სარწმუნო კორელაცია სისტოლურ არტერიულ წნევასთან მარილმგრძნობიარე ქვეჯგუფებში.

შედეგების ანალიზით ავტორებს გამოტანილი აქვთ დასკვნა, რომ მარილის ჭარბი (>200 მმლ) მოხმარება ხაგრძლივი დროის განმავლობაში, რაც დამახასიათებელია, როგორც ქართული ტრადიციული, ასევე სხვა დიეტებისთვის, აამოქმედებს ჰუმორულ და პათოფიზიოლოგიურ მექანიზმებს,რაც მარილმგრძნობიარე ესენციური ჰიპერტენზიის განვითარებას იწვევს. მარილის შეზღუდვა მარილმგრძნობიარე პირებში ესენციური ჰიპერტენზიის განვითარების პრევენციის საშუალებას წარმოადგენს, თუმცა, ჰიპერტენზია მარილრეზისტენტულ პოპულაციაშიც ვითარდება, მხოლოდ სხვა მექანიზმით, როგორიცაა რენინანგიოტენზინალდოსტერონის სისტემა.

ASSOSIATION OF ENDOGENOUS CARDIOTONIC STEROIDS WITH SALT-SENSITIVITY OF BLOOD PRESSURE IN GEORGIAN POPULATION

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Salt-sensitivity of blood pressure is a multifactorial cardiovascular dysfunction introduced by relatively decreased sodium excretion by the kidneys and defined by the different factors such as genetics [13]; Endogenous sodium pump inhibitors – digitalis –like compounds, also referred as endogenous cardiotonic steroids – Marinobufagenin (MBG) and Ouabain (OU). The main function of these compounds is inhibition of sodium pumps in the kidneys, blood vessels and heart, followed by reduced sodium reabsorbtion in the kidneys and increased natriuresis. Also, it can cause vasoconstriction by inhibition of Na/K pump and/or Na+/ Ca2+ changer activation in the smooth muscle of blood vessels. The first cardiotonic steroid found in human plasma was Endogenous Ouabain which has got affinity to the α2/α3 isoforms of Na/K-ATP-ase. As far as the target for natriuretic hormone is α1 isoform only, it cannot trigger natriuresis. But its role is essential in the pathogenesis of salt-sensitive hypertension [2,10]. The high concentration of ouabain is found in human plasma during acute as well as chronic salt loading. According to the data from experimental findings ouabain can stimulate Renin Angiotensin Aldosterone System (RAAS) in mammalian brain and thus increase the activity of sympathetic nervous system. As for marinobufagein, it belongs to bufadienolids family and was obtained from human placenta in 1996. MBG is the only bufadienolid in mammalians. It has got high affinity to α1 isoform of Na/K-ATP-ase and is found to be increased in acute as well as chronic salt loading in experimental models. MBG is elevated in volume expansion conditions such as congestive heart failure, Chronic Kidney Disease, Eclampsia, Primary Aldosteronism, Essential Hypertension etc. [3,4,6,14].

Three mechanisms can be discussed to explain the increased natriuresis by interacting cardiotonic steroids in nanomolar and subnanomolar concentrations with Na/K-ATP-ase.1. In normotensive salt-sensitive rats salt loading increases serum MBG concentration as well as MBG renal excretion, but decreases sodium pump activity followed by sodium retention. In salt-resistant rats salt loading increases sodium excretion, but MBG levels remain within the same range. 2. Cardiotonic steroids can be interacted with other natriuretic hormone such as natriuretic peptide, which can increase renal Na/K-ATP-ase sensitivity to MBG in salt-resistant rats. 3. Cardiotonic steroids induced natriuresis can be mediated by Na/K-ATP-ase endocitosis [1,5].

Except the direct inhibition of sodium pump Marinobufagenin has got ability to increase natriuresis by decreased Na+/H+ changer expression or interacting with specific Na/K-ATP-ase in proximal tubules [7-9,12].

According to the latest findings genetic mutations as well as genetic polymorphism of sodium pump plays a key role in the development of salt-sensitive hypertension. For instance, α-aducin gene Gly460Trp variation activates Na/K pump; Glucagone gene Arg40Ser variation decreases natriuresis by cAMP supression; SGK1 mutatioan increases aldosterone dependent expression of sodium pumps; Cyp4a10 gene mutation from Cytochrome P450 family also can lead development of salt-sensitive hypertension [11].

Material and methods. In the Case-Control study 185 subjects, 94 cases with Essential Hypertension stage 1 (JNC7) without prior antihypertensive treatment, and 91 controls – normotensive healthy individuals were involved.

Determination of circulating levels of endogenous sodium pump inhibitors was carried according to the following methodology: The disposable Sep-Pak C-18 columns was activated by 10 ml 100% acetonitrile and washed once with 5 ml distilled water. 0.5 ml plasma and 5 ml urine samples were then loaded to the columns. After another washing step with 5 ml distilled water, the columns were eluted by 7ml 20% acetonitrile followed by 7ml 80% acetonitrile. The eluates were combined, lyophilized and re-suspended in TBS buffer (50 mMTrizma, 150 mMNaCl, 7.7 mM NaN_3, pH 7.4). The concentration of MBG was then determined using a enzyme-linked immunosorbent assay (ELISA) based on a 4G4 anti-MBG monoclonal antibody. Briefly, 100 µl of MBG standards or sample eluates were mixed with 100 µl anti-MBG monoclonal antibody. The mixture was then added to MBG-thyroglobulin-coated and 1% BSA-blocked ELISA plate. After 1 h incubation, plates were washed 3 times and secondary anti-mouse antibody conjugated with alkaline phosphatase was added and incubated for another 1h. A fluorescent signal amplifier FDP was used to detect the signals after washing out the secondary antibody. The sample MBG concentrations were calculated based on the standard curve using purified MBG compound. The endogenous ouabain assay was based on the similar principal using the anti- ouabain monoclonal antibodies.

The paired t-test was used for the calculation of paired variables during high and low salt conditions. Differences between groups were assessed using Chi-square tests, ANOVA and multivariate logistic regression

Results and their discussion. The interesting data were obtained in the changes of concentrations of endogenous cardiotonic steroids during different sodium conditions (Fig. 1).

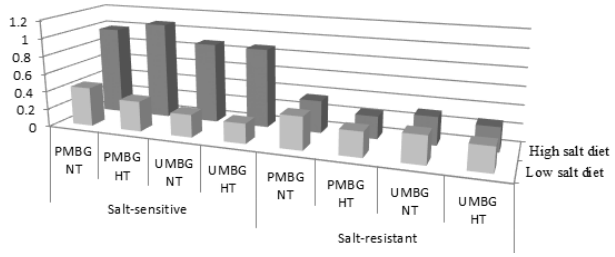

Fig. 1. MBG levels in blood and 24 hour urine samples during high and low sodium conditions in salt-sensitive and salt-resistant cases and controls

Ouabain concentration in plasma decreased almost 10 folds in salt-sensitive subjects compared to salt-resistant, where it remained at the same level (Fig.2).

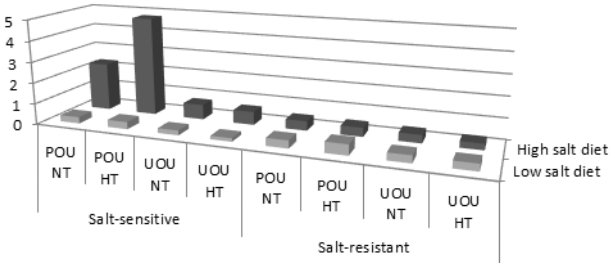

Fig. 2. Ouabain levels in plasma and 24 hour urine samples during high and low salt intake in salt-sensitive and salt-resistant hypertensive cases and normotensive controls

The results provide evidence that the difference of endogenous cardiotonic steroids levels in blood and 24 hour urine obtained during high and low sodium conditions are statistically significant in salt-sensitive group only $p<0.01$ (Table 1).

Presumably, endogenous Marinobufagenin raise in blood as an adaptive mechanism for the stimulation of natriuresis. In case of inapropriate excretion of sodium MBG overproduction inhibits vascular sodium pump and cause vasoconstriction. The similar changes were found in 24-hour urine concentrations of marinobufagenin. Statistically significant correlation was detected in salt-sensitive group $p<0.01$ (Table 2).

Likewise chages were found in Ouabain concentrations in blood as well as in 24-hour urine. $p<0.01$ in salt-sensitive group (Table 3,4).

The high levels of endogenous cardiotonic steroids and their decrease after low salt diet in salt-sensitive group compared to salt resistant strongly implicate their role in the development of so-called sodium induced, low renin, salt-sensitive hypertension.

One week low salt diet caused in reduction of MBG and OU levels in blood and urine samples and lowering of SBP in salt-sensitive group. MBG and OU levels positively correlated with SBP in salt-sensitive individuals but salt-resistant subjects didn't show any changes. It is important to note, that MBG and OU levels undergo the same changes in salt-

Table 1. Mean difference, confidence interval of the difference and significance of Endogenous Marinobufagenin in plasma during high and low salt diets

Sltsens	PMBG	Mean	95% Confidence Interval of the Difference		Sig. (2-tailed)
			Lower	Upper	
0	PMBGBs – PMBGLs	-0.013	-0.039	0.013	0.307
1	PMBGBs – PMBGLs	0.651	0.546	0.756	0.000

Table 2. Mean difference, confidence interval of the difference and significance of Endogenous Marinobufagenin in 24 hour urine during high and low salt diets

Sltsens	UMBG	Mean	95% Confidence Interval of the Difference		Sig. (2-tailed)
			Lower	Upper	
0	UMBGBs – UMBGLs	0.001	-0.020	0.022	0.900
1	UMBGBs – UMBGLs	0.648	0.568	0.727	0.000

Table 3. Mean difference, confidence interval of the difference and significance of Endogenous Ouabain in plasma during high and low salt diets

Sltsens	POU	Mean	95% Confidence Interval of the Difference		Sig. (2-tailed)
			Lower	Upper	
0	POUBs – POULs	0.01	-0.08	0.09	0.87
1	POUBs – POULs	3.28	3.00	3.57	0.00

Table 4. Mean difference, confidence interval of the difference and significance of Endogenous Ouabain in 24 hour urine during high and low salt diets

Sltsens	UOU	Mean	95% Confidence Interval of the Difference		Sig. (2-tailed)
			Lower	Upper	
0	UOUBs – UOULs	0.006	-0.023	0.036	0.678
1	UOUBs – UOULs	0.475	0.412	0.538	0.000

sensitive hypertensive cases as in normotensive controls, although their concentration is lower in controls. On the basis of aforementioned we suppose that in salt-sensitive individuals the levels of certain humoral compounds start to change at the normotensive stage and its' long term impact can lead to the development of salt-sensitive Essential Hypertension.

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SUMMARY

ASSOSIATION OF ENDOGENOUS CARDIOTONIC STEROIDS WITH SALT-SENSITIVITY OF BLOOD PRESSURE IN GEORGIAN POPULATION

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This investigation differentiates types of essential hypertension in a Georgian population as well as describes endogenous cardiotonic steroids in salt-sensitive and salt-resistant subjects. This case control study included 185 subjects: 94 cases with stage 1 essential hypertension (JNC7) naïve to antihypertensive treatment, and 91 controls. A salt-sensitivity test was used to dichotomize case and control groups into salt-sensitive and salt-resistant subgroups. Blood and

urine samples were obtained to categorize participants as consuming high and low salt diets. Endogenous cardiotonic steroids, sodium and plasma-renin activity (PRA) were measured in both samples at the different sodium conditions. Determinants of circulating levels of endogenous sodium pump inhibitors were carried out using the ELISA and RIA methods; PRA was assessed by radioimmunoassay. Descriptive statistics were used to analyze the data. Differences in variables between sodium conditions were assessed using paired t-tests. Salt-sensitivity was found in 60.5% of the total population investigated, with a higher proportion in females. A statistically significant positive correlation was found between salt-sensitivity and age in females ($r=0.262$, $p<0.01$), and with 24-hour urine sodium concentration changes ($r=0.334$, $p<0.01$). A significant negative correlation was found between salt-sensitivity and PRA. At the high sodium condition, endogenous MBG and OU were high in salt-sensitive subjects compared to those who were salt-resistant. These compounds decreased with a low-salt diet in both salt-sensitive cases and controls but remained the same in salt-resistant individuals. The MBG and OU levels positively correlated with systolic blood pressure in salt-sensitive individuals but no variability was evident among salt-resistant subjects. Our results show that MBG and OU levels start to increase at the normotensive stage and sustained high concentrations can lead to elevated systolic blood pressure, a risk factor for arterial hypertension in salt-sensitive subjects.

Keywords: endgenous cardiotonic steroids, marinobufagenin, ouabain, salt-sensitivity, essential hypertension.

РЕЗЮМЕ

ОПРЕДЕЛЕНИЕ ВЗАИМОДЕЙСТВИЯ ЭНДОГЕННЫХ КАРДИОТОННЫХ СТЕРОИДОВ В РАЗВИТИИ СОЛЬ-ЧУВСТВИТЕЛЬНОГО ТИПА АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИИ В ГРУЗИНСКОЙ ПОПУЛЯЦИИ

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Целью исследования явилось определение взаимодействия эндогенных кардиотонных стероидов в развитии соль-чувствительного типа артериальной гипертензии в грузинской популяции. Методом случай-контроль исследовано 185 лиц, из них 94 - с артериальной гипертензией в I (JNC7) стадии без предварительного антигипертензивного лечения и 91 здоровый волонтер. Определение уровней эндогенных ингибиторов натриевого насоса проведено методами ELISA и RIA с использованием моноклональных антител, специфических для уабаина (УА) и маринобуфагенина (МБГ). Различия показателей между диетами с высоким и низким содержанием соли оценивали с использованием парных t-тестов. Для описания разницы между группами были использованы тест Хи-квадрата, ANOVA и множественный регрессионный анализ.

Разница концентрации эндогенных кардиотонических стероидов в крови и 24-часовой моче, полученных в период диеты с высоким и низким содержанием соли, является статистически значимой только у соль-чувствительных лиц ($p<0,01$), что свидетельствует об их роли в развитии соль-чувствительной артериальной гипертензии. Следует отметить, что уровни МБГ и УА претерпевают одинаковые изменения как у соль-чувствительных лиц, так и у волонтеров. На основании вышеизложенного, следует предположить, что у соль-чувствительных лиц уровни УА и МБГ начинают изменяться уже на нормотензивной стадии и долгосрочное воздействие соли может привести к развитию соль-чувствительной артериальной гипертензии.

რეზიუმე

ენდოგენური კარდიოტონული სტეროიდების ურთიერთკავშირი ესენციური ჰიპერტენზიის მარილმგრძნობელობასთან ქართულ პოპულაციაში

ნ. ქანთარია, ი. ფანცულაია, ი. ანდრონიკაშვილი, გ. სიმონია

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, შინაგანი სნეულებათა დეპარტამენტი, საქართველო

ბოლო კვლევების მიხედვით, ნატრიუმის ტუმბოს ენდოგენური ინჰიბიტორები – მარინობუფაგენინი (მბგ) და უაბაინი (უა) მნიშვნელოვან როლს ასრულებენ ესენციური ჰიპერტენზიის განვითარებაში. კვლევის მიზანს წარმოადგენდა მარინობუფაგენინსა და უაბაინის ურთიერთკავშირის დადგენა ესენციური ჰიპერტენზიის მარილმგრძნობიარე ტიპის განვითარებაში.

შემთხვევა-კონტროლის მეთოდით გამოკვლეულია 185 პირი, 94 ესენციური ჰიპერტენზიით (I სტადია JNC 7), რომელთაც მანამდე მკურნალობა არ ჩატარებიათ და 91 ჯანმრთელი პირი. წყვილ მაჩვენებლებს შორის განსხვავებების სარწმუნოობა გამოთვლილი იყო წყვილი t-ტესტის საშუალებით. ჯგუფებს შორის განსხვავებების დადგენა განხორციელდა x-კვადრატ ტესტის, ANOVA-ს და მულტივარიაციული ანალიზის მეთოდით (ლოგისტიკური რეგრესია).

ენდოგენური კარდიოტონული სტეროიდების კონცენტრაციათა სხვაობა მაღალ- და დაბალ-

მარილოვანი დიეტების დროს სისხლსა და 24-საათიან შარდში სტატისტიკურად სარწმუნო იყო მხოლოდ მარილმგრძნობიარე ჯგუფში ($p<0.01$), რაც მიუთითებს ამ ნივთიერებების როლზე მარილმგრძნობიარე ესენციური ჰიპერტენზიის განვითარებაში. მნიშველოვანია აღინიშნოს, რომ მბგ და უა-ს კონცენტრაციები იგივე ცვლილებებს განიცდის მარილმგრძნობიარე ჰიპერტენზიულ პირებში, როგორც ნორმოტენზიულებში, თუმცა მათი დონე შედარებით დაბალია საკონტროლო ჯგუფში. ყოველივე აღნიშნულის საფუძველზე სავარაუდოა, რომ მარილმგრძნობიარე პირებში მბგ და უა-ს დონეები მატულობს ჯერ კიდევ ნორმოტენზიულ სტადიაზე,მათი ხანგრძლივი ზემოქმედება კი შესაძლოა გახდეს მარილმგრძნობიარე ჰიპერტენზიის განვითარების მიზეზი.

EPIDEMIOLOGY OF CLINICALLY MANIFESTED ACUTE HEPATITIS C CASES IN GEORGIA

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Hepatitis C is persistent viral infection of the liver, one of the most common reasons of chronic hepatitis, liver cirrhosis and/or Hepatocellular Carcinoma worldwide. Decompensated cirrhosis, as a consequence of chronic hepatitis C is the major reason of liver transplantation [3,7].

Hepatitis C virus is a major global health problem with an estimated 170 million people infected worldwide, out of which 350,000 die each year from liver damage associated with infection. 30-50% of individuals with cirrhosis progress to hepatocellular carcinoma. It is not clear completely why part of patients develop persistent infections while some patients have complete virus clearance after the infection.

Clinical manifestation of Hepatitis C is diverse. While some patients progress to severe fibrosis, cirrhosis and hepatocellular carcinoma after a rather short period from the infection, others develop mild fibrosis after many years of infection [1,4].

Due to importance of the disease and severe damage developed as a result of chronic hepatitis, it is very important to reveal cases of acute hepatitis timely and to establish a route of transmission that on its turn will help to prevent the future spread of the disease.

The prevalence in various countries varies between 1-10% of the population. For example, in USA, Australia and Scandinavia up to 1% of the population is infected, in West European countries – up to 5% of people, in Asian countries and Africa - 5-10%; very high rate of prevalence is in Egypt – where 30 % population is infected.

As for Georgia, according to Statistical Data of the National Center for Disease Control and Public Health (NCDC) the incidence rate (number of new cases per 100000 population at risk in a given time period) of hepatitis C has decreased in comparison with the year 2011 (55), but anyway it remains high (41). From newly revealed cases of hepatitis C, 9.4 % was presented as acute hepatitis and 90.4 % as the chronic hepatitis. Effective measures against hepatitis C epidemics would be impossible without drastic diminishment of its incidence rate.

The aim of the study was to reveal clinical-epidemiological peculiarities of acute hepatitis C cases.

Material and methods. In 2013-2015 we studied 31 hospitalized patients with diagnosis of acute hepatitis C. The research was conducted at the Infectious Diseases, Aids and Clinical Immunology Research Center.

Patients were diagnosed based on laboratory tests and clinical findings. For the diagnosis of hepatitis C, both serological (anti-HCV) and nucleic acid–based molecular assays are available (HCV-RNA). At the early stage of the acute hepatitis C, anti-HCV may be negative. Diagnosis of acute hepatitis C is defined in the case of confirmed seroconversion. Acute hepatitis C can be suspected by clinical signs and lab findings (elevation of Alanin-Aminotransferase (ALT), jaundice, elevation of bilirubin) in combination with the absence of history of chronic hepatitis [5,8]. Detection of HCV RNA is possible during acute phase of hepatitis C, however, existence of so called “window period” should not be excluded when HCV RNA is undetectable.

Results and their discussion. Among 31 hospitalized patients with acute hepatitis C, 19 patients were male (61%) and 12 were females (39%). Age of patients was 23-60 years and there was no age difference between females and males. From 19 males 11 (58%) were urban residents, 8 (42%) were rural residents of Imereti, Kakheti, Kartli, Mtskheta-Tianeti, Adjara, the regions where drug addiction is spread most of all. From

12 females - 6 (50%) were from Tbilisi and 6 (50%) were rural residents (Kvemo Kartli, Imereti, Adjara).

According to clinical manifestation of acute hepatitis in patients, dyspepsia, asthenic syndrome and arthralgia of various intensities were revealed in 100% of patients. Nevertheless, in every case, elevation of ALT 10-fold and more was revealed. History of chronic hepatitis was excluded. All of the cases were of mild or moderate severity, while severe cases of the disease were not found. In all cases diagnosis of acute hepatitis C was confirmed by anti-HCV antibodies seroconversion.

Elevation of total billirubin due to conjugated bilirubin was detected in 95% of cases. Reduction of prothrombin index and hypoalbuminemia was not revealed in any patient. According to ultrasound investigation of the liver and spleen, hepatomegaly was revealed in 85% of patients and splenomegaly was not revealed.

Suspected route of HCV transmission: routes of virus transmission in 11 urban male patients were inravenous drug use in 2 cases (18.11 %), risk factor cannot be indentified in 5 cases (45.45 %), and unsafe medical procedures 4 cases (36.44%) - dental procedure in 2 cases, operation - in 2 cases. Virus transmission route in 8 rural male patients: reason of infection was unsafe medical procedures in 3 cases (37,5%) and intravenous drug use identified in 1 case (12.5%) and unknown in 4 (50%) cases.

Among 6 females from Capital city Tbilisi route of transmission was not identified in 2 (33.3%) cases. 1 (16.6%) female had new sexual partner with chronic hepatitis C, 1 (16.8%) female was Healthcare worker (nurse) and in 2 (33.3%) cases route of transmission was identified to be the medical procedures (surgical interventions). Among 6 rural females the route of infection was unknown in 1 (16.6%) case; 1 (16,6%) female had new sexual partner with chronic hepatitis C and various medical procedures were identified as the infection route in 4 (66.8%) cases. Among them -2 cases were related with gynecological, 1 with dental and 1 with opthalmological procedures (Fig.).

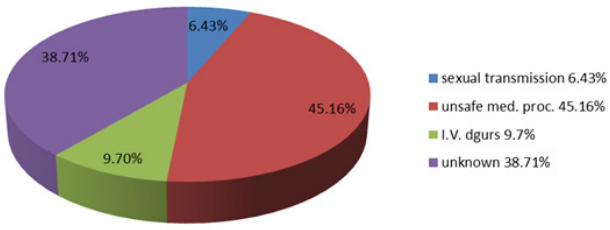

Fig. Routes of HCV transmission

Thus, according to our study, the most significant source of HCV infection is healthcare settings- unsafe medical procedures – in 14 (45.16%) cases. It is worthy to note that in 12 (38.71%) cases of the infected individuals cannot identify a source of their infection. It is believed that most of these cases are due to known risk factors – several occasions of intravenous drug use. However, in more than 10% of all cases, no risk factor can be identified. The third most behavior risk factor is particularly I.V. drug use, and is responsible for about – 3 (9.7%) cases of all identified cases of hepatitis C; sexual transmission revealed in 2 cases (6.43%). These all prove the significance of various unsafe medical procedures as causative agents in further increase of infected patients.

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SUMMARY

EPIDEMIOLOGY OF CLINICALLY MANIFESTED ACUTE HEPATITIS C CASES IN GEORGIA

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Hepatitis C virus is responsible for the majority of persistent viral infections of the liver, chronic hepatitis, liver cirrhosis and/ or hepatocellular carcinoma. Two strategies are important to curtailing the rising prevalence of disease: efficient

diagnosis of acute hepatitis and identification of the likely mode of transmission. The aim of this study was to identify the clinical and epidemiological hallmarks of acute hepatitis C. During 2013-2015, 31 patients were hospitalized with a diagnosis of acute C hepatitis. According to epidemiological data, the primary mode of transmission is during medical procedures, responsible for 14 (45.16%) of cases, followed by injection drug use 3 (9.7%) of cases and sexual transmission - 2 (6.43%) of cases. However, in 12 (38.71%) of cases the infected individual was unable identify the likely source of infection. Given that nearly half of all cases arise from nosocomial infection, it is imperative that infection control practices be reviewed and resources provided to prepare a sterile environment for patients and health care providers.

Keywords: Hepatitis C, liver cirrhosis, hepatocellular carcinoma, Georgia.

РЕЗЮМЕ

ЭПИДЕМПОЛОГИЧЕСКИЕ ДАННЫЕ КЛИНИЧЕСКИ ВЫЯВЛЕННЫХ СЛУЧАЕВ ОСТРОГО ГЕПАТИТА С В ГРУЗИИ

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Гепатит С – персистентная вирусная инфекция печени, является весьма частой причиной хронического воспаления, цирроза и/или гепатоцеллюлярной карциномы печени. Своевременное выявление случаев острого гепатита С и путей инфицирования весьма значимо для предотвращения дальнейшего распространения инфекции.

Целью исследования явилось установление клинико-эпидемиологических особенностей острого гепатита С в Грузии. С диагнозом острого гепатита С в 2013-2015 гг. стационарное лечение проводилось 31 пациенту. Согласно эпидемиологическому анамнезу, из 31 пациента у 14 (45,16%) причиной развития заболевания явились инвазивные медицинские манипуляции; в 3 (9,7%) случаях - интравенное употребление наркотиков; в 2 (6,43%) - половой путь инфицирования. У 12 (38,71%) пациентов пути инфицирования установить не удалось. Результаты проведенного исследования указывают, что самым частым путем инфицирования гипетитом С являются медицинских манипуляции.

რეზიუმე

კლინიკურად გამოვლენილი მწვავე C ჰეპატიტის შემთხვევების ეპიდემიოლოგიური მონაცემები საქართველოში

ე. ვაშაკიძე, ი. მიქაძე, ე. პაჭკორია

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ინფექციურ სნეულებათა დეპარტამენტი, საქართველო

C ჰეპატიტი წარმოადგენს ღვიძლის პერსისტულ ვირუსულ ინფექციას, ღვიძლის ქრონიკული ანთების, ღვიძლის ციროზისა და/ან ჰეპატოცელულარული კარცინომის ყველაზე ხშირ მიზეზს მთელ მსოფლიოში. მნიშვნელოვანია მწვავე შემთხვევების დროული გამოვლენა და ინფიცირების გზების დადგენა, რაც თავის მხრივ დაავადების შემდგომ გავრცელებას შეაფერხებს.

კვლევის მიზანს წარმოადგენდა დადასტურებული მწვავე C ჰეპატიტის შემთხვევების კლინიკურ-ეპიდემიოლოგიური თავისებურებების გამოვლენა. მწვავე C ჰეპატიტის დიაგნოზით 2013-2015 წწ-ში სტაციონარული მკურნალობა ჩაუტარდა 31 პაციენტს. ეპიდემიოლოგიური ანამნეზით ინფექციის გავრცელების მნიშვნელოვან ფაქტორად რჩება ინვაზიური სამედიცინო მანიპულაციები - 14 (45.16%) შემთხვევა; 3 (9.7%) შემთხვევაში დაავადება უკავშირდება ნარკოტიკების ინტრავენურ მომხმარებას; სქესობრივი გადაცემის გზა გამოვლინდა 2 (6.43%) შემთხვევაში; 12 (38.71%) შემთხვევაში ინფიცირების გზა უცნობია.

ამგვარად, ჩატარებული კვლევის შედეგები მიუთითებს, რომ C ჰეპატიტის განვითარების ძირითად მიზეზს უმრავლეს შემთხვევაში წარმოადგენს სამედიცინო მანიპულაციები.

GENOTYPIC DISTRIBUTION OF HPV AMONG WOMEN OF REPRODUCTIVE AGE IN GEORGIA

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Cervical cancer is one of major public health problems worldwide. In 2008, the reported number of cervical cancer cases was about 530,000 and the number of deaths was about 275,000 [6]. More than 85% of cervical cancer cases occur in developing countries [7]. Unfortunately, these diagnoses are typically made when the disease is already advanced. In developed countries, where cervical cancer screening programs exist, incidence and mortality related to cervical cancer have decreased dramatically. The benefits of screening and early intervention are clear. Implementation of breast and cervical cancer screening as well as HPV vaccination programs have been recently started in Georgia [10].

In majority of cases cervical and some other types of cancers are caused by persistent infection by high-risk human papillomaviruses (HPV). Persistence of HPV infections is the single greatest risk factor for malignant progression [3].

HPV are DNA viruses with the potential towards oncogenesis. More than 100 types of HPV exist, and approximately 35 of these infect the genital tract. The viruses are categorized as "high risk" and "low risk," depending on whether they are known to cause cancer or not. The most common high-risk types include types 16 and 18, which account for the majority (more than 70%) of cervical cancer cases. The low-risk HPV types cause benign tissue changes and warts [9]. Genital HPV infection is one of the most common sexually transmitted diseases worldwide; up to 75% of sexually active persons are estimated to be infected at some point. The public health importance of this infection is related to the ability of certain high-risk HPV types to cause cervical cancer in women.

In a previous pilot study being conducted by our group in collaboration with US scientists [2] cervical samples have been collected from 257 women with different gynaecological symptoms. 19.3 % of women have abnormal cytology test results. In the study among women in Georgia HPV prevalence was shown to be around 13.5 % [1]. This is higher than the average World estimate 9.2 % (HPV prevalence varied nearly 20 times between populations from 1.4 % in Spain to 25.6% in Nigeria [5].

In our recent study of women from the same population as for the current study, it was shown, that among women who agreed to be tested for anti-HPV antibodies, 21.1% were positive. Awareness of cervical cancer screening was significantly associated with HPV seropositivity. With multivariate analysis, both absence of condom use and lack of knowledge about cervical cancer screening were independently associated with HPV seropositivity [4].

The main goal of this study was to investigate genotypic distribution of HPV infections among ethnically homogenous Caucasian women population of reproductive age in Georgia.

Material and methods. The total sample included 2000 women recruited from different women's consultation centers (WCC) and National Screening Centers (NSC) in Tbilisi, Georgia. We recruited women visiting NSCs for screening on cervical or on cervical and breast cancers, to WCCs for routine gynecologic consultations and also to obstetricians for their post-natal visit and routine physical check-up, usually occurring around 6 to 8 weeks after delivery. The samples for HPV DNA investigation in most cases were collected in parallel with the pap tests, no additional visits were needed for the HPV DNA testing for these cases.

WCCs and NSCs were selected from different regions of Tbilisi based on their representation of the general population. Consecutive patients that meet the eligibility criteria (i.e., women of reproductive age who already have started the sexual life, speak Georgian or Russian) were invited to participate until we have selected the designated sample number.

250 women who had positive HPV DNA test were further investigated by the following methods: follow-up HPV DNA test at 18 months after the first HPV DNA investigation and identification of HPV genotypes from the positive HPV DNA samples.

Cervicovaginal samples for viral DNA extraction were obtained in parallel with the routine pap-test screening procedure or independently (in rare cases) by placing cervical brush into the appropriate buffer-containing tubes. HPV were isolated from the cervical samples and the consensus primer set Gp5+/Gp6+ were used for the amplification using PCR methodology. For positive HPV DNA specimens HPV genotyping was performed using type-specific primers [8,11].

Results and their discussion. The median age of respondents was 32 (range 18 to 48). The proportion of employed women was 49.6%. 64% of women reported ever having abortion.

Table. Distribution of HPV genotypes among women of reproductive age

Genotype	N (%)
6	98 (39.2)
11	27 (10.8)
16	64 (25.6)
18	47 (18.8)
33	23 (9.2)
45	19 (7.6)
66	9 (3.6)

250 samples positive on HPV DNA were investigated for determination of HPV genotypes. The genotype distribution was as follows: type 6 – 98 women (39.2 %), type 16 – 64 (25.6%), type 18 – 47 (18.8%), type 33 – 23 (9.2%), type 11 – 27 (10.8%), type 45 – 19 (7.6%), type 66 – 9 (3.6%). In 37 women (14.8%) presence of the mixture of the different HPV genotypes (coexistence of more than two virus types) has been documented (Table).

HPV genotypic profile among Georgian women is similar to the data generated from the studies conducted among the populations of the other European countries. Presence of the subset of HPV genotypes not covered by quadrivalent anti-HPV vaccine (types 33, 45 and 66) was demonstrated among the Georgian women.

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SUMMARY

GENOTYPIC DISTRIBUTION OF HPV AMONG WOMEN OF REPRODUCTIVE AGE IN GEORGIA

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Of the 100 types of human papillomaviruses (HPV), approximately 35 infect the genital tract. The viruses are categorized as "high risk" and "low risk" depending on whether they are known to cause cancer or not. Cervical cancer is an important cause of cancer mortality in Georgia, and worldwide. Only limited and incomplete data are available about the epidemiology of HPV infection and

related molecular and cellular changes in Georgia. Objectives of our study included the estimation of the prevalence and the distribution of HPV genotypes among women in Georgia. The study participants were women (~2000) aged 18-49 years randomly selected during a clinic appointment with a gynecologist for a regular check-up at one of the women's consultation centers (WCC) participating in the study. Venous blood (5 ml) was drawn and the prevalence of HPV evaluated by the detection of the HPV DNA. For genotyping, HPV DNA were extracted from the cervical samples, amplified first by consensus and then by primer-specific PCR, followed by a detection step on agarose gel. Of the total samples, 250 were positive for HPV DNA; these were further tested to identify the specific HPV genotype. The genotype distribution was as follows: type 6, 98 women (39.2 %); type 16, 64 (25.6%); type 18, 47 (18.8%); type 33, 23 (9.2%); type 11, 27 (10.8%); type 45, 19 (7.6%); and type 66, 9 (3.6%). In 37 women (14.8%), we found coexistence of several different HPV genotypes. The HPV genotypic profile among Georgian women is similar to data generated from studies conducted among the populations in other European countries. Presence of the subset of HPV genotypes not covered by quadrivalent anti-HPV vaccine (types 33, 45 and 66) was demonstrated among Georgian women.

Keywords: human papillomavirus (HPV), genotypes, women of reproductive age.

РЕЗЮМЕ

РАСПРОСТРАНЕНИЕ ГЕНОТИПОВ ПАПИЛЛОМАВИРУСОВ ЧЕЛОВЕКА СРЕДИ ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА В ГРУЗИИ

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Существует более чем 100 типов вируса папилломы человека (ВПЧ) и, приблизительно, 35 из них инфицируют генитальный тракт. В зависимости от того, вызывают или нет вирусы развитие опухоли, они классифицируются как «высокого» и «низкого риска». Рак шейки матки является значимой причиной смертности в Грузии. В стране имеются только ограниченные и неполные данные об эпидемиологии ВПЧ-инфекции и связанных с ней молекулярных и клеточных изменениях.

Целью данного исследования явилась оценка превалентности и распределения генотипов вируса папилломы человека среди женщин в Грузии.

Участники исследования отобраны из женских консультационных центров, в основном, женщины в возрасте 18-49 лет, посещавшие гинеколога для регулярной проверки. Венозная кровь (5 мл) забиралась в EDTA-побирки и посредством метода полимеразной цепной реакции оценивалась ДНК ВПЧ исследуемых женщин. Для генотипирования ДНК ВПЧ извлекали из цервикальных образцов, амплифицировали сначала консенсусной, а затем праймер-специфической ПЦР с последующей стадией детекции на агарозном геле. 250 образцов, положительных на ДНК ВПЧ, исследованы для определения генотипов ВПЧ. Генотипы были распределены следующим образом: тип 6 - 98 (39,2%) женщин, тип 16 - 64 (25,6%), тип 18 - 47 (18,8%), тип 33 - 23 (9,2%), тип 11 - 27 (10,8%), тип 45 - 19 (7,6%), тип 66 - 9 (3,6%). У 37 (14,8%) женщин подтверждено наличие смешанных различных генотипов ВПЧ (сосуществование более двух типов вируса). Данные генотипического профиля ВПЧ у женщин в Грузии схожи с таковыми исследований, проведенных среди населения других европейских стран. Следует отметить, что проведенные исследования подтверждают факт циркулирования таких генотипов ВПЧ, которые не охватываются четырехвалентной вакциной против ВПЧ (типы 33, 45 и 66).

რეზიუმე

ადამიანის პაპილომავირუსის სხვადასხვა გენოტიპის გავრცელება რეპროდუქციული ასაკის ქალებში საქართველოში

მ. ბუწაშვილი, მ. ქაჯაია, მ.კოჭლამაზაშვილი, მ. ზარანდია,თ. გაგუა,დ. მესხიშვილი,ნ. ავალიანი, გ. კამკამიძე

ჯანმრთელობის კვლევის კავშირი, თბილისი, საქართველო

სადღეისოდ აღწერილია ადამინის პაპილომავირუსის 100-ზე მეტი ტიპი, რომელთაგან 35-მდე გენიტალური ტრაქტის ინფექციას იწვევს. პაპილომავირუსები კლასიფიცირდება როგორც „მაღალი რისკის“ და „დაბალი რისკის“ იმის მიხედვით, იწვევს თუ არა ვირუსის კონკრეტული ტიპი სიმსივნის განვითარებას. საშვილოსნოს ყელის სიმსივნე წარმოადგენს საქართველოში სიმსივნით სიკვდილობის მნიშვნელოვან მიზეზს, თუმცა დღეისათვის ძალზედ მცირეა ეპიდემიოლოგიური მონაცემები საქართველოში პაპილომავირუსული ინფექციის გავრცელებისა და მის მიერ გამოწვეული პათოლოგიების შესახებ.

კვლევის მიზანს წარმოადგენდა საქართველოში ადამიანის პაპილომავირუსების გავრცელებისა და მათი გენოტიპური განაწილების შესწავლა. კვლ-

ევაში მონაწილე პირების შერჩევა განხორციელდა ქალთა საკონსულტაციო ცენტრებში. საკვლევი ჯგუფი მოიცავდა 18-49 წლის ქალებს,რომლებიც რეგულარულად ისინჯებოდნენ გინეკოლოგებთან. საკვლევ მასალას წარმოადგენდა პაპილომავიურუსის დნმ-ის აღმოსაჩენად 5 მლ ვენური სისხლი. პაპილომავირუსის გენოტიპირება ჩატარდა პოლიმერაზული ჯაჭვური რეაქციის (პჯრ) მეთოდით, ჯერ კონსენსუს-პრაიმერებით და შემდეგ აგაროზულ გელზე თვისობრივი დეტექციით. ზამოაღნიშნული მეთოდით მიღებული 250 სინჯის შემდგომი კვლევა ხორციელდებოდა გენოტიპ-სპეციფიკური პჯრ მეთოდით. პაპილომავირუსის გენოტიპები განაწილდა შემდეგნაირად: მე-6 ტიპის არსებობა დადგინდა 98 შემთხვევაში (39.2%), მე-16 ტიპი 64 (25.6%), მე-18 ტიპი – 47 (18.8%), 33-ე ტიპი – 23 (9.2%), მე-11 ტიპი – 27 (10.8%), 45-ე ტიპი – 19 (7.6%) და 66–ე ტიპი 9 (3.6%) შემთხვევაში. 37 ქალს (14.8%) დაუფიქსირდა ორი და მეტი გენოტიპის ერთობლივი არსებობა (შერეული გენოტიპები). საქართველოში პაპილომავირუსების გენოტიპური განაწილება აღმოჩნდა ევროპულ ქვეყნებში ჩატარებული კვლევების შედეგების ანალოგიური. საინტერესოა აღინიშნოს, რომ საქართველოში დაფიქსირდა პაპილომავირუსების ისეთი გენოტიპები (33, 45 და 66), რომლებიც არ შედის პაპილომავირუსების საწინააღმდეგო ოთხვალენტიანი ვაქცინის შემადგენლობაში.

ROLE OF VIRAL PATHOGENS IN INFANTS WITH SYSTEMIC INFECTION AT THE NEWBORN INTENSIVE CARE UNITS IN GEORGIA

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Neonatal and infant mortality remains the public health concern worldwide, particularly in developing countries [11]. The major cause of infant mortality in these countries is neonatal infections [12]. Different studies reported the role of viral pathogens (members of the Herpesviridae and Enteroviruses) in the development of generalized infections in newborns, while in Georgia in the majority of such cases the causative agents are not being identified [2,4,5,7,9]. Limited data from studies of generalized infections in neonates from other countries are available to provide some insight into the potential high incidences of these infections in the proposed high-risk study population.

Average infant mortality rate in Georgia is 14.3 deaths per 1000 live births [8]. Limited published data is available to estimate the potential incidence of viral infections among neonates with generalized infections in the country. The study by Macharashvili et al. is focusing mostly on the link of neonatal blood stream infections (BSI) and infant mortality; and determination antibiotic susceptibility of the isolated organisms [1,6].

This study synthesizes information on the role of viral pathogens and other risk factors in mortality of newborns and infants with generalized infections admitted to neonatal intensive care units (NICUs) of two largest pediatric hospitals in Tbilisi, Georgia.

Material and methods. A cross-sectional study was conducted among newborns and infants up to the age of eight weeks with severe acute illness who were admitted to the neonatal intensive care units (NICUs) of two largest pediatric hospitals in Tbilisi. 187 consecutive patients who met the inclusion criteria: (1) were eight weeks of age or younger (age group served by NICUs) and (2) had clinical manifestations of generalized infection of unknown origin (at the moment of investigation) were enrolled in the study.

Blood Samples and Laboratory Tests

Children suspected of having viral meningitis/encephalitis by CSF analysis were included in the study. Among participating individuals about 5 ml of venous blood was drawn. Where available, cerebrospinal fluid (CSF) was analyzed as well along with the blood samples. Cold boxes were utilized for transportation of the samples. The sample was further divided to three portions for testing by ELISA, PCR and RT-PCR.

Routine blood testing including biochemical analysis was done for all infants treated in NICUs. For detection of enteroviruses consensus PCR approach was used, which utilize primers corresponding to the 5' non-coding, conserved region of the enterovirus genome. Samples were screened for the presence of any serotype of enterovirus without their identification. PCR with two consensus primer pairs,

followed by restriction enzyme analysis with BamHI and BstUI was used for diagnosis of herpesviruses in infants. This method was validated for amplification and identification of all eight human herpesviruses.

Data were managed and statistically analyzed using IBM SPSS (version 20.0). Descriptive statistics was used for characterizing socio-demographic and clinical data. Prevalence ratios and 95% confidence intervals were computed to estimate the association of prevalence of viral infections with different factors. Statistical analyses by Poisson regression were utilized to identify factors predictive of neonatal/infant mortality. The specific factors included in the model were refined once univariate and bivariate analyses were completed.

Results and their discussion. During the one year study period 187 infants with clinical manifestations of generalized infection of unknown origin were admitted. Among them 98 (52.4%) were males and 89 (47.6%) females. Age at admission to the NICU was <7 days (early onset) among 51.3% of infants. 74.9% of admitted patients had the normal birth weight (2500 grams or more). For 66.3% of study participants Apgar score was normal (7 or above) and more than half (65.2%) were delivered by vaginal delivery.

The following distribution of enteroviruses and herpesviruses among participating infants with meningitis/encephalitis was observed: 66 (35.3%) had enterovirus infection and herpesvirus was detected in 68 (36.4%) cases. Among 53 (28.3%) patients the virus was not identified. 171 (91.4%) infants were treated with board - spectrum antibiotics, while only among 33 (17.6%) antiviral therapy was used.

Overall mortality rate of infants from the study group was 21.9 % (41/187). Neonatal outcome was more favorable when the infection was due to enteroviruses (2.9 % among those where enteroviruses were detected) compared to a herpesvirus infection (16.1 % among those where one of the herpesviruses has been detected) ($p<0.001$).

Bivariate analyses shown that for women 19 to 34 years living in an urban area whose baby were delivered by vaginal delivery and had premature birth there is a significant risk of mortality ($p=0.02$) for her baby treated in NICU for sepsis. Other factors such as education level, first child and umbilical discharge were not associated to the neonatal mortality.

In multivariate analyses babies' age <7 days (RR =2.04, 95% CI 1.13-3.69, $p=0.013$) and APGAR score ≤6 (RR=2.07, 95% CI 1.21 - 3.52, $p= .007$) were significantly associated with neonatal mortality.

Neonatal mortality was associated with the higher risk of neonatal infections. The results of this study suggest that death of an infant with generalized infection was conditioned by several risk factors. Namely, members of the Herpesviridae are responsible for the substantial portion of the death cases. Enteroviral infections are common in neonatal period, are associated with the sepsis-like illness, but are not fatal in most of cases. These results are similar to those found in other studies from developed countries. According to US National Enterovirus Surveillance System (NESS), fatal outcome due to viral infections was observed in 3.3% case, among them high rates of death reported in neonates (11.5%) [3]. The Netherland study found that viral infection was confirmed only in 1% of infants admitted to the neonatal intensive care unit. Mortality rate was 10% [10].

Our study found that clinical manifestation of HSV infection occurred in approximately 36% of cases. Mortality rate of this infant was 16.1 %. The actual absence of comprehensive data on the incidence of HSV infection among newborn in Georgia and the role of viral pathogens in sepsis-like illness in infants complicates the differentiation of the data with other studies.

In the current study, the mortality rate for patients with generalized infections of unknown origin was substantial (21.9 %), which made prognosis of the antiretroviral treatment and outcome poor enough. Further work is needed to improve the antiviral therapy in neonates in Georgia.
The primary limitation of our study was the small sample size, which complicated to find the statistically significant association between different subgroups. An additional limitation was the referral system, namely the infants were referred to NICU only from maternity houses and pediatricians' offices. Neonates/infants treated at home by family doctors could be missing in our study. Besides, study results would benefit from additional clinical findings such as mothers' viral test results but generally pregnant women are not screened for studied viral infections. Finally, the accurate outcome data of the infants discharged from the hospital would be also valuable information for our study but was limited.

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SUMMARY

ROLE OF VIRAL PATHOGENS IN INFANTS WITH SYSTEMIC INFECTION AT THE NEWBORN INTENSIVE CARE UNITS IN GEORGIA

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In Georgia, causative agents among infants with systemic infections are generally not identified and "neonatal sepsis" is usually diagnosed and treated without determining the etiology. The objective of this study was to estimate the role of viral pathogens (Herpesviridae and Enteroviruses) among neonates with generalized infections. A cross-sectional study was performed among neonates younger than <8 weeks admitted to a neonatal intensive care unit (NICU) at the two largest pediatric hospitals in Tbilisi, Georgia. Laboratory tests were performed by consensus and then by type-specific PCR methods. A total of 187 infants were recruited from the NICUs; most participants (74.9%) were of normal birth weight at admission to the NICU and half (51.3%) were younger than 7 days of age. Almost all babies (91.4%) were treated with a broad-spectrum antibiotic despite a lack of microbe identification. While the overall mortality rate of infants with a systemic infection was 21.9 %, neonatal outcomes were more favorable when the infection was due to enteroviruses (2.9% mortality rate) compared to a herpesvirus infection (16.1% mortality rate). Multivariate analyses identified independent predictors associated with neonatal mortality. These included etiology of infection, APGAR score and the type of delivery. Our investigation suggests that viral pathogens play a substantial role in systemic infections among NICU infants. Utilizing molecular-based testing in these cases could improve both the clinical management and outcomes of neonates with generalized infections.

Keywords: neonatal mortality, generalized infection, molecular diagnostics.

РЕЗЮМЕ

РОЛЬ ВИРУСНЫХ ПАТОГЕНОВ В РАЗВИТИИ СИСТЕМНЫХ ИНФЕКЦИЙ У ГРУДНЫХ ДЕТЕЙ В ОТДЕЛЕНИЯХ ИНТЕНСИВНОЙ ТЕРАПИИ ДЛЯ НОВОРОЖДЕННЫХ В ГРУЗИИ

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При проведении обследований грудных детей с системными инфекционными заболеваниями в медучреждениях Грузии часто не устанавливается возбудитель болезни, диагностируется "неонатальный сепсис" и лечение осуществляется без знания этиологии болезни.

Целью проведенного исследования явился анализ роли вирусных патогенов (вирусы группы герпеса и энтеровирусы) в развитии системных инфекций у грудных детей. Исследование проводилось среди новорожденных детей и младенцев в возрасте до 8 недель, которые были госпитализированы в отделениях интенсивной терапии двух ведущих педиатрических клиник г. Тбилиси. Диагностирование вирусов группы герпеса и энтеровирусов осуществляли на основе достаточно быстрого и экономически эффективного метода полимеразной цепной реакции (ПЦР). Обследование прошли 187 новорожденных детей и младенцев с генерализованной инфекцией неизвестной этиологии. Большинство детей (74,9%) были с нормальным весом при рождении (больше 2500 грамм), возраст 51,3% детей составил менее 7 дней. При лечении практически всех обследуемых детей (91,4%) назначались антибио-

тики широкого спектра действия, причем этиология болезни была неизвестна. Общая смертность грудных детей с системными инфекционными заболеваниями составила 21,9%, с вирусами группы герпеса - 16,1% и была намного выше, чем смертность детей, зараженных энтеровирусами (2,9%). Мультивариабельный анализ определил факторы, повышающие вероятность смертности среди грудных детей: этиология инфекции, состояние новорожденного по шкале Апгар и вид родов. Проведенное исследование позволило установить значимую роль вирусных патогенов в развитии генерализованных инфекций у грудных детей. Подобного рода обследования, проводимые с использованием молекулярной диагностики, позволят усовершенствовать методы лечения и повысить вероятность благополучного исхода у грудных детей с системными инфекциями.

რეზიუმე

ვირუსული პათოგენების როლი სისტემური ინფექციებით ახალშობილებში საქართველოს ჩვილ ბავშვთა ინტენსიური თერაპიის დაწესებულებებში

მ. კოჭლამაზაშვილი,ხ. ხატიაშვილი,მ. ბუწაშვილი, ო. ჩუბინიშვილი, შ. ხეცურიანი, გ. კამკამიძე

ჯანმრთელობის კვლევის კავშირი, თბილისი, საქართველო

საქართველოში გენერალიზებული ინფექციებით ახალშობილებში ვირუსული ინფექციის აგენტის დადგენა ხშირ შემთხვევაში ვერ ხერხდება და “ნეონატალური სეფსისის” დიაგნოზის დასმა და ანტიმიკრობული მკურნალობა ტარდება ეტიოლოგური ფაქტორის ცოდნის გარეშე.

კვლევის მიზანს წარმოადგენდა,ვირუსული პათოგენების,კერძოდ,ჰერპესის ჯგუფის ვირუსების და ენტეროვირუსების როლის დადგენა გენერალიზებული ინფექციებით ახალშობილებში. კვლევა ჩატარდა ჯვარედინ-სექციური მეთოდით. საკვლევ კონტიგენტს შეადგენდა საქართველოში გენერალიზებული ინფექციის სიმპტომებით 8 კვირაზე ნაკლები ასაკის ახალშობილები,რომლებიც ჰოსპიტალიზებული იყო თბილისის ორი ცენტრალური პედიატრიული კლინიკების ახალშობილთა ინტენსიური თერაპიის განყოფილებებში.ჰერპესის ჯგუფის ვირუსებისა და ენტეროვირუსების ლაბორატრიული დეტექციისთვის გამოყენებული იყო პოლიმერიზაციის ჯაჭვურ რეაქციაზე (პჯრ) დაფუძნებული მეთოდი. გამოკვლეულია 187 უცნობი ეტიოლოგიის გენერალიზებული ინფექციით ახალშობილი. სტაციონარში შემოსულ ბავშვთა შორის უმრავლესობა (74,9%) დაიბადა 2500 გრამზე მეტი წონით, 51,3% გამოკვლევის მომენტისათვის იყო 7 დღეზე ნაკლები ასაკის. ახალშობილთა უმრავლესობას (91,4%) მკურნალობა ჩაუტარდა ფართო სპექტრის ანტიბიოტიკებით, დაავადების ეტიოლოგიის ცოდნის გარეშე. გენერალიზებული ინფექციების მქონე ახალშობილების საერთო სიკვდილობა შეადგენდა 21,9%. მათგან,ჰერპესვირუსებით გამოწვეული ახალშობილთა სიკვდილობა (16,1%) გაცილებით უფრო მაღალი იყო ენტეროვირუსებით გამოწვეულ სიკვდილობასთან შედარებით, რომელიც შეადგენდა 2.9%, მულტივარიაციული ანალიზის შედეგად გამოვლინდა ნეონატალურ სიკვდილობასთან დაკავშირებული დამოუკიდებელი პრედიქტორული რიკს-ფაქტორები, კერძოდ, ინფექციის გამომწვევი ვირუსის სახეობა, აპგარის შკალის მაჩვენებელი და მშობიარობის ტიპი. კვლევის შედეგად დადგინდა, რომ სისტემური ინფექციებით ახალშობილებში ვირუსული პათოგენების როლი მნიშვნელოვანია. მოლეკულურ დიაგნოსტირებაზე დაფუძნებული ტესტირება საშუალებას იძლევა გენერალიზებული ინფექციებით ახალშობილებში გაიზარდოს ეტიოლოგიური ფაქტორის გამოვლენის ეფექტურობა და, შესაბამისად, გააუმჯობესდეს კლინიკური გამოსავალიც.

BACTERIOLOGICAL EXAMINATION OF THE ABDOMINAL EFFUSION IN BACTERIAL PERITONITIS

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The problem of fighting against surgical infection remains unsolved. Purulent-inflammatory processes of abdominal region are distinguished with extreme complexity. Despite using modern methods of intensive care measures, frequent non-satisfactory results of using existing methods in treatment of this disease, high frequency of purulent complications at post-operational period and still high mortality, requires more effective adequate antibiotic therapy together with improvement of operational methods of treatment [1,3].

It is to be admitted, that the spectrum of microorganisms causing peritonitis has been changed during the recent years. In most cases gram-negative bacteria are prevailing, antibiotic resistant strains have increased which worsens the treatment outcome [4-6,7].

The aim of the study was the bacteriological examination of aspirates taken during surgery from the abdominal cavity of patients with bacterial peritonitis, identification of microbes causing peritonitis and studying their sensitivity to antibiotics for the purpose of optimization of antibiotic therapy.

Material and methods. Bacteriological investigations were including isolation of poor culture, identifying microbes with rapid identification system (API20E, API Staph, API Strep, API Ana, bioMerieux). Rapid tests for identification of oxidase and catalase were also used.

Sensitivity of microorganisms to antibiotics was defined with disc-diffusion method using standard discs (EUCAST guidelines 2015) according to the standard of Clinical Laboratory Standard Institute (CLSI) (ATB strips: ATB G, ATB Staph, ATBANA, ATBPse, ATB-Strep. bioMerieux).

The study has involved 45 patients with bacterial peritonitis. 21 were women and 24 were men. Age varied between 18 and 75. In 26 cases the cause of peritonitis was acute destructive appendicitis, in 9 cases - acute destructive cholecystitis, and in 10 cases - perforation of ulcer. Material for bacteriological investigations was the aspirate taken from pathological foci (10-20 ml).

Results and their discussion. In 36 patients microbial growth **(**positive culture) has been documented by bacteriology, while negative culture was observed in 9 cases. In patients with negative culture, leukocyte count was in average 4-5 in visible area, epithelium cells 4-6 in visible area. In patients with positive culture, average number of leukocytes comprised 40-50, epithelium cells 8-10 in visible area.

Among 36 patients who showed presence of monomicrobial growth by bacteriological investigations, gram-negative bacteria prevailed, namely Escherichia coli was recovered in 14 patients and Enterobacter cloacae in 9 patients. Among gram-positive bacteria D-group Streptococcus were prevailing, Enterococcus faecalis was found in 6 patients, Staphylococcus aureus in 3 patients, Candida albicans - in 2 patients. As for anaerobes, in 1 case growth of gram-negative anaerobes Bacteroides fragilis and Fusobacterium spp were observed in one patient. Polymicrobial growth was shown in 3 cases. This was represented by combinations of Candida albicans and Escherichia coli, Enterobacter cloacae and Candida albicans, Escherichia coli and Bacteroides fragilis.

Despite the small sample size of the study, which does not allow us to generalize our results, it is important to mention, that the recovery rate from the clinical samples was pretty high, which by our opinion is related to immediate inoculation of study material into the thioglycollate broth which works both for aerobe and anaerobe bacteria.

Among 36 patients who showed presence of monomicrobial growth by bacteriological investigations, gram-negative bacteria prevailed, namely Escherichia coli was recovered in 14 patients and Enterobacter cloacae in 9 patients. Among gram-positive bacteria D-group Streptococcus were prevailing, Enterococcus faecalis was found in 6 patients, Staphylococcus aureus in 3 patients, Candida albicans - in 2 patients. As for anaerobes, in 1 case growth of gram-negative anaerobes Bacteroides fragilis and Fusobacterium spp were observed in one patient. Polymicrobial growth was shown in 3 cases. This was represented by combinations of Candida albicans and Escherichia coli, Enterobacter cloacae and Candida albicans, Escherichia coli and Bacteroides fragilis.

Antibiotics susceptibility testing showed that 12% of gram-negative bacteria was resistant to quinolones, 19% - to the third generation cephalosporins, while among gram-positive bacteria methicillin-resistant Staphylococcus aureus was not found. Relatively high resistance rate against cephalosporins and quinolones is mostly related with the wide ue of antibiotics of these groups for the prophylaxis and treatment of bacterial peritonitis [2].

In conclusion, timely identification of microbial spectrum causing bacterial peritonitis and determination of sensitiv-

ity and resistance to antibiotics are important contributing factors towards improvement of efficiency of treatment schemes for bacterial peritonitis.

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SUMMARY

BACTERIOLOGICAL EXAMINATION OF THE ABDOMINAL EFFUSION IN BACTERIAL PERITONITIS

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This study provides a contemporary epidemiology of aspirates taken during surgery from the abdominal cavity among patients with bacterial peritonitis to identify the isolates and study their sensitivity to antibiotics. Our bacteriology investigations included isolation of poor cultures, and detection of microbes was conducted using a rapid identification system (API20E, API Staph, API Strep, API Ana, BioMerieux). Rapid tests for detection of oxidase and catalase activity were also used. Susceptibility of microorganisms to antibiotics was defined by the disc-diffusion method using standard discs (EUCAST guidelines 2015) according to Clinical Laboratory Standard Institute (CLSI) protocols (ATB strips: ATB G, ATB Staph, ATBANA, ATBPse, ATBStrep. BioMerieux). The recovery rate from the clinical samples was good, likely because our protocol immediately inoculated study material into the thioglycollate broth which is an appropriate medium both for aerobic and anaerobic bacteria. Among the 36 patients with monomicrobial growth by bacteriological investigation, Gram-negative bacteria prevailed; *Escherichia coli* was recovered in 14 patients and *Enterobacter cloacae* in 9 patients. Among the Gram-positive bacteria, D-group Streptococci were prevalent, *Enterococcus faecalis* was found in six patients, *Staphylococcus aureus* in three patients, *Candida albicans* in two patients. In one patient, we observed dual colonization of two Gram-negative anaerobes *Bacteroides fragilis* and *Fusobacterium spp*. Polymicrobial growth was evident in three cases in the following combinations: *Candida albicans* and *Escherichia coli*, *Enterobacter cloacae* and *Candida albicans*, *Escherichia coli* and *Bacteroides fragilis*. Antibiotic susceptibility testing indicated that 12% of Gram-negative bacteria were resistant to quinolones and 19% to third-generation cephalosporins. No evidence of methicillin-resistant *Staphylococcus aureus* was found in Gram-positive specimens. The timely identification of microbes and administration of appropriate therapy based on antibiotic sensitivity profiles is important to optimizing clinical outcomes in bacterial peritonitis.

Keywords: aspirates, peritonitis, antibiotic treatment, resistance.

РЕЗЮМЕ

БАКТЕРИОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ ВЫДЕЛЕНИЙ БРЮШНОЙ ПОЛОСТИ ПРИ БАКТЕРИАЛЬНОМ ПЕРИТОНИТЕ

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За последние годы изменился спектр микроорганизмов, вызывающих перитонит - в большинстве случаев превалируют грамотрицательные бактерии, количество устойчивых к антибиотикам штаммов возросло, что значительно ухудшает результаты лечения перитонита.

Целью данного исследования явилось бактериологическое исследование аспирата, полученного интраоперационным путем из патологических очагов больных бактериальным перитонитом, а также идентификация микробов, вызывающих перитонит, изучение их чувствительности к антибиотикам для процесса оптимизации антибиотикотерапии.

Бактериологическое исследование включало: выделение чистой культуры, определение микроба с помощью

системы быстрой идентификации (API20E, API Staph, API Strep, API Ana, производитель: bioMérieux, США). Использованы также быстрые вспомогательные тесты на оксидазу и каталазу. Чувствительность микроорганизмов к антибиотикам установлена диско-диффузионным методом с применением стандартных дисков (EUCAST guidelines 2015) и в соответствии со стандартами Института стандарта клинических лабораторий - CLSI, ATB strips: ATB G, ATB Staph, ATBANA, ATBPse, ATBStrep, bioMerieux. Результаты исследования выявили достаточно высокий показатель частоты бактериологического высева при наличии инфекционного процеса, что связано с незамедлительным помещением материала в обогатительный бульон (тиогликолевый бульон для аэробных и анаэробных бактерий). Определение антибиотикочувствительности выделенных штаммов показало, что только 12% грамотрицательных бактерий были резистентными к хинолонам, 19% - к цефалоспоринам III поколения. Среди грамположительных бактерий метициллин-резистентный золотистый стафилокок не определился. Сравнительно высокая резистентность к цефалоспорину и хинолонам, по всей вероятности, связана с широким использованием антибиотиков данных групп для профилактики бактериального перитонита. Таким образом, результаты проведенного исследования позволяют сделать вывод о микробном спектре, вызывающем бактериальный перитонит и о чувствительности/резистентности этих микробов. Полученные данные послужат повышению эффективности лечения бактериального перитонита.

რეზიუმე

მუცლის ღრუს გამონადენის ბაქტერიოლოგიური კვლევა ბაქტერიული პერიტონიტის დროს

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თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, პირველი საუნივერსიტეტო კლინიკა, საქართველო

კვლევის მიზანს წარმოადგენდა ბაქტერიული პერიტონიტით პაციენტებში პათოლოგიური კერიდან ინტრაოპერაციულად აღებული ასპირატის ბაქტერიოლოგიური კვლევა, პერიტონიტის გამომწვევ მიკრობთა იდენტიფიკაცია და მათი ანტიბიოტიკომგრძნობელობის შესწავლა ანტიბიოტიკოთერაპიის ოპტიმიზაციისათვის.

ბაქტერიოლოგიური კვლევა მოიცავდა სუფთა კულტურის გამოყოფას, გამოყოფილი მიკრობის იდენტიფიკაციას სწრაფი საიდენტიფიკაციო სისტემით (API20E, API Staph, API Strep, API Ana, bioMérieux, აშშ) და ოქსიდაზას და კატალაზას განმსაზღვრელი სწრაფი დამხმარე ტესტებით. მიკროორგანიზმთა ანტიბიოტიკომგრძნობელობა განისაზღვრა დისკო-დიფუზური მეთოდით, სტანდარტული დისკების გამოყენებით (EUCAST guidelines 2015) და კლინიკური ლაბორატრიების სტანდარტის ინსტიტუტის სტანდარტების - ATB strips:ATB G, ATB Staph, ATBANA, ATBPse, ATBStrep, bioMerieu გათვალისწინებით. კვლევის პროცესში გამოვლინდა,რომ კლინიკურად ინფექციური პროცესის არსებობის პირობებში ბაქტერიების ზრდის გამოვლენის სიხშირემ იმატა, რაც უკავშირდება მასალის დაუყოვნებელ შეტანას გამამდიდრებელ ბულიონში (თიოგლიკოლის ბულიონი აერობული და ანაერობული ბაქტერიებისთვის). კვლევის პროცესში გამოყოფილ შტამებზე ლოკალური ანტიბიოტიკომგრძნობელობის განსაზღვრით გამოვლინდა, რომ გრამუარყოფითი ბაქტერიების მხოლოდ 12% იყო რეზისტენტული ქინოლონებზე, 19% - ცეფალოსპორინების III თაობის მიმართ, გრამდადებით ბაქტერიებს შორის მეტიცილინრეზისტენტული ოქროსფერი სტაფილოკოკი არ აღმოჩნდა. ცეფალოსპორინების და ქინოლონების მიმართ შედარებით მაღალი რეზისტენტობა უკავშირდება ბაქტერიული პერიტონიტის საპროფილაქტიკოდ ამ ჯგუფის ანტიბიოტიკების ფართო გამოყენებას. ამრიგად, კვლევის შედეგად მიღებული მონაცემები ხელს შეუწყობს ეფექტური ანტიბაქტერიული მკურნალობის სქემების შემუშავებას.

DETECTION OF CTX-M GENE ANTIBIOTICS RESISTANCE IN *KLEBSIELLA PNEUMONIA* ISOLATES OF HOSPITALS IN ADJARA (GEORGIA)

[1]Koiava T., [2,3]Gonçalves D., [2]Palmeira J., [4]Arobelidze K., [5]Tediashvili M., [1]Akhvlediani L., [2]Ferreira H.

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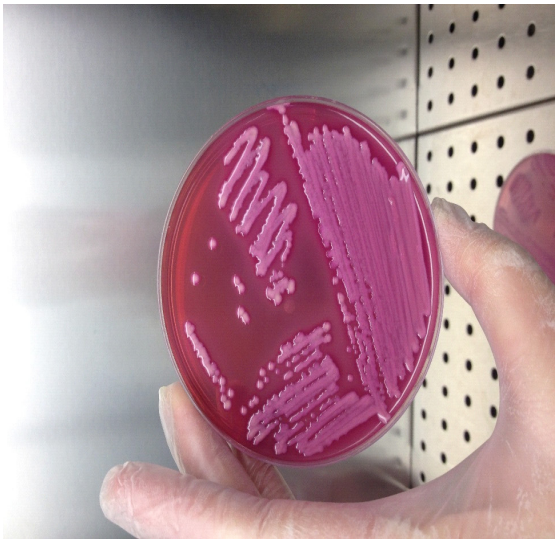
Extended-spectrum β-lactamases (ESBLs) represent a major threat among multidrug-resistant bacterial isolates. The production of ESBLs in *Enterobacteriaceae* confers resistance to all penicillins and cephalosporins (with the exception of cephamycins, in some cases), with the organisms generally remaining susceptible only to β-lactam–β-lactamase inhibitor combinations, such as amoxycillin–clavulanate, and the carbapenems, which are frequently the only therapeutic options available for treatment of hospital-acquired severe infections caused by these microorganisms [5]. CTX-M enzymes are a group of class A extended-spectrum β-lactamases (ESBLs) that are rapidly spreading among *Enterobacteriaceae* worldwide [6]. These CTX-M variants have been divided into 5 major phylogenetic groups, CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, or CTX-M-25 on the basis of their amino acid sequences [1,6].

Material and methods. Susceptibility profile and identification of the infection *Klebsiella pneumonia* (n=23) isolates collected in different hospital services (2013-2015) were performed by disc diffusion methods according to the CLSI guidelines and API 20E, E-Test, CIM-Carbapenem inactivation method, Blu Carba-Carba test (BCT), respectively. ESBL producers were detected and/or confirmed by the double disk synergy test use oximino-β-lactamic antibiotics with and without clavulanic acid. Genes of families *bla*TEM, *bla*OXA, blaSHV, CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, or CTX-M-25 (Table) were investigated by PCR. Sequencing was performed using group-specific primers for CTX-M group 1.

Identification of Klebsiella pneumonia.
Isolates The bacterial isolates were identified according to the cultural and biochemical properties. *Klebsiellae pneumonia* isolates were distinguished post growth on solid medium. It produces large, smooth, with pink color (lactose fermented), elevated and mucoid colony on Mac-Conkey. Picture 1. Furthermore, the biochemical tests were performed for the identification of *Klebsiellae pneumonia* isolates from other isolates [3].

Table. Primers used for the detection of beta-lactamases frequently encountered among Gram negative pathogens

Gene		Primers (5'- 3')	Reference	
		5′-GACCCCAAGTTTCCTGTAAGTG-3′		
***bla*$_{CTX-M-GRU-PO1}$**	multiplex	5′- AAAAATCACTGCGCCAGTTC-3′	[10]	Initial denaturation at 94єC5min; denaturation at 94єC-25s, annealing at 52єC-40s elongation at 72єC-50s, repeated for 30 cycles; Final extension at 72єC-6 minutes
		5 ′- AGCTTATTCATCGCCACGTT-3 ′		
***bla*$_{CTX-M-GRU-PO2}$**		5 ′- CGACGCTACCCCTGCTATT-3′	[11]	
		5 ′- CCAGCGTCAGATTTTTCAGG-3′		
***bla*$_{CTX-M-GRU-PO8}$**		5 ′- TCGCGTTAAGCGGATGATGC-3′	[12]	
		5′- AACCCACGATGTGGGTAGC-3′		
***bla*$_{CTX-M-GRU-PO9}$**		5 ′- CAAAGAGAGTGCAACGGATG - 3′	[13]	
		5′- ATTGGAAAGCGTTCATCACC-3′		
***bla*$_{CTX-M-GRU-PO25}$**		5′- GCACGATGACATTCGGG-3′	[14]	
		5′- AACCCACGATGTGGGTAGC-3′		

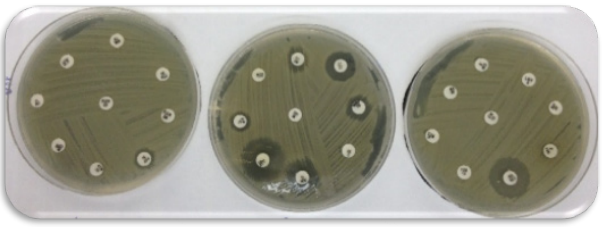


Pic. 1. Klebsiella pneumonia on the MacConkey agar

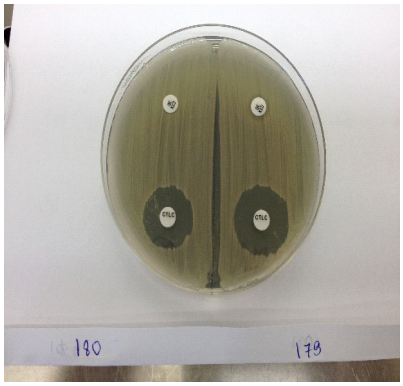
Antimicrobial Susceptibility Test. Disk diffusion method was performed to test the susceptibility of *Klebsiella pneumonia* isolates to common antibiotics on Mueller-Hinton agar, with an inoculum equal to 0.5 McFarland turbidity according to CLSI. The plates were incubated at 37°C for 18-24 hrs and the inhibition zone diameters around the antibiotic discs were measured. There were samples of sputum, urine and samples of biological fluids. All isolates were examined for the antibiotic resistance of the following antibiotics: β-LACTÂMICOS-AML Amoxicilina, CTX Cefotaxima, FOX Cefoxitina, FEP Cefepime. MONOBACTAMO- ATM Aztreonamo, EFT Ceftiofur, CAZ Ceftazidima, CPT Ceftarolina; β-LACTÂMICOS + INIB. β-LACT- AUG Amox. + Ac. Clavulanico, ETP Ertapenemo, TE Tetraciclina, IMI Imipenemo, CT Colistina, MRP Meropenemo, C Cloranfenicol, DOR Doripenemo, TGC Tigeciclina, PIP/TAZ Piperacilin/Tazobactam, CIP Ciprofloxacino, LEV Levofloxacin, STX Sulfamet. + Trimetrop, F Nitrofurantoina, FOS Fosfomicina, TOB Tobramicin, NET Netilmicin, AK Amikacin, CN Gentamicina (Pic. 2, Fig. 1).

Detection of ESBL Producing Isolates

The modified double-disc synergy test (m-DDST) was used to detect the extended spectrum β-lactamase-producing isolates, aztreonam, ceftazidime, cefotaxime and ceftriaxone discs (30 mg) were placed around an amoxicillin-clavulanic acid disc (10 mg) at interdisc distances (centre to centre) of 20 mm on Muller-Hinton agar inoculated by bacterial suspension equal to 0.5 McFarland, a clear extension of the edge of the aztreonam, ceftazidime, cefotaxime and ceftriaxone discs inhibition zone towards the disc containing clavulanic acid was interpreted as positive for ESBL production (Pic. 3) [7].



Pic. 2. Antimicrobial Susceptibility Test Klebsiella pneumonia



Pic. 3. Detection of ESBL Producing Isolates Imipenem-EDTA synergy test

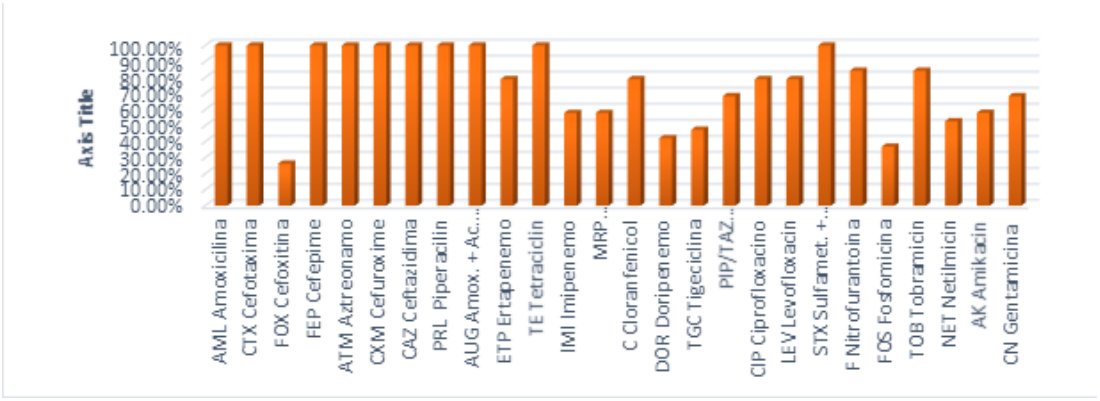


Fig.1. Antibiotic Resistance profile Klebsiella pneumoniae

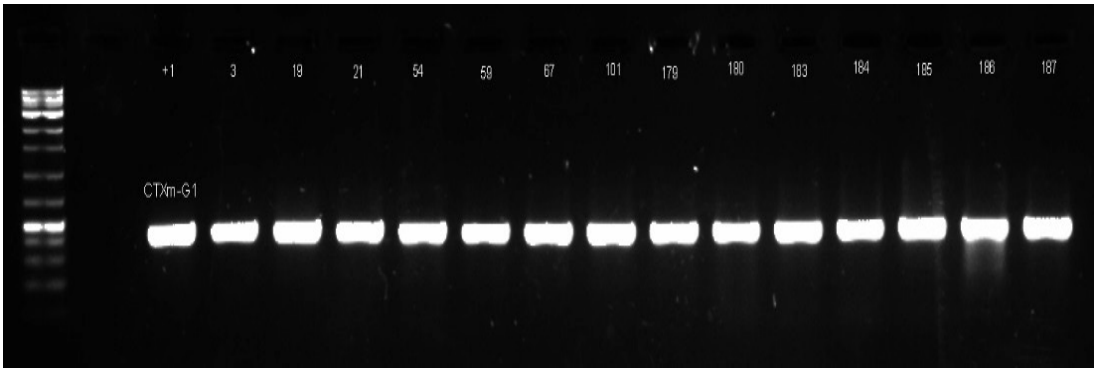


Fig. 2. PCR amplification of CTX-M gene of Klebsiella pneumonia isolates

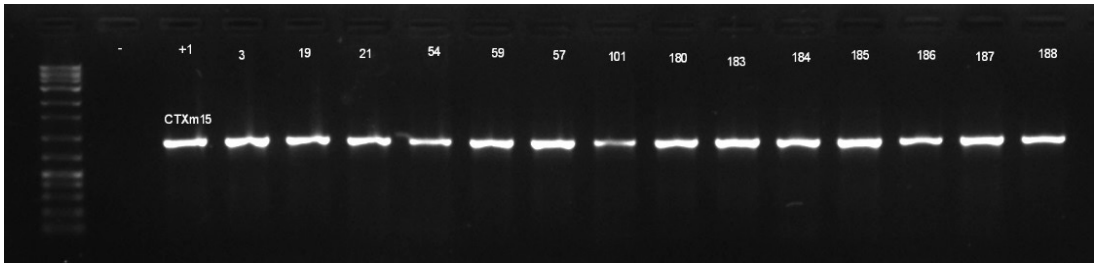
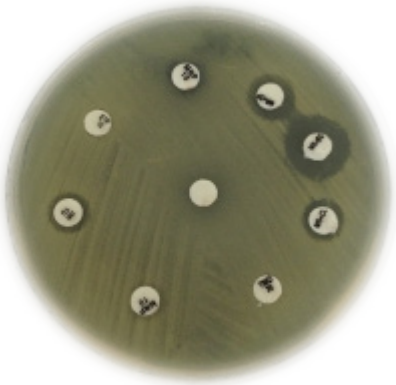


Fig. 3. PCR amplification of CTXm 15 genes of Klebsiella pneumonia isolates

EDTA (ethylene-diamine-tetraacetic acid) is a polyamino carboxylic acid that binds metal ions like zinc and can inactivate the metallo-beta-lactamases. Therefore, it is used for the phenotypic detection of MBL production in clinical isolates (Pic. 4) [4].



Pic. 4. EDTA synergy test-Klebsiella pneumonia

PCR Detection of β-lactamases

Polymerase chain reaction technique has been used to amplify genes encoding the CTX-M β-lactamases from genomic DNA of all *Klebsiella pneumonia*. isolates with specific forward and reverse primers; the lengths of amplified genes was 566 bp, The results of β- lactamase genes detection clarify that 14 isolates (100%) of ESBLs producers carrying CTX-M gene (Fig. 2).

Results and their discussion. Fourteen *Klebsiella pneumonia* producing CTX-M group 1 ESBL infection isolates were detected in different biological samples, namely in sputum (n=8), urine (n=5) and abdominal fluid (n=1), collected in different hospital services. The infection isolates showed an extended resistance profile to aminoglycosides, fluoroquinolones and tetracycline. CTX-M group 1 ESBL isolates showed specific amplification for $bla_{TEM,}$ bla_{OXA}, bla_{SHV}, aac(6′)-1b-cr and for the different variants of qnr, sul, tet families. This is the first report of CTX-M group 1 in infection *Klebsiella pneumonia* isolates in Adjara. This situation might represent the spread of these multidrug resistant Gram negative in acute care hospital in Adjara. Is urgent the implementation and/or reinforce of infection control measures, active antibiotic resistance surveillance and colonization screening of high risk patients in order to limit the dissemination of CTX-M ESBL producing *Klebsiella pneumonia* in health care institutions and to the community in this country. Colonization screening in elderly and/or dependent patients, upon admission at different health care institutions and their evaluation before discharge, are extreme importance to prevent the spread cycle of multidrug resistant *Klebsiella pneumonia* in the network of healthcare facilities and community in Georgia.

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SUMMARY

DETECTION OF CTX-M GENE ANTIBIOTICS RESISTANCE IN *KLEBSIELLA PNEUMONIA* ISOLATES OF HOSPITALS IN ADJARA (GEORGIA)

[1]Koiava T., [2,3]Gonçalves D., [2]Palmeira J., [4]Arobelidze K., [5]Tediashvili M., [1]Akhvlediani L., [2]Ferreira H.

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Research describing the epidemiology of antibiotic resistant microbes is vital to the proactive development of new antimicrobial agents. In the last years, CTX-M extended-spectrum β-lactamases (ESBLs) have emerged worldwide and have replaced classical TEM and SHV-type ESBLs in many countries. CTX-M-15 is currently the most frequent, with a pandemic distribution, and its rapid spread is facilitated by incorporation of resistance genes in mobile genetic elements. The ESBL is efficacious in Gram-negative bacteria and thus closely associated with nosocomial environments, often colonizing the intestines, particularly in older and dependent patients. Little is known about the CTX-M ESBLs among *Klebsiella pneumonia* in Adjara. Our paper describes the detected and characterized ESBLs among *Klebsiella pneumonia* isolates from patients in two different hospitals in Adjara.

Keywords: CTX-M-genes, Extended-spectrum β-lactamases ESBL, K. pneumonia.

РЕЗЮМЕ

ВЫЯВЛЕНИЕ ГЕНОВ АНТИБИОТИКОРЕЗИСТЕНТНОСТИ CTX-M В ИЗОЛЯНТАХ *KLEBSIELLA PNEUMONIA* В ГОСПИТАЛЯХ АДЖАРИИ (ГРУЗИЯ)

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В последние годы в мире появились CTX-M β-лактамазы широкого спектра (ESBL), которые заменили TEM и SHV-типы ESBL во многих странах. На сегодняшний день широко распространен CTX-M-15, который характеризуется пандемическим распространением. Быстрое распространение содействует объединению резистентных генов в мобильных генетических элементах. ESBL в грамм-отрицательных бактериях тесно ассоциируется с нозокомиальным окружением и производит колонизацию кишки, особенно у пожилых пациентов. В Аджарии исследований CTX-M генов ESBL в *Klebsiella pneumonia* по сей день не проводилось. В статье представлен материал по характеристике ESBL CTX-M-генов в изолянтах *Klebsiella pneumonia,* выявленных в двух госпиталях Аджарии.

Результаты проведенного исследования позволяют заключить, что выявление широкого спектра бета-лактамазного гена указывает на нерациональное использование антибиотиков.

რეზიუმე

Klebsiella pneumonia-ს ნიმუშებში ანტიბიოტიკორეზისტენტობის CTX-M გენის გამოვლენა აჭარის ჰოსპიტალებში (საქართველო)

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ბოლო წლებში მსოფლიოში გაჩნდა CTX-M ფართო სპექტრის β-ლაქტამაზები (ESBL), რომლებმაც ბევრ ქვეყანაში ჩაანაცვლეს TEM და SHV-ტიპის ESBL-ები. სადღეისოდ ფართოდ არის გავრცელებული CTX-M-15, რომელსაც ახასიათებს პანდემიური გავრცელება. სწრაფი გავრცელება ხელს უწყობს რეზისტენტული გენების გაერთიანებას მობილურ გენეტიკურ ელემენტებში. ESBL–ი გრამ-უარყოფით ბაქტერიებში მჭიდროდ ასოცირდება ნოზოკომურ გარემოცვასთან და ახდენს ნაწლავის კოლონიზებას, განსაკუთრებით, მოხუც პაციენტებში. აჭარაში დღემდე არ არის შესწავლილი CTX-M ESBL Klebsiella pneumonia–ში. სტატიაში დახასიათებულია აჭარის ორ სხვადასხვა ჰოსპიტალში Klebsiella pneumonia–ს იზოლანტებში აღმოჩენილი ESBL CTX-M- გენები..

კვლევის შედეგებიდან გამომდინარე შეგვიძლია დავასკვნათ,რომ ფართო სპექტრის ბეტა-ლაქტამაზური გენის გამოვლენა მიუთითებს ანტიბიოტიკების არარაციონალურ გამოყენებაზე.

SOME CLINICALLY IMPORTANT ERYTHROCYTE BLOOD GROUP ANTIGENS IN DONORS

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Erythrocytes, leucocytes, thrombocytes are the carriers of blood group antigens. For clinical medicine erythrocyte group antigens are very important in as much as they are they precondition blood compatibility and are the main reasons of post-transfusion complications. These antigens represent a genetically firmly-determined peculiarity. Erythrocyte group systems are sharply distinct features of immunogenetic polymorphism.

For today, 250 antigen determinants have been studied existing at erythrocyte membrane. Group glycoproteids determine trophical and regulatory functions of blood cells. They enter cell receptor composition and via their help hormones, vitamins, ferments and other biologically active albumens are transported into blood circulation. They are represented as the main structural elements of cellular membrane adhesion [7]. Human blood group antigens are glycoproteins and glycolipids expressed on the surface of red blood cells and a variety of human tissues, including the epithelium, sensory neurons, platelets and the vascular endothelium. Accumulating evidence indicate that ABO blood type is implicated in the development of a number

of human diseases, including cardiovascular and neoplastic disorders [4].

Blood group antigens determine adaptation of a human, as a biological species, to the surrounding environment. Frequency of blood group spreading is unequal for different races and ethnic groups and it is considered as a manifestation of gene-geographical adaptation in an ecosystem during the evolution process [5,6,9].

The clinical importance of blood group antigens is determined by their immunogenic nature – ability to form antibodies. The latter have ability to damage erythrocytes, leucocytes and thrombocytes. The mentioned antibodies cause post-transfusion complications, various types of reactions during transfusion of blood components, hemolytic diseases in new-born babies and neutropenia [1].

At present during blood transfusion, because of high immunogenetic nature, two (A, B) antigens of ABO system and 5 antigens of Rh system are taken into consideration. In persons with no such antigens, the theoretical risk of immunosensibilization is rather high. Unfortunately, screening is conducted throughout the region only according to three antigen composition. However, the above mentioned literary sources state that besides these three erythrocyte antigens, other dangerous or immunogenic antigens should also been considered during transfusion. The center of our interest is exactly this information. The investigation of the presented problem is vital for the newborn with heavy anamnesis (hemolysis diseases of newborn and Premature) when it is rather difficult to select the relevant donor in case of the need of transfusions.

The goal of the research is to study regional immunogenic features of official donors. We will study the frequency of spreading of A, B, C, c, D, E, e, K, k, M, N antigens in donor populations.

Material and methods. The blood of 656 donors has been investigated on erythrocyte blood group antigens. The sample has been provided from diagnostic laboratory of Medina Ltd Health Centre of Batumi. Lab analysis of the sample has been carried out on the basis of immunogenetics laboratory of Batumi Shota Rustaveli State University. In order to reveal the specific antigens of erythrocyte group plasma as well as the erythrocyte mass have been applied.

While carrying out the research the following internationally acclaimed immuno-serological methods was used:
- direct and cross-sectional reaction of ABO system determination;
- while determining rhesus system antigens:
a) express-method using universal reagents;
b) express method with the complete shape antibodies on the plane-table.

In order to reveal MN, Kell system antigens the express method with universal mono-clone antibodies was used. The following specific test-systems was used during the research: anti –AB, -B, -A, -D, -C, -c, -E, -e, -K, -k, -M, -N, standard O(I), (II), (III) group erythrocytes and standard O(I), A(II), B(III), AB(IV) serums.

For identification erythrocytes blood group antigens Also was used modern ID cards. We used different specify cards such as: ABO/D + Reverse Grouping, ID-System for Typing of Partial RhD (The ID-Partial RhD-Typing kit consists of 6 monoclonal anti-D which can aid in the further characterization of the RhD antigen after routine RhD typing), ID-System for Rh Phenotyping, Rh-Subgroups + Cw + K, ID-System for Blood Groups ABO and RhD with Double Determination of RhD (The first anti-D detects the presence of the DVI variant, the second anti-D is negative for the DVI variant. Both antibodies are monoclonal).

The obtained material will be studied and processed statistically.

Result and their discussion. Investigation of the donors on ABO system erythrocyte group markers revealed the following results: 50,2±1,95% of donors have O(I) blood group; 37,3±1,88% of donors -A(II) blood group. 9,5±1,14%of donors belong to - B(III) phenotypic group; 3,0±0,68% of the investigated population belong to AB(IV) blood group (Fig. 1).

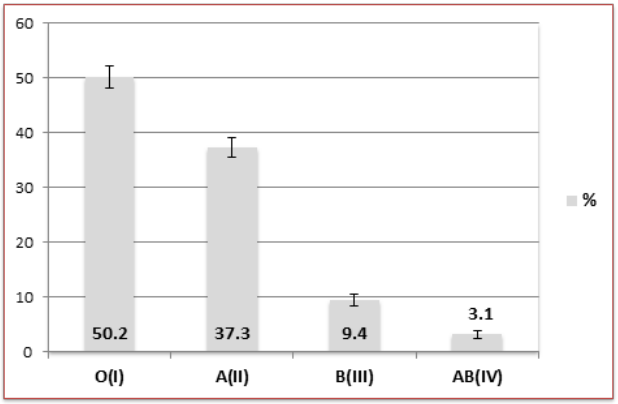

Fig. 1. Distribution of ABO blood group antigens in the donors

As it is known any population possesses specific genofond and genetic structure. The Unity of genotypes of individuals comprising population creates genofond of the relevant population. Thus, genofond is the unity of genes of population, while genetic structure of the population is the ratio of allele and genotypes in the population. We got curious about the genetic structure of the mentioned population according to each investigated characteristic.

The most important concepts in the population genetics are ratio of genotypes and frequency of allele. This concept considers the relative share of concrete genotypes and allele

in comparison with total amount of genotypes. Frequency of allele or genotypes is expressed in per cents or shares (actually, the amount of genotypes or allele is 100%, in other words their share equals to 1).

The frequency of allele of genes of erythrocytic ABO group system has been calculated with the application of the formula proposed by F. Bernstein and as it was mentioned above it is applied while investigation of three allele genetic system. The frequency of O, A and B genes in the given case was expressed with letters r, p and q. The results of the investigation of the frequency of allele of ABO system in donors revealed that r is the high frequency of allele spread and it equals to 0,70. Frequency of q allele appeared to be 0,23 whereas p allele was recorded as the allele with lowest frequency equaling to 0, 07 (Fig. 2).

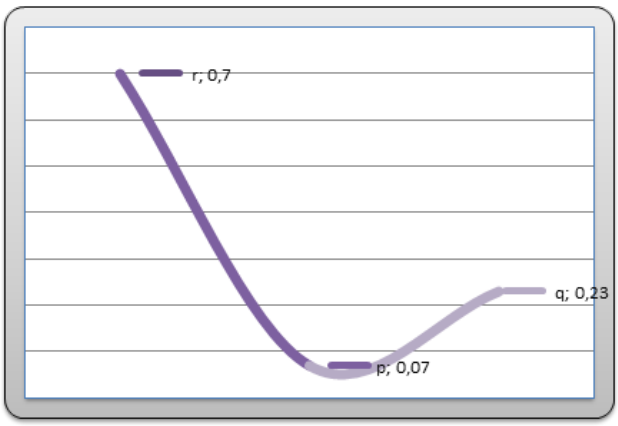

Fig. 2. The frequency of allele of genes of erythrocyte ABO group system

The results of investigation of phenotypes of rhesus system in donor populations displayed the following characteristics: 16,3±1,43% of investigated donors bear Rh(-) phenotypes; relevantly Rh(+) phenotype is found in 83,7±1,43% of donors (Fig. 3).

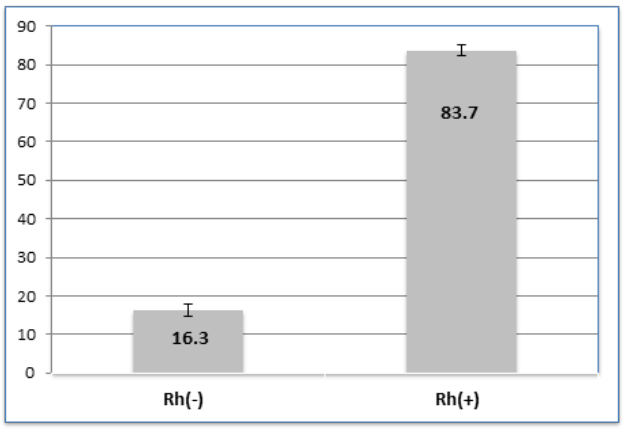

Fig.3. Distribution of Rh positive and negative blood group in the donors

It is possible to investigate some genotypes of RH system through application of Immunoserological method and monoclonal antibodies. High frequency of spread of ee genotypes can be observed in 68,4±1,81% of donors. The number of bearers of Cc genotypes is 47,9±1,94%. 33,4±1,84% is the frequency of cc genotype bearers. Spread frequency of Ee genotypes equals to 26,4±1,72%. CC genotype is observed in 18,7±1,51%; the lowest indicator with 5,2±0,86% was recorded in EE genotype (Fig. 4).

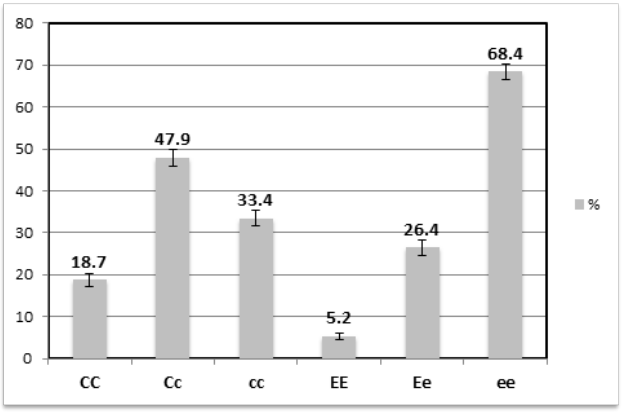

Fig. 4. Genotypes of Rh system

Application of biostatistics methods revealed e allele out of Rh system allele as the one with high level of frequency that equals to 0, 78; the frequency of C allele is also quite high and equals to 0,57. Among the donors investigated, C allele is presented with 0.43 frequency while the frequency of E allele is 0,18. Investigation results displayed that the frequency of D allele equals to 0, 6 and the frequency of s allele -0, 4 (Fig. 5).

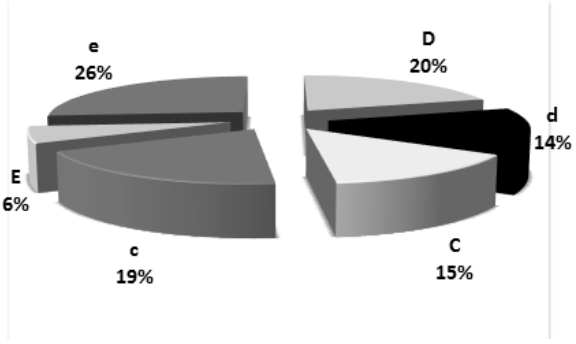

Fig. 5. Distribution of Rh system antigens in the donors

As it is well known, there are 18 phenotypic groups all over the world; They are: CDE; CDEe; Cde; CcDE; CcDEe; CcDe; Cde; cDEe; cDe; CD(-)E; CD(-)Ee; CD(-)e; CcD(-)E; CcD(-)Ee; CcD(-)Ee; CcD(-)E; Cd(-)Ee; Cd(-)e. The last eight phenotypes belong to rhesus negative group; The 18th phenotype complies with ccddee, which is the most spread Rh negative phenotype of the given phenotype. CcDee phenotype with its frequency that equals to 29,9±1,78% is frequently spread phenotype among phenotypic groups of rhesus system of the investigated donors. It is followed by CCD-ee - 17,2±1,47%, ccddee - 14,9±1,38% and CcD-Ee -

13,9±1,34%. ccD-Ee phenotype is the least spread phenotype with 11,1±1,22%; ccD-ee - 5,5±0,88%; same phenotype indicators -2,1±0,55 were observed for CcD-EE and ccD-EE; CCD-Ee phenotype frequency equals to 1,4±0,45%, CCD-EE phenotype frequency is 0,4±0,26% and frequency of Ccddee phenotype amounts 1,1±0,40%, ccddEe and CCddee phenotypes were recorded with the frequency of 0,2±0,17% (Fig. 6).

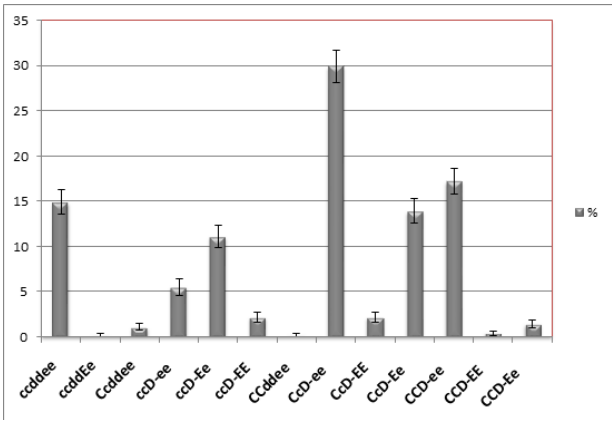

Fig. 6. Distribution of Rh phenotype in the donors

According to Rh system there are totally 8 genetic groups (Haplotype) CDE(Rz); Cde(R1); Cde(R2); cDe(R0); cde(r); Cde(R'); cdE(R''); CdE(Ry). Out of which some (Cde; Cde; cDe ϖ cde) are widely spread whereas the others are met rarely and some of them are observed hardly ever. Their frequency depends on probability of occurrence of chromosomal crossover.

In the donors to be investigated the frequency of Rh Haplotypes has been calculated with the formula proposed by A. E. Mourant (see statistics methods). Seven Haplotypes have been observed in donors (picture 20). The frequency of CDe Haplotype is 0,397; Haplotype of cde is 0,387 whereas Haplotype cDE is 0,137; Frequency of cDe is 0.064; frequency of Cde hich equals to 0,012, has been recorded; Frequency of CDE is 0,008; the lowest frequency was observed for the Haplotype cdE and it equals to 0,001 (Fig. 7).

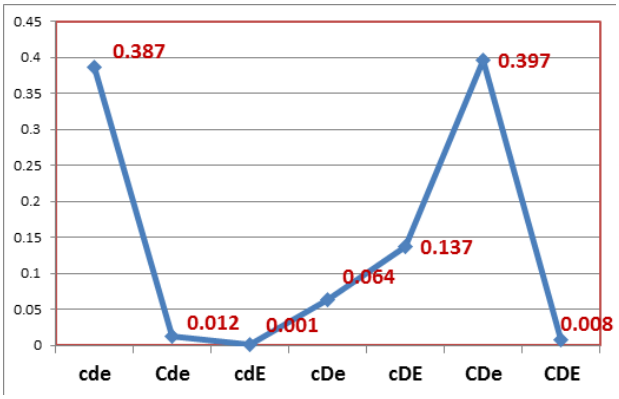

Fig. 7. Distribution of Rh Haplotypes in the donors

The frequency of Kell system genotypes has been distributed in the following way: the highest frequency 91,9±1,06% was observed in kk genotype; Kk genotype was observed to have the frequency that equals to 7,6±1,03% while the frequency of KK genotype equals to 0,5±0,24% (Fig. 8).

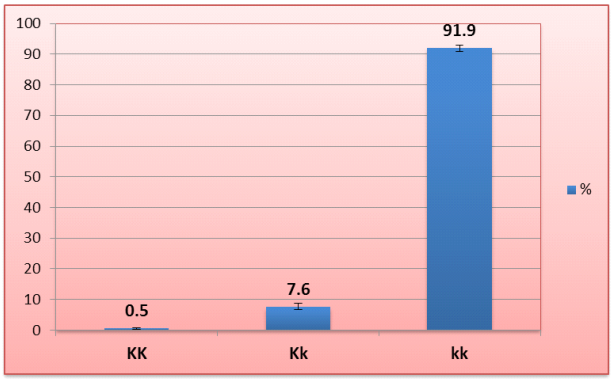

Fig. 8. Distribution of Kell system genotypes in the donors

Investigation of the frequency of Kell system allele revealed p allele low frequency equaling 0,05, whereas the frequency of q allele was observed to be 0,95 (Fig. 9).

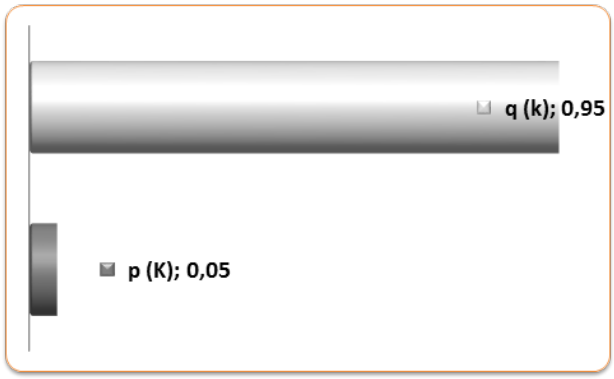

Fig. 9. The frequency of Kell system alleles

The research showed that according to the erythrocyte group antigens, the earlier stated population is characterized by rather high polymorphism. The study of the obtained data is of great importance for the rational preparation of blood components for the purpose of their use in transfusion. The obtained results can be used by medical institutions, especially hematological and transfusion centers.

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SUMMARY

SOME CLINICALLY IMPORTANT ERYTHROCYTE BLOOD GROUP ANTIGENS IN DONORS

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The distribution of erythrocyte blood group antigens was evaluated among 656 donors; samples were provided by the diagnostic laboratory "Medina" Ltd Health Centre of Batumi. Lab analysis of the sample was conducted by the immunogenetics laboratory at Batumi Shota Rustaveli State University. The frequency of the ABO allele system in donors was as follows: r (0.70), q (0.23), p (0.07). The distribution of Rhesus (Rh) factor in the donor population was as follows: Rh(-) was found among 16.3±1.43% of investigated donors; the Rh(+) phenotype was found in 83.7±1.43% of donors. Additionally, the CcDee phenotype frequency was 29.9±1.78%; CCD-ee was 17.2±1.47%; ccddee was 14.9±1.38%; and CcD-Ee was 13.9±1.34%; ccD-Ee phenotype was 11.1±1.22%; ccD-ee was 5.5±0.88%; same phenotype indicators -2.1±0.55 were observed for CcD-EE and ccD-EE; CCD-Ee was 1.4±0.45%, CCD-EE was 0.4±0.26%; and finally, the frequency of Ccddee phenotype amounts was 1.1±0.40%, ccddEe and CCddee phenotypes were both 0.2±0.17%. The analysis of the Kell system allele revealed a low frequency for the p allele at 0.05, whereas the frequency of the q allele was 0.95. This large epidemiologic analysis of donor blood provides valuable information for hematological and transfusion centers to inform the preparation of blood components for transfusion.

Keywords: erythrocyte blood group antigens, phenotypes, donors.

РЕЗЮМЕ

НЕКОТОРЫЕ КЛИНИЧЕСКИ ЗНАЧИМЫЕ ГРУППОВЫЕ АНТИГЕНЫ ЭРИТРОЦИТОВ ДОНОРОВ

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Исследовано 656 доноров крови на эритроцитарные групповые антигены. Материал для исследования предоставлен клинико-диагностической лабораторией центра здоровья г. Батуми „Медина“. Исследования проводились в иммуногенетической лаборатории Батумского государственного университета им. Шота Руставели. При исследовании аллельных концентраций АВО системы зафиксирована высокая частота (0,7) распространения r аллеллей. В популяции доноров q аллель встречается с частотой 0,23, с самой низкой частотой (0,07) встречается p аллель. 16,3±1,43% исследованных доноров являлись носителями Rh(-) фенотипа. Rh(+) фенотип выявлен в 83,7±1,43% случаях. Из фенотипных групп резус системы CcDee распространен в 29,9±1,78% случаев, за ним следует CCD-ee - 17,2±1,47%, ccddee - 14,9±1,38% и CcD-Ee - 13,9±1,34%. По сравнению с ними в более малом количестве встречается ccD-Ee фенотип 11,1±1,22% и ccD-ee - 5,5±0,88%, CcD-EE и ccD-EE фенотипы представлены равным показателем распространения 2,1±0,55. Частота встречаемости фенотипа CCD-Ee составляет 1,4±0,45%, CCD-EE фенотипа - 0,4±0,26%, Ccddee фенотипа - 1,1±0,40%, ccddEe и CCddee фенотипы представлены с частотой 0,2±0,17%. При исследовании частоты аллелей Kell системы выявлена низкая частота p аллеля - 0,05, q аллеля - 0,95. Результаты проведенного исследования позволяют оценить региональные иммуногенетические свойства доноров, что позволит избежать посттрансфузионные осложнения и индукцию аллоимунных антител.

რეზიუმე

დონორთა ზოგიერთი კლინიკურად მნიშვნელოვანი ერითროციტების ჯგუფური ანტიგენები

ი. ცინცაძე, თ. გორგოშაძე, ს. დონსკოვი, ლ. ახვლედიანი, მ. ნაგერვაძე

ბათუმის შოთა რუთაველის სახელმწიფო უნივერსიტეტი, საბუნებისმეტყველო მეცნიერებათა და ჯანდაცვის ფაკულტეტი, ბიოლოგიის დეპარტამენტი, საქართველო

656 დონორის სისხლი გამოკვლეულია ერითროციტურ ჯგუფურ ანტიგენებზე. საკვლევი მასალა მოწოდებულია ქ. ბათუმის შპს ჯანმრთელობის ცენტრ „მედინას“ კლინიკურ-დიაგნოსტიკური ლაბორატორიის მიერ. კვლევა ჩატარდა ბათუმის შოთა რუსთაველის სახელმწიფო უნივერსიტეტის იმუნოგენეტიკის ლაბორატორიის ბაზაზე. ABO სისტემის ალელების კონცენტრაციის კვლევისას დაფიქსირდა r ალელის გავრცელების მაღალი - 0,7 სიხშირე. დონორთა პოპულაციაში q ალელი აღმოჩნდა 0,23 სიხშირით, ყველაზე დაბალი სიხშირით - 0,07 დაფიქსირდა p ალელი. Rh(-) ფენოტიპის მატარებელია შესწავლილ დონორთა 16,3±1,43%, Rh(+) ფენოტიპი კი გვხვდება 83,7±1,43%. რეზუს სისტემის ფენოტიპური ჯგუფებიდან CcDee ფენოტიპი გავრცელებულია 29,9±1,78%. მას მოსდევს CCD-ee - 17,2±1,47%, ccddee - 14,9±1,38% და CcD-Ee - 13,9±1,34%. შედარებით ნაკლებად გვხდება ccD-Ee ფენოტიპი - 11,1±1,22%, ccD-ee - 5,5±0,88%, CcD-EE და ccD-EE ფენოტიპები გავრცელების თანაბარი მაჩვენებლებითაა წარმოდგენილი - 2,1±0,55. CCD-Ee ფენოტიპის სიხშირე შეადგენს 1,4±0,45%, CCD-EE ფენოტიპის - 0,4±0,26%, Ccddee ფენოტიპის - 1,1±0,40%, ccddEe და CCddee ფენოტიპები 0,2±0,17%-ით დაფიქსირდა. Kell სისტემის ალელთა სიხშირის კვლევისას გამოვლინდა p ალელის დაბალი სიხშირე - 0,05, q ალელის - 0,95. აღნიშნული კვლევის შედეგები საშუალებას იძლევა შეფასდეს დონორთა რეგიონული იმუნოგენეტიკური მახასიათებლები, რაც დაეხმარება ტრანსფუზიოლოგიურ სამსახურებს პოსტტრანსფუზული გართულებების და ალლოიმუნური ანტისხეულების ინდუქციის შემცირებაში.

SMOKING INCLINED GROUPS ACCORDING TO THE PHENOTYPE OF THE PTC GENE

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The ability of feeling the PTC (phenylthiocarbamide) bitterness represents one of the well-known and convenient genetic marker with regards to human populations and biomedical studies [3,7]. The scientists discuss two possible variants of PTC sensitivity inheritance – single-locus and two-locus models [11]. We can face two basic phenotypes in populations with the ability of PTC sensitivity - PTC sensitive or "tester" and PTC insensitive or "non-tester" phenotypes. The majority of the population on the earth (approximately 70%) belongs to the PTC sensitive phenotype, and the rest 30 % - to insensitive one [7,3,5]. It is interesting to know that according to these markers, different populations are characterized with different phenotype structure. For instance, the population of the older people in Southern Italy turned out to be PTC sensitive phenotype basically [1]. The other researches prove that PTC insensitive phenotype and the correspondent recessive allele of the gene are more inclined to the pathologies of thyroid glands (68%) than PTC insensitive phenotype (32%) [14]. According to the studies conducted in Missouri population, India, the researchers fixed the connection between PTC insensitive phenotypes and the inclination early onset of childhood obesity [13] and the body mass index of the PTC insensitive phenotypes exceeded the body mass index (BMI) of the PTC sensitive phenotypes 2,5 times [6]. They also registered the genetic connection to the PTC insensitive phenotypes in the population of smokers [2] and the researches are still conducting in order to study the haplotype structure of PTC sensitive gene.

Judging from the fact that the ability of PTC bitterness sensitivity represents the ethno-specific marker, the phenotype structure of this gene is specific for each particular population. The goal of our research was to study the phenotypic structure of PTC bitterness sensitivity among the cigarette smoker population living in Ajara Region and to reveal the risk groups addicted to drugs in the local population, besides, our goal was to provide the comparative analyses of the obtained results with the data received from the study of the general phenotype structure of the local population.

Material and methods. We have conducted our research among the cigarette smokers living in Ajara Region of

Georgia. 90 randomly selected volunteers of both sexes and different age groups were studied totally. They filled in special inquiry forms which included the questions of demographic and medical characters. The forms also included the questions on the duration and intensity of cigarettes smoking. The research was made under the ethic principles of Helsinki Declaration *(World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects)*. Every participant of the experiment confirmed their consents in the ritten form.

The research was based on Harris and Kalmus method [8]; we have used standard testers which contained 3,4 mg/kg concentration. The participants fixed the taste sensitivity data of the tester in written form. According to the bitterness sensitivity with the data obtained via PTC testing, the participants of the experiment were divided into two groups: PTC tester and PTC non tester phenotypes.

We have noted the dominant, heterozygous and recessive genotypes with the symbols applied in the scientific literature: T (taste) symbol indicates bitterness sensitivity; t – insensitivity. Consequently, TT is dominant homozygote according to the bitterness sensitivity; Tt – heterozygote; tt – recessive homozygote.

The obtained results were mathematically processed applying the statistical methods. We calculated the concentration of allelic PTC gene in the population and the frequency of its propagation applying Hardy-Weinberg equation - $q^2+2q(1-q)$, which reflects the distribution of genotypes in panmictic population. The authenticity of the obtained results was confirmed with the formula:

$$M = \pm\sqrt{(100-p)/n}$$

where, P denotes the percentage data, n – the number of the researched people

Experimental data were processed using of statistical analysis method where, the level of significance $\alpha=0.05$ and $p<0.05$.

Results and their discussion. The cigarette consumption is considered to be the provoking factor of lung cancer, respiratory, cardiovascular diseases, hypertension and many other diseases [4,9,12,15]. There are estimations on genetic inclination of the cigarette consumption of AVI haplotypes of PTC gene (PTC insensitive phenotype) [2]. At the same time, according to the ethno-specificity, the phenotype structure of this gene can be different in every particular population.

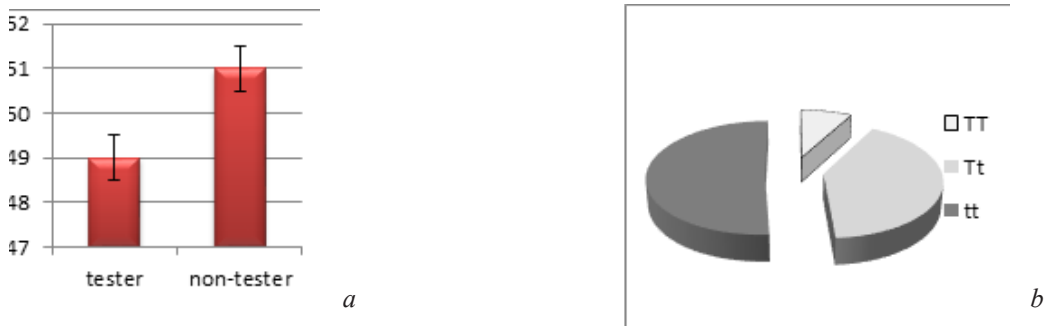
90 volunteers aged from 18 to 65, among them 23 women and 67 men were tested in the experiment. According to the obtained results, the part of the participants felt the established, universal, limited amount (3,4 mg/kg) of phenylthiocarbamide of the PTC tester sensibility for humans as very bitter, the part of them – as bitter, and the rest of them – as slightly bitter. Some of them could not feel the PTC bitterness at all. The PTC insensitive people (non-teser) fixed the taste of paper or could not feel any taste at all. As we can see, rather broad spectrum of PTC bitterness sensitivity was revealed in the research people. We conventionally divided them into three categories:

- Dominant homozygote (TT) – feels the PTC bitterness sharply;
- Heterozygote (Tt) – feels the PTC bitterness or fixes that slightly.
- Recessive homozygote (tt) – is unable to fix the PTC bitterness. They can perceive the paper taste or have neutral feeling.

Since the tester conducting the experiment could not provide the sensitivity gradation of different concentrations of Phenylthiocarbamide compound (did not have the appropriate scale), finally, the participants of the experiment were divided into two phenotypes according to their PTC sensitivity abilities: 1) PTC sensitive phenotype and 2) PTC insensitive phenotype.

According to the experimental data, 44 people from the studied population (90 people) were PTC sensitive, 46 – PTC insensitive. Therefore, the frequency of the propagation of the PTC sensitive phenotype in the smokers' population was $49\pm Z^{\alpha}/_{2}$-5,2 and the PTC insensitive people - $51\pm Z^{\alpha}/_{2}$-5,2 (Fig 1).

$TT(q^2)=0,08;\ Tt(2q(1-q))=0,41;\ tt(1-q)^2=0,51$



a *b*

Figure 1. The frequency distribution of phenotypes (a) and genotypes (b) in smoking population

Therefore, the recessive t-allele concentration of the coding gene of the bitterness sensitivity in the research population is:

$$1\text{-}q=\sqrt{0,51}=0,71$$

The dominant T-allele concentration in the population is:

$$q=1\text{-}(1\text{-}q)=1\text{-}0,71=0,29$$

Knowing the allele concentration enables us to calculate the distribution of genotypes in the research population (Fig.1).

We have studied the distribution of the phenotypes of the PTC sensitivity in the population according to the sex to reveal whether the sex affects to the PTC sensitivity or not. The experiment has shown that the majority of the cigarette smokers were males (74%). According to the outcomes of the experiment the PTC sensitive phenotype made up about 44,8% of the males of the research group, and the people having no PTC sensibility – 52,2%. In females, that composed 26% of the research group - 60,9% were PTC sensitive and 39,1% - PTC insensitive. The statistical processing has shown that the sex does not influence PTC sensitivity (α=0.05; χ^2=1,77; p-0.1828).

Concerning to the concentration of T and t alleles distribution in the research group amounts to 0,33 and 0,67. The frequency of genotypes is distributed as following: dominant homozygote TT-0,11; heterozygote Tt-0,44; recessive homozygote tt-0,45; (Fig. 2).

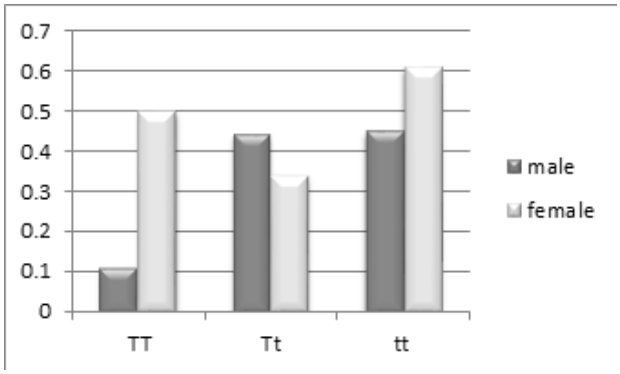


Fig. 2.The distribution frequency of PTC sensitivity genotype in men and women

As for the female research group the concentration of the dominant T and recessive t allele of the bitterness sensitivity coding gene in the research group is: 0,4 and 0,6. And the genotype frequency is distributed as following: TT – 0,5; T – 0,34; tt-0,61 (Fig. 2).

In connection with the age, the researchers' different views made us get concerned and analyze the structure of phenotype variations towards the PTC sensitivity in the research population. The people tested on the basis of the results obtained through the conducted experiment in the randomly selected research population were divided into three age groups: up to 20, from 20 to 50 and after 60 age groups.

The number of the participants of the experiment according to the age groups was divided as following: the first age group – 21%; the second age group – 62%; the third age group – 17%. The sensitive phenotype towards the PTC bitterness sensitivity amounts 46,3% for the first test group, and the insensitive phenotype – 52,6%. In the second age group the sensitive phenotype towards the PTC bitterness sensitivity is 37,5%, and the non-tester phenotype turned out to be 62,5%. In the third age group – 53,3% PTC sensitive and the insensitive phenotype – 46,7%.

The genotypes frequency according to the experimental age groups is represented on the schedule (Fig. 3).

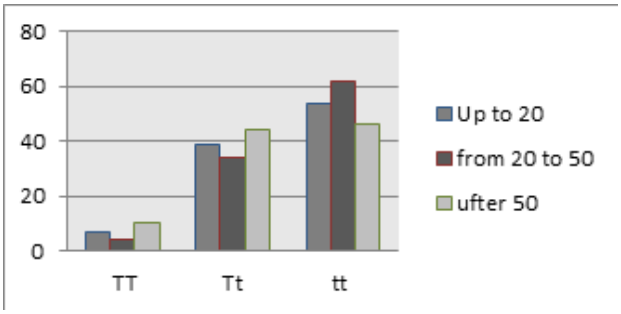


Fig. 3. The genotypes frequency according to the research age groups

The research population was analyzed in according to the intensity and duration of the cigarette consumption.

The research group addicted to smoking was divided into two groups: the first – light smokers who smoke about 10 cigarettes per day and second – intensive smokers who smoke more than 20 cigarettes a day. Research revealed 45,3% of the tested people from the first- light smokers' group (64 individuals) were PTC sensitive, and 54,7% - PTC insensitive; 30,7% of the tested people from the second group (26 individuals) were PTC sensitive, and 69,35% - PTC insensitive. The genotype frequency was following – for the first group - TT – 0,07; Tt – 0,38; tt - 0,55; For the second group: TT – 0,03; Tt – 0,28; tt-0,69 (Fig. 4.a).

During the experiment we attempted to find out whether the continuing consumption of cigarette has an influence upon PTC bitterness sensitivity or not. For this purpose we divided the experiment participants into two groups according to the cigarette consumption experience: we included the smokers with smoking experience of 1 to 10 years into the first group and those who had more than 10 years of smoking experience – into another.

The first group (50 individuals) made up 55,5% of the research group, and the second group (40 individuals) – 45,5%. 45,5% of the first group was PTC sensitive and 54,7% - PTC insensitive phenotype. In these groups the

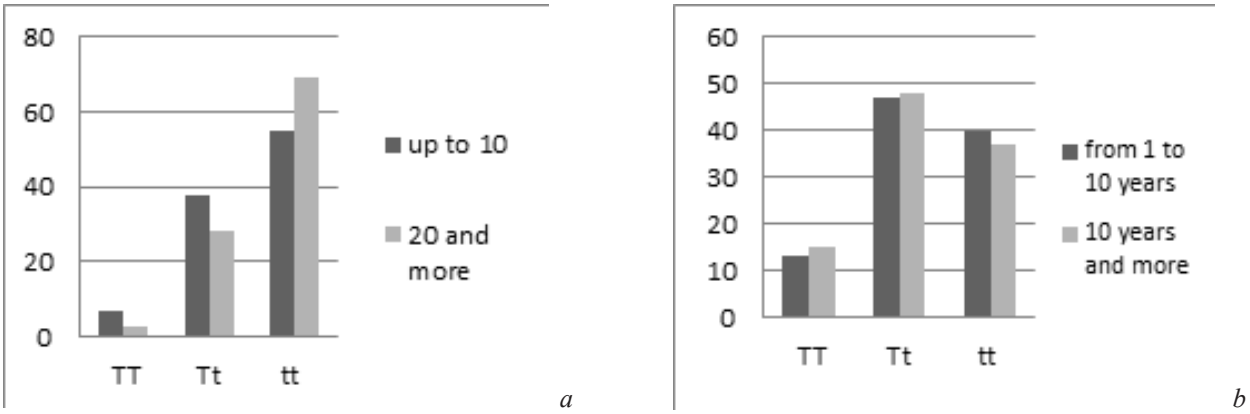


Fig. 4. The genotypes frequency according to the cigarette consumption intensity (a) and duration (b) in experimental groups

genotypes frequency has the following expression (schedule): the first group – TT-0,13; Tt-0,47; tt-0,4; the second group - TT-0,15; Tt-0,48; tt-0,37.

The conducted research shows that the genotypes frequency of the PTC sensitive phenotypes of the cigarette consumers in the research group is $49\pm Z^{\alpha}/_{2}$-5,2, and the insensitive phenotypes - $51\pm Z^{\alpha}/_{2}$-5,2. As we can see, both phenotypes of the PTC sensitivity in the tested group are almost equal. Though, it should be mentioned that the obtained results are different from the general structure of the population where PTC insensitive phenotype makes up only 32% of the entire population [10]. Presumably, depending of the statistical analysis it can be assume that PTC insensitivity somehow affect to the inclination to cigarette consumption ($Z\alpha_{2}$-0,5; Z=3.1057; p=0.0009).

According to the experiment, should be concluded that sex and age does not influence PTC sensitivity. The research groups separated according to the experience of the smoking intensity and consumption have almost similar phenotypic structure distribution. As we can see, the ability of PTC sensitivity is rather stable genetic sign and intensive consumption of cigarette is unable to change its phenotypic structure.

Depend to the comparison general and smoker population data should be concluded that PTC insensitive phenotypes can be considered as risk groups addicted to smoking and during preventive measures against smoking among adults this factor should also be considered.

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SUMMARY

SMOKING INCLINED GROUPS ACCORDING TO THE PHENOTYPE OF THE PTC GENE

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The ability to sense phenylthiocarbamide (PTC) bitterness represents a well-known and convenient genetic marker for human populations and biomedical studies. Two basic phenotypes can be dichotomized by PTC sensitivity: PTC-sensitive or “tester” and PTC-insensitive or “non-tester”. The majority of the population (approximately 70%) belong to the PTC-sensitive phenotype, while the remaining 30% are PTC-insensitive. The distribution of PTC sensitivities varies by consumption of alcohol, bitter coffee and cigarettes. This study was conducted among randomly selected 90 cigarette smokers living in the Ajara Region of Georgia. Our results indicate that PTC-insensitive phenotypes are correlated with cigarette consumption and should be considered as an important genetic proxy for cigarette use. This marker may prove very useful for identifying adolescents who might benefit from a focused smoking prevention intervention.

Keywords: phenylthiocarbamide, smoking, prevention.

РЕЗЮМЕ

ОПРЕДЕЛЕНИЕ ПРЕДРАСПОЛОЖЕННОСТИ К КУРЕНИЮ ПО ФЕНОТИПУ ГЕНА PTC

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Чувствительность восприятия вкуса к PTC (фенилтиокарбамид) является хорошо известным и распространенным генетическим маркером с точки зрения проведения биохимических исследований по изучению человеческих популяций. По степени восприятия вкуса выделяют два основных фенотипа PTC – восприимчивый или “дегустатор” и невосприимчивый или ”недегустатор”. Известно, что 70% населения относиться к фенотипу PTC “дегустатор” и только 30% -к фенотипу PTC ”недегустатор”. Имеются данные о различиях в структурном распределении этих фенотипов среди потребителей алкоголя, горького кофе, курильщиков. Исследовано 90 произвольно подобранных волонтеров, жителей Аджарского региона, потребителей табачных изделий. Результаты исследования выявили высокое распространение PTC нечуствительного фенотипа среди курильшиков. Исходя из этого, лица с фенотипом PTC “недегустатор” больше предрасположены к курению, что необходимо учитывать во время проведения превентивных мероприятий против курения среди подросков.

რეზიუმე

მწეველობისადმი მიდრეკილების განსაზღვრა PTC გენის ფენოტიპის მიხედვით

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ბათუმის შოთა რუთაველის სახ. სახელმწიფო უნივერსიტეტი, საქართველო

PTC (phenilthiocarbamide) სიმწარის შეგრძნების უნარი ერთ-ერთ კარგად ცნობილ და გავრცელებულ გენეტიკურ მარკერს წარმოადგენს ადამიანთა პოპულაციებისა და ბიოსამედიცინო კვლევების თვალსაზრისით. ადამიანებში PTC შეგრძნების უნარის მქონე ორი ძირითადი ფენოტიპი გვხვდება - PTC მგრძნობიარე ანუ „დეგუსტატორი“ და PTC არამგრძნობიარე ანუ „არადეგუსტატორი“. დედამიწის მოსახლეობის უმეტესობა (დაახლოებით 70%) PTC მგრძნობიარე ფენოტიპს განეკუთვნება,ხოლო 30% - არამგრძნობიარეს. ამავდროულად, არსებობს მონაცემები ალკოჰოლის, მწარე ყავის, სიგარეტის მომხმარებელთა შორის მგრძნობიარე და არამგრძნობიარე ფენოტიპების გადანაწილების განსხვავებული სტრუქტურის შესახებ. კვლევა ჩატარდა აჭარის რეგიონში მცხოვრებ სიგარეტის მწეველთა პოპულაციაში. გამოკვლეულია ორივე სქესის, განსხვავებული ასაკობრივი ჯგუფის რანდომულად შერჩეული 90 მოხალისე. მიღებული შედეგების თანახმად, PTC არამგრძნობიარე ფენოტიპები სიგარეტის მოხმარებისადმი მიდრეკილ რისკ-ჯგუფად შეიძლება იქნეს მიჩნეული. ამ გენეტიკური ფაქტორის გათვალისწინება მეტად მნიშვნელოვანია მოზარდებში სიგარეტის მოხმარების წინააღმდეგ გატარებული პრევენციული ღონისძიებების დროს.

IMPACT OF SOME ENVIRONMENTAL FACTORS ON HUMAN HEALTH

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According to the World Health Organization (WHO) almost ¼ of the diseases spread among humans nowadays is caused by the long term influence of polluted environment on human organism. Today it is clear that any activity of a human has an impact on the environment; deterioration of biosphere condition endangers human health. People's agricultural work is considered as a main source of biosphere pollution as a result of which environment is filled with gasses as well as liquid and hard substances that proliferates into water or air from the soil and then gets into human organism [2].

Human health cannot be discussed apart from the environment as he is part of nature. Therefore, changes in environment always effect human health. Health is a physical, psychical and social good will of a human. Other than that, not only is health a luxury that environment gives us but also a condition that humans live in.

As it is known, majority of human diseases is connected to the ecological condition of a living environment, however, it is quite difficult to find causal relationship between human health and atmospheric air, water and soil pollution. Although, nowadays, it becomes quite vivid that in a number of countries in the world environment pollution represents a potential risk factor of illnesses, disabilities and death.

Environment pollution and studying causal relationships between various diseases is a serious problem in our region as well.

Environment pollutants are quite diverse and have various negative results while affecting a human. Short term effect of substances of small concentration symptoms includes dizziness, nausea and coughing. High concentration pollutants might cause fainting, poisoning and death. These kinds of effects are characteristic, for instances, to industrial factory emission and smog.

Human reaction towards pollutants is also various and is determined by individual characteristic: age, gender, health condition, etc. While adapting to the negative ecological environment, human organism experiences tension and exhaustion. Tension is also followed by reduction of organism's functional, structural and energetic resources which finally results in exhaustion. According to the adaptation skills towards environment, there are 2 main types distinguished: 1. Sprinter – is characterized with high maintenance towards the effects of short-term extreme factors and long-term load; 2. Stayer – is a vice-versa type characterized with low maintenance.

If a small amount of toxic substances get into human organism systematically or periodically, chronic poisoning occurs that is characterized with the following symptoms: normal behavior disorder, neuro-psychical disorders, too much sleep or insomnia, lack of attention, etc. Poisoning of human organism by one and the same pollutants, might cause damage to kidneys, blood-producing organs, liver and nervous system. As for the biologically active chemical admixtures, they might cause chronic diseases of various organs, nervous system damage and disorders among infants.

Other than chemical substances, there are biological pollutants in the environment as well that lead to different kinds of human diseases. Among them there are microorganisms, viruses, helminthes and amoebic organisms. They can be found in atmosphere, water, soil, living organisms and humans too [1].

As it is known, the issue of air pollution from automobile fumes is one of the priorities for environment protection and necessary measures should be taken for its solution. During air pollution through sulfur and nitrogen oxides, the number of lung and respiratory system diseases increase 3.5-5 times. In case of the pollution with carbon oxide, the function of hemoglobin decreases resulting in the lack of oxygen in tissues as well as nervous and cardiovascular system disorders. Some of the hydrocarbons are is characterized with carcinogenic features [3].

Since 2002 the amount of vehicles and gasoline used has increased almost 2.5 times in Georgia as a result of which automobile fumes remain the main source of the air pollution. Toxic substances that proliferate in the air through them cause serious health problems to people.

Air pollution through automobile fumes depends on various factors, such as: the amount of vehicles, intensity of transportation, the year of production and technical condition of the vehicle, quality of the gasoline, law-institutional factors, etc [4].

The aim of our research was to determine statistic data of 2012-2015 of the diseases caused by atmospheric air and drinking water pollutants in Ajara Autonomous Republic, Georgia.

Material and methods. The main approach of the study was the determination of automobile impacts on atmospheric air; more specifically, of main atmosphere pollutants such as sulfur and nitrogen oxides, carbon oxide and dust concentration determination. Another approach was

Table 1. Ambient Air Quality Dynamics (2012-2015)

Harmful Substances	YEARS				Maximum permissible concentration mg/m³
	Concentration of harmful substances, mg/m³				
	2012	2013	2014	2015	
Dust	0,60	0,49	0,45	0,441	0,15
Carbon Dioxide, CO	2,8	3,1	2,34	2,1	3,0
Sulfur Dioxide SO_2	0,07	0,111	0,13	0,159	0,05
Nitrogen Dioxide NO_2	0,13	0,141	0,14	0,153	0,04

Table 2. Diseases Registered and Diagnosed in Ajara A.R. in 2012-2015 Related to the Atmospheric Air Pollution

Years	2012		2013		2014		2015	
Disease	Registered, diagnosed	Prevalence	Registered, diagnosed	Prevalence	Registered, diagnosed	Prevalence	Registered, diagnosed	Prevalence
Chronic and undetermined bronchitis	2430	646	2395	620	2720	690	2922	701
Asthma and asthma status	1503	399	1302	337	1151	292	1533	422
Allergic rhinitis	1681	447	1519	393	1369	347	1643	451
Malignant cancer of trachea, bronchi and lungs	301	80	242	63	250	63	282	72

studying statistical data of possible diseases caused by atmosphere pollution.

In our study we have determined the concentration of dust, sulfur and nitrogen dioxide and carbon oxide in atmospheric air together with Adjara Environment Monitoring Service of National Environment Agency.

Results and their discussion. The results of the study are given in Table 1. It is clearly shown that the maximum amount of the dust concentration was in 2012 (0.60 mg/m³) and the minimum – in 2015 (0.441 mg/m³). The maximum of average amount of carbon oxide was registered in 2013 (3.1 mg/m³), and the minimum – in 2015 (2.1 mg/m³). As for the maximum amount of sulfur dioxide average indicator, it was registered in 2015 (0.159 mg/m³) and the minimum in 2012 (0.07 mg/m³). Finally, the maximum of the average indicator of nitrogen dioxide was registered in 2015 (0.153 mg/m³) and the minimum – in 2012 (0.13 mg/m³).

We have also studied the statistical data of the diseases (chronic and undetermined bronchitis, asthma and asthma status, allergic rhinitis, malignant cancer of trachea/bronchi/lung) registered on Adjara A.R. territory in 2012-2015 which might be linked to the atmospheric air pollution (Table 2).

As we can see from Table 2, in 2012 there were 2430 registered and diagnosed cases of chronic and undetermined bronchitis; the prevalence was 646. In 2013 – 2395 cases; prevalence – 620. In 2014 – 2720 cases; prevalence – 690 and in 2015 – 2922 cases with 701 prevalence.

As for the asthma and the diseases with asthma status, in 2012 there were 1503 registered and diagnosed cases; prevalence – 399. In 2013 – 1302 cases; prevalence – 337; in 2014 – 1151; prevalence – 292, in 2015 – 1533 cases with the prevalence of 422. In 2015 – 1533 cases; prevalence – 422

Regarding allergic rhinitis, in 2012 there were 1681 registered and diagnosed cases with the prevalence of 447. In 2013 – 1519 cases; prevalence – 393. In 2014 – 1369 cases; prevalence – 347. In 2015 – 1643; prevalence – 451. In 2015 – 1642 cases; prevalence – 451.

As for the malignant cancer of trachea, bronchi and lungs, in 2012 there were 301 registered and diagnosed cases with the prevalence of 80. In 2013, there were 242 cases; prevalence – 63. In 2014 – 250 cases; prevalence – 63 and finally in 2015 there were 282 registered cases and the prevalence was 71.

Statistical data has shown that in 2015 in Adjara A.R. compared to the previous years the number of diseases connected to the atmospheric air pollution has increased. Therefore, it becomes vivid that the monitoring of atmospheric air should be improved and necessary preventive measures should be implemented.

As it has been determined, deterioration of ecological condition of environment is directly linked to the increased number of allergies, bronchial asthma and cancer. While atmosphere pollution by microbes, the airborne infection is spread by respiratory system. Due to the air pollution, new diseases emerge exact causes of which are hard to determine.

Based on the experimental data received by us, it becomes clear that more attention should be paid to the technical condition of the vehicles and the systematic observation of the chemical pollution of atmospheric air should continue in order to implement preventive measures in time. This topic becomes more urgent in terms of Batumi being one of the most important tourist attractions on the Black Sea Coast with its rich natural, medical and climate resources. Statistical data of Adjara Public Health Center points to the fact that the number of registered diseases which most likely are linked to the atmospheric air pollution, such as bronchitis, allergic rhinitis, asthma and asthma status diseases and the malignant cancer of trachea, bronchi and lungs, were most frequent among the people over the age of 40. Negative effects of the polluted environment are directly linked to the deterioration of the citizens' health conditions. In many of the countries of the world atmospheric air pollution is the main source of the diseases the specter of which is quite wide and mainly depends on the type of the air pollution, concentration, duration of exposition and organism condition.

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SUMMARY

IMPACT OF SOME ENVIRONMENTAL FACTORS ON HUMAN HEALTH

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This paper presents statistical data of 2012-2015 on the diseases caused by the atmospheric air and water pollutions in Ajara region. The research on the content of dust, sulfur dioxide and nitrogen dioxide as well as carbon monoxide in the atmospheric air was held together with the National Environment Agency Ajara Monitoring Service. The results of the research have shown that the average content of the dust reached its maximum in 2012 (0.60 mg/m^3) and it dropped to the minimum in 2015 (0.441 mg/m^3). As for average content of carbon monoxide the maximum was observed in 2013 (3.1 mg/m^3) and minimum in 2015 (2.1 mg/m^3). Average content of the sulfur dioxide was at maximum in 2015 (0.159 mg/m^3) and at minimum in 2012 (0.07 mg/m^3). The average content of nitrogen dioxide reached its maximum in 2015 (0.153 mg/m^3) and was found to be at its minimum in 2012 (0.13 mg/m^3). In parallel statistical research of the registered diseases (chronic and undetermined bronchitis, asthma, allergic rhinitis and trachea/bronchi/lung malignant cancer) in Ajara during 2012-2015 has been performed. These diseases were especially common among the population over the age of 40. It may be concluded that in 2015 the cases of diseases caused by the atmospheric air pollution in Ajara have become more frequent compared to the previous years. Therefore, it is evident that monitoring of atmosphere air should be improved and corresponding preventive measures should be undertaken.

Keywords: pollution, human health, sulfur dioxide, nitrogen dioxide, carbon monoxide.

РЕЗЮМЕ

ЗДОРОВЬЕ ЧЕЛОВЕКА И ОКРУЖАЮЩАЯ СРЕДА

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В статье представлены статистические данные возможных заболеваний, вызванных загрязнением питьевой воды и атмосферного воздуха, на территории Аджарской АР за 2012-2015 гг. Совместно с Национальным

агентством окружающей среды, Департаментом мониторинга окружающей среды Аджарской АР проведено исследование содержания диоксида азота, серы и оксида углерода в атмосферном воздухе. Исследования выявили, что средний максимальный показатель пыли зафиксирован в 2012 г. (0,60 мг/М^3), а минимальный - в 2015 г. (0,441 мг/М^3); средний максимальный показатель окиси углерода выявлен в 2013 г. (3,1 мг/М^3), минимальный - в 2015 г. (2,1 мг/М^3); средний максимальный показатель диоксида серы - в 2015 г. (0159 мг/М^3), минимальный - в 2012 г. (0,07 мг/М^3); средний максимальный показатель диоксида азота - в 2015 г. (0,153 мг/М^3), минимальный - в 2012 г. (0,13 мг/М^3).

Представлена статистика зарегистрированных заболеваний на территории Аджарской АР за 2012-2015 гг.: хронический и неуточненной этиологии бронхит; аллергический ринит; астма и заболевания, имеющие астматический статус; злокачественная опухоль легких, трахеи и бронхов, которые возможно связаны с загрязнением воздуха, особенно превалируют у лиц в возрасте старше 40 лет.

Анализ данных проведенного исследования позволяет заключить, что в 2015 г. в сравнении с предыдущими годами, в Аджарской АР возросло число случаев заболеваний, связанных с загрязнением воздуха. Вышеизложенное диктует необходимость усиления мониторинга атмосферного воздуха и принятия соответствующих профилактических мер.

რეზიუმე

ადამიანის ჯანმრთელობა და გარემო

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ნაშრომში შესწავლილია აჭარის ავტონომიური რესპუბლიკის (არ) ტერიტორიაზე ატმოსფერული ჰაერის და სასმელი წყლის დაბინძურებით გამოწვეული შესაძლო დაავადებათა სტატისკური მონაცემები 2012-2015 წწ. გარემოს ეროვნულმა სააგენტომ, აჭარის გარემოს მონიტორინგის სამსახურთან ერთად ჩაატარა ატმოსფერული ჰაერში მტვერის, გოგირდისა და აზოტის დიოქსიდის და ნახშირბადის ოქსიდის შემცველობის გამოკვლევა. გამოვლინდა, რომ მტვრის საშუალო მაჩვენებლის მაქსიმუმი აღინიშნა 2012 წ. (0,60 მგ/მ^3), მინიმუმი - 2015 წ. (0,441 მგ/მ^3). ნახშირბადის ოქსიდის საშუალო მაჩვენებლის მაქსიმუმი 2013 წ. (3,1 მგ/მ^3), მინიმუმი - 2015 წ. (2,1 მგ/მ^3), გოგირდის დიოქსიდის საშუალო მაჩვენებლის მაქსიმუმი – 2015 წ. (0,159 მგ/მ^3), მინიმუმი - 2012 წ. (0,07 მგ/მ^3), აზოტის დიოქსიდის საშუალო მაჩვენებლის მაქსიმუმი – 2015 წ. (0,153 მგ/მ^3), მინიმუმი - 2012 წ. (0,13 მგ/მ^3).

2012–2015 წწ. აჭარის არ ტერიტორიაზე ჩატარდა რეგისტრირებულ დაავადებათა (ქრონიკული და დაუზუსტებელი ბრონქიტი, ასთმა და ასთმური სტატუსი,ალერგიული რინიტი,ტრაქეის,ბრონქების, ფილტვის ავთვისებიანი სიმსივნე) სტატისტიკური გამოკვლევა. ალერგიული რინიტი, ასთმა და ასთმური სტატუსის დაავადებები, ფილტვის, ტრაქეის და ბრონქების ავთვისებიანი სიმსივნე, განსაკუთრებით ჭარბობდა 40 წელს გადაცილებულ პირებში.

კვლევის შედეგად დადგენილია, რომ 2015 წელს აჭარის ავტონომიურ რესპუბლიკაში წინა წლებთან შედარებით იმატა დაავადებათა იმ რიცხვმა, რომლებიც უკავშირდება ატმოსფერული ჰაერის დაბინძურებას. აუცილებელია რეგულარულად ჩატარდეს ატმოსფერული ჰაერის მონიტორინგი და სათანადო პრევენციული ღონისძიებები.

KNOWLEDGE, ATTITUDE TOWARD DISEASE AND PRACTICE SURVEY ON DIABETES IN GARDABANI DISTRICT

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Diabetes is one of the highly increasing non-communicable diseases in the world and it is associated with a number of disabling complications. According to International Diabetes Federation (IDF), it is estimated that 415 million people have Diabetes in the world and by 2040 it will rise to 642 million [1]. The global prevalence of Diabetes has doubled since 1980 and reached 8.5% in the world's adult population. Diabetes disease burden is tangible for both developed and developing countries, however, up to 60% of mortality from Diabetes is accounted in low and middle-income countries [4].

Diabetes is one of the key challenges for Georgia healthcare system. Prevalence and incidence rates are increasing from year to year. By 2014, 85 957 individuals were registered with diabetes, with the prevalence rate of 2 306.3 per 100 000 population [2]. In 2014, there were 1 783 hospitalization cases due to diabetes recorded national-wide in Georgia with the case fatality rate of 2.8 which is 2.1 times higher than in previous year [2,3]. According to IDF, one of the key factors favoring rapid growth and inadequate management of Diabetes is lack of awareness and knowledge about the disease.

Since 2014 Georgia Red Cross Society (GRCS) with financial and technical support from Danish Red Cross (DRC) and World Diabetes Foundation (WDF) has started implementation of the project: "Diabetes prevention in rural Georgia". The project aimed to improve diabetes prevention, detection and care in Gardabani district. The main objectives of the project were to improve detection, primary prevention and strengthening secondary prevention of Diabetes. To reach the project objectives Diabetes awareness baseline survey was conducted in 2014 to assess knowledge, attitudes and practices related to Diabetes among general population of Gardabani district. 2014 survey revealed significant gaps in knowledge, attitude and behavior practice related to Diabetes among general population of Gardabani district. In order to determine the achievements of the project goal and objectives, follow-up survey about Diabetes awareness, knowledge, attitudes and practices was conducted in 2016 among general population of Gardabani district. The goal of the follow-up Diabetes awareness survey was to determine the current level of knowledge, attitude and practices about Diabetes among general population in Gardabani district, compare it with the baseline survey findings and identify the trends between the two survey results.

Material and methods. Cross sectional study design was used for the survey. Target population included the general population (adults ≥18 years) of Gardabani district. Sampling from the target population was performed by multi-stage sampling approach. Sampling Frame was based on the 2014 census data. The Primary Sampling Units (PSU) were represented by the Geographic Clusters ea. all cities and villages in the Gardabani district. The number of study units for each cluster was defined by Probability Proportional to Size (PPS) approach. The Secondary Sampling Units (SSU) were the households selected from each clusters by simple random sampling. Kish methodology was used for selection of study participants from the selected household. Sample size calculation was done by the methodology for descriptive studies for the expected proportion 0.50 (maximizing the sample size), degree of accuracy (margin of error) +/- 0.05, the confidence level 95% and corresponding populations sizes. The sample size after subtraction of non-response cases (approximately 20% of the selected individuals) was estimated to be equal to 624.

Data were collected at households through face-to-face individual interviews by specially designed questionnaire. The questionnaire was available in Georgian and Azerbaijani language. The questionnaire was administered in participant's native language. Volunteers from Georgia Red Cross Society conducted interviews for the survey. Study participation was voluntary. Individuals who agreed to participate in the study signed an informed consent form and then were enrolled in the survey. Prior to the start of the survey the study protocol and survey tools were approved by the Institutional Review Board (IRB) of Health Research Union (HRU). Data entry, management and statistical analysis were conducted using statistical package SPSS v.22.0. Descriptive statistics procedures were applied for the evaluation of the study variables in the target population. The study variables were analyzed using t-test statistic for quantitative and chi-square tests for categorized data.

Results and their discussion. Totally 716 individuals were enrolled in the survey. Most of the respondents were Georgians (59.2%) and Azeri (37.7%). Among study participants 55.9% were females. Almost two thirds (58.4%) of the respondents were aged between 35 to 65 years. Nearly 70%percent of individuals interviewed reported themselves as currently married. Most of the respondents (43.3%) completed high school and only 13.2% reported having studied up to university or post-graduate degree.

By type of residence, the vast majority (83.5%) of study subjects lived in rural areas. Slightly more than half of study participants (51.1%) had families consisting of 2 to 4 members including themselves. Nearly 40% of the respondents were currently employed or self-employed, followed by unemployed (25.2%) and retired (23.4%) individuals. Majority of respondents (46%) reported family income between 400 to 800 GEL per month.

Almost all surveyed individuals (98.9%) reported of having heard about Diabetes. Hearing from the volunteers of Georgia Red Cross Society was one of the main sources of information on Diabetes among study subjects as 78.2% of respondents had heard about Diabetes from this source.

The vast majority of respondents (91.3%) considered the disease as an important or very important problem for Gardabani district. Compared to previous survey a significantly higher proportion of individuals think that Diabetes is an important problem for the region (91.3% in 2016 vs. 45% in 2014). The fact that Diabetes is a chronic disease was known by the vast majority (96.4%) of study participants. The level of knowledge on this issue was markedly increased from previous survey where only 18.1% of the respondents could identify that Diabetes is a disease. Compared to the previous survey the level of knowledge regarding Diabetes risk factors improved significantly. Much higher proportions of respondents consider overweight, family history of diabetes and unhealthy diet as risk factors for the disease. Most of study participants (85.3%) believed that Diabetes can be prevented and majority among them could correctly identify the measures of Diabetes prevention. Almost 80% of the respondents were aware that two types of Diabetes exist and among them majority (87.7%) knew that there is difference between type 1 and type 2 Diabetes but almost none of them could identify correctly what kind of difference is between the two different types of the disease.

All respondents who stated that they had heard about Diabetes were asked about the main signs and symptoms of the disease. Only 15.3% of the study subjects couldn't define any symptoms of Diabetes or stated incorrect symptoms. Considerably higher proportion of the respondents correctly identified the symptoms of Diabetes in 2016 than in 2014. 97.4% of study subjects have heard about Insulin. Among those who were aware of Insulin 92.9% knew that it is hormone or medicine regulating blood sugar and 90.4% was informed that not all people with Diabetes need to take Insulin. Majority of the respondents (91.5%) knew that Diabetes can be managed by healthy diet, blood sugar monitoring (81.1%) and diabetes medication or insulin therapy (84.5%).

A series of questions were asked to the study participants in order to assess their knowledge on the complications of Diabetes. The knowledge level about main complications of Diabetes between 2014 and 2016 surveys improved significantly. In 2016 survey higher proportion of respondents (92%) were aware that Diabetes complications can be avoided compared to 2014 (55.9%). 91.6% of respondents reported that Diabetes complications can be avoided by regular visits to the doctor. Nearly the same proportion of study subjects (87.9%) considered that taking medications (pills or insulin) will prevent complications. 25.2% of study participants reported that either they or their family member have Diabetes. Diabetes prevalence in families was slightly higher in 2016 compared to 2014 survey (17.1%). Further question specified who had Diabetes in respondent's family. 12 % of study participants had Diabetes themselves. Almost two thirds of respondents (58.2%) reported that they were diagnosed within the past two years. Those individuals who were diagnosed during the past two years were further asked if they have changed anything in their lifestyle after being diagnosed. The vast majority of them (93.5%) answered positively on this question. Among those who changed their lifestyle 83.7% started to eat healthier, 58.1% lost extra weight and 44.2% started more exercise. We tried to determine the factors that triggered the change of lifestyle among individuals diagnosed with diabetes within the past two years. 34.1% followed healthcare worker's advice and 41.5% of respondents reported that support from Georgia Red Cross Society's self-support groups helped them to change their lifestyle after being diagnosed with Diabetes. Regarding the barriers that these individuals faced after changing their lifestyle or the barriers for not changing anything in their lifestyle after diagnosis, for the vast majority of study participants (92.7%) the main barrier was cost of healthy diet.

Overall, 60.9% of respondents believed that they were well informed about Diabetes. Majority of participants (87.8%) wish to receive more information on Diabetes. Healthcare workers and volunteers from Georgia Red Cross Society were named as the preferred information sources for more than 80% of surveyed individuals. Majority of the respondents (43.4%) stated that prevention of Diabetes is the most important information to be provided to the society. Study participants were asked if they were aware about healthcare services for people with diabetes available in Gardabani district. Two thirds of respondents knew about such services but most of them (46.3%) could not specify what types of services are available. The awareness level about the availability of healthcare services for people with diabetes in Gardabani district improved from 2014 to 2016.

Study participant were also asked about their health-seeking and health-risk behavior, physical activity and healthy eating habits. Around half of the study subjects go to the doctor for medical check-up 2 to 4 times per year. Monthly family expenditure for medicines constitutes 20 to 100 GEL for about one third of the respondents and another one third stated that that their families spend for medicines less than 50 GEL per month. Among the respondents with Diabetes

41.2% go to doctor once per three months and for 41.7% family expenditure for medicines per month is 50 to 100 GEL. Around 70% of the study subjects never smoked cigarettes and around one third of the study participants were current or past alcohol users. Less than 10% of surveyed individuals reported that they do vigorous intensity physical activities. Among them 40% do vigorous intensity physical activities on 6-7 days a week and another 40%on 2-3 days a week. Higher proportion of study participants (49.9%) were involved in moderate intensity physical activities and most of them did it on 6 to 7 days per week (69.8%) and 30 to 60 minutes per day (59.9%). Assessing the diet, the survey revealed that two thirds of study participants eat meals two times a day on average. For 60.4% of the respondents fruit and vegetables are included in the diet 2 to 3 times a week. Only 10% of study subject reported that they eat fruit and vegetables every day.

Knowledge of Diabetes prevention (identification of all main measures that can prevent the disease including eating healthy food, regular physical activity and losing extra weight) was higher among individuals with higher education level (62.6% vs. 50.8%; p=0.05), higher family income (62.2% vs. 53.5%; p =0.03) and residing in rural settlements (58.6% vs. 25%; p<0.001) (Table 1). The level of knowledge about Diabetes prevention was also analyzed among study subjects with Diabetes by different socio-demographic characteristics. Statistically significant association was found between study subjects' ethnicity and the awareness of Diabetes prevention. More Azeri respondents could identify all measures of Diabetes prevention compared to Georgians (77.8% vs. 41.7%) (p=0.003). Marked difference in Diabetes prevention awareness was revealed between rural and urban respondents, but this association was also statistically not significant (57.4% vs. 22.2%) (p=0.07).

Bivariate analysis was also conducted about the knowledge of Diabetes management (correct identification of all main measures of Diabetes management, such as healthy eating, regular physical activity, Diabetes medication or insulin therapy and blood sugar monitoring) by different socio-demographic characteristics (Table 2). Type of residence was significantly associated with the awareness of Diabetes management as more representatives of rural population defined "how can the disease be managed" than in urban settings (50.3% vs. 28.4%) (p<0.001). Measures of Diabetes management was also more known among individuals with family income more than 500 GEL compared to those with family income less than 500 GEL (52.3% vs. 45.5%) (p=0.05). Difference was found by ethnicity and type of residence in knowledge level about Diabetes management among individuals with Diabetes. Azeri and rural respondents were more aware about how to manage Diabetes compared to Georgian and urban study subject (63.3% vs. 36.5% and 52.1% vs. 16.7%, respectively) and these associations were statistically significant.

Changing lifestyle after Diabetes diagnosis was related to older age, ethnicity (Azeri individuals were more likely to change lifestyle than Georgians), higher level of education, living in urban areas and higher family income, but none of these associations were statistically significant.

Table 1. Knowledge of diabetes prevention by socio-demographic characteristics

Characteristic	Knowledge of Diabetes Prevention					
	Yes		No		Total	*p* value
	n	%	n	%	n	
Age						
≤35 years	83	56.8	63	43.2	146	0.7
>35 years	256	54.7	212	45.3	468	
Ethnicity						
Georgian	200	54.5	167	45.5	367	0.2
Azeri	132	57.4	98	42.6	230	
Other	6	37.5	10	62.5	16	
Education Level						
College/ University	142	62.6	85	30.9	227	0.005
Other	196	50.8	190	49.2	386	
Type of residence						
Urban	16	25.0	48	75.0	551	<0.001
Rural	323	58.6	228	41.4	64	
Income in family (per month)						
≤500 GEL	222	53.5	193	46.5	415	0.03
>500 GEL	115	62.2	70	37.8	185	

Table 2. Knowledge of diabetes management by socio-demographic characteristics

Characteristic	Knowledge of Diabetes Management					
	Yes		No		Total	*p* value
	N	%	n	%	N	
Age						
≤35 years	75	46.9	85	53.1	160	0.5
>35 years	254	46.6	291	53.4	545	
Ethnicity						
Georgian	184	43.8	236	56.2	420	0.1
Azeri	135	51.5	127	48.5	262	
Other	11	50.0	11	50.0	22	
Education Level						
College/ University	127	49.0	132	51.0	259	0.3
Other	203	45.6	242	54.4	445	
Type of residence						
Urban	33	28.4	83	71.6	116	<0.001
Rural	297	50.3	293	49.7	590	
Income in family (per month)						
≤500 GEL	222	45.5	266	54.5	488	0.05
>500 GEL	103	52.3	94	47.7	197	

Analysis of the sources of information about Diabetes by different socio-demographic characteristics revealed that most of the respondents from both age groups reported similarly that they first heard about Diabetes form GRCS (78.3% among ≤35 years old and 76.0% among>35 years old). No difference was found on this issue between different ethnicities. The same proportion (77%) of Georgian and Azeri study participants stated that the first information source about Diabetes was GRCS. Respondents with higher and lower education levels equally received information about Diabetes from GRCS (78.2% and 75.7%, respectively). Similar finding was observed between higher and lower family income groups (76.1% and 77.3%, respectively). Statistically significant difference was found between rural and urban residents regarding the first source of information about Diabetes. More study participants residing in rural areas received information about Diabetes from GRCS than individuals living in urban settlements (78.6% vs. 66.1%) (p=0.004).

Awareness of Diabetes prevention was related to the first source of information about Diabetes. A higher proportion of respondents with knowledge on Diabetes prevention, named GRCS as a first source of information about Diabetes (85.5%) compared to those who were not aware of this issue (76.8%) (p=0.006). Another statistically significant association was found between the level of knowledge on Diabetes management and the first source of information. Among those who correctly listed all main measures of Diabetes management more individuals stated that they had heard about Diabetes from GRCS than among those who couldn't correctly define the management of the disease (83.6% vs. 71%).

Awareness and knowledge about Diabetes has significantly improved among general population of Garabani district from 2014 to 2016. The development and implementation of similar health intervention and education programs to increase the level of awareness and knowledge about Diabetes is required at national level to improve control and management of the disease throughout the country. The study findings also suggest that public health interventions related to diabetes should be targeted to certain target populations, particularly to those with lower education level and family income.

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SUMMARY

KNOWLEDGE, ATTITUDE TOWARD DISEASE AND PRACTICE SURVEY ON DIABETES IN GARDABANI DISTRICT

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In 2014-2016, the Georgia Red Cross Society (GRCS) implemented a project to improve diabetes prevention, detection and care in rural Georgia, namely in the Gardabani district. The KAP survey was conducted to determine current levels of knowledge, attitudes and practices regarding diabetes among the general population in the Gardabani district. We compared current findings with baseline results from a 2014 survey to identify trends. A cross sectional study design with multi-stage random sampling was used to select participants. Data were collected at households through face-to-face individual interviews using a specially designed questionnaire. In total, 716 individuals were surveyed, the majority of whom (98.9%) were aware of diabetes. Most respondents (85.3%) believed that diabetes can be prevented and correctly identified measures of diabetes prevention. Compared to the previous survey, the level of knowledge regarding risk factors, symptoms and complication of diabetes had improved significantly. Knowledge of diabetes prevention correlated positively with individuals having a higher education level (62.6% vs. 50.8%; p=0.05), higher family income (62.2% vs. 53.5%; p=0.03) and residing in rural settlements (58.6% vs. 25%; p<0.001). Knowledge of diabetes management was significantly associated with type of residence (rural 50.3% vs. urban 28.4%; p<0.001) and family income (high family income 52.3% vs. low family income 45.5%; p=0.05). Respondents identifying GRCS as a source of information tended to have a higher awareness of diabetes prevention and management. Knowledge of diabetes has significantly improved among the general population of the Garabani district from 2014 to 2016. The development and implementation of similar public health programs to increase the level of awareness and knowledge about diabetes is required in other parts of Georgia to improve control and management of the disease throughout the country.

Keywords: diabetes, KAP survey, awareness.

РЕЗЮМЕ

ОЦЕНКА ЗНАНИЙ О ДИАБЕТЕ, ОТНОШЕНИЯ К БОЛЕЗНИ И ПРАКТИКИ В ГАРДАБАНСКОМ РЕГИОНЕ ГРУЗИИ

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В 2014-2016 гг. Общество Красного Креста осуществило проект, целью которого явилось изучение осведомленности населения Гардабанского района о болезни сахарного диабета (СД). Для исследования использован поперечный дизайн и многоступенчатый метод отбора. Сбор данных проводился методом индивидуального интервьюирования, специально разработанным для данного исследования опросником. Всего опрошено 716 респондентов. Анализ их осведомленности о болезни сахарного диабета, отношения к данному заболеванию, а также знаний по уходу за подобными больными показал, что большинство респондентов (98,9%) осведомлены о данной болезни, причем большая часть опрошенных (85,3%) знала, что является превенцией СД. Показатель уровня осведомленности респондентов о факторах риска возникновения СД, его клинических симптомах и осложнениях в 2016 г. был выше, чем в 2014 г. Осведомленность была больше у респондентов с более высоким общим образованием (62,6% по сравнению с 50,8%, p=0,05) и с более высоким экономическим доходом (62,2% по сравнению с 53,5%, p=0,03).

Интересной находкой можно назвать, что уровень осведомленности о сахарном диабете оказался выше в сельской местности Гардабанского района (50,3%) по сравнению с городом Гардабани (28,4%) (p<0,001). Большинство респондентов отметили, что информацию о данном заболевании в свое время получили от Общества Красного Креста. Исходя из вышеизложенного, вытекает необходимость проведения подобных программ, что, в свою очередь, приведет к улучшению превенции и контроля данного заболевания.

რეზიუმე

დიაბეტის შესახებ ცოდნის, დამოკიდებულებისა და პრაქტიკის შეფასება გარდაბნის რაიონში

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ჯანმრთელობის კვლევის კავშირი,თბილისი; საქართველოს წითელი ჯვრის საზოგადოება; დანიის წითელი ჯვრის საზოგადოება

წითელი ჯვრის საზოგადოებამ 2014-2016 წწ. განახორციელა პროექტი, რომლის მიზანი იყო შეესწავლა გარდაბნის რაიონში მოსახლეობის ინფორმირების საკითხი შაქრიანი დიაბეტის დაავადების შესახებ. გამოკვლევებისათვის გამოყენებულ იქნა ჯვარედინი დიზაინი და მრავალსაფეხურიანი შერჩევის მეთოდი. მონაცემთა შეგროვება წარმოებდა ინდივიდუალური გასაუბრების მეთოდით,სპეციალურად ამ კვლევისთვის შემუშავებული კითხვარით. გამოკითხული იყო 716 რესპონდენტი. რესპონდენტების დიაბეტის დაავადების ინფორმირების, დაავადების მიმართ დამოკიდებულების, აგრეთვე, ასეთი ავადმყოფების მოვლის ცოდნის ანალიზმა გამოავლინა,რომ რესპონდენტების უმრავლესობა (98.9%) საკმაოდ კარგად არის ინფორმირებული დაავადებისა და მისი პრევენციის შესახებ.

რესპონდენტების ინფორმირების დონე დაავადების განვითარების რისკ-ფაქტორებთან, კლინიკურ სიმპტომებთან და გართულებებთან დაკავშირებით 2016 წ. უფრო მაღალი აღმოჩნდა, ვიდრე 2014 წ. მაღალი ინტელექტითა და ეკონომიკური შესაძლებლობის რესპოდენტების ინფორმირებუ-ლება უფრო მაღალი აღმოჩნდა.

აღსანიშნავია ის ფაქტი,რომ გარდაბნის რაიონის მოსახლეობის ცნობიერება ამ დაავადების შესახებ (50.3%) უფრო მაღალია,ვიდრე ქ. გარდაბანში (28.4%), p<0.001. რესპონდენტების უმრავლესობა აღნიშნავს,რომ ინფორმაციას ღებულობდნენ წითელი ჯვრის საზოგადოების საშუალებით.

ყოველივე ზემოაღნიშნულიდან გამომდინარე, ავტორებს მიზანშეწონილად მიაჩნიათ ასეთი პროგრამების ჩატარება დაავადების პრევენციისა და კონტროლის გაუმჯობესებისათვის.

CLOSTRIDIAL PARAPROCTITIS WITH GAS GANGRENE OF FRONT-LATERAL ABDOMINAL WALLS AND NECROTIC FASCIO-MYOSITIS (CASE REPORT)

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Anaerobic clostridial infection is the most severe form of the paraproctitis. The incubation period is very short, from 3 to 6 hours, sometimes it lasts for 1-2 days. Clostridial infection spreads rapidly and induces gas gangrene, causes destruction of cells and other intermediate substances, destroys blood circulation [1].

Unlike other species of Clostridium (eg. Clostridium perfringens), Clostridium septicum is aerotolerant, mobile and releases four powerful toxins: alpha, beta, gamma and delta. Alpha toxin causes intravascular hemolysis, tissue necrosis, metabolic acidosis, renal failure and fever. Clostridium septicum affects muscular-fascial tissues, causing gas generation, tissue destruction in form of amorphous mass and decay. Skin takes a whitish color first and then brown and finally gray-black appearance as a result of tissue swelling.

Severe pain, severe general condition, tissue swelling and discoloration of the skin, crepitation or appearing the gas bubbles' "clacking" indicates to the development of the gas gangrene.

Clostridium infection can only be confirmed through bacteriological examination of the necrotizing tissue (muscle and/or fascia).

Case report. 38 years old man, who was treated at TSMU's First University Clinic in Proctologic, Surgery and Intensive Care Departments for the anaerobic clostridial paraproctitis, has developed gas gangrene with spread on abdominal front and lateral walls. A few days before he was operated on forhisacute, horseshoe shapes, pelviorectalperiproctitis. Infections were caused by gram-negative bacilli (Enterobacter cloacae). On the third day the patient was discharged from the clinic in improved condition.

Five days later, the patient entered the Emergency department at the University Clinic with a strong abdominal pain and bloating, nausea, the hectic fever (39-40^0), dizziness, with severe pain.

By physical examination the patient had a dry tongue, free swallowing, moderately swollen abdomen, the swollen front wall, skin was pale, painful on palpation. Liver was coming out of the costal arch, the spleen could not been examined, Bloomberg symptoms were negative. Minor serous-hemorrhagic discharge from the wound between perineum and anus channel has been observed with swollen edges, ampoules were free.

Abdominal and pelvic ultrasound, computer tomographic studies as well as clinical, biochemical, ELISA studies were conducted urgently which was followed by the study of markers determining infection and inflammation indicators. On the basis of these laboratory-instrmental investigation the gangrene with the spread on extraperitoneal basis on the abdominal front and lateral walls was suspected.

An emergency operation was conducted through endotracheal anesthesia: a tunnel between the abdominal front wall and the perineum was formed by a mid-lower extraperitoneal section on the abdominal front wall and the section towards the left groin and from the perineum through pelviorectal space. Infection center was opened where the stinking, bubble shaped, brownish mass poured out; necrosis-destruction fasciomyositis of the abdominal direct and indirect muscle was observed, a “dirty” gray color bleeding was not observed due to ischemic necrosis. There were conducted excision of damaged tissues, necrectomy, curettage, wide drainage, readjustment with antiseptics. Penrose drains were installed in the wound. Drainage tube was installed in the abdominal-perineal tunnel. Removed muscle-fascial biopsy material was sent for bacteriological examination. Bacterial inoculation was made for clostridium septicum 107/ml.

Treatment was continued in intense care department in the isolated room. The patient was connected to a respirator, the general condition was extremely severe, the patient experienced severe intoxication and septic shock (Apache scale - 38 points, C-reactive protein - 360 mg/l).

The intensive resuscitation measures (by infusion of protein components, hemotransfusion, etc.), wound drainage care was carried out with the antiseptics and oxygenation.

Antibiotic therapy regimen included vancomicin, imipenem, celastatin based on the results of bacteriological investigations of the urine, blood, wound material, sputum. The level of patient’s severity was evaluated in accordance with the Apache II scale and infection and inflammation markers including procalcitonin and CRP. Intraabdominal pressure monitoring was conducted on a regular basis.

Some additional surgical interventions (10 in total) were conducted with the CT control (eight CT scans in total) on the abdominal front and lateral surfaces, pararectic, Spiegel, the front and rear axillar lines. The drainage of the abscess through the wide sections, readjustment, necrectomy, fasciomyonecrectomy were performed. Permanent bacteriological monitoring of the materials taken from the wounds and shipped in special containers, in oxygen-free conditions, for the bacteriological examination. On 15th day tracheostomy was carried out.

Based on surgical and intensive care measures, positive dynamics was observed from the 25th day. The wounds cleaned out, hemodynamic data become stable, diuresis was adequate, patient moved on enteral feeding, defecation was managed using lactulose.

On the 28thday, the edges of wounds came closer to nodular stitches, on 30th day removed from inotropes support, on 33th days tracheostomy tube was removed, airways were free, patient was transferred to the surgical department. The wounds were totally sewed by nodular stitches. On 37th day the patient was discharged in satisfactory condition, with the proper instructions and recommendations.

We think that this clinical case is important for the following reasons: 1. This was an extremely severe form of anaerobic infection – clostridial paraproctitis with spontaneous gas gangrene, cellulitis, fasciomyositic necrosis, strong intoxication and septic shock on the abdominal front and lateral surfaces; 2. Presence of severe infection with Clostridium septicum, a rare and highly toxic gram-positive, spore forming, obligate anaerobic bacillus that progresses and migrates rapidly, affects all soft tissues (muscle, fascia), provokes four toxins, causes gas gangrene, intravascular hemolysis, tissue necrosis, septic shock. Mortality rate is 79-80% (2). 3. The positive clinical outcome was determined by a) Immediate surgery, wide excision of damaged tissues, necrectomy, curettage, wide drainage, readjustment, oxygenation through drainages, further additional surgical correction through CT control (necrectomy, readjustment, oxygenation, totally 10 operations), with wide bandages in operating block, with the support of anesthesiology; b) Identification of the damaged sections through properly constructed diagnostic algorithm, ultrasound and CT studies, infectious agent assessments, bacteriological monitoring carried out on the 1st-2nd-5th-7th-12th-15th-21st-25th days; C) Adequately evaluated markers for the evaluation of severity (Apache scale), infection (prokalcitonin), inflammation (CRP) and other markers; D) Rational antibiotic therapy with permanent susceptibility testing.

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SUMMARY

CLOSTRIDIAL PARAPROCTITIS WITH GAS GANGRENE OF FRONT-LATERAL ABDOMINAL WALLS AND NECROTIC FASCIO-MYOSITIS (CASE REPORT)

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Anaerobic clostridial infection is the most severe form of paraproctitis. The incubation period is very short, from 3 to 6 hours, sometimes lasting for 1-2 days. Clostridial infection spreads rapidly and induces gas gangrene, causes destruction of cells and other intermediate substances, and impedes blood circulation. This paper presents a case study of an extremely severe form of anaerobic infection with spontaneous gas gangrene, cellulitis, fasciomyositic necrosis, severe intoxication and septic shock on the abdominal front and lateral surfaces. This patient presented as infected with *Clostridium septicum*, a rare and highly toxic Gram-positive, spore-forming, obligate anaerobic bacillus that progresses and migrates rapidly, affecting all soft tissues (muscle, fascia), and produces four toxins which cause gas gangrene, intravascular hemolysis, tissue necrosis, and septic shock. The mortality rate is typically 80%. In this case study, a positive clinical outcome was achieved by aggressive identification of the microbe, appropriate and immediate therapy, and vigorous surgical intervention. Specifically, immediate surgery was conducted to ensure a wide excision of damaged tissues, necrectomy, curettage, wide drainage, readjustment, oxygenation through drainages, further additional surgical corrections through CT control with wide bandages in the operating area. Further, the diagnostic workup was thorough, identifying the microbe through a properly constructed diagnostic algorithm, ultrasound and CT studies, infectious agent assessments, and bacteriological monitoring carried out on the 1st-2nd-5th-7th-12th-15th-21st-25th days. Rational antibiotic therapy with permanent susceptibility testing informed the selection of an appropriate agent. Finally, markers for the evaluation of severity (Apache scale) were assessed, as they were for stage of infection (prokalcitonin), inflammation (CRP) and other indicators.

Keywords: clostridial paraproctitis, gas gangrene, anaerobic infection.

РЕЗЮМЕ

КЛОСТРИДИАЛЬНЫЙ ПАРАПРОКТИТ С ГАЗОВОЙ ГАНГРЕНОЙ ПЕРЕДНЕЙ И БОКОВЫХ БРЮШНЫХ СТЕНОК. НЕКРОТИЧЕСКИЙ ФАСЦИОМИОЗИТ (СЛУЧАЙ ИЗ ПРКТИКИ)

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Больной 38 лет поступил в приемное отделение Первой университетской клиники ТГМУ с жалобами на боли в животе и повышение температуры до 39-40) с ознобом. При осмотре - состояние тяжелое, выражены общие признаки интоксикации, живот вздут, при пальпации болезненный, на передней брюшной стенке - гиперемия. В анамнезе 5 дней назад произведена операция по поводу острого парапроктита. Больной срочно переведен в операционный блок; проведена операция – санация, удаление поврежденых тканей и дренирование. Больной продолжил лечение в реанимационном отделении. Курс лечения включал хирургиргическое лечение (в общей сложности 10 операций), реанимационные мероприятия (аппаратная поддержка дыхательных функций, противошоковая терапия), антибактериальную терапию и симптоматическую терапию. В ходе лечения применялись методы: компьютерной томографии, ультразвукового и рентгенологического исследования, лабораторные методы диагностики: бактериологические, биохимические тесты. Посредством бактериологического исследования поврежденных тканей выделен возбудитель инфекции - Clostridium septicum, весьма редкий, с выраженной токсичностью и спорообразующей способностью микроб, который характеризуется подвижностью и быстрым распространением, проявляет тропность к мягким тканям и продуцирует 4 экзотоксина, которые вызывают интраваскулярный гемостаз, некроз тканей и септический шок. Согласно данным литературы, смертность при этой инфекции составляет 70-80%. Положительный исход данного клинического случая, на наш взгляд, был обеспечен: вовремя, в неотложном режиме проведением операций (ломпастные разрезы, некрэктомия, санация и непрерывное дренирование поврежденного участка); все манипуляции проводились под контролем компьютерной томографиии с последующей хирургической коррекцией. В общей сложности выполнено 10 операций, ежедневные перевязки проводились в операционном блоке при анастезиологической поддержке.

Выздоровлению способствовали адекватно проведеные реанимационные мероприятия, оценка инфекционных маркеров в динамике. Адекватная антибактериальная терапия (вначале лечения деэскалационный режим, в последующем - смена антибактериальной терапии с учетом бактериологических исследований и антибиотикограммы).

რეზიუმე

კლოსტრიდიული პარაპროქტიტი მუცლის წინა და გვერდით კედლებზე გავრცელებული აიროვანი განგრენით, ნეკროზული ფასციომიოზიტით (კლინიკური შემთხვევა)

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თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, პირველი საუნივერსიტეტო კლინიკა, საქართველო

ანაერობული კლოსტრიდიული ინფექცია პარაპროქტიტის ყველაზე მძიმე ფორმაა. დაავადების საინკუბაციო პერიოდი ძალზე მოკლეა და შეადგენს 3-დან 6 საათს,ზოგჯერ 1-2 დღეს. კლოსტრიდიული ინფექცია ვრცელდება დიდი სისწრაფით და წარმოქმნის აიროვან განგრენას, იწვევს უჯრედების და სხვა შუალედური სუბსტანციების დესტრუქციას, სისხლის მიმოქცევის მოშლას. აღწერილი კლინიკური შემთხვევა საინტერესოა, ვინაიდან წარმოადგენს ანაერობული ინფექციის უკიდურესად მძიმე ფორმას – კლოსტრიდიულ პარაპროქტიტთან მუცლის წინა და გვერდით ზედაპირებზე გავრცელებული სპონტანური აიროვანი განგრენით, ცელულიტით, ნეკროზული ფასციომიოზიტით, ძლიერი ინტოქსიკაციით და სეპტიკური შოკით; ინფექციის გამომწვევი – Clostridium septicum, უიშვიათესი და უაღრესად ტოქსიური გრამდადებითი,სპორის წარმომქმნელი ობლიგატური ანაერობული ჩხირია, რომელიც სწრაფად პროგრესირებს და მიგრირებს,აზიანებს ყველა რბილქსოვილოვან სტრუქტურას (კუნთი, ფასცია), აპროდუცირებს ოთხ ტოქსინს, იწვევს აიროვან განგრენას, ინტრავასკულურ ჰემოლიზს, ქსოვილების ნეკროზს, სეპტიურ შოკს. ლეტალობის მაჩვენებელი შეადგენს 79-80%; კლინიკური შემთხვევის პოზიტიური გამოსავალი განპირობებული იყო დაუყოვნებლად (ურგენტულ რეჟიმში) წარმოებული ოპერაციით: ფართე განაკვეთებით დაზიანებული ქსოვილების ამოკვეთა, ნეკრექტომია, კიურიტაჟი, დრენირება, სანაცია, ოქსიგენაცია დრენაჟების საშუალებით. კტ კონტროლით, შემდგომი დამატებითი ქირურგიული კორექციით,გაფართოებული შეხვევებით საოპერაციო ბლოკში, ანესთეზიოლოგიური მხარდაჭერით; სწორად აგებული დიაგნოსტიკური ალგორითმით,ულტრაბგერითი და კომპიუტერილ-ტომოგრაფიული კვლევებით,დაზიანებული უბნების იდენტიფიკაციით, ინფექციური ფლორის ხარისხის შეფასებით,1–2–5-7-12-15-21-25 დღეებში ჩატარებული ბაქტერიოლოგიური მონიტორინგით; სიმძიმის ხარისხის (აპაჩის შკალა) ადეკვატურად წარმოებული მასშტაბური რეანიმაციული ღონისძიებებით, ინფექციის პროკალციტონინის, ანთების (CRP) მარკერების და სხვა კლინიკურ-ლაბორატორიული მაჩვენებლების გათვალისწინებით; ანტიბიოტიკოთერაპიით, მაღალი დოზებით (ტიენამი, ვანკომიცინი, კოლომიცინი, მეროპენიმი, კოლისტინი), ნიმუშის ანტიბაქტერიულ პრეპარატების მიმართ მგრძნობელობის პერმანენტული განსაზღვრით.

CONFLICT IN EASTERN UKRAINE: STRATEGY FOR TUBERCULOSIS

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The aim of this paper is to describe the health service delivery challenges and potential humanitarian interventions in the areas affected by the conflict in Eastern Ukraine

Ukraine with its population of 45 million is one of the former Soviet Union (FSU) republics located in the Eastern Europe [1]. Ukraine as well as other FSU countries like Georgia and Moldova has been trying to establish closer ties with the western world since gaining independence in early 90es of the last century. In the end of 2013, Ukraine's pro-Russian president suddenly refused to sign an agreement with the European Union. This led to mass protests and clashes between pro- and anti-government forces. In February 2014 pro-Russian president left the country

and forces favoring closer ties with the western countries came into power [2]. These events were followed by the military conflict, which started in March, 2014 affecting up to 5 million people in the region of Donbass [3].

There are several state and non-state players as well as layers of root causes intersecting in this conflict. It has a global and internal dimension. Therefore, this conflict is a hybrid of both an old and a new war. On a global level Russia is trying to defend its "legitimate" area of influence - FSU countries and Russian speaking people from the western influence [2]. According to the current Russian leader "*The breakup of the Soviet Union* was the *greatest* geopolitical tragedy of the 20th century." [4]. On an internal level weak government, fragile democratic institutions and slow economic growth were the major factors fueling the tensions between primarily pro-Russian and pro-western groups of people [2]. Greed-driven influential oligarchs and corrupt politicians have created and deepened divisive lines between the groups of people with different linguistic preferences (Russian or Ukrainian). Through these years they have been promoting grievances among Ukrainian citizens with Russian language as their mother tongue. Political elites have failed to reconcile the different groups of people by offering better future through effective government, modernized economy and equity irrespective of linguistic preferences.

Political instability worsened drastically Ukraine's economic situation with local currency depreciating against US dollar by 50% and inflation reaching 25% [5]. Economic hardship was aggravated by armed conflict on the territory of self-proclaimed "newly independent states" in Donbass forcing populations to flee their homes either to government controlled territories or to neighboring countries of Russia and Belarus. At present fragile ceasefire is in place, but incidents of shelling and fighting are still reported. As of March, 2015 according to the Ukraine's Ministry of Social policy 1.1 million IDPs are already registered on the government controlled territories [5]. People in conflict affected areas are in need of safe shelter and food. Ukraine has a Semashko style centralized healthcare system offering guaranteed healthcare services at the point of use for everyone. Despite, cross-sectional survey conducted before the conflict initiation, revealed that almost 100% of interviewed Ukrainians made out-of-pocket payment for outpatient, inpatient care and pharmaceuticals [6]. Both sides of conflict were violating International Humanitarian as well as War Laws endangering civilians, healthcare and humanitarian personnel [5]. Healthcare system is in desperate need of reconstruction and adequate staffing in the areas where fighting took place. Due to water supply cuts and sanitation deterioration cases of viral hepatitis A increased. Children population in war affected areas was not getting vaccination coverage offered through state immunization calendar which created the risk of vaccine-preventable diseases re-emergence. People affected by chronic non-communicable and communicable diseases like cancer, diabetes, TB and HIV are not getting adequate healthcare due to healthcare staff absenteeism, inadequate stocks of drugs and medical supplies, and lack of financial means to cover healthcare and drugs [5].

The focus of this paper is tuberculosis (TB) among IDPs and improved health care delivery for IDPs with TB. TB is an infectious disease spread by TB bacilli. It affects primarily lungs but other organs can be affected as well. People are infected by inhaling the air with bacilli droplets [7]. Overcrowding, inadequate ventilation and poor nutritional status are the risk factors for acquiring and developing disease. In case of inadequate treatment bacilli become resistant to at least 2 drugs from the panel of first line antibiotics and more complex (with second line antibiotics) and longer treatment is needed to reach the disease resolution. According to WHO Ukraine is not a high TB burden country but it is listed among 27 countries with highest Multidrug-resistant TB (MDR-TB) rates. Re-emergence of TB as a major public health problem which is characteristic for the FSU countries experiencing social and economic hardship and break down of the public health infrastructure after the dissolution of the Soviet Union. According to WHO Compared to other countries TB patients from the FSU countries have ten times more chances to have MDR-TB [8]. Tuberculosis ranked 18th in 1990 went up to 9th place in 2010 in the ranks for years of life lost (YLLs) due to premature mortality in Ukraine [9]. In 2013, most cases of extensively Drug-Resistant (XDR) TB were reported in Ukraine. In case of XDR TB cases both 1st and 2nd line treatments are useless. Thus, XDR-TB treatment is really limited. Additionally, Ukraine is ranked second among European countries with TB and HIV co-infection [8]. Even before conflict emergence Ukraine has struggled to provide adequate healthcare delivery due to outdated case detection and treatment regimens. In general Tuberculosis treatment due to its rate of side-effects is quite complex and requires patience and dedication from both patients and healthcare personnel in order to avoid treatment interruption contributing to the emergence of MDR and XDR TB bacilli. Direct observation therapy (DOT) is used to supervise and help patients to continue treatment and reach the recovery, but so far Ukraine had only piloted this approach in selected sites. Before conflict provision of adequate number of quality-ensured anti-TB drugs was an issue due to the corruption and inefficient government [10].

In-depth IDP health status and health system analysis should be conducted in order to elaborate a detailed plan of action for TB in conflict affected areas. Data on proportional morbidity and mortality should be assessed in order to have a general understanding of the IDPs' health status. Additionally TB incidence and prevalence as well as specific mortality rates should be assessed including the

rates for MDR as well as XDR –TB. Then using Sphere minimum standards for humanitarian response and clinical care provision in humanitarian crisis data on TB population survival needs should be collected - Are there sufficient food supplies per each IDP to ensure adequate nutrition, i.e. 2,100 kilocalories per person per day? Do they have access to the sufficient quantities of safe water? Do they have shelter at all? Is the shelter overcrowded? Is there enough space to isolate TB, MDR and XDR-TB cases whom have active diseases and are able to transmit the disease to the healthy people? In order to plan for interventions in TB healthcare delivery the status of current TB and primary healthcare facilities should be evaluated. Are they still functional in the areas were military hostilities took place? What proportion of medical staff is still in place there? Are they qualified to provide healthcare services to the TB patients - Do they have specialized training in TB diagnosis and treatment? do they have laboratory equipment and supplies essential for TB and MDR TB rapid diagnosis? Are ratios of existing medical facilities and healthcare personnel to the IDP population meeting Sphere minimum standards of healthcare provision? In order to gather this information both primary and secondary data should be used. Both quantitative and qualitative data is useful to assess the situation. Data from state, local and international TB NGOs, advocacy groups, humanitarian organizations, and IDPs should be collected in order to have an understanding of the problem magnitude and also to be aware of already planned or ongoing interventions to ensure coordination between state or non-state actors' similar activities.

In order to answer all the questions above TB case registration system set-up is required. Both existing healthcare setting (TB centers, primary health care centers) and special mobile units of trained healthcare personnel in order to gather TB prevalence data can be used. Ideally, unified electronic registry for both stationery and mobile settings should be developed allowing case registration cross-checking. Mobile units should be fully funded so that provided care is free of charge. In order to ensure healthcare system long-term strengthening medical and laboratory personnel for the mobile units should be recruited from both primary healthcare and specialized TB treatment centers. Special schedule of visits should be developed to ensure that all IDP shelters are covered. Additionally, mobile units will be able to access the sites were health infrastructure is disrupted and provide both case registration as well as treatment. In order to increase TB awareness among IDPs state and rebel government structures as well as IDP community leaders should be engaged in awareness campaigns. IDPs should be informed of the sites where they can seek care. This will also increase TB detection rate. Linking TB treatment (DOT) with the operations of the mobile units and the existing healthcare settings will be very useful. Those IDPs whom already know that they are TB positive should be encouraged to continue treatment with nutritional or financial incentives given at the DOT point of care (either stationary or mobile). Additionally, the mobile units as well as the TB and primary healthcare settings should be equipped with rapid MDR-TB diagnostic capacities. This will ensure timely detection of MDR-TB cases in order to isolate them from the other IDPs. Both state and rebel governments should be convinced that special shelters should be allocated to the active TB cases and MDR-TB cases in order to curb transmission. Based on the collected data and available resources clear set of objectives and relevant indicators should be compiled to monitor the implementation and evaluate these interventions after their implementation. Main objectives of these interventions will be reduced TB and MDR TB incidence and mortality rates, improved treatment completion rates. Main indicators will be reduction of the incident and lethal cases by certain percent for a given time period. Another indicator will be an increase in the number of cases with treatment completion by certain percent. In order to meet the above mentioned goals state and rebel government, international and local donor organizations should have both political and financial commitment to fight against TB; quality diagnostic capacity, quality drugs and quality treatment regimens should be available through qualified medical staff. Monitoring should take place at pre-determined time points and if necessary ad hoc monitoring may be required as well (e.g.: in case of epidemics). Such monitoring system allows the project managers and the sponsors to be aware of the ongoing situation. After completion of the project collected data both quantitative and qualitative should be analyzed and interpreted. One of the major reasons of such evaluation is to learn about major success and failure stories of the project. Quite often this evaluation is sponsor driven. Ideally, availability of the laboratory driven TB and rapid MDR TB diagnostic capacity, internationally approved standard treatment regimens and DOT for every TB patient should be the part of the national TB strategy in Ukraine. This project can be regarded as a pilot project that can be expanded to other non-conflict areas. Expansion of the DOT may face challenges in the post-conflict situation since TB specialists society may not like if primary healthcare setting is kept involved in the anti-TB strategy. Additionally, many of them may not be convinced of the benefits of new drug regimens over the old ones and their professional recommendations may influence policy making. Due to high corruption rate and inefficient government policy makers may not have adequate political will to upgrade existing anti-TB system and even if funding is available it may be used improperly. Communicating facts about the disease transmission and individual stories of patients whom achieved treatment success due to the DOT with the government, TB and primary healthcare professional societies, and TB patient advocacy groups may help to engage major policy makers in favor of spreading more efficient WHO advocated TB control program in the whole country.

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SUMMARY

CONFLICT IN EASTERN UKRAINE: STRATEGY FOR TUBERCULOSIS

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This root cause analysis concerns the conflict in the Eastern Ukraine and its impact on healthcare delivery in the context of treating internally displaced persons (IDPs). Inadequate treatment of tuberculosis (TB) was selected as a major topic for intervention planning in conflict areas in Ukraine. With respect to treating TB among IDPs, rapid diagnosis and adequate nutrition and shelter are important components of care and disease control. The DOT, supported by trained primary healthcare providers equipped with rapid MDR TB diagnostic capacities, need to provide appropriate shelter and nutrition to IDPs. In addition to active disease management, this paper discusses the important role of ongoing project monitoring and communicating evaluation findings with all the major stakeholders shaping the national TB strategy in Ukraine. A comprehensive strategy is essential for successful transitioning and re-structuring of TB healthcare delivery both during after conflict resolution.

Keywords: tuberculosis, internally displaced persons, Ukraine.

РЕЗЮМЕ

КОНФЛИКТ В УКРАИНЕ: СТРАТЕГИЯ ПО БОРЬБЕ С ТУБЕРКУЛЕЗОМ

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В статье представлен краткий анализ основных медицинских потребностей вынужденных переселенцев из зоны конфликта в Восточной Украине.

Целью исследования явилось обсуждение поставки медицинских услуг для вынужденных переселенцев, больных туберкулезом, и планируемых мероприятий в пострадавших от конфликта районах Украины. В целях борьбы с туберкулезом среди вынужденных переселенцев обсуждены вопросы внедрения стратегии терапии "под непосредственным наблюдением" при поддержке первичного медико-санитарного персонала, своевременного выявления и диагностики заболевания, привлечения всех поставщиков медицинских услуг, а также предоставления соответствующих убежищ и адекватного питания для вынужденных переселенцев. В статье обсуждается необходимость своевременного информирования о результатах осуществляемого в регионе противотуберкулезного проекта всех заинтересованных лиц, которые в будущем ответственны за формирование национальной стратегии лечения туберкулеза в Украине.

რეზიუმე

კონფლიქტი უკრაინაში: ტუბერკულოზის საწინააღმდეგო სტრატეგია

თ. ქუჩულორია, თ. ახვლედიანი, ნ. ახვლედიანი

კვლევითი და საგანმანათლებლო ჯგუფი საქართველოში, თბილისი, საქართველო

აღნიშნულ სტატიაში განხილულია აღმოსავლეთ უკრაინაში კონფლიქტის დროს იძულებით გადაადგილებულ პირთა ძირეული საჭიროებები

და კონფლიქტის პირობებში სამედიცინო მომსახურების თავისებურებები. კვლევის ძირითად მიზანს წარმოადგენს ტუბერკულოზის საწინააღმდეგო სამედიცინო მომსახურების ნაკლოვანებების გამოვლენა უკრაინის კონფლიქტით დაზარალებულ რეგიონებში. სტატიაში განხილულია ისეთი საკითხები როგორიცაა "თერაპია უშუალო დაკვირვების ქვეშ" სტრატეგიის განხორციელება სათანადო მომზადების მქონე პირველადი სამედიცინო რგოლის სპეციალისტების ჩართულობით, სწრაფი და თანამედროვე დიაგნოსტიკის მეთოდების ხელმისაწვდომობის გაზრდით, დაავადებულთათვის სათანადო თავშესაფრებისა და საკვების უზრუნველყოფით. სტატიაში განხილულია კონფლიქტით დაზარალებულ რეგიონებში განხორციელებული პროექტის შედეგებით იმ დაინტერესებული პირების ინფორმირების საკითხი, რომლებიც განავითარებენ ტუბერკულოზის საწინააღმდეგო ეროვნულ გეგმას,როგორც წარმატებულ ინსტრუმენტს კონფლიქტის შემდგომ პერიოდში ტუბერკულოზის საწინააღმდეგო სისტემის გარდაქმნისათვის.

IMPORTANT ASPECT OF HEALTH CARE ASSOCIATED INFECTIONS IN GEORGIA WITH THE FOCUS ON VENTILATOR-ASSOCIATED PNEUMONIA (REVIEW)

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Health care associated infections (HAIs) are infections acquired in healthcare setting and constitute the most frequent adverse event in health-care delivery worldwide [16]. Each year, approximately 4 100 000 patients are estimated to acquire a healthcare-associated infection in the European Union. The number of deaths occurring as a direct consequence of these infections is estimated to be at least 37 000 [6]. The respiratory tract infections have been recognized as the most common healthcare-associated infection in acute hospitals [5]. Ventilator-associated pneumonia (VAP) is a major cause of mortality and morbidity in critical care patients [1] and we will focus on this pathology. VAP is defined as pneumonia occurring more than 48 hours after the patient has been intubated and received mechanical ventilation [10]. Approximately 20% of ICU patients are affected by VAP [2].

After the collapse of the Soviet Union, per capita public expenditures on health in Georgia rapidly declined from around US $13.00 in 1990 to less than US $1.00 in 1994 [14]. Georgia has made significant efforts to adapt the health policy to the new environment and there has been a clear progress but it still remains a developing country with limited resources. Hospital acquired infections has been a neglected problem in our healthcare sector. The country still does not have any HAI surveillance in place. The hospitals have no incentive to report their HAI cases because there is no requirement to report and if reported, the HAI cases are not covered by insurance. Besides, there is a large gap in knowledge at every level. Butsashvili at Al. reports how little health care professionals know about the HAI and infection control: 31.4% of health care workers never received any type of training in HAI. The main education activities reported by those who had received training were just short seminars or presentations. Even though this article focuses on maternity clinics, the similar situation is in all the healthcare facilities [3].

Regarding the most significant pathogen causing VAP, since there is no national surveillance and no official data available, we can only speculate about it, based on the knowledge in the medical community and personal communications with intensive care unit (ICU) and infectious diseases (ID) physicians as well as on very few publications in this field. *Klebsiella pneumoniae* is probably the most common cause of VAP in Georgia. According to Kandelaki et al., *klebsiella* is the most commonly isolated microorganism (26.5%) in their patient setting [9].

Klebsiella is a gram-negative bacterium, member of the family of enterobacteriaceae. It can cause different types of healthcare-associated infections, including pneumonia [11]. This pathogen is usually found in human intestines but in healthy people it normally does not cause disease. In healthcare settings, *Klebsiella* bacteria can be spread through person-to-person contact – from patient to patient via contaminated hands of healthcare personnel, or other persons, or less commonly by contaminated environment [11].

As mentioned above, the risk of getting a respiratory infection, including with *Klebsiella pneumoniae* is increased when the patient is on mechanical ventilation. In Georgia, until recently, obsolete Soviet-time mechanical ventilation

equipment was being used, which increases the risk of complications, including VAP.

It is a common knowledge that hands should be washed and gloves should be used when taking care of the ICU patient. But these practices are often neglected. When the medical staff is not receiving any training about infection control for years and when this problem is not recognized, like it is in Georgia, hospital infection becomes something typical and expected at the hospital setting, especially in patients who spend a long time at the ICU. When the nursing staff is not adequate at the ICU, it becomes almost impossible to follow infection control rules. In Georgia, the medical personnel, especially nurses, are underpaid. They have no incentive or encouragement to perform their duties and no professional development opportunities to refresh knowledge. So, unqualified staff is definitely a risk for developing these infections.

Poor mouth Hygiene can contribute to the development of HAI pneumonia, including VAP. Besides, it is very important to control sedation. Oversedation can lengthen ventilator time, which raises the risk of VAP. Undersedation, on the other hand, may cause patients to try to self-extubate or injure themselves and others [2].

One of the risks is the limited space in the ICU. If the patient has VAP, it is important to move him to an isolated ward and preferably have a dedicated nurse, taking care of him. This will protect other patients.

Poor laboratory capacity of the hospital creates a risk of complication of HAIs and makes it difficult to prescribe an adequate antibiotic treatment. The laboratory should be able to isolate the pathogen, when possible, and do the antibiotic susceptibility testing. If resistance pattern is not defined correctly, the adequate antibiotic treatment will not be prescribed. At the same time, doctors should be able to interpret results received from the laboratory.

The limited financial resources is a risk factor for developing VAP. When sterile gloves are not available in the hospital, the knowledge of personnel about what should be done is of little help.

Patients at the ICU spend a lot of time in the same supine position which contributes to developing VAP.

The infection of lower respiratory tract often takes place through the passage of oropharyngeal contents into the trachea. To control this process, it is necessary to regulate the cuff pressure. Maintaining cuff pressure of endotraeal tubes at >=20 mm Hg reduces nosocomial pneumonia by minimizing the changes of getting oropharyngeal content into the trachea [2].

All the above-mentioned factors have a role in developing VAP at the ICU. There are no risk factors specific for VAP caused by *Klebsiella*.

Infection control strategy to reduce the burden of HAIs, including VAP caused by klebsiella
Controlling *klebsiella* infection is not different from controlling every other cause of VAP in the ICU. First of all, it is important to recognize the problem of VAP in the ICU and be determined to tackle it. Nowadays, hospitals in Georgia have budget and have basic infrastructure to put in place at least a very simple infection control plan. It is important that the willingness to control this infection comes from the management and even higher – from the Ministry of Health. The surveillance system is necessary in order to have a true picture of these infections, to evaluate whether or not *klebsiella* really is a major responsible pathogen for VAP in the given clinic.

In order to implement an infection control plan, an infection control team should be established, with an infection control nurse, whose full time job will be to gather information, observe, conduct audits, have contact with laboratory, etc. For the successful implementation of the infection control plan, the laboratory should be well trained and equipped.

Intensive training at all levels is very important. I think it would be reasonable to invite colleagues from countries with well established infection control strategy and successful experience of reducing VAP. Also, it is critical to mobilize experts in the country and engage them as trainers. It is important to draw everybody's attention on the seriousness of the problem, give numbers, other country's examples, and set realistic concrete goals. First goal would be to clearly define what percentage of patients on mechanical ventilation develop VAP and when it develops, how many days after starting mechanical ventilation; describe the spectrum of bacteria causing VAP. We are only giving a hypothesis that most of the VAP cases are caused by *Klebsiella pneumonia* but it is just a speculation until the robust surveillance network proves it or rejects it.

Sometimes there is scepticism among our colleagues that our country is so backwards that nothing makes sense and nothing can be changed. It is assumed that everything needs huge financial resources that we can not afford at our clinics. It is important to convince the whole multidisciplinary team involved in the infection control that it is not so expensive to have a simple infection control plan. What is important is determination and taking responsibility, believing that the team effort can make marked changes.

Specific procedures and activities:
It is well-known that the semi-recumbent position is associated with a lower risk of VAP and oral rather than nasal intubation has a lower risk of VAP [10]. This practice is already established in the ICUs in Georgia and it should be continued [2].

The proper maintenance, cleaning and decontamination of the ventilator equipment should be done by the staff [10]. Some VAP is contracted from inhalation of bacteria through the ventilator circuit and may be a result of contaminated aerosols or suction catheters. Traditionally, ventilator circuit changes have been on a regular schedule and recommendation often was to do it daily. However, the data examining this practice reveal that there is no benefit from changing the circuit on a regular basis, and the present recommendations are to only change the circuit when soiled [2].

It would be reasonable to offer a simple and easy to follow guideline for the ICU to control VAP:
1. First and foremost, avoid risk – intubate only if absolutely necessary
2. Reduce aspiration. For this purpose, keep patient in a semi-recumbent position; perform subglotic drainage;
3. Reduce bacterial load by using antibiotics;
4. Implement bundles (see below).

The bundle in HAI control is specifically selected care elements selected from evidence-based guidelines and when implemented together, provide improved outcomes compared to individual elements alone [11]. The bundle should not have too many components, otherwise it will be difficult to implement it. A simple bundle can include the following components:
- and hygiene
- cuff pressure control
- oral care (oral decontamination with chlorhexidine reduces incidence of VAP presumably reducing oropharyngeal colonization)
- and sedation control.

Hand hygiene is simple and universally known necessary component for the control of infection both in the community and the hospital level. WHO has hand hygiene guidelines which distinguish 5 moments for hand hygiene: 1- before patient contact, 2- before aseptic task, 3- after body fluid exposure risk, 4 – after patient contact, and 5 – after contact with patient environment. It is important that staff is fully engaged in implementing hand hygiene rules, have a mechanism to delicately remind each other, and it is important to have easily accessible hand hygiene products at the point-of-care [7].

These types of measures for prevention of VAP are well-documented and evidence-based. Significant improvements in outcomes can be achieved with high compliance in implementing these measures [4,12]. In one study, the VAP incidence rates before and after implementation of the education program was reduced by 51% [15].

The VAP prevention plan is very important. However, to achieve success we need to make sure that the plan is implemented in due manner. The staff compliance with proper infection control methods is often poor and inconsistent. Therefore, it is very important to have a right approach to ensure compliance. Significant improvements in outcomes can be achieved with high compliance in implementing a care bundle package for VAP prevention [12].

There are key success factors, so called 4E [8]: Engage, educate, execute and evaluate.
1. Engagement: the infection control plan should involve a multidisciplinary team. And the whole team should be well connected to each other. Local champions should be engaged and their example followed. Peer networks should be utilized.
2. Educate: training should be a continuous process. It is very important to realize from the very beginning that the hospital infection including VAP is a medical error and not something unavoidable at the ICU. Developing evidence based guidelines, bundles and protocols, conformed to each ICU;
3. Execute: care procedures should be standardized to make sure that everybody is doing the same thing. The staff should be learning from examples. Every case of infection should be discussed and lessons should be learned.
4. Evaluate: performance should be measured. Infection control team will play a key role in this. Frequent audits and monitoring should be conducted by the infection control nurse. It is important to realize that the purpose of audits and evaluation is not to punish. The mindset which encourages hiding or not even recognizing the problem should be changed. It is important that audits and monitoring give quick feedback and it should be available for the whole team.

When thinking about challenges of implementation of such a program, the main difficulty, in our opinion, will be a laboratory support. Unfortunately, we have a shortage of experienced microbiologists and laboratories are often not well equipped. Or sometimes, the equipment is there but consumables are not regularly supplied. Another potential challenge will be some senior doctors who are often resistant to changes and training. Also, it will be a challenge to convince hospital managers that it is really worthwhile to allocate resources in HAI control even though the cost of HAI is much higher than the cost of HAI control program.

It is very promising that the ministry of Health of Georgia recognizes the necessity of having HAI surveillance. Recently, it has become one of the priorities and the National Centre for Disease Control and Public Health of Georgia is working on an establishment of surveillance network, taking consultations with CDC and ECDC. Hopefully, soon Georgia will have its first national surveillance system on HAI.

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SUMMARY

IMPORTANT ASPECT OF HEALTH CARE ASSOCIATED INFECTIONS IN GEORGIA WITH THE FOCUS ON VENTILATOR-ASSOCIATED PNEUMONIA (REVIEW)

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Health care associated infections are the most frequent adverse event accompanying healthcare delivery worldwide. Of these, respiratory tract infections, including ventilator-associated pneumonia (VAP), have been recognized as the most common infections in acute hospitals. Sparse anecdotal and epidemiologic data from intensive care units (ICU) and infectious diseases physicians, as well as several publications in this field, suggest that the etiology of VAP in Georgia is most likely *Klebsiella pneumoniae*. This review article discusses the challenges of infection control in the Georgian health care system, with a focus on VAP. We present the most significant risk factors as well as potential strategies to remediate infection control practices and reduce the prevalence of VAP.

Keywords: ventilator-associated pneumonia, Health care associated infections.

РЕЗЮМЕ

ИНФЕКЦИИ, СВЯЗАННЫЕ С ОКАЗАНИЕМ МЕДИЦИНСКОЙ ПОМОЩИ В ГРУЗИИ, С АКЦЕНТОМ НА ПОСТВЕНТИЛЯЦИОННУЮ ПНЕВМОНИЮ (ОБЗОР)

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Заболеваемость инфекциями, связанными с оказанием медицинской помощи (ИСМП), происходит во время оказания медицинской помощи и представляет собой самое частое побочное явление в мире. Инфекции респираторного тракта, включая поствентиляционную пневмонию (ПВП) признаны самыми частыми ИСМП в больницах неотложной терапии. В секторе здравоохранения Грузии ИСМП длительное время не признавалась как проблема. В результате изучения ретроспективных и текущих научных источников выявлено, что самой частой причиной ПВП в Грузии является *Klebsiella pneumoniae*. Обзорная статья рассматривает задачи, связанные с инфекционным контролем в системе

здравоохранения Грузии, с акцентом на ПВП; описаны основные риск-факторы и разработана потенциальная стратегия сокращения числа случаев неблагоприятных исходов, связанных с ИСМП.

რეზიუმე

საქართველოში სამედიცინო მომსახურებასთან ასოცირებული ინფექციების მნიშვნელოვანი ასპექტები,აქცენტით პოსტვენტილაციურ პნევმონიაზე (მიმოხილვა)

თ. ახვლედიანი, ნ. ახვლედიანი, თ. ქუჩულორია

კვლევითი და საგანმანათლებლო ჯგუფი საქართველოში, თბილისი, საქართველო

სამედიცინო მომსახურებასთან ასოცირებული ინფექციებით (სმაი) დასნებოვნება ხდება სამედიცინო მომსახურების პირობებში და წარმოადგენს მსოფლიოში სამედიცინო მომსახურების თანმხლებ ყველაზე ხშირ გვერდით მოვლენას. სასუნთქი გზების ინფექციები, მათ შორის, პოსტვენტილაციური პნევმონია (პვპ), მიჩნეულია ყველაზე ხშირ სამედიცინო მომსახურებასთან ასოცირებულ ინფექციად გადაუდებელი თერაპიის პროფილის საავადმყოფოებში. საქართველოს ჯანდაცვის სექტორში სმაი, როგორც პრობლემა, დიდი ხნის მანძილზე უგულვებელყოფილი იყო. სამედიცინო საზოგადოებაში არსებული ცოდნის, ასევე ინტენსიური თერაპიის ექიმებთან და ინფექციონისტებთან პირადი საუბრების და არსებული რეტროსპექტული და მიმდინარე სამეცნიერო ლიტერატურის შესწავლის საფიძველზე სავარაუდოა,რომ საქართველოში პვპ-ის ყველაზე ხშირ მიზეზს წარმოადგენს Klebsiella pneumoniae. სტატიაში განიხილულია საქართველოს ჯანდაცვის სისტემაში ინფექციის კონტროლთან დაკავშირებული გამოწვევები პვპ-ზე განსაკუთრებული აქცენტით, აღწერილია ყველაზე მნიშვნელოვანი რისკ-ფაქტორები და პოტენციური სტრატეგია სმაი-ის ტვირთის შემცირებისთვის.

IMMUNOGENETIC FACTORS INFLUENCING CLINICAL COURSE OF HCV INFECTION (REVIEW)

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Hepatitis C virus (HCV) infection remains one of the most important blood-borne diseases worldwide with about 130-170 million people chronically infected with hepatitis C virus, and more than 350 000 people die from hepatitis C-related liver diseases each year [1]. HCV is endemic in most areas of the world. Infection with HCV becomes chronic in approximately 80% of cases. Chronic infections of hepatitis C can, and often do, lead to end-stage liver diseases such as cirrhosis and hepatocellular carcinoma; in fact, HCV infection is the leading chronic blood borne infection in the US and several other countries. The great variation in prevalence of HCV infection is dependent upon the various risk factors for infection. The highest prevalence of HCV infection is found among those with large or repeated direct percutaneous exposures to blood (injecting drug users, recipients of transfusions from HCV-positive donors, and persons with hemophilia). Moderate or low prevalence is found among persons with smaller or inapparent percutaneous or mucosal exposure, such as hemodialysis patients, health care workers, and persons with evidence of high-risk sexual practices. As the prevalence of HCV rises, there is a corresponding increase in the number of people in all risk groups [1].

HCV is primarily transmitted parenterally in adulthood by intravenous drug use, blood transfusion, or other medically-related parenteral exposures [2]; HCV is rarely transmitted through the placenta, breast-feeding, or sexual contact [3]. HCV RNA can be detected in blood, saliva, tears, seminal fluid, and cerebrospinal fluid. The main route of transmission of HCV virus is transfusion. Prior to 1987, when the screening of blood donations for HCV began, 17% of HCV infections in the USA were caused by transfusion [4]. In developed countries, intravenous drug use is the major risk factor for HCV infection; other parenteral routes include hemodialysis, organ transplantation, and tattooing.

The prevalence of viral hepatitis in Georgia is high. A study of blood donors in Georgia found very high prevalence of HCV (6.9%) and HBV (3.4%) [5]. The prevalence rates are much higher in high-risk groups. In a study of intravenous drug users, 70.4 % were positive for HCV [6]. The risk factors for the spread of viral hepatitis are high prevalence of intravenous drug use, low infection control awareness among health care workers, lack of disposable materials in health care settings, lack of disinfectants and sterilization equipment. There are cases of outbreaks of hepatitis in health care facilities.

Currently drug addiction and drug use are highly prevalent in the country [7]. Different studies conducted among drug users in Georgia and other Former Soviet countries reported various types of intravenous drug use behavior potentially related to transmission of blood borne viruses - high prevalence (about 69%) of needle and syringe sharing [8], including sharing for expressing trust to the friends, refilling syringes from larger syringes provided by dealers, adding human blood to drug during manufacturing, using newly prepared drug, such as Koknar extracted from poppy straw from common bowl etc. According to the data of Georgian Institute of Narcology, the most frequently used drug is heroin.

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Only a minority (about 20%) of HCV acutely infected patients present with clinical symptoms, having greater chance of eliminating the virus, whereas an asymptomatic course of infection usually results in chronic HCV. Host immune responses to HCV play an important role in both viral control as well as disease pathogenesis. As many other viruses, HCV does not kill the cells if infects, but triggers an immune-mediated inflammatory response that either rapidly clears the infection or slowly destroys the liver. The innate immune system is the first line of defense in response to pathogen infection. Natural Killer (NK) cells are important component of the innate immune system; they participate in early responses against virally infected or transformed cells by production of cytokines and direct cytotoxicity. The HLA molecules present the viral peptide to CD8 and CD4 T cells. The virus somehow escapes T cell recognition and activation (Fig.).

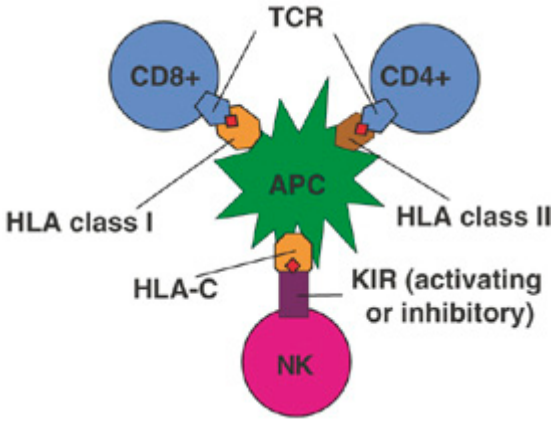

Fig. Interactions of APC, NK, CD4+ and CD8+ cells

Populations of NK-cells are functionally heterogeneous as the result of differential cell surface expression of various inhibitory and activating receptors. Three main receptor families are: KIR, C-type lectin, and NCR (Natural cytotoxicity receptors). HLA and KIR are the most diverse, polymorphic genetic systems in the human genome, both molecules are associated with immune response, therefore, strong selection, and viral infections among them can shape their distribution [9].

HLA complex has long been shown to be an important host genetic risk factor in infectious diseases, their association in susceptibility or protection with more than 50 different diseases is well documented and published during more than 4 decades. Today more than ten thousands (Class I (A, B, C) and Class II (DR, DQ, DP) different alleles are described worldwide. Unfortunately, very little is known about their distribution in Georgian population, which should be useful for finding unrelated bone marrow donor, because those are the same genes that have to be matched in patient/donor setting.

KIR system is relatively new, and the discovery of an unexpected level of diversity has led to search for their role in human disease. Their polymorphism and binding to HLA highlights their importance in physiopathology of numerous diseases, and mostly in viral models.

The human KIR gene family is both polygenic and polymorphic. There are at least 14 closely positioned functional human KIR loci and two pseudogenes (2DP1 and 3DP1) located on chromosome 19q. The inhibitory receptor for HLA-C (lys80) is KIR2DL1, and those for HLA-C (asn80) are KIR2DL2 and KIR2DL3. Both HLA-A and HLA-B alleles provide additional epitopes for interactions with primarily KIR3DL2 and KIR3DL1 receptors, respectively. For most activating KIR receptors the ligand is still unknown [9, 10, 11].

Variability of KIR haplotypes are mostly related with the presence or absence of activating KIR, while the most of inhibitory KIR are present in majority of haplotypes [12]. Several studies have shown association of HLA/KIR profiles with the development of different diseases – both infectious, such as HIV [13, 14], HCV [15], HPV [16, 17], CMV [18], HBV [19] and non-infectious [reviewed in 9]. In a study of African female sex workers resistance to HIV-1 infection was found to be associated with the absence of HLA ligands for inhibitory KIR [20].

In some cases HCV infection does not develop in individuals exposed to HCV and they do not seroconvert [21]. This can be explained by the complete clearance of HCV by the innate immune responses. Existence of such a phenomenon is supported by the data of recent studies showing presence of INF-gamma secreting specific T cells to the HCV structural antigens [22] among HCV negative individuals. It is difficult to "catch" these cases for investigation because such HCV acute infections cause almost no clinical symptoms and leave no evidence of their occurrence [9]. Those cases where adaptive immune responses are developed and HCV-specific antibodies are produced can be evaluated more straightforwardly. Approximately 20% of HCV infected persons develop spontaneous clearance of HCV involving genetic and immunologic mechanisms not completely understood at this moment. Several stud-

ies support the fact that vigorous HCV-specific CD4+ and CD8+ T cell responses are critical for the clearance of HCV infection [23], but there remains a question to be answered – why some individuals can develop a strong HCV-specific T-cell responses, while in others these responses are very weak [24]. Investigation of regulatory function of the innate immune system can provide a key to the solution of this problem.

Several recent studies have shown that MHC class I and class II as well as natural killer (NK) cell's immunoglobulin-like receptors (KIR) loci can be associated with the HCV protection and clearance as well as with disease progression and responsiveness to interferon treatment. It is known that NK cells play an important role in the liver [25].

In a study of a large HCV clearance panel in parallel with the carefully matched controls associations of HLA-A*11:01, -B*57 and –Cw*0102 with HCV clearance and of HLA-A*23:01 and -Cw*04 with HCV persistence were documented [26]. In different studies ethnic and geographical differences in HLA associations with the outcome of HCV infection were shown.

Among individuals infected by injection or injury with a contaminated needle, homozygosity for both KIR2DL3 and group C1 allotypes was shown to be associated with the clearance of HCV infection, explained by the reduced inhibition of NK cells [15]. This fact was supported by some authors [27], but opposite results were obtained by others. So the influence of HLA/KIR homozygosity on the clearance or persistence of HCV infection needs further clarification.

In one recent study [28] of intravenous drug users an association of inhibitory KIR2DL2 and KIR2DL3 genes with HLA-C1 in the presence of the activatory KIR2DS4 gene was associated with the protection against HCV infection. Thus the role of KIR activating signaling should be further investigated for its potential role in the development of resistance against HCV infection.

Our previous studies included HLA and KIR distribution in Georgian population, analyzing healthy blood donors [29], where it was shown that HLA-C1, C2 distribution was under Hardy-Weinberg equilibrium, and distribution of genotypes was: C1C1=40%, C1C2=45% and C2C2=13 %, the most frequent HLA-C antigen was C*07 (C1 group) and C*12 (C1+C2 group depending on the HLA-C allele). C1 is the dominant HLA-C group in most populations, it was interesting to compare Georgians with other ethnic groups (Data from IHWG).

We found several HLA-B alleles which are very rare in other ethnic groups. Sequencing results for HLA-B reveled various alleles for B*15 and B*35: *35:01 (5%), *35:02 (2%), *35:03 (5%) and *35:08 (3%), *15:01,*15:09, and frequent *15:17 (3%). Interestingly, B*35:03 and B*35:08 is found both Europe and Asia, could be result of migrations from neighboring countries. B*15:09 and B*15:17 alleles are not common. B*35 alleles differ from each other by 1 or 2 SNP. However, B*15:09 and B*15:17 are clearly recombinant alleles. Their exon 2 has multiple SNP compared with possible parental B*15:01 alleles. B*15:09 belongs to B70 (Bw6) serological group, whereas B*15:17 is B63 (Bw4). Their African origin could corroborate the archeological findings in Georgia, which clearly shows African affinity, and may represent the species that first migrated out of Africa. HLA-Bw4 and Bw6 distribution was analyzed also. The ligand for Bw4 is KIR 3DL1. We analyzed the same samples for 3DL1/3DS1 alleles [30]. Georgian 3DS1 allele frequency is very similar to other Caucasian populations, higher than in Africans and lower comparing with Amerindians. Overall KIR-3DL1 allele high diversity in Georgian population was comparable to other ethnic groups, Africans had the highest diversity of this gene [30].

In general, further studies are needed to identify the real nature of the associations between genetic markers and HCV infection especially among different ethnic populations. Potential confounding factors including age, gender, alcohol consumption, drug abuse, etc. also need further detailed investigations.

Currently our research group is conducting the study of the immunogenetic factors influencing clinical course of HCV infection in the cohort of HCV infected patients who are enrolled in the HCV Elimination State Program which was launched in April 2015 in Georgia [32].

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SUMMARY

IMMUNOGENETIC FACTORS INFLUENCING CLINICAL COURSE OF HCV INFECTION (REVIEW)

[1]Kamkamidze G., [1]Butsashvili M., [2]Gendzekhadze K.

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Hepatitis C virus (HCV) infection remains one of the most important blood-borne diseases worldwide with about 130–170 million people chronically infected with hepatitis C virus, and more than 350 000 people die from hepatitis C-related liver diseases each year. Infection with HCV becomes chronic in approximately 80% of cases, while in up to 20% of cases hepatitis C virus is cleared from the human organism. Chronic infections of hepatitis C often leads to the end-stage liver diseases such as cirrhosis and hepatocellular carcinoma. The clinical course and the outcome of the HCV infection is determined by the complex interplay between the viral replication and the host defense mechanisms. Several recent studies have shown that MHC class I and class II as well as natural killer (NK) cell's immunoglobulin-like receptors (KIR) loci can be associated with the HCV protection and clearance as well as with disease progression and responsiveness to antiviral treatment. Current status of our knowledge about the influence of immunogenetic factors on the clinical course of HCV infection is presented in the paper. Plans to investigate these factors among HCV infected patients enrolled in the HCV Elimination Program (launched in April 2015 in Georgia) are discussed.

Keywords: hepatitis C virus, clearance, immunogenetic factors, HLA, KIR.

РЕЗЮМЕ

ВЛИЯНИЕ ИММУНОГЕНЕТИЧЕСКИХ ФАКТОРОВ НА КЛИНИЧЕСКОЕ ТЕЧЕНИЕ ИНФЕКЦИИ ВИРУСОМ ГЕПАТИТА С (ОБЗОР)

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Инфекция вирусом гепатита С (HCV) является одной из самых значимых инфекций, передающихся через кровь. Количество лиц с хронической инфикцией вирусом гепатита С достигает 130-170 миллионов; от болезней, связанных с HCV, ежегодно погибает 350 000 больных. HCV переходит в хроническую форму в 80% случаев, в 20% случаев имеет место клиренс вируса из организма человека. Хроническая инфекция часто прогрессирует и переходит в конечные формы печеночной патологии, такие как цирроз печени и гепатоцеллюлярная карцинома. Клиническое течение и исход инфекции вирусом гепатита С зависит от комплексного взаимодействия между вирусной репликацией и защитными механизмами организма. Современные исследования показывают, что определенные локусы комплекса гистосовместимости I и II классов и иммуноглобулиноподобных рецепторов натуральных киллеров могут быть ассоциированны с клиренсом вируса, а также с иходом антивирусного лечения инфекции. В статье обсуждаются современные достижения по изучению влияния иммуногенетических факторов на клиническое течение HCV инфекции и намечаются пути исследования вышеуказанных факторов среди пациентов, вовлеченных в программу элиминации гепатита С в Грузии, которая вошла в действие в апреле 2015 года.

რეზიუმე

იმუნოგენეტიკური ფაქტორების ზეგავლენა C ჰეპატიტის ვირუსით ინფექციის კლინიკურ მიმდინარეობაზე (მიმოხილვა)

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C ჰეპატიტის ვირუსით (HCV) ინფექცია წარმოადგენს ერთ-ერთ ყველაზე მნიშვნელოვან სისხლის გზით გადამდებ ინფექციას მთელს მსოფლიოში, სადაც დღეისათვის C ჰეპატიტის ვირუსით ქრონიკული ინფექციით 130-170 მილიონი ადამიანი ცხოვრობს და ყოველწლიურად დაახლოებით 350 000 ათასი ადამიანი იღუპება C ჰეპატიტთან დაკავშირბული პათოლოგიით. C ჰეპატიტის ვირუსით ინფექცია ქრონიკულ ფორმაში გადადის შემთხვევათა 80%-ში, მაშინ როდესაც, დანარჩენ 20%-ში ადგილი აქვს C ჰეპატიტის ვირუსის კლირენსს ორგანიზმიდან. C ჰეპატიტის ვირუსით ქრონიკული ინფექცია ხშირ შემთხვევაში პროგრესირებს და გადადის ღვიძლის ტერმინალურ დაავადებებში,როგორიცაა ღვიძლის ციროზი და ჰეპატოცელულარული კარცინომა. HCV ინფექციის კლინიკური მიმდინარეობა და მისი გამოსავალი მნიშვნელოვანწილად განპირობებულია ვირუსის რეპლიკაციისა და მასპინძელი ორგანიზმის დაცვითი მექანიზმებს შორის კომპლექსური

ურთიერთქმედებით. უკანასკნელი კვლევებით ნაჩვენებია,რომ ჰისტოშეთავსებადობის კომპლექსის I და II კლასის და ნატურალური კილერების იმუნოგლობულინის მსგავსი რეცეპტორების გარკვეული ლოკუსები შეიძლება ასოცირებული იყოს C ჰეპატიტის ვირუსის კლირენსთან,ასევე ამ ინფექციის ანტივირუსული მკურნალობის გამოსავალთან. სტატიაში განხილულია დღეისათვის არსებული მიღწევები C ჰეპატიტის ვირუსით ინფექციის მიმდინარეობაზე იმუნოგენეტიკური ფაქტორების ზეგავლენის შესწავლის სფეროში და დასახულია გეგმები აღნიშნული ფაქტორების შესწავლის თაობაზე C ჰეპატიტის ვირუსით ინფიცირებულ პაციენტებში, რომლებიც ჩართული არიან საქართველოში 2015 წლის აპრილის თვიდან მომქმედ C ჰეპატიტის ელიმინაციის პროგრამაში.

New Technologies

RAPID IDENTIFICATION OF THE ETIOLOGICAL FACTORS CAUSING DIARRHEAL DISEASES

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Diarrheal diseases pose significant disease and financial burden internationally. Diarrheal diseases account for 1 in 9 child deaths worldwide, making diarrhea the second leading cause of death among children under the age of 5 [6].

In Georgia the burden is mostly expressed though growing number of unnecessary/preventable hospitalizations, especially among children.

In 2013 publicly funded healthcare system covered over 10 thousand cases of diarrheal diseases, from which over half was in-patient cases and expenditures to the system accounted for more then 1 650 thousand GEL (~1 million USD). Notably, in most of the cases diagnostics was not done in line with the best international practice due to high cost and limited availability of the service.

Over 19 thousand cases of diarrheal diseases are reported annually through formal registration system in Georgia (NCDC, 2012). Over 70% of all registered cases are among children and majority of cases are reported in Tbilisi (≈20%) and in Adjara and adjunct seaside regions (≈33%).

In over 80% of in-patient cases of diarrheal diseases covered by the public system in 2013 specific causing agent was not identified, signifying the lack of access to proper diagnostics and limitation of targeted treatment [10].

Conventional methods for routine diagnostics of agents causing gastrointestinal infections include bacteriological culture with microscopy, enzyme immunoassays and immunochromatographic tests [3]. However, these conventional methods are labor intensive and some of them have low sensitivity and specificity.

Using molecular methods based on polymerase chain reaction (PCR and RT-PCR) substantially improve detection of enteric pathogens [1,4]. In recent years Multiplex real-time PCR and RT-PCR has been successfully applied for simultaneous identification and differentiation of pathogens causing diarrheal diseases [2,5,7-9]. Currently commercially available multiplex platforms have extremely high price which make them unaffordable for many clinical settings.

Further work is needed to develop affordable and highly sensitive and specific test-systems for simultaneous molecular diagnostics of several infections agents responsible for gastrointestinal infections in the clinical settings.

The main objectives of the work was to develop and pilot the real-time Polymerase Chain Reaction diagnostic systems for rapid and simultaneous identification of number of pathogens with a particular emphasis on diarrheal disease diagnostics as these diseases are one of the primary public health priorities in Georgia and worldwide.

Approximately 500 stool samples collected at the collaborating hospitals and clinics were analyzed by real-time PCR method using the Neo_PCR_Diagnostics primers. The selection of the pathogens for detection is based on their epidemiological and clinical importance.

The following bacterial pathogens were targeted for detection by the panel of Neo_PCR_Diagnostics: Salmonella spp., Campylobacter spp., Shigella spp., Clostridium difficile (Toxin A/B), Escherichia coli (ETEC, STEC and O157), Yersinia enterocolitica and Vibrio cholerae. The comparison of the results obtained by our molecular technology was performed with the conventional methods – bacterial culture (for bacterial growth) and ELISA (for bacterial toxins).

The following viral pathogens were targeted for Neo_PCR_Diagnostics: Adenoviruses, Rotaviruses and Noroviruses. The comparison of data gained by application of our methodology was done with the data obtained from the clinical testing using immunochromatographic assays for direct detection of viral antigens in the stool samples or with the data obtained by use of home-made end-point PCR (where available).

Genetic material (DNA) of the following parasites was the target in our study: Giardia lamblia, Entamoeba histolitica and Cryptosporidium spp. Results of our study was compared with the following conventional methods used in clinical settings based on the light and/or fluorescent microscopy or by direct immunochromatographic assays for direct detection of parasite antigens in the stool samples

Neo_PCR_Diagnostics tests' performance and accuracy was evaluated by comparison with the data obtained using conventional microbiological methods. The sensitivity and specificity of our method was within the 95-100% range.

Advantages of the proposed technology over existing conventional technologies include the ability of simultaneous identification of diarrheal infections by multiple pathogens. The proposed test-systems allow the detection of pathogenic agents directly from the fecal samples and could be completed within one working day.

In general, the spectrum of pathogens detected by our approach was wider than those detected by the conventional methods used in the clinical settings tacking into consideration the list of pathogenic agents requisitioned by physicians within the framework of the routine clinical investigations.

On the basis of the Neo_PCR_Diagnostics tests' performance and accuracy we could recommend their use for molecular microbiological diagnostics in clinical and/or research settings.

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SUMMARY

RAPID IDENTIFICATION OF THE ETIOLOGICAL FACTORS CAUSING DIARRHEAL DISEASES

[1,2,3]Kamkamidze G., [4]Migriauli I., [5]Razmadze D., [1,2]Kochlamazashvili M., [3]Mulkijanyan K., [1,2,3]Butsashvili M.

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The main objective of this investigation was to develop and pilot a real-time Polymerase Chain Reaction (rt-

PCR) diagnostic system for rapid and simultaneous identification of pathogens with a particular emphasis on diarrheal disease diagnostics. The diarrheal diseases were selected as a target for the pilot because they constitute a primary public health priority in Georgia and worldwide. The product developed by our research team "Neo_PCR_Diagnostics" represents an original system for the identification of pathogens associated with gastrointestinal tract infections and diarrhea. The advantages of the proposed technology over existing conventional methods include the ability of simultaneous identification of multiple pathogens and the detection of pathogenic agents directly from the fecal samples. For the evaluation of the new diagnostic system, **s**tool samples were collected at collaborating hospitals and clinics and analyzed by real-time PCR using the Neo_PCR_Diagnostic system. The selection of the pathogens for detection was based on their epidemiological and clinical importance. The following bacterial pathogens were targets for detection: *Salmonella spp., Campylobacter spp., Shigella spp., Clostridium difficile* (Toxin A/B), *Escherichia coli* (ETEC, STEC and O157), *Yersinia enterocolitica* and *Vibrio cholerae*. The following viral pathogens were studied: adenoviruses, rotaviruses and noroviruses. Genetic material (DNA) of the following parasites were targets in our study: *Giardia lamblia, Entamoeba histolitica and Cryptosporidium spp*. We also compared the results obtained by our molecular technology with the conventional methods – bacterial culture (for bacterial growth) and ELISA (for bacterial toxins). For viral and parasitic pathogens, comparison tests were performed with immunochromatographic assays for direct detection of antigens in the stool samples or with the data obtained by use of home-made end-point PCR (where available). Advantages of the proposed technology over existing conventional technologies include the ability of simultaneous identification of diarrheal infections by multiple pathogens. The proposed test system allows the detection of pathogenic agents directly from the fecal samples and can be completed within one working day.

In general, the spectrum of pathogens detected by our approach was wider than those detected by the conventional methods used in the clinical setting, taking into consideration the list of pathogenic agents requisitioned by physicians within the framework of the routine clinical visit. Given these promising results, Neo_PCR_Diagnostics test performance and accuracy may be sufficient for use in molecular microbiological diagnostics in clinical and/or research settings.

Keywords: diarrheal diseases, Polymerase Chain Reaction, multiplex detection.

РЕЗЮМЕ

БЫСТРАЯ ИДЕНТИФИКАЦИЯ ЭТИОЛОГИЧЕСКИХ ФАКТОРОВ РАЗВИТИЯ ДИАРЕЙНЫХ ЗАБОЛЕВАНИЙ

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Основными задачами проведенного исследования явилась разработка и пилотирование диагностических систем на основе метода полимеразной цепной реакции в реальном времени (rt-PCR) для быстрого и одновременного выявления определенного количества патогенных микроорганизмов с особым акцентом на диагностике диарейных заболеваний, так как эти заболевания являются одним из основных приоритетов в области общественного здравоохранения в Грузии и во всем мире. Продукт, разработанный специалистами исследовательской группы "Neo_PCR_Diagnostics", представляет собой оригинальную систему для идентификации патогенов, связанных с инфекциями желудочно-кишечного тракта и диареей. Преимуществом предлагаемой технологии, в сравнении с существующими традиционными способами, является возможность одновременной идентификации патогенов. Технология позволяют обнаружить патогенные агенты непосредственно из образцов фекалий. Из образцов стула (около 500 обрацов) методом ПЦР в реальном времени с использованием системы "Neo_PCR_Diagnostic" осуществлялась экстракция генетического материала. Селекция патогенов производится с учетом их эпидемиологического и клинического значений. Мишенью для выявления были бактериальные патогены: Salmonella spp., Campylobacter spp., Shigella spp., Clostridium difficile (Toxin A/B), Escherichia coli (ETEC, STEC and O157), Yersinia enterocolitica и Vibrio cholerae. Сравнение результатов, полученных с помощью предложенной молекулярной технологии проводили с использованием традиционных методов - бактериальной культуры (для роста бактерий) и ELISA (для бактериальных токсинов). Изучены следующие вирусные патогены: Adenoviruses, Rotaviruses и Noroviruses. Данные, полученные посредством предложенной нами методики, сравнены с таковыми, полученными в результате клинических испытаний с использованием иммунохроматографического анализа для прямого выявления вирусных антигенов в образцах кала или с показателями, полученными при использовании обычного ПЦР. Исследован генетиче-

ский материал - ДНК следующих паразитов: Giardia lamblia, Entamoeba histolitica и Cryptosporidium spp. Результаты исследования сравнены с обычными методами, используемыми в клинических условиях, на основе световой и/или люмисесцентной микроскопии или иммунохроматографических анализов для выявления антигенов паразитов в образцах кала. Преимуществом предлагаемой технологии, в сравнении с существующими традиционными технологиями, является предоставление возможности одновременного выявления желудочно-кишечных инфекций с множественными патогенами. Предлагаемые тест-системы позволили обнаружить возбудителей болезней непосредственно из образцов фекалий и завершить анализ в течение одного рабочего дня. Спектр патогенов, выявленных указанным методом, был шире выявленных с помощью традиционных методов, используемых в клинических условиях, принимая во внимание перечень патогенных агентов, запрошенных врачами в рамках рутинных клинических исследований. На основе производительности и точности "Neo_PCR_Diagnostics" тестов авторы считают целесообразным использовать их для молекулярной микробиологической диагностики в клинических и/или научно-исследовательских условиях.

რეზიუმე

დიარეული დაავადებების ეტიოლოგიური ფაქტორების სწრაფი იდენტიფიკაცია

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შრომის ძირითად ამოცანას წარმოადგენდა რეალურ დროში პოლიმერაზულ ჯაჭვურ რეაქციაზე დაფუძნებული სადიაგნოსტიკო ტესტ-სისტემის შემუშავება,რომელიც გამიზნული იქნებოდა რიგი პათოგენების სწრაფი და ერთდროული აღმოჩენისათვის. მოცემული კვლევა ფოკუსირდებოდა აღნიშნული სისტემის გამოყენებაზე დიარეული დაავადებების დიაგნოსტიკისათვის, რომლებიც წარმოადგენს საზოგადოებრივი ჯანდაცვის ერთერთ უპირველეს პრიორიტეტს როგორც მთელს მსოფლიოში, ისე ჩვენს ქვეყანაში. ჩვენს მიერ შემუშავებული პროდუქტი „Neo_PCR_Diagnostics” წარმოადგენს ორიგინალურ სადიაგნოსტიკო ტესტ-სისტემას,რომელიც გამიზნულია გასტროენტერალური ინფექციებისა და დიარეის გამომწვევი პათოგენების იდენტიფიცირებისათვის. მოწოდებული ტექნოლოგიის უპირატესობას არსებულ ტრადიციულ მეთოდებთან შედარებით წარმოადგენს მისი საშუალებით პათოგენების ერთობლივი იდენტიფიცირების შესაძლებლობა უშუალოდ საკვლევი მასალიდან (ფეკალური მასა). შემუშავებული ტესტ-სისტემის შესაფასებ-ლად საკვლევი მასალიდან (დაახლოებით, 500 სინჯი) ხდებოდა პათოგენების გენეტიკური მასალის ექსტრაქცია. პათოგენების შერჩ44
ევა,რომელთა აღმოჩენაც წარმოებდა “Neo_PCR_Diagnostics” სისტემის საშუალებით, ხორციელდებოდა მათი ეპიდემიოლოგიური და კლინიკური მნიშვნელობის გათვალისწინებით. შესაბამისად, შერჩეული იყო შემდეგი ბაქტერიული პათოგენები: Salmonella spp., Campylobacter spp., Shigella spp., Clostridium difficile (Toxin A/B), Escherichia coli (ETEC, STEC and O157), Yersinia enterocolitica და Vibrio cholerae. აღნიშნული პათოგენური აგენტების შემთხვევაში ავტორების მიერ მოწოდებული მეთოდოლოგიით ჩატარებული ტესტების შედეგების შედარება წარმოებდა ისეთ ტრადიციულ მეთოდებთან, როგორებიცაა ბაქტერიული კულტურა (ბაქტერიების ზრდის შეფასება) და იმუნოფერმენტული ანალიზი (ბაქტერიული ტოქსინების განსაზღვრა). შესწავლილია შემდეგი ვირუსული პათოგენები: ადენოვირუსები, როტავირუსები და ნოროვირუსები. აღნიშნული პათოგენების შემთხვევაში შედარებითი ანალიზი წარმოებდა იმუნოქრომატოგრაფიულ ტესტებით და კონვენციურ პჯრ მეთოდით ჩატარებულ კვლე-ვებთან მიმართებაში. ასევე, კვლევის სამიზნეს წარმოადგენდა შემდეგი პარაზიტების დნმ: Giardia lamblia, Entamoeba histolitica and Cryptosporidium spp. აღნიშნული მეთოდის შედეგები შედარდა ფლუორესცენტული მიკროსკოპიითა და პირდაპირი იმუნოქრომატოგრაფიული ტესტებით მიღებულ შედეგებს. გამოვლინდა, რომ ჩვენს მიერ შემუშავებული ტესტ-სისტემით შესაძლებელი იყო რიგი პათოგენების ერთობლივი დიაგნოსტირება პირდაპირ საკვლევი მასალიდან, ანალიზის ხანგრძლივობა არ აღემატებოდა ერთ სამუშო დღეს და მისი საშუალებით გამოვლინდა ინფექციური აგენტების გაცილებით ფართო სპექტრი, ვიდრე ტრადიციული მეთოდებით. "Neo_PCR_Diagnostics" სისტემის მახასიათებლების გათვალისწინებით, ავტორები რეკომენდაციას უწევენ აღნიშნული მეთოდის გამოყენებას მოლეკულური დიაგნოსტიკისათვის როგორც კლინიკურ,ისე სამეცნიერო დაწესებულებებში.