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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

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GEORGIAN MEDICAL NEWS

No 12 (165) 2008

The special issue editor – the Director-General of Infectious Diseases, AIDS and Clinical Immunology Research Center, the Head of the Faculty of Medicine, Iv. Javakhisvhili Tbilisi State University Tsertsvadze T.

Редактор специального выпуска - генеральный директор Научно-практического центра инфекционных заболеваний, СПИДа и клинической иммунологии, председатель департамента инфекционных заболеваний и клинической иммунологии медицинского факультета Тбилисского государственного университета им. Ив. Джавахишвили, профессор Тенгиз Николаевич Церцвадзе

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

ТБИЛИСИ - НЬЮ-ЙОРК

“Georgian Medical News” is a Georgian-Russian-English-German monthly journal and carries original scientific articles on medicine and biology, which are of experimental, theoretical and practical character.

“Georgian Medical News” is a joint publication of GMN Editorial Board and The International Academy of Sciences, Education, Industry and Arts (U.S.A.).

“Georgian Medical News” is included in the international system of medical information “MEDLINE” which represents the central electronic database of the world medical scientific literature. The journal is stored in the funds of US national library. It is listed in the catalogue of The Central Scientific-Medical Public Library of Russian Federation and world-wide catalogues: *“Ulrich’s International Periodicals Directory”* and *“Medical and Health Care Serials in Print”*. Articles from the bulletin are under review of *scientific and technological informative journal of the Russian Academy of Sciences*.

“Georgian Medical News” - ежемесячный научно-медицинский рецензируемый журнал, в котором на русском, английском и немецком языках публикуются оригинальные научные статьи экспериментального, теоретического и практического характера в области медицины и биологии, статьи обзорного характера, рецензии; периодически печатается информация о проведенных научных мероприятиях, новшествах медицины и здравоохранения.

“Georgian Medical News” является совместным изданием с Международной Академией Наук, Образования, Искусств и Естествознания (IASEIA) США.

“Georgian Medical News” включен в международную систему медицинской информации “MEDLINE”, которая является центральной электронной базой данных мировой медицинской научной литературы. Журнал хранится в фондах библиотеки конгресса США; входит в каталог Государственной Центральной научно-медицинской библиотеки Российской Федерации и Всемирные каталоги *Ulrich’s International Periodicals Directory и Medical and Health Care Serials in Print.* Статьи из журнала реферируются в реферативном журнале *Всероссийского института научной и технической информации Российской академии наук (ВИНИТИ РАН)* и хранятся в его базе данных по медицине.

“Georgian Medical News” - არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, რომელშიც რუსულ, ინგლისურ და გერმანულ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინისა და ბიოლოგიის სფეროში, მიმოხილვითი ხასიათის სტატიები, რეცენზიები.

“Georgian Medical News” წარმოადგენს ერთობლივ გამოცემას აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიასთან (IASEIA) ერთად.

“Georgian Medical News” შეყვანილია სამედიცინო ინფორმაციის საერთაშორისო სისტემა “MEDLINE”-ში, რომელიც წარმოადგენს მსოფლიოს სამედიცინო სამეცნიერო ლიტერატურის ცენტრალურ ელექტრონულ მონაცემთა ბაზას. ინახება აშშ-ის კონგრესის ბიბლიოთეკის ფონდებში; შესულია რუსეთის ფედერაციის სახელმწიფო ცენტრალური სამეცნიერო ბიბლიოთეკის კატალოგსა და საერთაშორისო კატალოგებში *“Ulrich’s International Periodicals Directory”* და *“Medical and Health Care Serials in Print”*. ჟურნალში გამოქვეყნებული სტატიები რეფერირდება *რუსეთის მეცნიერებათა აკადემიის სამეცნიერო და ტექნიკური ინფორმაციის ინსტიტუტის* რეფერატულ ჟურნალში და ინახება მედიცინის მონაცემთა ბაზაში.

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With computer-printed texts please enclose a diskette carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume, must be at least 5 pages and not exceed the limit of 10 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.

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

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12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე, დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე,3სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი Times New Roman (**Кириллица**); შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს დისკეტი სტატიით. ფაილი დაასათაურეთ ლათინური სიმბოლოთი.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 5 გვერდზე ნაკლებსა და 10 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეს ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

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5. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული და ტუშით შესრულებული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით. თითოეული სურათის უკანა მხარეს ფანქრით აღნიშნეთ მისი ნომერი, ავტორის გვარი, სტატიის სათაური (შემოკლებით), სურათის ზედა და ქვედა ნაწილები. სურათების წარწერები წარმოადგინეთ ცალკე ფურცელზე მათი N-ის მითითებით. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი.

6. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით; კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით.

7. 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.

8. 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.

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

10. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ და რუსულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: შესავალი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

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

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

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

Содержание:

НАУКА

TB/HIV CO INFECTION IN GEORGIA

Gabunia[1] P., Salakaia[2] A., Kiria[2] N., Kandelaki[1] G., Tsertsvadze[1,3] T.

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HIV is the most powerful factor known to increase the risk of Tuberculosis (TB) and TB is one of the important cause of HIV-related deaths worldwide [6]. The interaction between tuberculosis (TB) and human immunodeficiency virus (HIV) infection is complex. HIV infection weakens the immune system and increases likelihood of acquisition of TB, progression of latent TB infection (LTBI) to active TB (ATB) and more aggressive course of the disease. It also alters the clinical presentation of TB and compromises the response to anti-TB treatment [2]. The diagnosis is also complicated; HIV patients, especially those with low CD4 count are anergic to PPD, CXR findings are often subtle or absent, sputum smears are more often negative then in non-HIV individuals and etc.

The lifetime risk of developing active TB once infected, in absence of HIV infection, is about 10% [5]. However, it increases tenfold in HIV infected individuals. This has resulted in reemergence of TB epidemy [13,4]. TB can occur at any time in the course of progression of HIV infection [3].

Globally there were 700,000 TB cases among HIV positives in 2006, and an estimated 230,000 people living with HIV will die as a result of TB in 2008 despite the fact that TB is curable [7,8].

Georgia is the country with low prevalence of HIV and high prevalence of TB. The knowledge about the rate of HIV infection in TB patients and vice versa, the prevalence of ATB among HIV infected persons is very important for effective disease prevention and control planning and resource allocation.

The aim of this study was to determine the rate of TB among newly diagnosed HIV infected persons and on the other hand to determine the rate of HIV infection among TB patients in Georgia.

Materials and methods. The prospective observational study has been conducted in Georgia since January 01 2006. Started from January 2006 screening of all HIV/AIDS cases for TB (active and latent infections) were initiated at Georgian Infectious diseases, AIDS and Clinical Immunology research center. In the current study we included only newly diagnosed HIV patients.

All HIV positive persons with or without TB symptoms underwent tuberculin skin test (TST). Patients with positive TST with no clinical, x-ray or microbiological evidence of ATB, were diagnosed with latent TB infection (LTBI) and received TB preventive treatment (TPT) according to National Clinical Guideline [6].

The diagnosis of ATB among HIV/AIDS patients was based on the "algorithm for assessing TB risk and disease in HIV positive person", outlined in National Clinical Guideline "Management of TB/HIV co infection"[6]:

Patients presenting with symptoms suggestive of PTB who had productive cough for three weeks or more with at least two positive sputum smears or one positive smear and x-ray findings consistent with active PTB were classified as smear-positive PTB cases.

Patients presenting with cough of three weeks or more with initial three negative smears and no clinical response to a course of broad-spectrum antibiotics, three negative smear results after a course of broad-spectrum antibiotics, x-ray findings consistent with active PTB and decided by a clinician to be treated with anti-TB chemotherapy, were classified as smear-negative PTB cases.

Patients presenting with symptoms suggestive of TB other than the lungs (pleural effusion, lymphadenopathy, pericardial disease, miliary disease, meningitis, disseminated TB with mycobacteraemia) which did not respond to a course of broad-spectrum antibiotics

and decided by a clinician to be treated with anti-TB chemotherapy were classified as EPTB cases.
From January 2006 we also conducted HIV screening among TB patients at the following TB care institutions: National Center for Tuberculosis and Pulmonary Diseases in the capital of Georgia – Tbilisi, Center for Tuberculosis and Pulmonary Diseases of Western Georgia - Kutaisi, Hospital of Tuberculosis and Pulmonary Diseases in Adjara - Batumi, Hospital of Tuberculosis and Pulmonary Diseases in Samegrelo Region - Zugdidi.

Patients with ATB were offered the opportunity for HIV evaluation. The initial assessment of a patient's HIV status included: HIV pretest counseling.

Serological ELISA test (Vironostica ® Uniform II Ag/Ab BIOMÉRIEUX). All sera found reactive on ELISA were confirmed by a western blot confirmatory test (2.2 Genelabs Diagnistics, Singapore). Post-test counseling.

Results and their discussion. In 2006, 276 HIV positive patients were included in the study. Of these, 201 (72.8%) were men and 75 (27, 2%) - women. 46 (16, 7%) patients were diagnosed with ATB. Among them 43 (93.5%) were men and 3(6.5%) were women. LTBI was revealed in 90 (32.6%) HIV/AIDS cases and TPT was started.

In 2007, 344 HIV positive patients were investigated. Of these, 245 (71.2%) were men and 99 (28.8%) were women. 57 (16.6%) patients were diagnosed with ATB. Among them 51 (89.5%) were men and 6 (10.5%) - women. LTBI was diagnosed in 77 (22.4%) HIV/AIDS cases and TPT was started.

In 2008 (January 01 – September 30) 296 HIV positive patients were included in the study. Of these, 210 (72.8%) were men and 86 (27.2%) were women. In 65 (22%) patients were diagnosed with ATB. Among them 60 (92.3 %) were men and 5 (7.7 %) were women. LTBI was diagnosed in 77 (26 %) HIV/AIDS cases and TPT was initiated.

In 2006, 841 ATB patients were investigated on HIV. Of these 595 were from National Center for Tuberculosis and Pulmonary Diseases in Tbilisi and HIV positive results were received in 15 (2.5%) cases; 106 were from Center for Tuberculosis and Pulmonary Diseases of Western Georgia and HIV positive results were received in 4 (3.8%) cases, 30 ATB patients were from Hospital of Tuberculosis and Pulmonary Diseases in Adjara and all of them were HIV negative. 92 ATB cases were investigated from Hospital of Tuberculosis and Pulmonary Diseases in Samegrelo Region and HIV positive results were received in 2 (2.2%) cases. In total the prevalence of HIV among ATB patients in 2006 was 2.3% (21 out of 841).

In 2007, 1483 ATB patients were included in the study. 1012 were from National Center for Tuberculosis and Pulmonary Diseases in Tbilisi and HIV positive results were received in 21 (2.1%) cases. 127 were from Center for Tuberculosis and Pulmonary Diseases of Western Georgia and HIV positive results were received in 2 (1.6%) cases. 49 ATB patients were from Hospital of Tuberculosis and Pulmonary Diseases in Adjara and HIV positive results were received in 3 (6%) cases. 161 ATB cases were investigated from Hospital of Tuberculosis and Pulmonary Diseases in Samegrelo Region and HIV positive results were received in 5 (3.1%) cases. In total the prevalence of HIV among ATB patients in 2007was 2.1% (31 out of 1483).

In 2008 (January 01 – September 30) 1705 ATB patients were included in the study. 1169 were from National Center for Tuberculosis and Pulmonary Diseases in Tbilisi and HIV positive results were received in 20 (1.7%) cases. 204 were from Center for Tuberculosis and Pulmonary Diseases of Western Georgia and HIV positive results were received in 3 (1.5%) cases, 116 ATB patients were from Hospital of Tuberculosis and Pulmonary Diseases in Adjara and HIV positive results were received in 1 (0.86 %) cases; 216 ATB cases were investigated from Hospital of Tuberculosis and Pulmonary Diseases in Samegrelo Region and HIV positive results were received in 5 (2.3%) cases. In total the prevalence of HIV among ATB patients in 2008 was 1.7 % (29 out of 1705 respectively).

Our study has clearly shown the striking prevalence of TB (both active and latent TB) among HIV patients. Up to 22% (16.7-22%) according to years of HIV positive individuals had ATB, and 22.4 - 32.6% had LTBI. Since HIV patients are frequently anergic to TST, have atypical CXR findings and sputum smears are often negative, the real prevalence of TB (both active and latent infections) is most prob-

ably underestimated by current study. Based on the abovementioned statistics, it is hard to overemphasize the need of meticulous screening for LTBI and ATB among HIV patients. All HIV positive individuals should be screened for TB by TST. TST positives (whether symptomatic or asymptomatic) should have scrupulous work-up to identify the possibility of ATB. Patients diagnosed with ATB should be treated accordingly. If ATB is excluded, LTBI should be treated with 9 months of INH. TST negative patients should be screened annually with repeated TSTs. Since TST is not very sensitive test in HIV patients, interferon gamma release assays (IGRA) and T-SPOT might be helpful in the future, but because of the associated cost, they cannot be recommended on a routine basis at this time without further data regarding their performance.

The prevalence of HIV among TB patents was not very high, ranging from 1.7 to 2.2%, but we think the rate is high enough to justify routine screening for HIV in this cohort of patients, considering the adverse influence of HIV on TB and also the vital importance of timely diagnosis of HIV.

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SUMMARY

TB/HIV CO INFECTION IN GEORGIA

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HIV and TB co-infection is a considerable problem worldwide. HIV significantly increases the morbidity and mortality from TB and often makes the diagnosis more challenging. In this study we attempted to evaluate the prevalence of TB among newly diagnosed HIV infected persons and determine the rate of HIV infection among active TB patients in Georgia. The prospective observational study has been conducted in Georgia since January 01, 2006. All newly diagnosed HIV positive persons were screened for active and latent TB infection and the prevalence of TB was identified. During the same time period HIV screening was performed in all identified active TB cases. Up to 22% (16.7 to 22%) of HIV positive individuals were found to have active TB, and 22.4 to 32.6% had LTBI. The prevalence of HIV among TB patents ranged from 1.7 to 2.2%. The study showed significant prevalence of TB (both active and latent TB) among HIV patients. Because of problems with TB diagnosis in HIV patients, the real prevalence may be underestimated. The alarming statistical data should force us towards meticulous and scrupulous screening for tuberculosis among HIV positive individuals. The prevalence of HIV among TB patents was not very high, ranging from 1.7 to 2.2%, but we recommend routine screening for HIV in all active TB patients.

Key words: HIV, tuberculosis, co-infection, prevalence.

РЕЗЮМЕ

КОИНФЕКЦИЯ ТУБЕРКУЛЕЗА И ВИЧ/СПИДа В ГРУЗИИ

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Коинфекция ВИЧ/СПИДа и туберкулеза - серьезная проблема во всем мире. ВИЧ способствует повышению заболеваемости туберкулезом и смертности от него, а также связан с диагностическими проблемами. В данной работе исследована превалентность туберкулеза среди ВИЧ-инфицированных и количество ВИЧ-инфекции среди больных активным туберкулезом в Грузии. Проспективное обзорное исследование проводилось в Грузии с 2006 г. Все новые ВИЧ-инфицированные пациенты были обследованы на наличие активного и латентнго туберкулеза. Идентифицирована превалентность туберкулеза. В этот же период проводился скрининг ВИЧ-инфекции среди больных с активным туберкулезом. У ВИЧ-позитивных лиц был диагностирован активный туберкулез в пределах 16,7-22% случаев. Латентный туберкулез диагностирован в пределах 22,4-32,6% случаев. Превалентность ВИЧ-инфекции среди больных туберкулезом варьировала в пределах 1,7-2,2%. Анализ результатов проведенного исследования выявил значительную превалентность туберкулеза (активного и латентного) среди ВИЧ-инфицированных пациентов. В связи с диагностическими проблемами реальная превалентность туберкулеза среди ВИЧ-позитивных больных может быть недооценена. Настораживающая статистика указывает на необходимость скрупулезного скрининга туберкулеза среди ВИЧ-позитивных лиц. Превалентность ВИЧ среди больных туберкулезом не высока, однако рекомендуем рутинный скрининг на ВИЧ антитела всех больных активным туберкулезом.

ANTIRETROVIRAL TREATMENT IN GEORGIA

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Human immunodeficiency virus (HIV) is among the top 10 leading causes of infectious disease death worldwide.

HIV infection is pandemic, affecting almost all countries. The Joint United Nations Programme on AIDS (UNAIDS) and the World Health Organization (WHO) estimate that approximately 33.2 million people were living with HIV worldwide in 2007, including 2,5 million children younger than 15 years [7].

The first case of HIV infection in Georgia was reported in 1989. As of October 2008, a total of 1796 HIV/AIDS cases were registered in the country, among them 75% were male. The estimated number of people living with HIV/AIDS (PLHA) is around 3500 as per Spectrum modeling. Annual number of newly reported HIV infections has risen each year. Of note, more than half of 1796 registered HIV cases were reported in the past three years (2005-2008) (Figure).

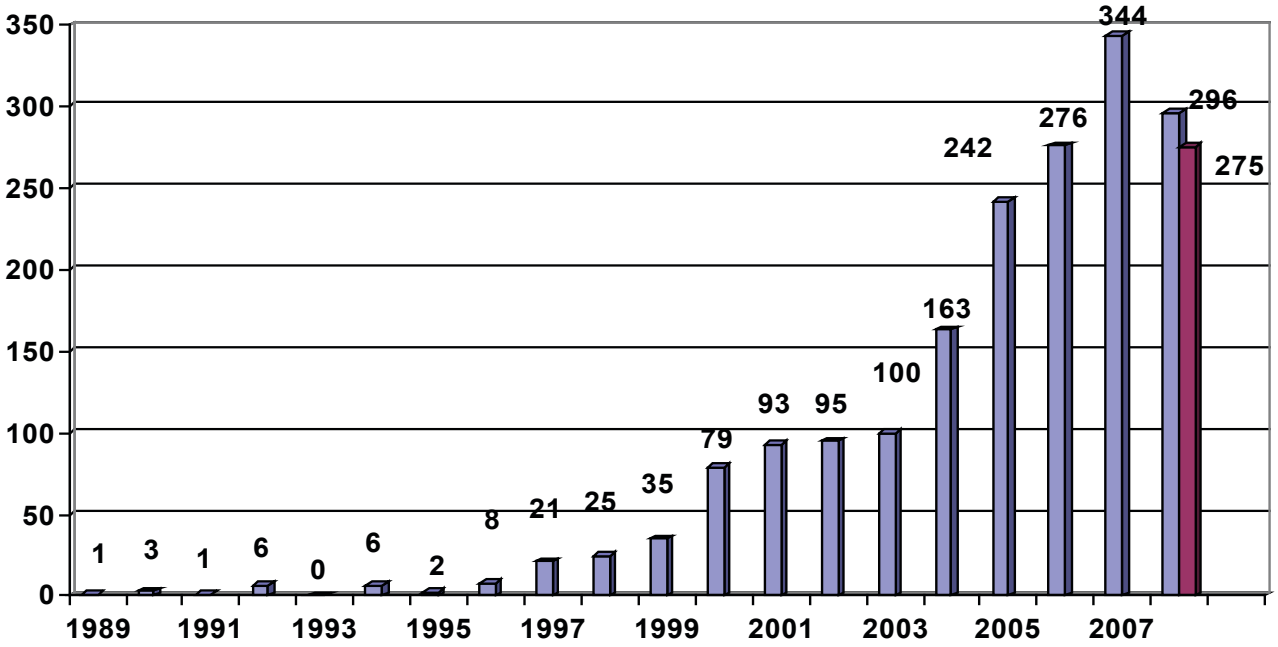

Fig. Number of new cases of HIV infection by year

The HIV epidemic in Georgia is mostly concentrated among the injecting drug users (IDU) accounting for 60% of cumulative reported number. It's worth to mention that up to 51% of HIV infected individuals are co-infected with hepatitis viruses. Although the HIV epidemic is in its nascent phase, Georgia is believed to be at risk for an expanding epidemic due to the widespread IDU; high rates of sexually transmitted infections (STI) and hepatitis B and C; and intense population movement between neighboring high-prevalence countries such as Ukraine and Russia.

Georgia was the first among the former Soviet Union (FSU) countries to attain universal access to free-of-charge ART for all PLHA. According to the recent WHO, UNAIDS and UNICEF joint report, Georgia is among the 9 low- and middle-income countries that reached treatment coverage of at least 75 percent [5].

Diagnostics and treatment of AIDS patients in Georgia are provided at the highest attainable standards based on adaptation of the WHO, European, British HIV Association, DHHS of USA, IAS-USA Panel and other guidelines [1,2,4,5,12].

The aim of this study is to evaluate effectiveness of ARV treatment principles in Georgia, including treatment and monitoring methods.

Materials and methods. The investigation was conducted at the Infectious Diseases, AIDS & Clinical Immunology Research Center (IDACIRC) of Georgia. Study included all HIV/AIDS patients, who have been registered at IDACIRC and available in Georgia since 2004. Out of cumulative 1796 HIV/AIDS cases registered at the IDACIRC, 387 died and 357 were out of country. Therefore1052 patients were included in the study, 736 men and 316 women. All study subjects were investigated by special algorithm to evaluate the need of ARV treatment. Patients were aged from 3 months to 64 years. 51% of study subject were co-infected with HCV, HBV or both.

To ensure universal access to ARV therapy all 1052 HIV/AIDS individuals included in the study were offered to have following examinations: Investigation of all HIV infected persons by special algorithm: Physical examination; Complete blood count; Immunological investigation (including CD4 count); Viral Load (HIV RNA by quantitative PCR); Clinical chemistry (wider range); Ultrasound; X-ray; CT-scan. Monitoring of CD4 cell count, HIV RNA viral load dynamic and appearance of opportunistic infections were scheduled in every 3-4 months.

Identification all patients requiring ARV therapy according to special criteria described in National Guidelines on HIV/AIDS treatment: Symptomatic HIV infection (AIDS or severe symptoms); CD4 count < 200/mm3; If CD4 is 201- 350/mm3, decision is made upon CD4 decline rate, viral load, presence of hepatitis co-infection [6,9,10].

Offering ARV therapy to all patients requiring treatment.

At present in Georgia the National Guidelines recommend as a first-line ARV regimen combination of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). Second-line ARV regimen includes one of the Protease Inhibitors (PIs) boosted with Ritonavir and two NRTIs. Salvage regimen includes Fusion Inhibitor Enfurvitide (ENF) and the new PIs – Tipranavir (TPV) or Darunavir (Prezista). Another option is a combination of two PIs, except TPV, which is not to be combined with any other PIs. It's worth to mention during selection of ARV regimen following factors has been taken into account: hepatotoxicity of non-nucleoside reverse transcriptase inhibitors (NNRTIs) (such as with Nevirapine (NVP)) and presence of hepatic injury, teratogenicity of Efavirenz in first trimester of pregnancy, use of opioid substitution therapy (pharmacokinetic interaction between NNRTIs and methadone or buprenorphine), coexistent medical or psychiatric conditions and etc.

Monitoring of all patients receiving ARV treatment was done at baseline, after 8 weeks of treatment initiation and then in every 3-4 months. Clinical symptoms, CD4 T-cell count, HIV RNA level, side-effects and adherence was monitored during the follow-up visits [8,12]. Possible side effects of particular drugs could require more frequent monitoring and was decided for individual patients. Treatment failure was defined according to the National Protocols: Virologic failure: a) VL>400 c/ml at 24 weeks, b) VL>50 c/ml at 48 weeks, c) after viral suppression VL rebound to >400 c/ml at two occasions in 4 to 8 weeks.

Immunological failure: a) Increase in CD4 cell count $\leq$ 50 cells/mm^3 during a first year; b) >50% fall from on therapy CD4 peak level; clinical failure: a) Occurrence of new Opportunistic Infections (OI); b) Recurrence of prior OI; c) onset of WHO stage III conditions.

In case of severe Opportunist Infectious like TB, Pneumocystic Pneumonia etc., treatment was deferred for few days or weeks until disease stabilization, to avoid overlapping toxicity of drugs and possibility of Immune Reconstitution Syndrome (IRIS). However, this was done with caution in patient with CD4 count below the 50 cells/mm^3, where ARV treatment was initiated as soon as possible.

In case of treatment failure adherence was reassessed and genotypic resistance test was performed to determine whether failure was due to HIV drug resistance or other causes (e.g., inadequate adherence, drug side effects) and consequently second-line or salvage ARV regimen was initiated.

Detection of HIV antibodies was performed by ELISA (third or fourth generation) with further confirmation by Western Blot Assay.

HIV-1 RNA in plasma was measured by commercially available quantitative Polymerase Chain Reaction (PCR) method (AMPLICOR HIV-1 MONITOR Test, version 1.5, Hoffmann-La Roche, Inc.) and run according to the protocol of the manufacturer. Since 2006 The COBAS TaqMan HIV-1 test (Real time PCR) has been used for quantification of HIV-1 RNA in Hunam EDTA plasma, using the High Pure System Viral Nucleic Acid Kit for manual specimen preparation and the COBAS TaqMan 48 Analyzer for automated amplification and detection. The test can quantify HIV-1 RNA over range of 40-10,000,000 copies/mL.

For determination of percentages and absolute count of T lymphocyte subpopulations (T helpers, T cytotoxic,) specific markers were measured according to the standard CDC recommended single-platform immunophenotyping technique using the Becton-Dickinson FACSCalibur flow cytometer. Complex of following monoclonal antibodies was used CD3-FITC/ CD8PE/ CD45PerCP/ CD4APC. In brief this method consists of steps as follows:1) incubation of sample with relevant monoclonal antibodies in True Count tubes; 2) lysing of erythrocytes using lysing solution; 3) incubation and following analyzing on flow cytometer using appropriate software. Data for a minimum of 5,000 lymphocytes was acquired for each sample.

For resistance testing TRUGENE *HIV-1* Genotyping Kit with the OpenGene DNA Sequencing System (Siemens) was used. Resistance testing involves the following steps:
1. HIV-1 RNA extraction; 2. Reverse Transcription and PCR Amplification of the target sequence in the *pol* gene; 3. CLIP/Sequencing Reaction; 4. Gel electrophoresis of CLIP reaction products; 5. Sequence Analysis. The Guidelines™ software of the TruGene system was used to interpret the genotype

for drug resistance and producing a clinical report. The sequences obtained were also analyzed using the software available http://hivdb.stanford.edu/

Results and their dscussion. Of 1052 patients investigated 595 were in need of ARVs and treatment was offered to all of them. 594 patients started treatment, 1 patient refused. Out of treated patients 22 self-discontinued treatment, 111 patients died and 461 patients (438 adult and 23 children) remained on ARV treatment. Out of treated patients 406 adults and 21 children are receiving first-line treatment and 31 adults and 2 children are already on second-line treatment, 1 adult is receiving salvage regimen.

Distribution of various first-line and second-line ARV regimens among adult patients are presented in Table 1 and Table 2 accordingly (Tables 1 and 2). 1 patient is receiving salvage regimen including Darunavir and Enfuvirtide.

Table 1. Distribution of patients receiving first-line ARV treatment

First-line ARV regimens	Number of adults	Number of children
ZDV + 3TC + EFV	213	8
ZDV + 3TC + NVP	45	9
TDF + FTC + EFV	16	-
TDF + FTC + NVP	3	-
ABC + 3TC + EFV	120	2
ABC + 3TC + NVP	9	2

Table 2. Distribution of patients receiving second-line ARV treatment

Second-line ARV regimens	Number of adults	Number of children
LPV/r (SQV/r or FPV/r) + TDF + ABC	9	-
LPV/r (FPV/r or ATV/r) + ddI + ABC	19	2
LPV/r + TDF + (ZDV + 3TC)	3	-

Our results indicate that ARV treatment in Georgia is quite successful. Out of 461 patients receiving ARV treatment, 427 patients are remained on first-line regimen. In this group of patients the adherence and compliance is up to 95%. As a result viral replication is suppressed, CD4 count is increased and quality of life is improved.

Reasons of switching ARV from first to second-line were analyzed. Treatment failure was reported in 55 cases. Among them immunological failure was observed in 7 cases, clinical failure in 1 case and virologic failure in 47 cases. In all virologic failure cases adherence was reassessed and genotypic resistance test was performed while patient was still on treatment. Of 47 cases in 34 (72%) failure was attributable to drug resistance. Of 34 patients with HIV drug resistance 1 patient has three-class drug resistance (NRTI, NNRTI and PI); 30 patients had two-class drug resistance, and 3 patients found to be resistant to one class: NNRTIs. In 13 cases (28%) virologic failure due to non-adherence was found that was confirmed by detectable viremia, without resistance mutations. Out of these 13 cases 2 patients discontinued treatment and in 11 cases adherence was ensured. Out of 34 cases with drug resistance all patients were switched to second-line regimen. Out of them currently 31 adult patients and 2 children were switched and remained on second-line ARVs and 1 was switched to salvage regimen.

Common NRTI mutations reported in treatment experienced patients included: the signature Lamivudine mutation M184V has been detected in 31 subjects; 2 subject was found to be resistant to Tenofovir (TDF) due to K65R mutation; 3 subjects had intermediate resistance to Zidovudine (AZT) and possibly to Stavudine (d4T) due to T215Y/F+K219Q; 2 subject had possible resistance to Zidovudine (AZT) and Stavudine (d4T) due to T215Y/F, which also limits the effectiveness of Abacavir (ABC), Didanosine (ddI) and particularly Tenofovir (TDF).

As for NNRTI mutations: 13 subjects had K103N mutation causing resistance to Nevirapine (NVP), Delaviridine (DLV) and Efavirenz (EFV); 20 subjects were resistant to Nevirapine (NVP) and Efavirenz (EFV) due to G190S/A mutation with

presence K101E mutation in two cases; 4 subjects were resistant to Nevirapine (NVP) and Delaviridine (DLV) due to Y181C mutation, one subject had resistance to same drugs caused by V106I mutation; In addition, M230, A98G, V179D/E and V108I mutations were detected in these subjects, which reduced susceptibility to each of the available NNRTIs.

Table 3. Causes of death among patients receiving ARV

Reasons of death among patients receiving ARV	Number of deaths
AIDS related (total)	*25*
Toxoplasmic encephalitis	2
Cryptococcal meningitis	5
Tuberculous meningoencephalitis	6
Wasting syndrome	3
Meningoencephalitis of unknown etiology	4
Lung Tuberculosis	5
Cancers (total)	*27*
Lymphoma	8
Kaposi's Sarcoma	3
Colon cancer	5
Lung cancer, Respiratory failure	11
Non-AIDS related	*59*
Myocardial Infarction	5
Stroke	6
Trauma	5
Acute renal failure	5
Cirrhosis and end-stage liver disease	38
Total	*111*

Fatality rate and reasons of death was studied among treated patients. Overall fatality rate of ARV treated patients was 19% (111 patients out of 594). Of died cases in 25 was death attributable to the late stage of HIV/AIDS complicated with life-threatening diseases, 27 cases were due to incurable malignancies and 59 were due to non-AIDS related death. Table 3 summarizes reasons of deaths among patients receiving ARV. It's worth to mention that the highest number of deaths was due to liver failure in HIV/HCV and/or HBV co-infections. This fact once more emphasizes that co-infection with hepatitis viruses is of major concern in HIV patients (Table 3).

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SUMMARY

ANTIRETROVIRAL TREATMENT IN GEORGIA

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HIV infection is the major public health, social and economic problem in Georgia. The aim of this study is to evaluate effectiveness of ARV treatment system in Georgia.

Study included 1052 people living with HIV/AIDS in Georgia registered at Infectious Disease, AIDS and Clinical Immunology Research Center since 2004. To ensure universal access to ARV therapy all HIV/AIDS individuals included in the study were investigated by special algorithm, all identified patients requiring ARV therapy were offered treatment and monitored during therapy on treatment effectiveness and side effects. Detection of HIV antibodies was performed by ELISA with further confirmation by Western Blot Assay. HIV-1 RNA in plasma was measured by quantitative Polymerase Chain Reaction. For determination of percentages and absolute count of T lymphocyte subpopulations single-platform immunophenotyping technique using the Becton-Dickinson FACSCalibur flow cytometer was applied. For resistance testing TRUGENE *HIV-1* Genotyping Kit with the OpenGene DNA Sequencing System (Siemens) was used.

Treatment was offered to 595 HIV/AIDS patients. 594 patients started treatment, 1 patient refused. Out of treated 594 HIV/AIDS patients 22 patients discontinued, 111 patients died and 461 patients are currently on ARV treatment. Out of treated patients 406 adults and 21 children are receiving first-line treatment, 31 adults and 2 children are on second-line treatment and 1 adult is receiving salvage regimen. Treatment failure was defined in 55 cases. Among them immunological failure was observed in 7 cases, clinical failure in 1 case and virologic failure in 47 cases. Prevalence of drug resistance among virologic failure cases accounted for 72% and inadequate adherence for 28% cases. Majority of death cases among ARV treated patients was due to non-AIDS related or incurable conditions, while deaths due to AIDS related conditions mainly were associated to the delayed referral of patients in already advanced stage of disease. It's worth to mention that highest number of death cases was due to liver failure in HIV/HCV and/or HBV co-infected patients.

Key words: HIV/AIDS, ARV treatment, drug resistance, adherence, fatality.

РЕЗЮМЕ

АНТИРЕТРОВИРУСНАЯ ТЕРАПИЯ В ГРУЗИИ

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Целью данного исследования явилось определение эффективности антиретровирусной терапии в Грузии. Исследования проводились в Научно-

практическом центре инфекционных заболеваний, СПИДа и клинической иммунологии. В исследовании принимали участие 1052 ВИЧ-инфицированных больных, зарегистрированных в Центре СПИДа с 2004 г. Для достижения универсальной доступности был разработан специальный алгоритм. ВИЧ-инфекцию диагностировали методом иммуноферментного анализа и Вестерн Блота. Число CD4 клеток измеряли методом иммунофенотипирования, используя проточный цитометр FACSCalibur.

Антиретровирусная терапия (АРВ) была предложена 595-и ВИЧ-инфицированным больным, среди которых 594 начали АРВ терапию, 1 отказался. Из 594-х пациентов 22 прекратили лечение, 111 - умерло, а 461 находятся на терапии. Из них 427 пациентов принимают лекарства первой линии (взрослые - 406 и дети -21); 33 пациента - лекарства второй линии (взрослые - 31 и дети - 2) и один больной принимает резервные препараты.

Терапия была неуспешной в 55-и случаях, среди которых 7 случаев были иммунологическими, 1 - клинический и 47 - вирусологической неудачей. Среди 47-и вирусологических неудач резистентность наблюдалась в 72% случаев. Следует отметить, что в большинстве случаев смертность была вызвана патологией печени, а не СПИД-ассоциированными заболеваниями.

SUCCESSFUL APPLICATION OF LABORATORY TOOLS FOR THE DETECTION OF HIV DRUG RESISTANCE IN ROUTINE CLINICAL CARE IN GEORGIA

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Advent of highly active antiretroviral therapy (HAART) in the mid 1990s had the dramatic impact on the course of the AIDS epidemic. HAART has changed the perception of AIDS from a death sentence to a chronic, manageable disease, through decline in HIV-related morbidity and mortality [12] However, the effectiveness of HAART can be compromised by the emergence of drug resistant HIV variants. The mutations have been described for all currently available drug classes: nucleoside reverse transcriptase inhibitors (NRTI), nonnucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitors (PI), entry inhibitors and integrase inhibitors [9]. Drug resistance develops progressively and eventually patients may harbor multidrug-resistant variants, which limit further treatment options and is associated with unfavorable disease outcomes [1,13].

Antiretroviral drugs (ARV) were introduced in Georgia in the 1990s. Until recently, the use of ARVs was limited because of economic reasons. Since 2004, however, through increased resource allocation from the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), Georgia has been able to ensure universal access to free-of-charge HAART [16]. As of October 1, 2008 a total 585 HIV infected individuals initiated HAART. Currently, 461 persons remain on therapy, 34 of these are receiving second line ARV regimens. Treatment is provided in accordance with the National HIV/AIDS Treatment and Care guidelines developed based on the protocols of WHO and leading Western countries [5,6,10,14]. Recommended initial regimen consists of two NRTIs and one NNRTI or, in special circumstances, one PI. Whereas the second line regimen generally consists of ritonavir boosted PI in combination with two NRTIs.

As per National guidelines, the standard of HIV care in the country relies upon laboratory monitoring of immune system using CD4 counts, viral suppression

by measuring plasma HIV RNA level and development of resistance with genotypic drug resistance test. CD4 counts and viral load are measured on a quarterly basis, while drug resistance test is performed in cases of suspected virologic failure.

Emergence of HIV drug resistance has been recognized as a threat to sustained impact of HAART on the epidemic as the world witnesses the expansion of resistance through transmission of drug resistant viruses [8]. However, it is believed that the driving force of resistant HIV epidemic is the evolution of resistant variants in patients failing on HAART [3]. It is therefore important to study the drug resistance in treated populations, especially in those showing evidence of virologic failure.

The objectives of this study was to explore the utility of routine use of viral load and HIV drug resistance testing for the timely detection of HIV drug resistance in clinical care and to describe the patterns of genotypic drug resistance.

Materials and methods. HIV infected individuals enrolled in HAART program since 2004, who experienced virologic failure and underwent HIV genotypic drug resistance testing are the focus of present analysis. Subjects were identified through laboratory and medical records. Data on demographic and clinical characteristics were abstracted through chart review.

The virologic failure was defined as plasma HIV RNA level >400 copies/mL 6 months or >50 copies/mL 12 months after commencing treatment in patients that remain on HAART, or if patient had viral rebound confirmed by two consecutive measurements following the undetectable plasma HIV RNA levels while on therapy. Date of suspected virologic failure was defined as date of blood drawn for resistance test.

For genotypic resistance testing the TruGene HIV-1 Genotyping Kit (Bayer HealthCare LLC, Tarrytown, NY) was employed according to the manufacturer's instructions using OpenGene DNA Sequencing System. Resistance mutations, defined as substitutions as compared to a reference wild type HIV-1_{HXB2} sequence, were classified according to the International AIDS Society (IAS)-USA Panel definitions [9]. The viral strain was considered resistant to a given drug class if at least one of the IAS-USA mutations for nucleoside and nonnucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs) or one of the major mutations for PI was evidenced in the sequence.

For the subtype classification sequences of the *pol* region were aligned using the ClustalW multiple sequence alignment program. Phylogenetic analyses were conducted using MEGA version 4.0. The neighbor-joining method and Kimura two-parameter model were used for tree construction, with reliability estimated from 1000 bootstrap replicates. Subtype reference sequences were obtained from the Los Alamos HIV Sequence database.

Statistical analyses were carried out using SAS 9.1 (SAS Institute, Cary, NC, USA). Standard descriptive statistics were performed to describe patient characteristics. Values of plasma HIV RNA level were $\log_{10}$ transformed to stabilize the variance. Differences between continuous variables were tested using Wilcoxon Signed-Rank Test or Mann-Whitney U Test where appropriate, whereas Fisher's exact test was utilized for categorical data. All statistical tests were carried out at a significance level of 0.05.

Results and their discussion. Overall, virologic failure was suspected in 47 patients based on viral load monitoring. In two patients genotyping could not be performed and thus were excluded from the analysis. Main characteristics of remaining 45 patients are summarized in Table 1. Briefly, 73.3% were males, the mean age was 33.7 and predominant exposure risk category was injection drug us (IDU) - 57.8%. At the time of HAART initiation the mean CD4 count was 187 cells/mm^3 and mean viral load was 5.2 $\log_{10}$ copies/mL. HAART consisted of two NRTIs – Zidovudine (AZT) or Abacavir (ABC) or Stavudine (d4T) and Lamivudine (3TC) in combination with one NNRTI – Efavirenz (EFV) or Nevirapine (NVP). The most frequently prescribed initial regimen was AZT + 3TC + EFV (42.2%). Nine (20%) patients had been exposed to ARVs prior to enrollment in the program. Thirty-nine (86.7%) patients were infected with HIV subtype A1 strains, which clustered with A variants circulating in the Eastern European countries.

Patients were exposed to HAART on average for 15 months. At the time of virologic failure mean viral load was 4.3 $\log_{10}$ copies/mL, with nearly 30% of patients having viral load less then 10000 copies/mL and overall 85% having viral

load below the 100 000 copies/mL; mean CD4 count was 239 cells/mm^3. At least one resistance mutation was detected in 34 (75.6%) patients (table 1). Median number of mutations was 2. Resistance to a single drug class was found in three (6.7%) patients, dual-class drug resistance – in 30 (66.7%) patients. One (2.2%) patient carried triple-class resistance mutations.

Table 1. Main characteristics of 45 patients with virologic failure included in the analysis

Characteristic	Value
Gender, n (%)	
Male	33 (73.3)
Female	12 (26.7)
Age at seroconversion, mean (SD)	33.7 (7.4)
Exposure risk category n (%)	
Injection drug use	26 (57.8)
Heterosexual Contact	11 (24.4)
Other	8 (17.8)
CD4 count at HAART initiation, mean cells/mm^3 (SD)	186.9 (109.3)
Viral load at HAART initiation, mean $\log_{10}$ copies/mL (SD)	5.2 (0.8)
Initial HAART Regimen, n (%)	
AZT+3TC+EFV	19 (42.2)
AZT+3TC+NVP	14 (31.1)
d4T+3TC+EFV	7 (15.6)
d4T+3TC+NVP	2 (4.4)
ABC+3TC+EFV	3 (6.7)
Time (months) to virologic failure, mean (SD)	15.4 (8.4)
CD4 count at virologic failure, mean cells/mm^3 (SD)	238.7 (147.3)
Viral load at virologic failure, mean $\log_{10}$ copies/mL (SD)	4.3 (0.6)
Viral load at virologic failure, n (%)	
< 10 000	13 (28.9)
10 000 to 100 000	25 (55.6)
> 100 000	7 (15.5)
Drug Resistance, n (%)	
At least one resistant mutation	34 (75.6)
No resistant mutations	11 (24.4)
Number of resistant mutations, median (IQR)	2 (1-3)
HIV Subtype, n (%)	
A	39 (86.7)
B	6 (13.3)

SD = Standard Déviation ; IQR = Interquartile Range

The frequency of mutations in the reverse-transcriptase (RT) gene is shown in figure. The most commonly detected NRTI mutation was M184V/I (68.9%). The frequency of mutations selected by thymidine analogues (M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) was relatively low, with only 10 (22.2%) patients having any thymidine analogue mutation (TAM). Most prevalent TAM was T215Y/F detected in 6 (13.3%) patients. Only three (6.7%) had ≥3 TAMs. G190S/A was the most frequent NNRTI mutation (42.2%), followed by K103N (28.9%). One patient (2.2%) had PI mutation D30N.

The difference in frequency of mutations and resistance patterns between patients who were receiving their first HAART regimen and those with prior ARV therapy experience did not reach statistical significance. Patients with prior exposure to ARVs were more than two times as likely to develop any TAM compared to those first starting HAART. However the difference was not statistically significant. Similarly, no statistically significant difference was found between these two groups with regard to selecting ≥3 TAMs (table 2).

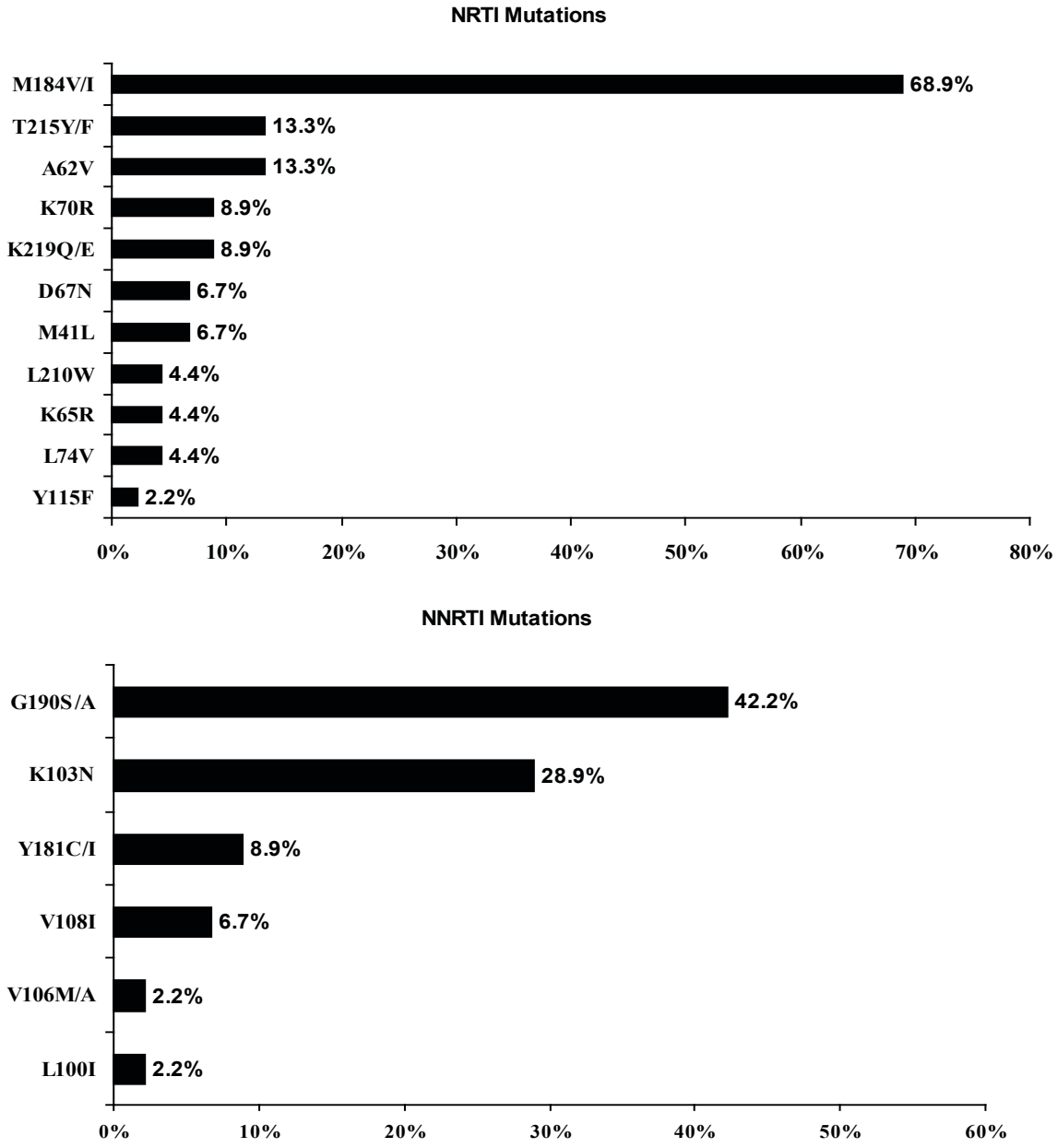

Figure. Frequency of resistant mutations in reverse transcriptase region of pol gene

Table 2. Comparison of drug resistance patterns between patients with prior exposure to ARVS and those first starting HAART

	Prior ARV exposure	First HAART regimen	*P* value
Number of mutations, median	2	2	*0.86**
Any resistance mutation, %	88.9	76.2	*0.42*†
Dual class resistance, %	77.8	66.7	*0.70*†
At least one TAM, %	44.4	16.7	*0.09*†
≥3 TAMs, %	11.1	5.6	*0.50*†

**Mann-Whitney U Test; †Fisher's exact test*

All patients with drug resistance mutations were switched to second line regimens guided by resistance profile of the virus. To study the virologic and immunological outcomes among patients with resistance, we analyzed data on 23 patients who had at least two follow-up measurements of CD4

and viral load. Nearly half of these patients were switched to Abacavir (ABC), Didanosine (ddI), Lopinavir/ritonavir (LPV/r) regimen. A mean decrease of 2.4 $\log_{10}$ was observed in viral load by the first follow-up measurement with the mean time to measurement of 6 months. A mean gain in CD4 count at the first follow-up was 61 cells/mm^3. Statistically significant virological and immunological improvements have been further sustained. At a mean time of 19 months after resistance testing mean decrease in plasma viremia was 2.5 $\log_{10}$ copies/mL and mean increase in CD4 counts - 181 cells/mm^3 compared to values observed at the time of treatment failure (table 3).

Table 3. Virologic and immunological outcomes in 23 patients with drug resistance, who had at least 2 follow-up visits after drug resistance test (mean time to first follow-up visit: 6 months, mean time to last follow-up visit: 19 months)

	Virologic failure	First Follow-up	Difference (p value)*	Last follow-up	Difference (p value)*
Viral load, mean $\log_{10}$ copies/mL	4.3	1.9	2.4 (<0.01)	1.8	2.5 (<0.01)
CD4, mean cells/mm^3	209	270	61 (<0.01)	391	181 (<0.01)

**Compared to mean value at virologic failure (Wilcoxon Signed-Rank Test)*

Access to HAART is expanding rapidly worldwide. However, this progress might be threatened by the epidemic with resistant variants of HIV. Accumulation of drug resistant mutation is a sequential process [4,11] and, therefore, monitoring of viral load is an essential tool for predicting resistance, while the use of HIV drug resistance testing is important for future treatment decision. Since 2004 Georgia, first among former Soviet Union (FSU) countries, ensured universal access to free-of-charge ARVs. While effective selection algorithm ensures sustainability of the universal coverage, comprehensive laboratory monitoring builds the basis for the long-term success of HAART. In present paper we report on successful application of viral load and HIV genotypic resistance testing in clinical care in Georgia. It should be mentioned that Georgia first among FSU countries implemented HIV genotypic resistance testing in HIV clinical care.

Routine monitoring of viral load allowed for early identification of patients failing on HAART, thus reducing the opportunity for mutations to accumulate. Overall small number of mutations and low frequency of TAMs is suggestive of shorter exposure to failing regimens. Preventing accumulation of resistant variants through timely regimen modification has major implications for the therapeutic success [15]. In Georgia, a treatment decision during virologic failure is based on genotypic resistance testing and this approach warrants the effective design of subsequent regimens. Resistance test guided modification of HAART led to favourable virologic and immunologic responses. Challenges should also be mentioned: although majority of patients achieved durable viral suppression after regimen switch, part of the patients had measurable viremia at the last follow-up, emphasizing the need of continuous provision of adherence support.

The present analysis revealed the high prevalence of mutations in codon 184, which is consistent with previous reports, demonstrating rapid emergence of M184V after commencing HAART [7]. Unlike previous studies, G190S/A but not K103N was the most common NNRTI mutation. It is possible that G190S/A is the favoured NNRTI mutation in HIV subtype A1. Further investigation of clade-specific resistance pathways is needed to provide more detailed picture, which may have important implications for the clinical management of infection with HIV subtype A1. Changes in resistance profile should be expected in future in terms of increased frequency of mutations in RT positions 65 and 74 as a result of 2007 revision of national treatment guidelines, which recommends ABC and Tenofovir (TDF) as preferred NRTIs for the initial HAART regimen.

In conclusion, incorporation of viral load and resistance testing in routine clinical care in Georgia allowed for early identification of virologic failure caused by HIV drug resistance. Clinical utility of resistance testing has been already shown after only four years of universal availability of ARVs in the country. It will gain more clinical relevance as epidemic matures and mutations accumulate on a population level.

In addition to apparent advantages on an individual level, routine use of sophisticated laboratory methods should become part of the strategy to combat HIV drug resistance. The evolution of resistance during treatment failure is an important determinant of the risk for transmitted resistance, therefore surveillance of HIV drug resistance, including resistance patterns, among ARV-experienced patients is necessary to guide future regimen selections, to limit emergence of resistance and to inform public health actions.

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SUMMARY

SUCCESSFUL APPLICATION OF LABORATORY TOOLS FOR THE DETECTION OF HIV DRUG RESISTANCE IN ROUTINE CLINICAL CARE IN GEORGIA

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Since 2004, Georgia the first among Eastern European countries ensured universal access to highly active antiretroviral therapy (HAART). Laboratory monitoring of HAART using CD4 count, viral load (VL) and HIV genotypic resistance testing was carried out in according with National HIV/AIDS Treatment Guidelines. Georgia the first among former Soviet Union countries implemented HIV genotypic resistance testing in HIV clinical care. The present paper reports on successful application of laboratory tools in routine clinical care for the early detection of HIV drug resistance. For genotypic resistance testing the TruGene HIV-1 Genotyping Kit (Bayer HealthCare LLC, Tarrytown, NY) was used according to manufacturer's instructions. Analysis included 45 patients with virologic failure. Of them 34 (75.5%) had at least one resistant mutation. Dual-class drug resistance was found in 30 (66.7%) patients. One (2.2%) patient carried triple-class resistance mutations. Median number of resistant mutations was 2. Most commonly detected

NRTI mutation was M184/V/I (68.9%). G190S/A was the most frequent NNRTI mutation (42.2%), followed by K103N (28.9%). All patients with drug resistance mutations were switched to a second line regimens. Analysis of virologic and immunological outcomes among 23 patients who had at least two follow-up measurements of CD4 and VL after resistance test, showed statistically significant decrease in VL by 2.5 $\log_{10}$ and mean gain of 181 cells/mm^3 in CD4 count by the last available measurement. Routine monitoring of VL and subsequent use of HIV drug resistance testing allowed for early identification of HIV drug resistance, reducing the opportunity for mutations to accumulate. Routine use of sophisticated laboratory methods for HAART monitoring has beneficial impact on clinical outcomes and should be used as part of the strategy to combat resistance.

Key words: HAART, HIV drug resistance, virologic failure.

РЕЗЮМЕ

ПРИМЕНЕНИЕ ЛАБОРАТОРНЫХ МЕТОДОВ ДЛЯ ВЫЯВЛЕНИЯ РЕЗИСТЕНТНОСТИ ВИЧ В РУТИННОЙ КЛИНИЧЕСКОЙ ПРАКТИКЕ В ГРУЗИИ

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С 2004 г. Грузия первая в Восточной Европе обеспечила универсальный доступ к высокоактивной антиретровирусной терапии (ВААРТ). Лечение проводится в соответствии с Национальными гайдлайнами по лечению СПИДа, предусматривающими лабораторный мониторинг ВААРТ с помощью CD4 числа, вирусной нагрузки (ВН) и генотипной резистентности ВИЧ. Грузия первая среди стран бывшего Советского Союза внедрила тест на генотипную резистентность ВИЧ в клинической практике. Представленная работа описывает успешное применение лабораторных методов в рутинной клинической практике для раннего выявления резистентности ВИЧ. Комплект генотипирования TruGene HIV-1 (Bayer HealthCare LLC, Tarrytown, NY) был использован в соответствии с инструкциями производителя для выявления резистентности. 45 пациентов с вирусологической неэффективностью были включены в анализ. У 34-х пациентов была обнаружена, по крайней мере, 1 мутация. Резистентность к двум классам медикаментов была выявлена у 30-и пациентов, один пациент имел резистентность к трем классам медикаментов. Среднее число резистентных мутаций равнялось 2-м. У пациентов, принимающих нуклеозидные ингибиторы обратной транскриптазы наиболее часто выявляемой мутацией была M184/V/I (68,9%). У пациентов, принимающих ненуклеозидные ингибиторы обратной транскриптазы наиболее часто выявляемой мутацией была G190S/A (42,2%), за которой следовала K103N (28,9%). Все пациенты с резистентностью были переведены на режимы второй линии. Анализ вирусологических и иммунологических результатов среди 23-х пациентов, у которых после теста на резистентность, по крайней мере, 2 раза измеряли CD4 и ВН, показал статистически достоверное снижение ВН на 2.5 $\log_{10}$ и повышение числа CD4 в среднем на 181 клеток/mm^3. Рутинный мониторинг ВН с последующим применением теста на резистентность способствовал раннему выявлению ВИЧ резистентености, снижая вероятность накопления мутаций. Рутинное применение высокотехнологических лабораторных методов для мониторинга ВААРТ имеет положительное влияние на клинические исходы и должно быть использовано в рамках стратегии по борьбе с резистентностью.

IMPLEMENTATION OF PMTCT IN GEORGIA

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Implementation of PMTCT strategies in developed countries reduced transmission rate from 30-45% to less than 2% [2,4,5,10].

Prevention of mother-to-child transmission (PMTCT) of HIV is one of the strategic priorities as defined by the National Strategic Plan of Action (NSPA) of Georgia in 2006-2010 years.

First case of management of HIV infected pregnant woman for prevention of HIV vertical transmission took place in 1999. Before 2005 access to VCT was limited to the capital - Tbilisi, where it was provided in 2002- 2005 by the Elizabeth Glaser Pediatric AIDS Foundation (EGPAF) and was operated by the Maternal and Child Care Union.

The comprehensive PMTCT program was implemented in 2005. Georgia was the first among the former Soviet Countries that ensured the universal access to PMTCT of all pregnant women throughout the Country. PMTCT service is provided free of charge and is supported by the Government of Georgia, the Vishnevskaya-Rostropovich Foundation (VRF) and the Global Fund To Fight Aids, Tuberculosis and Malaria (GFATM).

All pregnant women with confirmed HIV diagnosis are involved in the PMTCT program implemented by the Infectious Diseases, AIDS and Clinical Immunology Research Center (IDACIRC) in partnership with Georgian Obstetricians and Gynecologists Association. The comprehensive approach to PMTCT is based on existing evidence [1,6,7,12-14,16] and includes: provision of prophylactic ART in pregnant and newborn; counseling and support for delivery and infant feeding; including infant PCP prophylaxis by TMP-CTX.

Materials and methods. There are 116 VCT Centers for pregnant women in Georgia located at Women Health Centers, maternity hospitals, and at different regional hospitals of Georgia.

Testing on HIV/AIDS was based on identification of HIV antibodies by screening method, using Rapid Anti-HIV (1&2) Test InTec PRODUCTS, INC. By protocol, all reactive samples were sent for confirmation by Western Blot method to IDACIRC. Confirmation were done by retesting of sample using Vironostica HIV Uni-Form II Ag/Ab, bioMerieux, The Netherlands by ELISA with further confirmation by Western Blot method using HIV BLOT 2.2 Western Blot Assay. After confirmation, results were sent back to the councilors for post-test consultation. Post-test counseling was held in the same institution. All HIV-positive women were referred to IDACIRC for follow-up, psychological support and clinical/ prophylactic interventions.

According to the present national guideline, PMTCT is initiated from the 24th-28th week of gestation for ARV naive pregnant women (without clinical/laboratory indications for HAART). ARV regimens are following: 1. If Viral load is less than 10 000 c.ml, AZT monotherapy is administrated. Delivery is performed vaginally (viral load is less than 1000c/ml) with AZD+3TC+NVP oral combination in woman and in newborn or with caesarean section (viral load is more than 1000c/ml) at the 38th week of pregnancy with i/v AZT during delivery and child is given AZT.
2. If viral load is more than 10 000 c/ml, AZT+ 3TC+ SQV/rtv is administered. Delivery is performed vaginally (if viral load is less than 1000c/ml) or with caesarean section (viral load is more than 1000c/ml) at the 38th weeks of pregnancy with i/v AZT during delivery. Child is given AZT.
3. In case pregnant women' CD4 is less than 200/mm^3, AZT+3TC+NVP is administered with caesarean section with i/v AZT during section. Child is given AZT.
4. In case pregnant woman requires ARV treatment with CD4 200-350/mm^3, AZT+ 3TC+ SQV/rtv is administered with caesarean section with i/v AZT during section. Child is given AZT.
5. In case pregnant woman is on ARV therapy, regi-

men continues unchanged. In case regimen contains EFV, it is switched to ABC or SQV/r or NVP in the 1st trimester. Caesarean section is performed with i/v AZT during section. Child is given AZT.

Mothers are recommended not to breast-fed infants. PMTCT program considers also PCP prophylaxis of HIV exposed children.

All exposed infants are screened for HIV infection twice using virological test (HIV DNA PCR) at ages 48 hours and 4 months of age by two positive consecutive PCR Test results done by AMPLICOR HIV-1 DNA PCR TEST V1.5 of whole blood (Roche diagnostics).

Data collection was made retrospectively, using information from IDACIRC National HIV/AIDS Data Base, VRF for the period 1999–2008.

Results and their discussion. In 2005-2008 years the prevalence of HIV among pregnant women was 0.03%. A total of 84 pregnancies in 78 HIV-infected women were reported in Georgia from 1999 to 2008. Among them 5 pregnancies were ceased intentionally and 2 pregnant women leave country before delivery. 77 pregnancies (75 HIV positive women) were monitored by IDACIRC. Majority of women were in the age group of 20 to 30 at the time of HIV diagnosis, were unemployed (72%) and married (84%) (Table 1). Nine women were internally displaced persons.

Table 1. Demographic Characteristics of HIV positive pregnant women in Georgia

Variables	No of pregnant women (%)
Age, year	
17-19	4 (5 %)
20 – 24	22 (29 %)
25-29	33 (45 %)
30 – 49	16 (21 %)
Unemployed	49 (77 %)
Employed	12 (19 %)
Student	3 (4%)
Married	63 (84 %)
Divorced	3 (4 %)
Single	5 (7 %)
Widow	4 (5 %)

Distribution of HIV positive pregnant women by regions of Georgia correlated with HIV prevalence in these regions, with most of cases reported from the capital city of Tbilisi.

Sixty nine women (92%) acquired infection through heterosexual contact and in 6 cases (8%) mode of transmission was not ascertained. None of the HIV-positive pregnant women reported intravenous injection of illicit drugs. The majority of women had one sexual partner (91%). There was low demand and use of condoms and contraceptives in this population. With regard to sexual partners: 62 (83%) were HIV-positive, 6 (8%) were HIV- negative and in 7 (9%) cases partner HIV status was not detected. Among 62 positive partners 44 (71%) were infected through injecting drugs intravenously and 12 (19%) - through heterosexual contacts.

All HIV pregnant women admitted at the IDACIRC underwent the comprehensive clinical and laboratory investigations. CD4+ cell count in 40 cases of pregnancy was more than 500 cells/mm^3, in 29 cases 200–500 cells/mm^3, and in 8 cases were below 200 cells/mm3. AIDS was determined in 8 cases of pregnancy. Viral load was above 1,000 cop/ml in 70 cases and below this level in 7 cases.

Anemia was frequent problem in HIV-positive pregnant women in Georgia. Main reason of anemia was iron deficiency.

Prophylactic strategy was tailored individually according to the acting national guideline, women gestation age, HIV disease stage, availability of ARV's, patients' desire to receive prophylactic service etc.

Table 2. Results of laboratory investigation of HIV positive pregnant women

Laboratory tests & co-infections	No of cases of pregnancy (%)
CD4 count, cells/mm^3	
>500	40 (52%)
200-500	29 (38%)
<200	8 (10 %)
Viral Load	
>1 000	70 (91 %)
<1 000	7 (9 %)
Hemoglobin level >100 g/l	57 (74 %)
100 -80 g/l	18 (23 %)
<80g/l	2 (3 %)

Table 3. Cases of Pregnant women undergone PMTCT program in Georgia

No of cases	ARV			Mode of delivery	ARV of infant	Infant feeding	No of HIV infected children	Comments
	During pregnancy		During delivery					
	Before/From 28th week of gestation	Later than 28th week of gestation						
14	-	-	-	caesarian section or vaginal	NVP or AZT+NVP or AZT+3TC or No ARV	Infant formula or breast feeding	2	HIV status was defined short before delivery or ARV was not available or according to the pregnant women's desire.
13	AZT	-	NVP or i/v AZT	caesarian section	NVP +/ - AZT	formula or breast feeding	0	ARV was not fully available
31	AZT+3TC+ SQV/rtv	-	I/v AZT	caesarian section	AZT + NVP	formula	0	
5	AZT+3TC+ NVP	-	I/v AZT	caesarian section	AZT + NVP	formula	0	
6	-	AZT+3TC+ SQV/rtv	I/v AZT ±NVP	caesarian section	AZT + NVP	formula	1	Admission in the late stage of pregnancy.
5	AZT+3TC+ SQV/rtv							

36 pregnant women received full prophylaxis therapy and all children were negative for HIV infection. Among this group 5 women began ARV regimen consisting AZT+3TC+NVP for their HIV status before or during pregnancy.

In 33 cases pregnant women received PMTCT services partially. The reason was: late HIV diagnosis (6 cases), limited access to ARV from 1999 till 2004 (26 cases), refusal by women to receive PMTCT service (1 case). In 3 cases children were HIV-infected. Two cases were registered before 2004, no ARV was used, delivery was performed vaginally and children received infant feeding. Another HIV infected child was born from the woman who admitted IDACIRC in the 3rd trimester of pregnancy. In addition, in one case HIV status of the child could not be determined due to loose of follow-up.

In case of 13 cases when pregnant women received partial prophylactic therapy due to limited access to ARV,

regimen was consisted of AZT from about the 28th week of pregnancy. During delivery they received NVP or i/v AZT. Caesarean section was performed in all cases. Newborns received combination of NVP and AZT. They were fed by infant formula or breast. No cases of HIV transmission was recorded in this group.

As of November 2008, 8 women are still pregnant. Five of them receive ARV prophylaxis with AZT+3TC+SQV/r. Three women are under 28 weeks of gestational age (Table 3).

Over the last several years the national response to AIDS in Georgia achieved significant progress. Implementation of nationwide program on PMTCT should be regarded as another major step towards universal access (UA) to HIV prevention, treatment, care and support along with the achievement of universal coverage with ART. All services are implemented in compliance with UA basic principles, implying that services have to be equitable, accessible, comprehensive and sustainable over the long-term [9].

Provision of comprehensive packages of PMTCT services in Georgia has been shown to minimize the risk of vertical transmission. PMTCT programs are indisputably the main entry point not only for HIV-related care and treatment for women, but also for other comprehensive care and prevention.

Although most of the pregnant women were infected through heterosexual contacts, majority of them acquired infection from their IDU partner. In Eastern European countries, with more mature epidemics and high prevalence in injecting drug users, sexual transmission from users to non-injecting sexual partners has long been the dominant mode of heterosexual transmission [3]. This interpretation is very important as it confirms that as in early years, Georgia's HIV epidemic is driven by IDU [11,15]. At the same time, female sexual partners of IDU may serve as bridging population for expansion of HIV epidemic. Implementation of universal access to PMTCT in Georgia along with prevention interventions for IDUs can serve as key element for halting spread of the infection from population at risk to general public and avoiding wide-scale epidemic in the country.

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SUMMARY

IMPLEMENTATION OF PMTCT IN GEORGIA

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To review the existing experience in prevention of Mother-To-Child Transmission (PMTCT) of HIV in Georgia the comprehensive PMTCT state program was started in 2005. Georgia was the first among the former Soviet Countries that ensured the universal access to PMTCT throughout the Country.

According to the National PMTCT protocol, all pregnant women are offered Voluntary Counseling and Testing for HIV infection at Women Health Centers, maternity hospitals, and regional hospitals of Georgia. Positive results are referred to the Infectious Diseases, AIDS and Clinical Immunology Research Center (IDACIRC) for the confirmation and management that implies: antiretroviral therapy, caesarian section, infant feeding by formula and PCP prophylaxis by TMP-CTX. Data were collected using National HIV/AIDS Data Base.

Prevalence of HIV among pregnant women attending VCT services in 2005-2008 years was 0.03%. Throughout the period 1999–2008 total 84 pregnancies were registered at the IDACIRC, among them 77 pregnancies were monitored by IDACIRC. Prophylactic strategy was tailored individually according to the national acting guideline, women gestation age, HIV disease stage, ARV's availability, etc. Totally 36 pregnant women received full PMTCT service. In this group no vertical transmission of HIV infection was recorded. 33 pregnant women received partial PMTCT service. The reasons were: late HIV diagnosis, limited access to ARV (from 1999 till 2004), refusal by pregnant woman. Number of HIV transmission cases was 3 in this group. As of November, 2008 eight women are still pregnant.

Since 2005 Georgia ensured comprehensive and sustainable PMTCT service throughout the Country and universal access for all pregnant women. Provision of full package of this service minimized the risk of vertical transmission.

Key words: Mother-To-Child Transmission of HIV, prophylaxis, pregnant women, vertical transmission of HIV infection.

РЕЗЮМЕ

ВНЕДРЕНИЕ ПРОГРАММЫ ПРОФИЛАКТИКИ ВЕРТИКАЛЬНОЙ ПЕРЕДАЧИ ВИЧ-ИНФЕКЦИИ В ГРУЗИИ

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Профилактика вертикальной передачи ВИЧ-инфекции является одной из стратегических программ в Грузии, которая осуществляется с 2005 года. Протокол национальной программы по профилактике вертикальной передачи ВИЧ-инфекции предусматривает: добровольную консультацию и тестирование всех беременных на ВИЧ-инфекцию, конфирмацию положительного результата в Центре инфекционной патологии, СПИДа и клинической иммунологии (Western Blot Essay) и последующий менеджмент ВИЧ инфицированных беременных.

Материал для ретроспективного обзорного исследования был предоставлен базой данных Центра инфекционной патологии, СПИДа и клинической иммунологии. Превалентность ВИЧ-инфекции среди беременных женщин, про-

шедших добровольную консультацию и тестирование в 2005-2008 гг. составила 0,03%. Среди ВИЧ-инфицированных беременных 83% были инфицированы гетеросексуальным путем. Среди них не было интравенных наркоманов. Большинство инфицированных женщин (91%) имели одного полового партнера. В 1999-2008 гг. 36 женщин получили полную профилактическую терапию, в результате чего дети были ВИЧ негативными. 33 женщины получили неполную профилактическую терапию. Причинами этого явились поздняя диагностика ВИЧ-инфекции, недоступность антиретровирусных препаратов (в 1999-2004 гг.), отказ беременных от получения профилактической терапии. ВИЧ трансмиссия отмечалась в трех случаях. На сегодняшний день на учете состоят 8 женщин. Среди них 5 получают профилактическую терапию, а 3-е находятся на ранних этапах беременности. Грузия одна из первых среди бывших Советских республик осуществила полноценную национальную программу по профилактике вертикальной передачи ВИЧ-инфекции на всей территории страны. Исследование показывает высокую эффективность программы по профилактике вертикальной передачи ВИЧ-инфекции, которая до минимума снизила риск вертикальной передачи ВИЧ-инфекции.

DISTRIBUTION OF HIV-1 RESISTANT POLYMORPHISMS AMONG HIV INFECTED PATIENTS IN GEORGIA

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Various factors including prevalence and spread of sexually transmitted diseases (STD), frequency of intravenous drug use (IVDU), as well as a variety of socioeconomic factors influence the rate and severity of HIV/AIDS epidemic in a particular country.

An important role in contributing to the rapid or slow spread of HIV in some areas of the world and in the rate of progression of AIDS in particular patients is contributed by host genetic factors such as chemokine receptors CCR5 and CCR2, and chemokine SDF1[10].

The CCR5- Δ32 mutation, a 32-basepair deletion from the coding region of the CCR5 gene, creates a premature stop codon, producing a defective receptor that is not expressed at the cell surface. Homozygotes are resistant to HIV infection with R5 viruses, because they lack the requisite HIV-1 entry coreceptor on their lymphoid cells [4]. Therefore, individuals homozygous for the mutant allele, are resistant to HIV-1 infection [4,13,14]. Heterozygotes express less than half the wild-type levels of CCR5 receptors, slowing HIV-1 replication, and delaying progression among infected persons. [4,7,11,12,13,14].

Some research studies indicate that the CCR2-64I protein can preferentially dimerize with CXCR4 polypeptides (the HIV-1 receptor that replaces CCR5 as an entry receptor at later stages), whereas the wild-type CCR2 peptides do not. Thus, this mechanism suggests that CCR2-64I delays AIDS by limiting the transition from CCR5 to CXCR4 using viruses in HIV infected individuals, a turning point in the destruction of the CD4-T lymphocyte cell population and a prelude to AIDS-defining disease [2]

Another HIV resistance mutation is the mutation in the stromal-derived factor-1 (SDF-1), which is the chemokine ligand of CXCR4, the co-receptor used by the more pathogenic X4HIV-1 strains for cell entry [3,9]. Co-receptor usage of primary human

immunodeficiency virus type I isolates varies according to biological phenotype. A G-to-A substitution at position 801 in the 3 prime untranslated regions of the SDF-1 gene has been reported to slow HIV disease progression [16], although this finding is still controversial [6].

CCR2-64I and SDF1-3'A mutations appear to be common in all ethnic groups [5,15,] while Δ32 allele is most common in Caucasian populations, where the frequency of the mutant allele ranges from 4-16%; approximately 20% of Caucasians are heterozygous and 1% are homozygous for CCR5 Δ32 [11]. This allele, however, is less common among African Americans and Latinos/Hispanics, and was absent in African and Asian populations studied in anthropological surveys and investigations of non-HIV-related conditions [11].

Mutations of CCR5, CCR2 and SDF1 genes have been studied in various ethnic groups and populations, but the study of these host genetic factors has been rare in the countries of former Soviet Union (FSU). The frequency of genetic variants of the CCR5, CCR2 chemokine receptors, and SDF1 chemokine genes among HIV infected population has not been studied in Georgia.

The larger study of CCR5-32, CCR2-64I and SDF1-3A mutations involving general population and HIV exposed seropositive individuals is underway and the results will be published later.

This paper article describes the frequency of the HIV-1 resistance conferring polymorphisms, i.e. CCR5-32, CCR2-64I and SDF1-3A mutations among 120 HIV-1 seropositive individuals as well as the correlations between CCR5Δ32, CCR-64I and SDF1-3A genotypes and HIV disease progression in our country.

Materials and methods. Study Population. 120 HIV infected individuals from different region of Georgia were enrolled in the study of CCR5, CCR2 and SDF1 genetic variants. HIV infected patients registered at Infection Diseases, AIDS and Clinical Immunology Research Center (IDACIRC) were enrolled in the study during their monthly follow up visits. Interviews with the HIV infected patients were followed by their signing an informed consent and a blood draw. The basic demographic data included were their age, gender, and ethnicity. Subjects were interviewed to determine their self-reported drug use, sexual behavior, and history of exposure to blood or blood products. A 2 ml blood specimen was obtained from each study subjects for CCR5, CCR2 and SDF1 genotyping. Collected blood was spotted on a filter paper and dried at room temperature for 24 hours before further processing.

The group of HIV infected patients who were enrolled in the study consisted of 47 subjects infected by heterosexual contact, 5 by homosexual transmission, and 59 subjects infected by parenteral exposures/ injection of illicit drugs. Among the latter group, 4 were infected by transfusion of HIV-1 contaminated blood or medical injections, 4 patients were infected vertically. The route of acquisition of HIV-1 in one person was unknown.

Laboratory methods. Genomic DNA was extracted from the dried blood spots using Qiagen kits according to the manufacturer's instruction. Extracted genomic DNA was subjected to the PCR reaction for genotyping. Genotyping of CCR5 Δ 32, CCR2 64I, and SDF1 3´A alleles was performed as reported previously with minor method modifications [1,8]. The primers used for PCR reaction were as follows: CCR5 Δ32 forward: 5´- GCCAGGACG-GTCACCTTTGG -3´, reverse: 5´- CCACAGAT-ATTTCCTGCTCCCCAG -3´; CCR2 64I forward: 5´- CATCTCGTTCTCGGTTTATCAG-3´, reverse: 5´- GATGATTCCTGGGACAGAAGC -3´; SDF1 3´A forward: 5´- CAGTCAACCTGGGCAAAGCC-3´, reverse: 5´- AGCTTTGGTCCTGAGAGTCC-3´ (Table 1). All genomic DNA amplification reactions were carried out with appropriate negative controls in parallel to detect contamination at each step of the procedure. The PCR cycling conditions were the same for all three amplification assays.

Statistical analysis. Allele frequencies were estimated by Hardy –Weinberg equilibrium. SPSS 13.0 version was used for the data processing and statistical analysis. CCR5, CCR2 and SDF1 genotypes was categorized into 3 groups: wild type, heterozygous and homozygous. Analyses were performed by comparing these 3 groups in overall and according to their HIV disease stage (HIV infection or AIDS). Chi-Square Test was used to assess the relationship between HIV disease stage and prevalence of CCR5, CCR2 and SDF1 genotypes.

Table 1. Primer sequences and digestion enzymes

	Primer sequence	Digestion enzyme	Digested product size	Source
CCR-5	F- 5'-GCCAGGACGGTCACCTTTGG-3'	EcoR1	W - 267, 370	pesonal comunication
			W/M - 267, 338, 370	
	R- 5'CCACAGATATTTCCTGCTCCCCAG-3'		M – 267, 338	
SDF 1-3A	F –5'CAGTCAACCTGGGCAAAGCC-3'	Fok I-	W - 302	Apostolakis et al
			W/M - 302, 202, 100	
	R- 5'AGCTTTGGTCCTGAGAGTCC-3'		M- 202, 100	
CCR 2-64 I	F-5'CATCTCGTTCTCGGTTTATCAG-3'	HPAII	W - 134,387	Lewandovs-ka et al
			W/M - 134,183,204,387	
	R-5'GATGATTCCTGGGACAGAAGC-3'		M - 134,183,204	

Briefly, 5 µl- 10× PCR buffer, 6 µl- 25Mm $MgCl^{2+}$, 4 µl- 10mM dNTP mix, 0.1 µl- of each primer, 2.5 unit of Taq polymerase, 29.3 µl-dd-H_2O and 5 µl of the purified genomic DNA was added to a total mastermix volume of 45 µl.

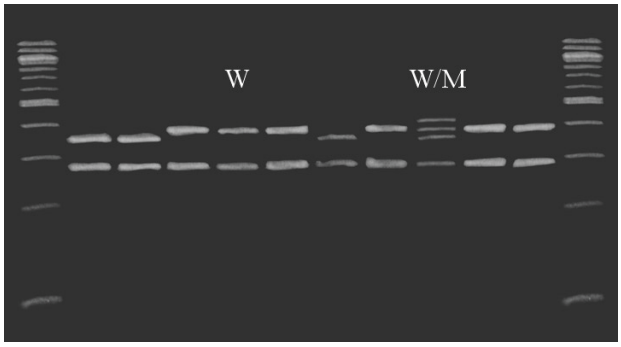
Amplification reactions were performed in a thermocycler GeneAmp PCR system 9700 and the program for DNA amplification consisted of one cycle of initial denaturation (94°C for 2 min), 35 cycles of amplification (94°C for 30 sec, 58°C for 30 sec, and 72°C for 30 sec) followed by a final extension (72°C for 10 min).

For evaluation, CCR5 Δ32, CCR2 64I and SDF1 3´A alleles, amplified products were digested with the restriction endonuclease EcoR1, HPAII and Fok I respectively (Table 1), and the digested products were separated by electrophoresis on 2.0% agarose gel. Based on the characteristic product sizes for the wild type and variant alleles, the genotypes for CCR5 Δ32, CCR2 64I, and SDF1 3´A were determined for each subject.

Results and their discussion. The wild type of CCR5 produced two fragments of 267 bp and, 370 bp, and CCR5-Δ32 hoterozygous formed three fragments of 267, 338, 370 bp (Fig. 1). The wild type of CCR2 produced a fragment of 134, 387 bp, while CCR2-64I homozygous allele produced three fragments of 134 bp, 183 bp, and 204 bp (Fig. 2). SDF1-3′G formed a fragment of 302 bp, while SDF1-3′A formed two fragments of 202, 100 bp (Fig. 3).

Frequencies of the CCR5 Δ 32, CCR2 and SDF1 genotypes. Genotypes and allelic frequency of CCR5-Δ32, CCR2 and SDF1-3′G allele were evaluated in a group of 120 HIV infected patients in Georgia.

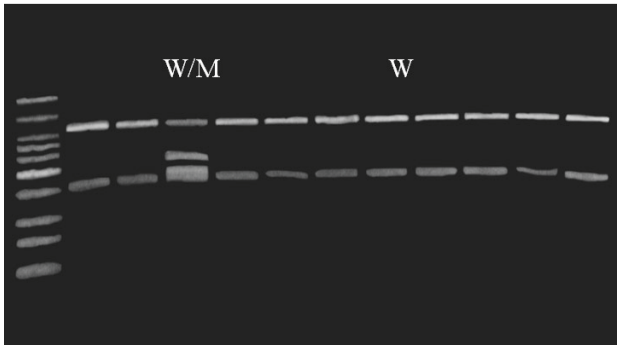
100 bp ladder



W- Wild type homozygotes
W/M - Heterozygotes

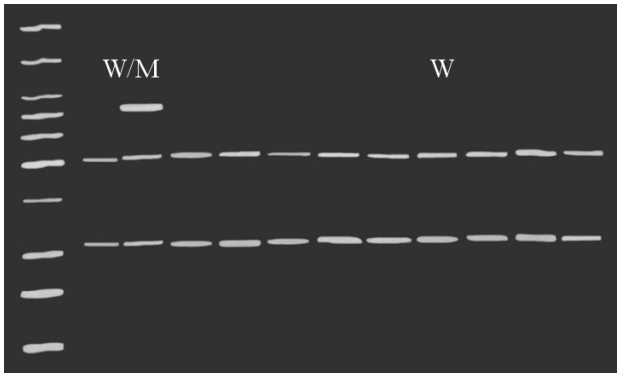
Fig. 1. Analyses of CCR5 gene on the 2% agarose gel electrophoresis with ethidium bromide staining

100 bp ladder



W- Wild type homozygotes
W/M - Heterozygotes

Fig. 2. Analyses of CCR2 64I gene on the 2% agarose gel electrophoresis with ethidium bromide staining



W- Wild type homozygotes
W/M - Heterozygotes

Fig. 3. Analyses of SDF1-3A gene on the 2% agarose gel electrophoresis with ethidium bromide staining

Six subjects (5.85%) were found to be heterozygous for CCR5 Δ32 mutation giving an allele frequency of 2.4 (Table 2). As expected, no subjects were detected who were homozygous for CCR5-Δ32 deletions. Among 6 patients with heterozygous mutation 5 had HIV infection and only one had developed AIDS. There was a correlation between the HIV disease stage (HIV infection or AIDS) and presence of CCR5 Δ32 mutation ($p<0.05$).

The above allele has been reported to be quite common among Caucasian- European populations but rather rare in Middle East and completely absent in Africa. The CCR5-Δ32 polymorphism is found all across Europe at different allele frequencies, with a North to South downhill gradient and lower distribution in the regions of Southeast Mediterranean.

Table 2. Genotypes and allelic frequencies of HIV/AIDS protective mutations among HIV infected Georgian population

	W/W[a]			W/M[b]			M/M[c]		Mutation allele frequencies (%)
	n	%		n	%		n	%	
CCR5	114.07	95.05		5.85	4.8		0	0	2.4
CCR2	95.26	76.38		23.3	19.4		1.42	1.18	10.88
SDF1	55.3	46.08		52.3	44.4		12.3	10.25	32.45

a- *Wild type homozygotes*
b- *Heterozygotes*
c- *Mutant type homozygotes*

Genotyping of CCR2 64I polymorphism revealed 2 homozygous (1.18%) and 22 heterozygous (19.4%) subjects among 120 HIV/AIDS patients giving the allele frequency of 10.88 % (Table 2). Among 2 patients with a homozygous mutation 1 had HIV infection and the other had developed AIDS. Among 22 patients with a heterozygous mutation 8 had HIV infection and 14 had developed AIDS. Our study did not reveal significant relationship between the HIV disease stage (HIV infection or AIDS) and the presence of the CCR2 64I mutation.

The CCR2 64I polymorphisms vary considerably across different racial groups. The distribution within European countries is from 6.5-17 %. The frequency of 10.88% among Georgians is similar to that reported among European populations.

Genotyping of SDF1 polymorphism revealed 53 heterozygous (44.1%) and 12 homozygous subjects (10%) among the study population. The overall allele frequency was 32.45%. Among 12 patients who were homozygous for the mutation 5 had HIV infection and 7 had developed AIDS. Among 53 patients who were heterozygous for the mutation 28 had HIV infection and 25 had developed AIDS. Our study did not reveal a significant relationship between the HIV disease stage (HIV infection or AIDS) and the presence of the SDF1 mutation. The prevalence of SDF1 mutant allele has substantial global variation, with the highest frequency observed in Oceanic populations (54-71%). The highest prevalence in the European countries was observed among Greeks living in Crete and it is less than 32.45%.

Overall frequency of CCR2 and CCR5 mutations is comparable to the frequency among European population. Moreover, to our knowledge, the frequency of SDF1-3A allele frequency in Georgians is higher than reported in European countries. We found a delay

in the progression of HIV infection among persons who were between heterozygous for the CCR5 Δ32 mutation. In order to explore the impact of host genetic factors on the HIV epidemic in Georgia, host genetic studies involving different groups like general population and HIV exposed seronegative individuals would be of interest.

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SUMMARY

DISTRIBUTION OF HIV-1 RESISTANT POLYMORPHISMS AMONG HIV INFECTED PATIENTS IN GEORGIA

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Host genetic factors are believed to play an important role in the pathogenesis and natural history of HIV disease along with determining the rate and severity of HIV epidemic in a particular country. CCR5, CCR2 and SDF1 genes are known to influence the susceptibility to HIV-1 infection and to be involved in the rate of disease progression. Unlike CCR5 Δ32 mutation, mutations in CCR2-64I and SDF1-3A do not provide full protection against HIV-1 acquisition,

however, they are believed to delay the onset of AIDS defining illness.

The objectives of this study were to evaluate the prevalence of host genetic factors among HIV infected patients in Georgia in order to define the correlations between CCR5Δ32, CCR-64I and SDF1-3A genotypes and HIV disease progression in our country.

120 HIV infected individuals were enrolled in the study. Mutations were detected by the polymerase chain reaction/restriction fragment length polymorphism method.

We have studied the DNA polymorphisms at the loci that encode these proteins in 120 HIV infected individuals. As expected, no CCR5 homozygous 32 base pair mutation was found among HIV infected persons, however 6 heterozygous patients produced allele frequency 2.5%. Allele frequency of CCR2 and SDF1 allele was equal to 10.75% and 32 % respectively.

The overall frequency of CCR2 and CCR5 mutations is comparable to their frequency among European populations. However, to our knowledge, the frequency of SDF1-3A allele frequency in Georgians is higher than has been reported in European countries. We found a delay in the progression of HIV infection among persons who were between heterozygous for the CCR5 Δ32 mutation. In order to explore the impact of host genetic factors on the HIV epidemic in Georgia, host genetic studies involving different groups would be of interest.

Key words: Host genetic markers, HIV infection, HIV natural history.

РЕЗЮМЕ

РАСПРОСТРАНЕНИЕ ВИЧ-1 УСТОЙЧИВИХ ПОЛИМОРФИЗМОВ СРЕДИ ВИЧ-ИНФИЦИРОВАННЫХ ПАЦИЕНТОВ В ГРУЗИИ

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Генетические факторы играют значимую роль в патогенезе и клиническом лечении ВИЧ-инфекции, а также в распространении и тяжести ВИЧ-эпидемии в конкретной стране. Как известно, маркеры CCR5, CCR2 и SDF1 имеют защитное воздействие против ВИЧ-инфекции, а также задерживают прогрессирование заболевания. В отличие от CCR5, CCR2 и SDF1 не обеспечивают полной защиты от ВИЧ инфицирования, однако известно, что они задерживают развитие СПИДа. Целью настоящего исследования явилось установление генетических факторов среди ВИЧ-инфицированных пациентов в Грузии и их корреляции с прогрессированием заболевания.

Исследования были проведены среди 120-и ВИЧ-инфицированных пациентов. Мутации были обнаружены путем полимеразной цепной реакции. У 120-и пациентов обследовали полиморфизм ДНК в тех локусах, где происходит кодирование данных протеинов. Как и ожидалось, CCR5 гомозиготная мутация у пациентов не обнаружена, а частота аллелей составила 2,5%. Частота аллелей CCR2 – 10,75%, а SDF1 - 32%.

В целом, частота CCR2 и CCR5 мутаций схожа с таковой среди населения Европы. По нашим данным, частота аллеля SDF1 выше, чем у европейских народов. Проведенные исследования выявили взаимосвязь между стадией заболевания СПИДа и гетерозиготной формой мутации CCR 5.

С целью определения роли генетических факторов в развитии ВИЧ-эпидемии в Грузии, особый интерес представляет определение генетических факторов в общей популяции и риск группах.

NEUROLOGICAL COMPLICATIONS IN PATIENTS WITH HIV/AIDS

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Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS), which is a multi-system disorder including the central nervous system (CNS). Neurological diseases are the first manifestations of AIDS in 7-20% of people with HIV [2]. The spectrum of neurological disorders is broad and involves the CNS and the peripheral nervous system (PNS). Neurological disorders related to HIV often result in reduced quality of life and shortened survival, especially in people with more advanced HIV disease. Nevertheless, some neurological conditions are mild, readily treatable, or reversible. Several have become less common since the introduction of highly active antiretroviral therapy (HAART) [5,10].

HIV-related neurological disorders may develop directly from infection with HIV, or indirectly as a result of opportunistic illnesses (OIs) or treatment complications. For example, OIs such as toxoplasmosis often arise from reactivation of previous infections when immune system defenses break down. Viruses that cause progressive multifocal leukoencephalopathy (PML) may be activated by HIV itself. Toxic side effects of certain anti-HIV medications may affect peripheral nerves and muscle [4].

CD4 lymphocytes number determines the risk of development of certain opportunistic infections. CD4 lymphocytes count is the best predictor of immediate or short-term risk of developing of new opportunistic diseases. It is known that most complications occur with increasing frequency at lower CD4 cell counts. It has been also suggested correlation between the neurological disorders in HIV infected patients and course of HIV infection. However this issue relationship of neurological manifestations of HIV infections with CD4 cell need further investigation.

HIV associated neurological disorders often complicate later stages of HIV disease and occur in up to 40%-60% of all patients with AIDS during their lifetime. HIV enters the CNS at the early phase of infection [9], persists in that system for decades and induces multiple symptoms of motor, cognitive dysfunction and behavioral changes. Many factors can contribute to the neuropathology of AIDS, particularly opportunistic brain infections such as cryptococcus, Toxoplasma gondii, JC virus, cytomegalovirus, Epstein-Barr virus, Varicella zoster virus, and human herpes virus type 6 [2, 6-8].

The aim of the study was to determine the prevalence of HIV-related neurological (CNS) disorders in HIV positive patients and its relationship to CD4 cell counts in Georgia.

Material and methods. The investigation was conducted at the Infectious Diseases, AIDS & Clinical Immunology Research Center (IDACIRC) of Georgia. This study included HIV/AIDS patients, who have been admitted to the in-patient Department of IDACIRC since 2006. Therefore, 388 patients were included in the study, 302 men and 86 women.

The HIV/AIDS patients were divided into two groups: antiretroviral treatment naïve and ARV treated patients. In both groups we studied the prevalence of the following neurological diseases: HIV associated dementia, CNS Toxoplasmosis, tuberculosis (TB) meningitis, criptoccocosis meningitis, progressive multifocal leukoencephalopathy (PML), cytomegalovirus (CMV) encephalitis, bacterial meningitis, primary CNS lymphoma.

Diagnose of neurological disorders was made based on clinical symptoms and instrumental-laboratory investigations. All subjects had a complete physical examination and carried out the necessary diagnostic procedures by consultation with the neurologist. Detection of HIV antibodies was performed by ELISA (third or fourth generation) with further confirmation by Western Blot Assay.

Measurement of CD4 lymphocyte count was performed by indirect Immunofluorescent assay by using the monoclonal antibodies.

HIV associated dementia was diagnosed according to the history, physical examination, screening with HIV dementia scale and in the absence of other causes of dementia (toxic and metabolic encephalopathy, progressive multifocal leukoencephalopathy, other opportunistic CNS infections) [3]. Magnetic resonance imaging (MRI) showed cerebral atrophy, typically with rarefaction of white matter.

CNS Toxoplasmosis was diagnosed in patients with focal signs and symptoms, multifocal enhancing mass lesions in MRI or computerized tomography (CT), positive toxoplasma antibodies by ELISE method and improvement of symptoms after initiation of unidirectional antitoxoplasmosis therapy [1].

Cryptococcal meningitis was considered in the presence of typical symptoms and detection of the cryptococcal antigen by ELISE method or direct identification of cryptococcal organisms by India ink staining.

TB meningitis was presumed by typical symptoms of basal meningitis or focal sign and symptoms in addition to a) hypodense or isodense rounded lesions with irregular walls of varying thickness, oedema and mass effect, cortical location, ring or nodular enhancement, increased basal meningeal enhancement; b) pulmonary TB on Chest X-ray (CXR) and/or acid-fast bacilli (AFB) on sputum microscopy; c) increased protein, decreased glucose, pleocytosis, positive TB antibodies by ELISE in the cerebrospinal fluid (CSF); d) response to TB treatment - clinically and radiologically.

PML was defined in patients with progressive focal signs and symptoms, a decline in cognitive function, multifocal non-enhancing white matter lesions in the CT and positive JC virus polymerase chain reaction (PCR) in CSF.

Diagnosis of primary CNS lymphoma was based on CT and MRI. MRI showed single or multiple lesions that are isodense or hypodenseand usually homogeneous, but sometimes ring forms. With contrast, CT and MRI scans showed enhancement that was usually irregular (due to rapid growth).

CMV encephalitis was assumed in the presence of focal signs and symptoms, positive CMV-DNA in plasma and CSF, and nonspecific pathological MR findings such as ventriculomegaly, periventricular enhancement, or cortical and subcortical inflammation.

Bacterial meningitis was diagnosed by detection of bacteria in GRAM stain and positive latex for bacterial antigens or culture and pleocytosis in CSF.

Results and their discussion. Among 388 patients admitted to the in-patient department of AIDS Center, CNS neurological complications were detected in 76 patients. 13 patients had two or more neurological complications. It is very interesting that out of HIV/AIDS patients with neurological complications only two patients were on ARV treatment. The median time of HAART use in this two patients was two months (range: 1-3 months), and none of them were virologically suppressed at admission (detection limit of HIV-1 viral load = 400 copies/mL). The median age of patients with neurological complications was 36 years (range: 25-49 years) and 42 (89%) were men.

Tuberculosis meningitis were the most common neurological disorders 26 (34%), followed by CNS toxoplasmosis 17 (22%), cryptococcal meningitis 11 (15%), presumed CMV encephalitis 5 (7%), PML 4 (5%), primary CNS lymphoma 4 (5%) and bacterial meningitis 3 (4%). AIDS related dementia was detected in 18 patients (24%). Distribution of neurological disorders of HIV/AIDS patients are presented in Figure.

The median CD4+ T lymphocyte count was 47 cells/mm^3 (range: 2-183 cells/mm^3). There was correlation between the CD4 T lymphocyte count and type of neurological manifestation. Namely, in the patients with HIV associated dementia median CD4 T lymphocyte count was 164 cells/mm^3, in the patients with CNS toxoplasmosis median CD4 count was 83 cells/mm^3, in the patients with cryptococcal meningitis median CD4 T lymphocyte count was 34 cells/mm^3 and in the patients with CMV encephalitis median CD4 T lymphocyte count was 26 cells/mm^3. Some neurological disorders such as TB meningitis and bacterial meningitis can occur at any CD4 level. PML and primary CNS lymphoma occurred when CD4 T lymphocyte count < 50 cells/mm^3.

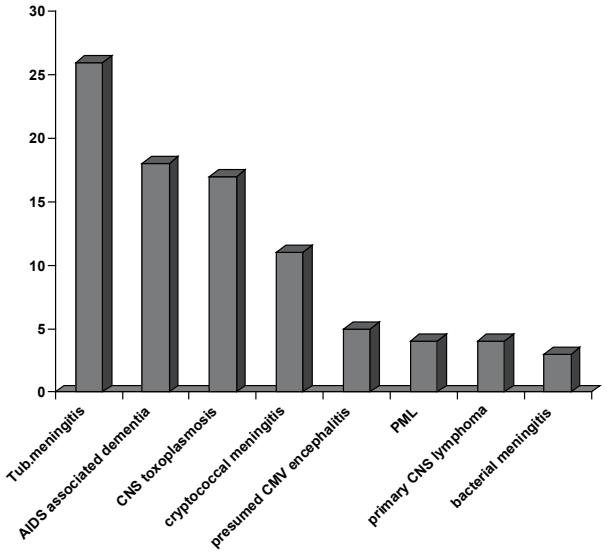

Fig. Distribution of neurological disorders of HIV/ AIDS patients

There is a significant correlation between the level of CD4 T lymphocyte count and type of neurological manifestation of HIV infection. Presence of Neurological complications as well as other clinical manifestations is associated with decreased CD4 T lymphocyte count, while asymptomatic patients usually have high CD4 T lymphocyte count. Thus, level of CD4 T lymphocyte count can serve as an indicator for initiation of prophylaxis treatment of certain opportunistic infections of nervous system in HIV infected patients.

The most common clinical manifestations of neurological disorders in HIV infected patients were headache (91%), fever (75%), focal neurological deficits (61%), speech disturbances (42%), cognitive dysfunction (41%), visual disturbances (36%), impaired coordination (29%) and seizures (15%).

Results of this study provide convincing evidence that neurological disorders with HIV infection might serve as an indicator for advanced HIV infection, immunosuppression and decreased CD4 cell counts. Our data have shown correlation between the type of neurological manifestations of HIV infection and CD4 T lymphocyte count.

Neurological manifestations of AIDS patients may appear atypical, they may be widespread, have prolonged course and the response to treatment may be poorer than expected.

The neurologist's role in the care of HIV-infected patients is to be familiar with HIV-associated neurological disorders, their diagnoses, and management. It is also a part of the extensive interdisciplinary knowledge necessary for any physician who takes care of HIV-infected patients.

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SUMMARY

NEUROLOGICAL COMPLICATIONS IN PATIENTS WITH HIV/AIDS

Bolokadze[1,2] N., Gabunia[1] P., Ezugbaia[1] M., Gatserelia[1] L., Khechiashvili[1] G.

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The aim of the study was to determine the prevalence of HIV-related neurological disorders in HIV positive patients and its relationship to CD4 cell counts in Georgia.

This study included 388 HIV/AIDS patients (302 men and 86 women), who have been admitted to the in-patient Department of Infectious Diseases, AIDS & Clinical Immunology Research Center (IDACIRC) of Georgia since 2006. Diagnose of neurological disorders was made based on clinical symptoms and instrumental-laboratory investigations. CNS Neurological complications were detected in 76 patients; 13 patients had two or more neurological complications. Tuberculosis meningitis were the most common neurological disorders 26 (34%), followed by CNS toxoplasmosis 17 (22%), cryptococcal meningitis 11 (15%), presumed CMV encephalitis 5 (7%), PML 4 (5%), primary CNS lymphoma 4 (5%) and bacterial meningitis 3 (4%). AIDS related dementia was detected in 18 patients (24%). The median CD4+ T lymphocyte count was 47 cells/mm3 (range: 2-183 cells/mm^3) in HIV patients with neurological complications. There was correlation between the CD4 T lymphocyte count and type of neurological manifestation. Namely, in the patients with HIV related dementia median CD4 T lymphocyte count was 164 cells/mm^3, in the patients with CNS toxoplasmosis median CD4 count was 83 cells/mm^3, in the patients with cryptococcal meningitis median CD4 T lymphocyte count was 34 cells/mm^3 and in the patients with CMV encephalitis median CD4 T lymphocyte count was 26 cells/mm^3. Some neurological disorders such as TB meningitis and bacterial meningitis can occur at any CD4 level. PML and primary CNS lymphoma occurred when CD4 T lymphocyte count < 50 cells/mm^3. The most common clinical manifestations of neurological disorders in HIV infected patients were headache (91%), fever (75%), focal neurological deficits (61%), speech disturbances (42%), cognitive dysfunction (41%), visual disturbances (36%), impaired coordination (29%) and seizures (15%). The study provide convincing evidence that neurological disorders with HIV infection might serve as an indicator for advanced HIV infection, immunosuppression and decreased CD4 cell counts. Our data have shown correlation between the type of neurological manifestations of HIV infection and CD4 T lymphocyte count.

Key words: HIV infection, CD4 T lymphocyte count, HIV-related neurological disorders.

РЕЗЮМЕ

НЕВРОЛОГИЧЕСКИЕ ОСЛОЖНЕНИЯ СРЕДИ ВИЧ-ПОЛОЖИТЕЛЬНЫХ ПАЦИЕНТОВ

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Целью данного исследования явилось определение превалентности неврологических расстройств у ВИЧ-положительных лиц и их взаимосвязи с количеством СД4 лимфоцитов. Исследовано 388 пациентов в боксированном отделении Научно-практического центра инфекционных заболеваний, СПИДа и клинической иммунологии. Диагностика неврологических расстройств проводилась на основании клинических симптомов и лабораторно-инструментальных исследований. Неврологические осложнения центральной нервной системы были выявлены у 76-и пациентов. В 13-и случаях было обнаружено сочетание более двух неврологических осложнений. Туберкулезный менингит являлся наиболее частым неврологическим заболеванием и был выявлен в 26-и (34%) случаях, токсоплазмоз ЦНС выявлен в 17-и (22%) случаях, криптококковый менингит – в 11-и (15%), вероятный CMV энцефалит - в 5-и (7%), ПМЛ - в 4-х (5%) случаях, первичная лимфома ЦНС - в 4-х (5%) и бактериальный менингит - в 3-х (4%) случаях. СПИД ассоциированная деменция

была обнаружена у 18-и (24%) больных. Среди ВИЧ положительных пациентов с неврологическими осложнениями среднее количество CD4 лимфоцитов составило 47 cells/mm^3 (диапазон - 2-183 cells/mm^3). У больных с ВИЧ деменцией среднее количество CD4 + Т лимфоцитов составило 164 cells/mm^3, у больных токсоплазмозом - 83 cells/mm^3, у больных криптококковым менингитом - 34 клеток/mm^3 и у больных CMV энцефалитом - 26 cells/mm^3. Некоторые неврологические заболевания, такие как туберкулезный менингит или бактериальный менингит не проявляют корреляцию с количеством CD4. Результаты проведенного исследования выявили, что неврологические осложнения ассоциированы с низким количеством CD4 лимфоцитов. Обнаружена корреляция между числом CD4 лимфоцитов и типом неврологического заболевания.

ASSESSMENT OF LIVER FIBROSIS AND CIRRHOSIS BY TRANSIENT ELASTOGRAPHY AMONG PATIENTS WITH CHRONIC HBV AND HCV INFECTION IN GEORGIA

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Transient elastography (TE) using Fibroscan (Echosens, Paris, France) is a new reproducible, painless and only non-invasive method for assessing liver fibrosis (LF). The device evaluates LF by measurement of liver stiffness (LS). Fibroscan measures the stiffness of the right liver lobe by the intercostal approach. TE can be performed for the staging of LF independent from the underlying liver disease. LS is correlated with the quantity of fibrosis in patients suffering from the following chronic liver diseases: viral hepatitis, chronic alcoholic liver disease, primary billiary cirrhosis, primary sclerosing cholangitis, etc. [5].

Liver fibrosis is caused by the hystopathological changes due to the chronic liver diseases. As a result of chronic inflammation, excess amount of collagen is produced in the liver, which reduces hepatic elasticity without alteration of liver architectonic and functions [1].

Liver cirrhosis is a consequence of chronic liver disease, characterized by replacement of liver tissue by fibrous scar tissue as well as regenerative nodules and connective tissue septas; with altered hepatic architectonic and portal hemodynamic, development of intra – and extra hepatic portocaval anastomosis leading to progressive loss of liver sinthetic and desintoxication functions [1].

Currently liver biopsy is the gold standard to assess the degree of liver damage in chronic liver diseases. A liver biopsy is clearly crucial to establish a diagnosis and to provide prognostic information, but it has limitations. The main limitation of the liver biopsy is that it represents a very small part of the liver (1/50000) and therefore, sampling error can occur. In addition, histological examination is prone to intra- and interobserver variation, which may occur even when widely validated systems are used to score liver damage. Finally, liver biopsy is an invasive procedure with different complications: pain occurs in 20% of patients and bleeding or hemobilia in 0.5%. For this reason, liver biopsy has poor acceptance. Patients are currently aware of noninvasive methods and are reluctant to undergo an invasive procedure if similar information can be obtained using new methods. As-

sessment of liver damage using non-invasive methods is currently an important topic in hepatology [6]. Nowadays TE has being used widely and becomes one of the most reliable methods for assessing the liver fibrosis and cirrhosis; though the method has some limitations. Factors associated with LS measurement non-applicability generally are high body mass index (BMI) and narrow intercostal spaces [5,7]. TE also is contraindicated in pregnant women, in patients with active implantable devices such as a pacemaker or defibrillator and on wounds [5]. Most of these limitations are already resolved by using new, specially modified transducers.

Fibroscan device is composed of 3 key parts: a dedicated probe, an ultrasound system coupled to an elastometry system in the Fibroscan frame, and a specific software program that makes it possible to transcribe information obtained in the exam. Fibroscan using ultrasound is based on pulsed elastography technology and operates according to the following principle: The electrodynamic transducer of the probe generates a low-amplitude mechanical pulse. This mechanical pulse generates a low-frequency elastic wave referred to as a shear wave. The propagation rate of this elastic wave is measured by the ultrasound transducer of the probe and is directly associated with the stiffness of the medium. The ultrasound signals are used to measure the propagation rate of the elastic wave in the liver in order to deduce its stiffness while the elastic wave is passing through the skin, adipose tissue and the liver, ultrasound acquisitions are carried out [5].

The measurement of the stiffness is carried out on the right lobe of the liver by intercostal route. In order to do this, the patient must be lying down on the examination table in supine position with the right arm in maximum abduction. It is recommended to choose a hepatic zone at least 7 cm thick, without big vessels and away from the edges of the liver to obtain reliable measurements. The system displays two ultrasound images: one in A mode (current ultrasound signal) and one in TM mode (time movement signal) which are used to localize the measurement zone that satisfies these criteria. In general, the region chosen for a transparietal biopsy is appropriate [5].

The rationale for the use of Fibroscan in chronic liver diseases depends on the presence of fibrosis within the liver that leads to an increase in the organ's stiffness. The volume of liver parenchyma which can be studied by FibroScan is about 100 times greater than that obtained by biopsy, and has therefore a potentially lower sampling error [10].

Liver stiffness is correlated with the fibrosis stages by Metavir scoring system and also allows to predict such complications of cirrhosis as the presence of varicose, ascites, hepatocellular carcinoma and others [4,6]. E.g. LS – 19 kpa is considered as the cut-off value for predicting the presence of esophageal varicoses grade II or III, LS – 37 kpa is the cut-off value for predicting liver cirrhosis B or C by Child-Pugh, LS 49 kpa and 54 kpa – are cut-off values for predicting ascites and hepatocellular carcinoma, accordingly [4,6].

The most common cause of liver fibrosis and cirrhosis are viral hepatitis. About 180 mln people are infected with hepatitis C virus (HCV) and 300 mln people with hepatitis B virus (HBV) worldwide, among them about 50% have advanced fibrosis and cirrhosis [9].

According to the epidemiological studies performed in Georgia the prevalence of HCV infection in general population is 6.7% [1]. The prevalence of HBsAg in general adult population of Georgia is 1,7% and prevalence of anti-HBc total is 11.4% [8].

Due to the high prevalence of viral hepatitis in Georgia as well as high rate of advanced fibrosis and cirrhosis among HCV and HBV infected persons worldwide, study of prevalence of fibrosis stages in patients with hepatitis C and B in Georgia was considered reasonable.

The aim of the study was to evaluate liver fibrosis and cirrhosis using TE in patients with chronic HBV and HCV infection in Georgia.

Materials and methods. The study was conducted at the Georgian-French joint hepatology clinic "Hepa". 525 patients with chronic HCV infection and 105 patients with chronic HBV infection were investigated from November 2007 till November 2008.

Diagnosis of HCV infection was made based on detection of antibodies against HCV in serum by Enzyme-Linked Immuno Sorbent Assay (ELISA) using ORTO HCV 3.0 test and further confirmed by Recombinant Immunoblot Assay (RIBA), using CHIRON RIBA HCV 3.0 SIA. Detection of HCV RNA was done by PCR method (qualitative) using AMPLICOR HCV

RNA 2.0 test (Roche Diagnostics, Switzerland) and HCV RNA viral load was measured by- Real Time PCR technique using the COBAS TaqMan HCV-2.0 Test, respectively. HCV genotypes were identified among HCV RNA positive specimens by Reverse Hybridization Line Probe Assay (Inno Lipa) using VERSANT HCV Genotype kit 2.0 (Innogenetics, Belgium).

The diagnosis of chronic HBV infection was made based on detection of HBsAg by ELISA using ImmunoLISA HBsAg 2 step kit. HBV DNA viral load was measured by Real Time PCR technique using the Cobas TaqMan HBV Testing (Roche Diagnostics, Switzerland).

TE was performed using the Fibroscan device, which consists of a 5-MHz ultrasound transducer probe mounted on the axis of the probe. Mild amplitude and low-frequency vibrations (50Hz) were transmitted to the liver tissue, inducing an elastic shear wave that propagates through the underlying liver tissue. The tip of the transducer was covered with a drop of gel and placed perpendicularly in the intercostal space with the patient lying in dorsal decubitus position with the right arm in the maximal abduction. Under control TM and A-mode a liver portion within the right liver lobe at least 7 cm thick, free of large vascular structures and gallbladder was chosen. The operator was a staff physician who had previously performed at least 100 determinations in patients with chronic liver diseases. The median value of 10 successful acquisitions, expressed in kilopascal (kpa) with a success rate of at least 60% was kept as representative of the liver stiffness measurement (LSM).

LS<5.5 kpa was considered as no fibrosis, LS - 5.5-8.0 kpa – as mild fibrosis, LS - 8.0-14.0 kpa – as severe fibrosis and LS>14.0 kpa was considered as liver cirrhosis.

In particular, LS<5.5 kpa was considered as fibrosis stage F0-F1 by Metavir, 5.5 -8.0 kpa – fibrosis stage F2, 8.0-10.0 kpa – fibrosis stage F2-F3, 10.0-12.5 kpa – fibrosis stage F3, 12.5-14 kpa – fibrosis stage F3-F4 and LS>14.0 kpa – fibrosis stage F4 by Metavir. In case of necessity liver biopsy was performed.

Results and their discussion. Among investigated 105 patients with chronic HBV infection 65 (61.9%) had no fibrosis (LS<5.5 kpa), 23 (21.9%) had mild fibrosis (LS – 5.5-8.0 kpa), 9 (8.6%) had severe fibrosis (LS – 8.0-14.0 kpa) and 8 (7.6%) had liver cirrhosis (LS > 14.0 kpa).

Distribution of fibrosis stages by Metavir among patients with HBV infection was the following: 65 (61.9%) patients had fibrosis stage F0-F1 (LS<5.5 kpa), 23 (21.9%) patients had fibrosis stage F2 (LS – 5.5 – 8.0 kpa), 4 (3.8%) patients had fibrosis stage F2-F3 (LS – 8.0 – 10.0 kpa), 3 (2.9%) patients had fibrosis stage F3 (LS – 10.0 – 12.5 kpa), 2 (1.9%) patients had fibrosis stage F3-F4 (LS – 12.5 – 14 kpa) and 8 (7.6%) patients had fibrosis stage F4 (cirrhosis, LS > 14.0 kpa).

Among investigated 525 patients with chronic HCV infection 200 (38.1%) had no fibrosis (LS<5.5 kpa), 139 (26.5%) patients had mild fibrosis (LS – 5.5-8.0 kpa), 87 (16.5%) patients had severe fibrosis (LS – 8.0-14.0 kpa) and 99 (18.9%) patients had liver cirrhosis.

Distribution of fibrosis stages by Metavir among patients with HCV infection was the following: 200 (38.1%) patients had fibrosis stage F0-F1 (LS < 5.5 kpa), 139 (26.5%) patients had fibrosis stage F2 (LS – 5.5 – 8 kpa), 50 (9.5%) patients had fibrosis stage F2-F3 (LS – 8.0 – 10.0 kpa), 26 (5.0%) patients had fibrosis stage F3 (LS – 10.0 – 12.5 kpa), 11 (2.0%) patients had fibrosis stage F3-F4 (LS – 12.5 – 14.0 kpa) and 99 (18.9%) patients had liver fibrosis stage F4 (cirrhosis, LS>14 kpa).

TE was characterized with an excellent accuracy. LF by Metavir measured using Fibroscan were well correlated with the clinical signs (spider angiomata, palmar erythema, hepatosplenomegaly, oesophageal varices, ascites, caput medusa, etc.) as well as with the results of laboratory and instrumental investigations (ALT, AST, leukocyte count, platelet count, prothrombin time, INR, albumin, Fibrotest, Fibromax, abdominal ultrasound, gastroscopy, etc).

It is worth to mention that in 14 patients (9 with chronic HCV infection and 5 with chronic HBV infection) liver biopsy was performed. From the total of 14 patients LF stages by Metavir using Fibroscan were completely corresponded with the liver biopsy results in 12 (85.7%) patients. In 2 patents insignificant discordant results were obtained. Particularly, in one patient with HCV infection LS was correlated

with the fibrosis stage F2-F3 by Metavir as liver biopsy showed fibrosis stage F3; and in another person with chronic HBV infection, LS was correlated with the fibrosis stage F3-F4 by Metavir as liver biopsy showed fibrosis stage F3.

TE using Fibroscan is a new, simple, non-invasive, reliable and easily reproducible method for assessing liver fibrosis and cirrhosis in patients with chronic HBV and HCV infections.

TE is characterized with an excellent accuracy. TE results are well correlated with the clinical signs as well as with the results of laboratory and instrumental investigations. Fibrosis stages by Metavir measured using Fibroscan well corresponds with the liver biopsy results. Considering the high prevalence of fibrosis and cirrhosis among patients with chronic HBV and HCV infection, TE is a very valuable method for detecting early stages of fibrosis allowing to avoid the progression of liver damage, as well as end-stage liver disease.

TE is easy to perform and therefore allows regular follow-up of the course of LF.

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SUMMARY

ASSESSMENT OF LIVER FIBROSIS AND CIRRHOSIS BY TRANSIENT ELASTOGRAPHY AMONG PATIENTS WITH CHRONIC HBV AND HCV INFECTION IN GEORGIA

Tsertsvadze[1,2,3] T., Dolmazashvili[1,2,3] E., Abutidze[1,3] A., Sharvadze[1,2,3] L., Karchava[1,3] M.

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The aim of the study was to evaluate liver fibrosis (LF) and cirrhosis using Transient elastography (TE) using Fibroscan in patients with chronic HBV and HCV infection. The device evaluates LF by measurement of liver stiffness (LS).

525 patients with chronic HCV infection and 105 patients with chronic HBV infection were included in the study. These patients were investigated at the Georgian-French joint hepatology clinic "HEPA" from November 2007, till November 2008. Among investigated HBV infected 105 patients 65 (61.9%) had no fibrosis (LS<5.5 kpa), 23 (21.9%) had mild fibrosis (LS – 5.5-8.0 kpa), 9 (8.6%) had severe fibrosis (LS – 8.0-14.0 kpa) and 8 (7.6%) had liver cirrhosis (LS > 14.0 kpa).

Among investigated HCV infected 525 patients 200 (38.1%) had no fibrosis (LS<5.5 kpa), 139 (26.5%)

patients had mild fibrosis (LS – 5.5-8.0 kpa), 87 (16.5%) patients had severe fibrosis (LS – 8.0-14.0 kpa) and 99 (18.9%) patients had liver cirrhosis.

It is concluded that transient elastography (TE) using Fibroscan is simple, non-invasive, reliable and easily reproducible method for assessing liver fibrosis and cirrhosis in patients with chronic HBV and HCV infection. TE is characterized with an excellent accuracy. TE results are well correlated with the clinical signs as well as with the results of laboratory and instrumental investigations. Fibrosis stages by Metavir measured using Fibroscan well corresponds with the liver biopsy results. Considering the high prevalence of fibrosis and cirrhosis among patients with chronic HBV and HCV infection, TE is a very valuable method for detecting early stages of fibrosis allowing to avoid the progression of liver damage, as well as end-stage liver disease. TE is easy to perform and therefore allows regular follow-up of the course of LF.

Key words: transient elastography, fibroScan, liver fibrosis, cirrhosis, chronic HBV infection, HCV infection.

РЕЗЮМЕ

ОЦЕНКА ФИБРОЗА И ЦИРРОЗА ПЕЧЕНИ МЕТОДОМ ЭЛАСТОГРАФИИ СРЕДИ ПАЦИЕНТОВ С ХРОНИЧЕСКОЙ HBV И HCV ИНФЕКЦИЕЙ В ГРУЗИИ

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Эластография печени с применением «ФиброСкана» (Франция, фирма «Эхосенс») является новым, безболезненным методом для оценки фиброза и цирроза и может проводиться у пациентов с хронической HBV или HCV инфекцией для оценки жесткости печени (ЖП). Эластография характеризуется высокой точностью. Результаты эластографии хорошо коррелируют с клиническими данными пациента, а также с результатами лабораторных и инструментальных исследований. «ФиброСкан» определяет фиброз путем измерения ЖП.

Целью исследования явилась оценка фиброза и цирроза печени методом эластографии у больных хронической HBV или HCV инфекцией в Грузии.

В грузино-французской совместной гепатологической клинике «Гепа» в 2007-2008 гг. исследовано 525 пациентов с хронической HCV инфекцией и 105 пациентов с хронической HBV инфекцией. Среди 105-и пациентов, инфицированных вирусом гепатита В, 65 (61,9%) не имели фиброза (ЖП <5,5 кпа), у 23-х (21,9%) выявлен фиброз средней тяжести (ЖП - 5.5-8.0 кпа), 9 (8,6%) пациентов имели тяжелую форму фиброза (ЖП-8,0-14,0 кпа), в 8-и (7,6%) случаях был диагностирован цирроз печени (ЖП>14,0 кпа).

Среди 525-и пациентов, инфицированных вирусом гепатита С, 200 (38,1%) не обнаружен фиброз (ЖП<5,5 кпа), у 139-и (26,5%) выявлен фиброз средней тяжести (ЖП - 5.5-8.0 кпа), 87 (16,5%) пациентов имели тяжелую форму фиброза (ЖП-8,0-14,0 кпа) и в 99-и (18,9%) случаях был диагностирован цирроз печени (ЖП>14,0 кпа).

Стадии фиброза по Metavir, диагностированные с помощью «ФиброСкана», хорошо совпадают с результатами биопсии печени. Учитывая высокую распространенность фиброза и цирроза печени у больных хронической HBV или HCV инфекцией, эластография печени является весьма ценным методом для выявления ранних стадий фиброза, позволяющим избежать осложнения циррозом печени. Эластография печени легко выполни, что позволяет регулярно исследовать пациентов для оценки состояния болезни.

ACUTE/RECENT HCV INFECTION. CLINICAL COURSE, VIRAL REPLICATION KUNETIC AND DISEASE OUTCOME

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HCV infection is one of the major public health problems worldwide. It is associated with very high rates of morbidity, mortality, hospitalization and disability, increasing in proportion to the growing epidemic. Infection with HCV proceeds to chronicity in about 80% of cases and 20-40% of these individuals develop end-stage liver diseases: cirrhosis and hepatocellular carcinoma after 15-20 years of HCV infection [1,-3,5,9,17].

Georgia is among the countries with relatively high prevalence of HCV infection, mostly concentrated among injecting drug user (IDUs). HCV seroprevalence was found to be 68.1 % in IDUs, 2.4% in blood donors (in 2004) and 6.7% in general population of Georgia (2004). Due to these reasons Hepatitis C infection is considered as a top priority problem for the Georgian Health Care System [1,9,11,12,14].

After infection with HCV, as it was mentioned above, most of patients will develop chronic infection with consequent complications. During the acute stage of infection the patient mostly completely asymptomatic. Only 25% of acute HCV infected patients are jaundiced and others remain asymptomatic. Most of symptomatic patients with acute HCV infection (about 40-50%) recover from the virus, while asymptomatic acute HCV patients mostly develop chronic infection [1,2,4,6,9,13,16].

The knowledge about the natural disease course from the very early stage of acute/recent HCV (before seroconversion) infection is very limited and the mechanisms that determine the disease outcome are not well understood as well. Although it is widely assumed that host (HLA, virus-specific immune response) and viral (genotype, replication kinetic) characteristics can play an important role [4-8,10,13-16].

Studies addressing these scientifically very interesting and important questions in humans are also very restricted and more research is needed to clarify the natural history of acute/recent HCV infection

The problem of studying the natural history of acute/recent HCV infection is that HCV has long-lasting clinically silent incubation period and it is practically impossible to catch and identify the patients before the onset of clinical symptoms. Consequently there are very restricted possibilities to observe the natural history of acute HCV infection and relationship of its outcome with host genetic factors, viral replication kinetic, immune response and molecular characteristics of the virus from the very early days of infection.

The objecties of the presented study were: to reveal and investigate Acute/Recent HCV infection from the very first days (before seroconversion) of the infection in order to asses clinical laboratory variations of infection, viral replication kinetic, disease outcome, host and virus characteristics

The specific aims were: Detection of acute /recent HCV infection by PCR testing among ELISA negative blood donors and IDUs and follow up of revealed infection.

Our study was one of the first attempts worldwide to investigate the natural history of acute/recent HCV infection after the very first days of exposure and host and viral factors related to it.

Materials and methods. A prospective two-year follow-up study was performed in two groups: Anti-HCV ELISA seronegative 7000 blood donors and Anti-HCV ELISA seronegative 3000 IDUs with high risk practice (needle and syringe sharing).

For detection of HCV infection from the very first days the special study design was elaborated. Mini-pool method was used for investigation: a pool of 6 was applied for blood donors' a pool of 5 for IDUs (fig.1). The sample and pool sizes were selected based on the current epidemiological data on HCV prevalence in blood donors (2.4%) and IDUs population (68.1%) in Georgia.

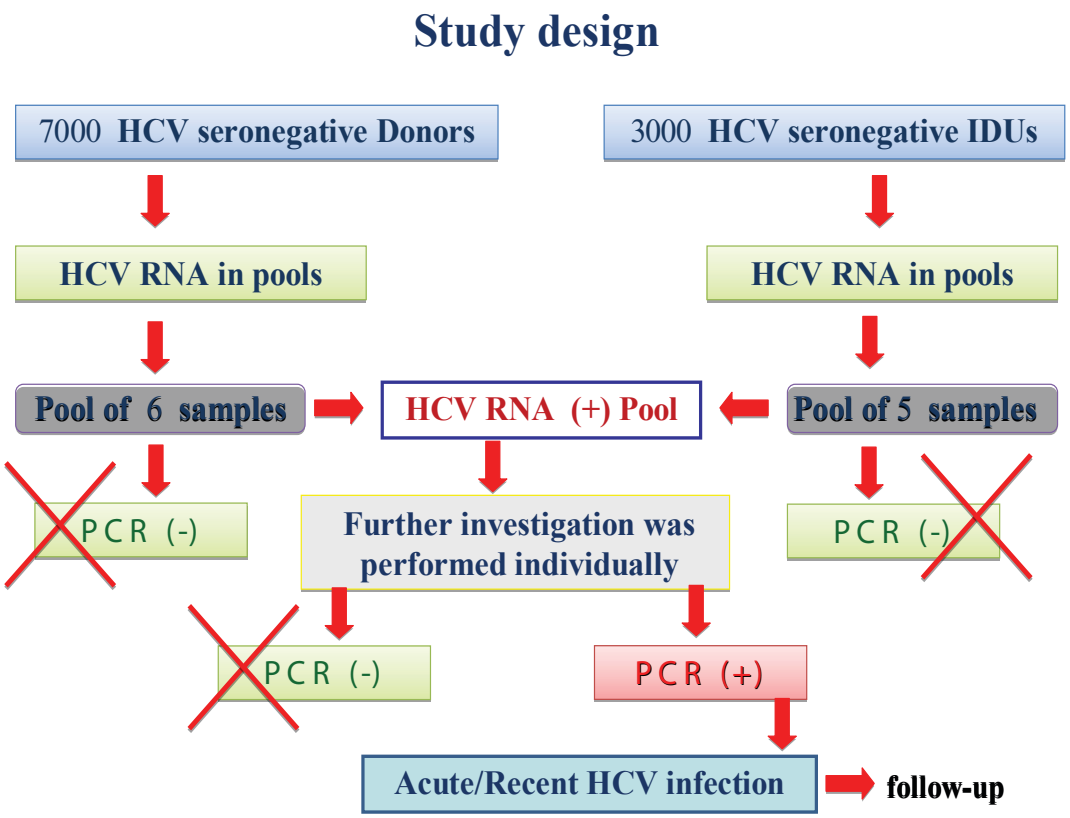

Fig.1 Study design

PCR negative pools were excluded from the further study, while PCR positive pools of blood donors and IDUs were examined on individual samples.

Detection of HCV RNA by PCR in ELISA negative persons gave us unique possibility to detect early HCV infections (acute/recent HCV infection) in the seronegative window period (before seroconversion) and to follow the disease course.

All HCV RNA positive persons (persons with acute / recent HCV) revealed within the study were followed on viral load dynamic, clinical course and disease outcome at detection moment and at 2, 4 and 8 weeks and after 3, 6 months from the possible exposure.

The following lab and clinical investigations were performed at every visit of acute/recent HCV patient: Quantitative HCV RNA (viral load); clinical chemistry, ultrasound investigation, physical examination. Besides we investigated HCV genetic types and HLA for determining host genetic factors.

Diagnosis of HCV infection was made based on detection of antibodies against HCV in serum by enzyme-linked immunosorbent assay (ELISA) method using ORTO HCV 3.0 test and further confirmed by recombinant immunoblot assay (RIBA), using CHIRON RIBA HCV 3.0 SIA.

Detection of HCV RNA was performed by PCR method (qualitative) using AMPLICOR HCV RNA 2.0 test (Roche Diagnostics, Switzerland).

Detection of HCV RNA viral load was measured by Real time PCR technique using COBAS TaqMan HCV-2.0 Test.

HCV genotyping was performed by reverse hybridization line probe assay (Inno lipa) using VERSANT HCV Genotype kit 2.0 (Innogenetics, Belgium).

HLA typing was performed by Sequence Specific Primer Amplification (SSP).

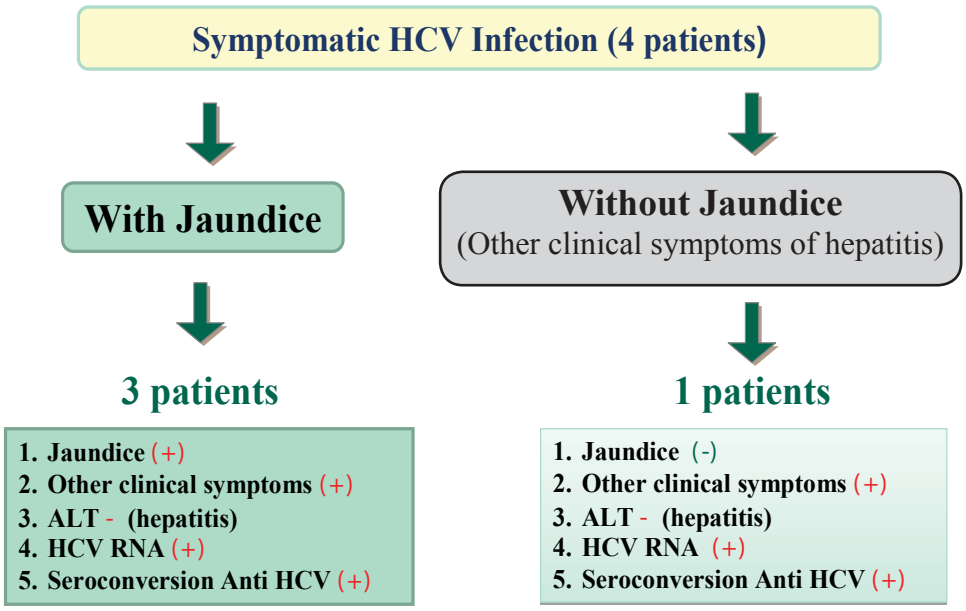

Fig.2 Clinical laboratory variants of acute/recent HCV infection

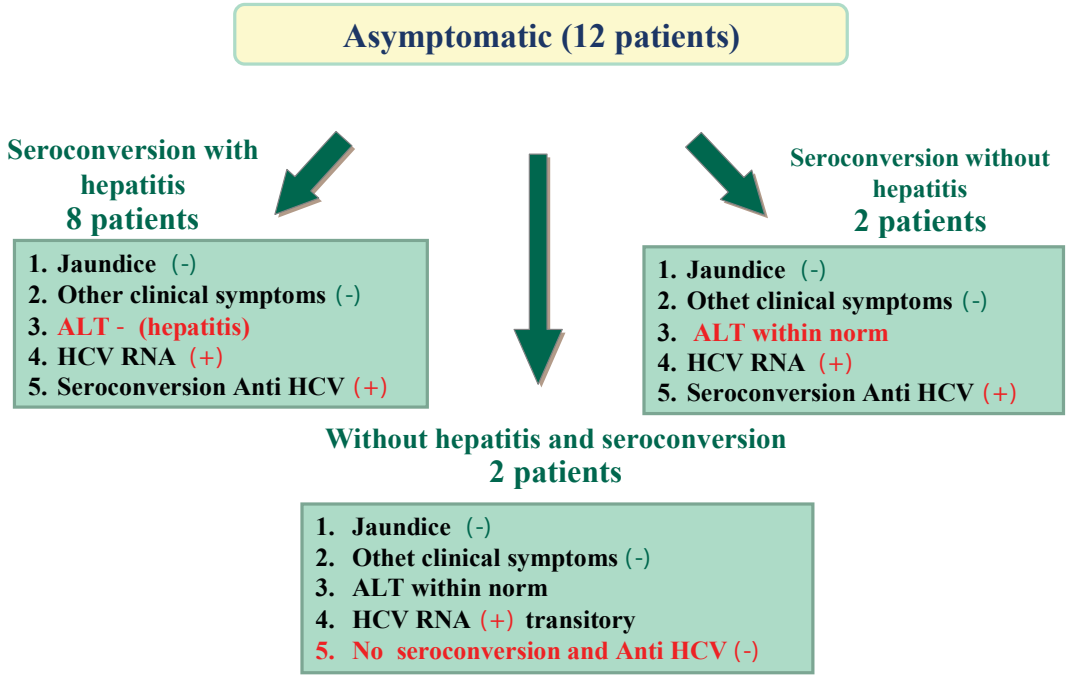

Fig.3. Clinical laboratory variants of acute/recent HCV infection

Results and discussion. Totally, among investigated Anti-HCV seronegative 7000 blood donors and Anti-HCV seronegative 3000 IDUs we identified 16 patients with acute/resent HCV infection: 7 patients were from blood donors and 9 patients -from IDUs.

The following clinician laboratory variants of acute/recent HCV infection were identified: 4 patients out of 16 were symptomatic (25%), while others 12 were asymptomatic.

Among 4 symptomatic patients: 3 patients were with jaundice and with other clinical symptoms of hepatitis, with elevated ALT, positive HCV RNA and with further anti HCV seroconversion.

Out of 4 symptomatic patients 1 patient was without jaundice, but with other clinical symptoms of hepatitis (fatigue, malaise, dizziness, nausea, fever and etc.), with elevated ALT and with anti HCV seroconversion (fig. 2).

Among 12 asymptomatic patients: 8 patients had elevated ALT with further anti HCV seroconversion.

2 patients had neither elevated ALT, nor any other symptoms, but subsequently showed anti-HCV seroconversion. So, these two patients developed chronic HCV infection without any clinical or biochemical evidence of hepatitis during the observation period.

2 patients were most interesting due to the fact that they had only detectable HCV RNA at the beginning without any clinical or biochemical indices of hepatitis. During the observation period HCV RNA gradually disappeared without further Anti-HCV seroconversion (fig.3)

Therefore we might assume that these 2 patients had transitory HCV infection. Of course we understand that more evidence is necessary to prove this hypothesis.

Clearance or chronisation of infection (disease outcome).

Spontaneous clearance of virus (recovery from the disease) was observed in 4 patients out of 16 patients with acute/recent HCV infection.

Among these 4 recovered patients 2 were symptomatics and 2 asymptomatics:

Among 12 patients with chronic infection: 10 were asymptomatics and 2- symptomatics.

Totally overage recovery rate in whole group was 25% and rate of chronization 75% accordingly.

Spontaneous clearance of virus (recovery from the disease) was observed in 2 out of 4 symptomatic patients and only in 2 patients out of 12 asymptomatics. The latter two recovered patients (asymptomatics) both had elevated ALT.

Rate of recovery was 50% in symptomatic patients and about 16% in asymptomatics.

Distribution of genotypes were as follow: Among 4 recovered patients: 2 patients with genotype 1b, 1- genotype 2a/2c and 1 patient with genotype 3a.

Among 12 patients with chronic infection: 7 with genotype 1b, 1 –genotipe 1a, 2- genotype 2a/2c and 2- with genotype 3a.

Viral replication kinetics of Acute/Recent HCV infection.

In all patients with acute/recent infection viremia was detectable 2 weeks after inoculation, it increased very rapidly and reached a peak titer by week 4. The viral titer was remarkably stable for the next 5-6-7 weeks, falling only two or three fold by week 9. After week 10 the viremia rapidly decreased: ≈>4 logs or ≈>5 logs by week 12 and it became either undetectable by weeks 14-15-16 (viral clearance), or virus was not eliminated and persisted in all follow up period (chronic infection) (fig. 4).

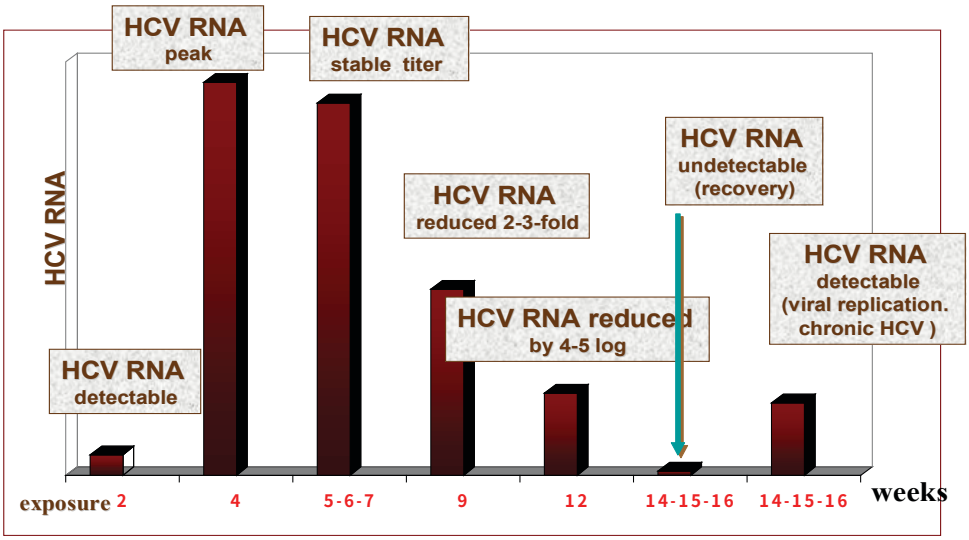

Fig. Viral replication kinetics of acute/recent HCV infection

Based on the received results of HLA investigation the following relationship between HLA alleles and natural history of HCV revealed: HLA DRB1 1101, DQB1 0301 and DRB1 1301/DQA1 0103 alleles were associated with clearance of HCV whereas DRB1 0301 was associated with chronic infection.

Prevalence of HCV among seronegative blood donors was 0.1% and among IDUs 0.3%.

Among acute/recent HCV infected patients rate of chronicity was 75% (50% in symptomatics and 83% in asymptomatics).

Rate of recovery was 50% in symptomatic patients and about 16% in asymptomatics.

Acute/recent HCV infection might have following clinical laboratory forms: symptomatic disease with or without jaundice, asymptomatic with or without elevated ALT, but with further anti-HCV seroconversion.

It remains unclear whether enigmatic form of disease - acute/recent HCV infection without further seroconversion exists or not.

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SUMMARY

ACUTE /RECENT HCV INFECTION. CLINICAL COURSE, VIRAL REPLICATION KUNETIC AND DISEASE OUTCOME

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The aim of the study was to reveal and investigate acute/recent HCV infection at the very early stage in seronegative blood donors and seronegative Injecting Drug Users (IDUs) and to assess clinical laboratory variants of infection, viral replication kinetic, disease outcome, host and viral characteristics. Two groups of patients were included in this study. The first group consisted of ELISA negative 7000 blood donors; the second group included 3000 Injecting Drug Users (IDUs). All patients were investigated on HCV RNA by qualitative PCR using mini pool method. A pool of 6 was applied for blood donors' and a pool of 5 for IDUs. PCR negative pools were excluded from the study, while PCR positives were examined on individual samples. Anti-HCV was detected by ELISA and RIBA. Detection HCV RNA was performed by Real time PCR technique using COBAS TaqMan Test. HCV genotyping –by INNO-Lipa. HLA typing - by Sequence Specific Primer Amplification (SSP). 16 patients with acute/recent HCV were revealed: 7 from blood donors, 9 from IDUs. Among them: 4 were symptomatics and 12 asymptomatics. Out of 4 symptomatics 3 were with jaundice. Among 12 asymptomatics: 8 – had elevated ALT; 2 – neither elevated ALT nor symptoms but developed anti-HCV; 2 – were with normal ALT and without further anti-HCV seroconversion. Among 16 subjects: 9 – had genotype -1b, 1 – genotype 1a, 3 – genotype 2a/2c and 3 – genotype 3a. Out of 16 cases 4 cleared the virus; 12 – developed chronic infection. Spontaneous clearance (recovery from the disease) was observed in 2 out of 4 symptomatic patients and only in 2 patients out of 12 asymptomatics. In all patients viremia increased rapidly and reached a peak by week 4. Viral titer was remarkably stable for the next three weeks, followed by two or three fold decrease by week 9. After week 10 the viremia rapidly decreased: 4 or 5 logs by week 12 and it became either undetectable by weeks 16-18 (viral clearance), or virus was not eliminated and viral titer persisted in all follow up period (chronic infection). HLA DRB1 1101, DQB1 0301 and DRB1 1301/DQA1 0103 alleles were associated with clearance of HCV whereas DRB1 0301 was associated with chronic infection. Prevalence of HCV among seronegative blood donors was 0.1% and among IDUs 0.3%. Among acute/recent HCV infected patients rate of chronicity was 75% (50% in symptomatics and 83% in asymptomatics). Rate of recovery was 50% in symptomatic patients and about 16% in asymptomatics. Acute/ recent HCV infection might have following clinical laboratory forms: symptomatic disease with or without jaundice, asymptomatic with or without elevated ALT, but with further anti-HCV seroconversion. It remains unclear whether enigmatic form of disease - acute/recent HCV infection without further seroconversion exists or not.

Key words: Acute/recent HCV infection, seronegative blood donors, seronegative Injecting Drug Users (IDUs), seroconversion.

РЕЗЮМЕ

ОСТРАЯ РАННЯЯ HCV ИНФЕКЦИЯ. КЛИНИЧЕСКОЕ ТЕЧЕНИЕ, КИНЕТИКА ВИРУСНОЙ РЕПЛИКАЦИИ И ИСХОД ЗАБОЛЕВАНИЯ

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Целью исследования было выявление и изучение острой ранней HCV инфекции среди серонегативных доноров крови и серонегативных потребителей инъекционных наркотиков (ПИН); оценка клинико-лабораторных вариантов инфекции, кинетики вирусной репликации, исхода заболевания, особенностей хозяина и вируса путем наблюдения

за инфекцией с первых дней инфицирования. Исследование проводили в двух группах. Первую группу составили 7000 доноров крови, вторую – 3000 ПИН. Всех пациентов обследовали на наличие HCV РНК с применением качественного PCR метода объединения образцов крови в мини-пулы. Для доноров крови пул состоял из 6-и образцов, а для ПИН – из 5-и. PCR отрицательные пулы были исключены, а PCR положительные пулы были обследованы на уровне индивидуальных образцов. Тестирование на Anti-HCV проводили методами ELISA и RIBA. Наличие HCV РНК определяли методом PCR в реальном времени с помощью COBAS TaqMan Test. Генотипы HCV определяли методом INNO-LiPA. HLA тип определяли методом Sequence Specific Primer Amplification (SSP). Обнаружено 16 больных с острой ранней HCV инфекцией: 7 доноров крови и 9 - ПИН. Из них 4 имели симптоматическую инфекцию и 12 – асимптоматическую. Из 4-х симптоматических больных 3-ое болели желтухой. Из 12-и асимптоматических больных у 8-и отмечалось повышение ALT, у 2-х - anti-HCV без повышения ALT и без клинических симптомов. У 2-х больных с нормальным показателем ALT, anti-HCV сероконверсия не обнаруживалась. Из 16-и больных один имел генотип 1a, девять – генотип 1b, трое – генотип 2a/2c и трое – генотип 3a; 4 выздоровели, а у 12-и развилась хроническая инфекция. Спонтанное выздоровление наблюдалось у 2-х из 4-х симптоматических больных и у 2-х из 12-и асимптоматических больных. У всех больных виремия быстро повысилась и достигла пика к 4-ой неделе. Вирусный титр оставался стабильным на протяжении следующих 3-х недель, с последующим снижением на 2 или 3 раза к 9 неделе. Спустя 10 недель виремия резко понизилась на 4 или 5 log к 12-ой неделе и к 16-18-ой неделе (выведение вируса) не определялась, или вирус не был удален и вирусный титр оставался определяемым до конца наблюдения (хроническая инфекция). HLA аллели DRB1 1101, DQB1 0301 и DRB1 1301/DQA1 0103 были ассоциированы с выведением вируса, а аллель DRB1 0301 - с хронической инфекцией. Превалентность HCV среди серонегативных доноров крови и ПИН составил 0,1% и 0,3%, соответственно. Среди больных с острой ранней HCV инфекцией частота хронизации была 75%, частота выздоровления - 50% у симптоматических и приблизительно16% - у асимптоматических больных. Острая ранняя HCV инфекция может проявляться в симптоматической форме с или без желтухи и асимптоматической форме с или без повышения ALT, однако с последующей anti-HCV сероконверсией. По сей день остается неясным существует ли загадочная форма заболевания – острая ранняя HCV инфекция без последующей anti-HCV сероконверсии.

IMPORTANT ASPECTS OF NOSOCOMIAL BACTERIAL RESISTANCE AND ITS MANAGEMENT

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Nosocomial infections represent one of the most important and dramatically growing problems in healthcare. They are associated with substantial morbidity, mortality and cost. By the estimation of United States Centers for Disease Control and Prevention (CDC) approximately 1.7 million hospital acquired infections have occurred in USA during the year 2002. 98,987 people died from the consequences of nosocomial infections. 35,967 deaths were associated with pneumonia and 30,665 with bloodstream infections.

Urinary tract and surgical site infections accounted for 13,088 and 8,205 lethal outcomes respectively [15]. In United Kingdom more then 100,000 nosocomial infections are responsible for about 5000 deaths per year. Globally 1.4 million people suffer from hospital acquired infections at any point in time. It is estimated that US expenses for these infections alone comprise striking $4.5-5.7 billion annually. The cost for UK is around £1 billion yearly [34].

During last several decades we have witnessed remarkable increase in multi-drug resistant organisms in hospitals. Both gram positive and gram negative bacteria developed diverse, exceedingly effective ways to evade the action of antibiotics. These "super-bugs", as they are called by many, not only are resistant to multiple antibiotics, but it is getting more and more difficult to detect their true susceptibilities by conventional microbiologic methods, making them even more dangerous. Below we will try to illustrate this on the examples of *Staphylococcus aureus* and gram-negative bacteria and provide with certain prospects in the management of these infections.

Once readily susceptible to penicillin, *staphylococcus aureus* has evolved into increasingly resistant and difficult to treat organisms: methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediately resistant *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA). Shortly after the introduction of methicillin in early 1960s, the first MRSA was reported [12]. The genetic locus - staphylococcal cassette chromosome *mec* (SCC*mec*) - a cluster of genes, containing *mec*A gene, is responsible for methicillin resistance [14]. *Mec*A encodes for membrane bound penicillin-binding protein 2a (PBP2a) [36]. PBPs are involved in cross-linkage of cell wall peptidoglycans. β-lactams bind to PBPs and inhibit this vital step of cell wall synthesis. PBP2a is a mutant form with low affinity towards β-lactams, thus abolishing their effect on bacterial growth [18]. There are five types of SCC*mec*: types I, II and III are associated with hospital aquired MRSA (HA-MRSA) and types IV and V with comunity aquired MRSA (CA-MRSA) [5]. The most common type of HA-MRSA is SCC*mec* type II. MRSA is resistant to all β-lactams, including carbapenems. Type II and III SCC*mec*s are large enough to carry other resistant genes to non β-lactam antibiotics, confering multi-drug resistance. The spread of MRSA is mostly clonal, with little evidence of horizontal transfer (between the strains of same species or between different species) of resistance [6]. VISA strains evolve from MRSAs as a consequence of vancomycin exposure. These strains contain chromosome mediated genes which hyper-produce the peptidoglycan layer of cell wall, decreasing the effect of vancomycin. VISA may show susseptible MICs in 1.5-2 range by disk diffusion method, thus confusing physicians. Complete vancomycin resistance was transferred to VRSA from another resistant gram positive bacterium – *Enterococcus faecalis*, via plasmid encoded *van*A gene [33], which is very efficacious way to spread resistance horizontally.

The major mechanism of resistance to antibiotics in Gram negative rods (GNR) are β-lactamases. β-lactamases represent diverse group of enzymes, which hydrolyses β-lactam ring of antibiotics. They can be classified in two ways: according to structural similarities (groups A, B, C and D) [1] or functional characteristics (substrate and inhibitor types) [4]. Each group has subgroups and families. We will use Ambler's structural classification in this article. With the introduction of novel β-lactams (cephamycins, oxyimino-cephalosporins and carbapenems), there was a drastic evolution of β-lactamases, (extended spectrum β-lactamases (ESBLs), AMP-C β-lactamases and carbapenemases), with increasing resistance to new antibiotics.

ESBLs are plasmid encoded enzymes, mainly of group A (TEM, SHV, CTX-M and other minor families) and group D (OXA family). They were first identified in early 1980s. ESBLs can hydrolyze and confer resistance to first, second and third generation cephalosporins and aztreonam. They remain susceptible to cephamycins (Cefoxitin and Cefotetan) and carbapenems, and are inhibited by β-lactamase inhibitors (e.g. clavulanic acid) [27]. The sensitivity to cefepime, the forth generation cephalosporin, is variable between different classes of ESBLs (some OXA and CTX-M families conferring resistance to cefepime). ESBL producing organisms by definition are resistant to oxyimino-cephalosporins (ceftriaxone, cefotaxime, ceftazidime) and aztreonam, so these antibiotics should never be used, even if in vitro tests show sensitivity to one or several of them. Cefepime and piperacillin/tazobactam may show in vitro activity but they tend to produce an inoculum effect, decreasing their efficacy with increased inoculum of bacteria from 10X5 to 10X7 colony forming units [11,35], and their use should be discouraged. Carbapenems are associated with the best results, but cephamycins, aminoglycosides and floroquinolones can also be used [10].

AMP-C β-lactamases (group C β-lactamases) were originaly encoded on the chromosomal genes in *Enterobacter* spp*, Proteus, Citrobacter* spp., *Morganella morganii*, *Serratia marcescens* and etc. Chromosomal AMP-C β-lactamase can be inducible (by different β-lactam antibiotics) or noninducible (derepressed) - producing large amounts of enzymes constantly. During the evolution, these genes were transferred to plasmids and via them were spread to other organisms - *Klebsiella* spp., *Escherichia coli*, *Salmonella* spp and etc. Plasmid encoded AMP-C genes are derepressed, producing broad spectrum resistance not only to oxyimino-cephalosporins but also to cephamycins and are not inhibited by clavulanic acid. The latter two properties distinguish AMP-C from ESBL. Induced resistance is very difficult to detect by current microbiologic methods, and treatment failure can occur during therapy despite initial report of in vitro sensitivity to these antibiotics. AMP-C producing organisms remain susceptible to cefepime. Carbapenems are the best choice for treatment of these infections [25].

Carbapenemases have the broadest spectrum activity among all β-lactamases. They confer resistance to all cephalosporins (including cephamycins), aztreonam (except for metallo-β-lactamases) and carbapenems. There are two basic groups of these enzymes: 1. class B β-lactamases (metallo-carbapenemases) of IMP, VIM, SPM and GIM families, which use zinc ion at the active site and are inhibited by EDTA, 2.Class A (KPC family) and class D (OXA family) serine containing carbapenemases. These genes are now transferred to plasmids, facilitating their interspecies spread. IMP metallo-β-lactamase is mainly confined to *Pseudomonas aeruginosa,* OXA carbapenemase to *Acinetobacter baumannii* and KPC to *Klebsiella pneumoniae* [30]. The literature on the treatment options for carbapenemase producing organisms is rather limited. Potential options include aminogycosides, floroquinolones, colistin, tigecycline. Metallo-β-lactamase producing bacteria can be susceptible to aztreonam too. Detection of carbapenemases by conventional methods can be a problem. Imipenem MICs of KPC-producing *K. pneumoniae* can often be as low as 2 [22] (susceptible MIC range is 4 and less), thus producing falls sense of security.

The transfer of these highly resistant genes (ESBL, AMP-C, carbapenemases) from chromosomes to plasmids enabled them to spread very rapidly, not only by clonal expansion, but also through interspecies dispersion, increasing exponentially the proliferation of MDR gram negative organisms. The deficiencies in laboratory methods of identification and scarce treatment armamentarium for these infections make the problem especially hazardous.

Unfortunately the rapid spread of MDR organisms is accompanied by severe shortage of newly developed antibiotics on pharmaceutical market [32]. Pharmaceutical companies are shifting their interest towards more profitable medications required for chronic conditions, as opposed to antibiotics which usually are used for short periods of time. It is becoming more and more difficult to treat these infections especially when empiric antibiotics are required, before the microbiological culture results are available. This results in delay of effective therapy. Reports have shown the vital importance of timely administration of effective antibiotics. Excessive mortality was found to be associated with delay of appropriate antibacterial therapy in studies of ventilatory associated pneumonia (VAP) [20] and severe sepsis [9]. Hence it is especially important to use more efficiently the existing resources to maximize the positive clinical outcome.

We need to take advantage of pharmacokinetic and pharmacodynemic properties of antibiotics. Concentration dependent antibiotics (aminoglycosides, floroquinolones and daptomycin) should be dosed with the highest possible dose within the therapeutic window, in order to achieve high serum peak levels. The higher Cmax (maximal serum concentration of the drug)/MIC ratio, is associated with better results [2]. The frequency of dosing can be reduced to once a day in most instances, since these drugs have post-antibiotic effect, enabling them to suppress bacterial growth even several hours after their clearance from the blood. Once a day dosing was associated with lower toxicity and better convenience then conventional ways of administration [3]. In contrast, to be effective, time dependent antibiotics (penicillins, cephalosporins, carbapenems, macrolides etc.) need to achieve concentrations above MIC for sufficient amount of time (at least 40-50% of the time). The magnitude of the concentration is not that important as long as it stays above the MIC. This can be accomplished either by more frequent dosing or continuous infusion. This method of administration may possibly target MDRO strains with MICs in intermediate resistance range, when there are no other fully active antibiotic options. Some studies have shown positive effect [13,29], while others were not

very encouraging [8,25]. The magnitude of antibiotic penetration in tissues should also be considered. For example, floroquinolones achieve much higher concentration in lung tissues [17] and urine [28] then in plasma and this may be beneficial in treatment of pathogens with relatively high MICs. Tissue penetration may be one reason why linezolid was found to be superior to vancomycin in treating of MRSA nosocomial pneumonia [31]. Another reason could be pharmacokinetic and pharmacodynemic properties of vancomycin: treatment of MRSA pneumonia is associated with better response when 24 hour area under the concentration-time curve (AUC24)/MIC ratio is >400 [21]. To achieve this for MRSA strains with 1.5-2 MICs, vancomycin trough level should be at least 15-20 mg/L, and for VISA strains 30-40 mg/L may be required [29]. Such high levels are often associated with toxic side effects and may not be achievable. Thus, the use of alternative antibiotics (e.g. linezolid, Tigecycline) should be considered. In serious infections (e.g. endocarditis and meningitis) the use of bactericidal antibiotics are supported by some data [7]. Since certain antimicrobials are characterized with excessive bactericidal activity (e.g. daptomycin), [23] it may be prudent to use them as a first line antibiotics in serious, lifethreatening infections. Numerous studies have evaluated potential synergistic effects of different antibiotic combinations (e.g. β-lactams, aminoglycosides, rifampin, polymyxins). There is some evidence to support the idea that combination therapy may be clinically effective [16,26,37,38].

In conclusion, the rising numbers of nosocomial infections, with increasingly prevalent MDROs, present significant challenge to physicians. New types of antibiotics are drastically needed to fight resistant infections. Creative approach to existing antibacterial therapy is to be considered also. More sophisticated microbiologic methods, to rapidly identify the MDROs are required as well.

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SUMMARY

IMPORTANT ASPECTS OF NOSOCOMIAL BACTERIAL RESISTANCE AND ITS MANAGEMENT

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The article reviews management of nosocomial bacterial resistance aspects. Nosocomial infections are associated with substantial morbidity, mortality and cost. During the last several decades multi-drug resistant organisms increased in number considerably. Methicillin-resistant staphylococcus aureus, Vancomycin-intermediately resistant staphylococcus aureus and fully vancomycin-resistant staphylococcus aureus evolved as a consequence of methicillin and vancomycin use. The introduction of third generation cephalosporins were followed by emergence of extended spectrum and AMP-C β-lactamases among gram negative bacteria, and carbapenems were targeted by carbapenemases. The poor diagnostic yield of current microbiologic methods in identifying certain resistant organisms, combined with decreasing numbers of newly developed antibiotics pose a significant challenge to physicians. We reviewed some of the approaches which can be followed to maximize the positive clinical outcome in patients with resistant nosocomial infections, using currently available antibiotics. More sensitive microbiological methods and new types of antibiotics are needed to adequately address the problem in the future.

Key words: infection by the nosocomial pathogens, nosocomial infections, multi-drug resistant organisms, carbapenemases, Methicillin-resistant staphylococcus aureus, Vancomycin-resistant staphylococcus aureus.

РЕЗЮМЕ

АСПЕКТЫ БАКТЕРИАЛЬНОЙ РЕЗИСТЕНТНОСТИ ВАЖНЕЙШИХ ФОРМ НОЗОКОМИАЛЬНЫХ ИНФЕКЦИЙ И ИХ ЛЕЧЕНИЕ

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Внутрибольничные инфекции представляют собой быстро развивающуюся проблему в медицине. Они связаны с высокой заболеваемостью и смертностью, а также существенными затратами. За последние десятилетия значительно увеличилось число лекарственно-резистентных организмов. Устойчивый к метицилину золотистый стафилококк, средне резистентный и полностью устойчивый к ванкомицину золотистый стафилококк развились как следствие применения метицилина и ванкомицина. Внедрение третьего поколения цефалоспоринов сопровождалось появлением бета-лактамаз расширенного спектра и AMP-C бета-лактамаз среди грамотрицательных бактерий, при этом карбапенемы были нейтрализованы карбапенемазами. Ограниченные диагностические возможности современных микробиологических методов в области идентификации некоторых устойчивых организмов, а также замедление темпов появления новых антибиотиков представляют собой значительный вызов для врачей. Целью исследования была максимизация положительных клинических результатов у пациентов со стойкими внутрибольничными инфекциями с помощью доступных в настоящее время антибиотиков. Исследование показало, что для адекватного решения этой проблемы необходимы более чувствительные микробиологические методы и новые типы антибиотиков.

PREVALENCE OF HEPATITIS B AND C AMONG HIV POSITIVE PATIENTS IN GEORGIA AND IT'S ASSOCIATED RISK FACTORS

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HIV is one of the most serious health care problems in the world. Base on World Health Organization (WHO) and Joint United Nations Program on AIDS (UNAIDS) data, at the end of 2007 approximately 33.2 mln people are infected with HIV [11,12]. Prevalence of Hepatitis B and C is much higher in the world. Hepatitis B is one of the infections, which has a leading role on developing liver diseases worldwide [8,9]. According to the UNAIDS about 350-400 mln persons have a chronic Hepatitis B [8,9]. Hepatitis B is mostly spread among 20-50 years old population. Like Hepatitis B, prevalence of Hepatitis C is high among general population in the world. WHO estimates that about 180 mln people (3% of the world's population) are infected with Hepatitis C virus. There are about 4 million carriers in Europe alone [12].

Georgia belongs to the countries with a low HIV prevalence. At the end of 2007, prevalence rate of HIV infection is 25.63 and incidence rate is 6.82 per 100 000 population. Since 2004 Georgia has 100% access to universal treatment and care of HIV patients (all HIV positive people who are in need of ART by WHO protocols are under the treatment), which is the one reason that prevalence of HIV increased about 2.5 times from 2004 [1]. The major route of transmission is drug injection, followed by sexual contacts. According to statistics last years transmis-

sion of HIV infection by sexual contact started to increase (Figure 1).

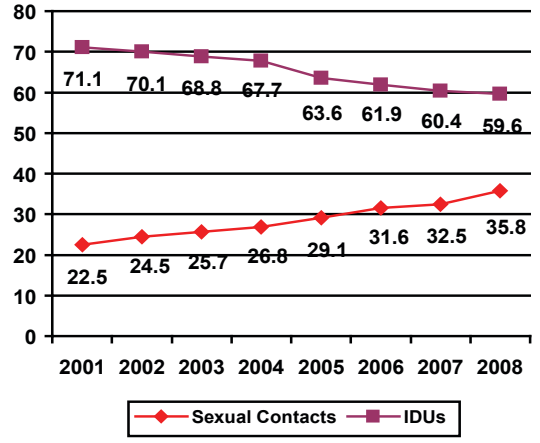

Figure 1. Percentage of HIV Transmission by Route of Transmission (2001-2008 years)

Source – Infectious Diseases, AIDS and Clinical Immunology Research Center (2008)

At the end of 2007, among registered HIV positive people 59.6% were infected by injecting dugs, 35.8% by sexual contacts (33% heterosexual and 2.8% homosexual contacts), 2.6% by vertical and 0.6% by blood and blood product transmission. In 1.4% the route of transmission is unknown [1].

Like other countries, prevalence of Hepatitis B and C in Georgia is relatively high compare to HIV infection. According to National Center for Diseases Control and Public Health (NCDCPH) incidence rate of Hepatitis B and C in 2006 was 20.1 and 24.2 per 100 000 population. Increase rate of both viral hepatitis (B and C) was observed in last years (Figure 2).

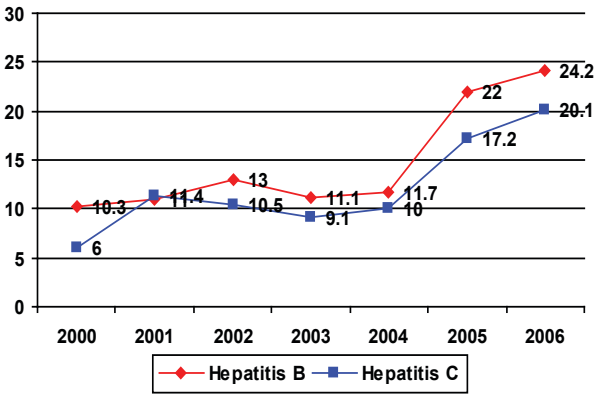

Figure 2. Incidence rate of Hepatitis B and C in Georgia (2000-2006)

Source NCDCPH (2007)

Epidemiological surveillance data, done by AIDS Center in 2003-2004, showed that prevalence of HCV in Georgia among general population reached 6.7%. Prevalence of HCV was 10 times higher (68%) among injecting drug users (IDUs) compare to general population [10]. Prevalence of HBsAg and Anti HBc (total) among adult general population were 1.8% and 11.4% accordingly [8]. Like HCV infection, HBV is more concentrated among IDUs (Prevalence of HBsAg among drug users was 11.9%, and the prevalence of anti HBc (total) - 63%). (Figure 3).

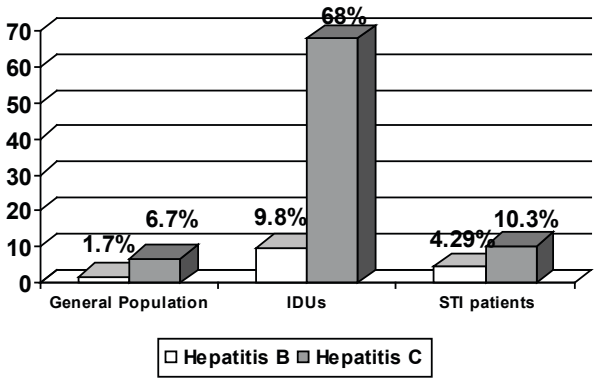

Figure 3. Prevalence of Hepatitis B (HBsAg) and Hepatitis C Viruses Among Different Risk Groups of Georgia (2003)

As it is known, way of HIV and viral hepatitis B and C is similar and viruses can be transmitted by using contaminated medical equipment, blood or blood products transfusion, by vertical way from infected mother to child and through sexual contact with infected patients. Although, it is acknowledged that transmission of Hepatitis C by heterosexual contact is less then 2% among monogamous partners. The rate of HCV transmission through heterosexual contact can be increased to 4-6% among people, who have multiple sex partners or have sexually transmitted infections (STIs) [3,7].

Because of common way of transmission, co-infection with HIV /HBV, and HIV/HCV is quite common and is the major public health, social and economic problem worldwide. Prevalence of HIV/HBV co-infection reaches 10% worldwide [4,7]. Risk of transmission is mostly associated with injection drug use and unprotected sex. HIV/HCV co-infection is different to different countries. In US it varies from 15-30% and in Europe from 23-47% [7].

Treatment and care of HIV patients co-infected with HBV or HCV is more complicated and need advanced management strategies [5-7].

To identify prevalence of HIV/HBV and HIV/HCV co-infection in Georgia, Infectious Diseases, AIDS and Clinical Immunology Research Center (IDACIRC) conducted the cross-sectional study among newly diagnosed HIV positive patients.

Design of the study was cross-sectional. Selection of study participants continued for 5 months. Study participants were recruited from IDACIRC Voluntary Counseling and Testing (VCT) unit (according to the National AIDS Prevention program protocols, voluntary counseling is offered to all clients, who would like to undergo testing on HIV).

Study participants inclusion criteria were: HIV positive status, verified by Western Blot method, age -18 year and alder and voluntary participation. Testing on HIV was done by ELISA method (Vironostica HIV Uni-Form II Ag/Ab, bioMerieux, Netherlands) and for confirmation was used HIV BLOT 2.2 (Western Blot Assay, Vironostica).

From 191 HIV positive patients, who were diagnosed with HIV infection during study period, 175 agree to participate and undergo interview. Specially designed questionnaire was developed to identify risk factors/behaviors of infected patients. Interview was done privately, in counseling room by trained interviewer. Questionnaire included demographic (sex. age, gender, marital status) variables, risk factors of HIV/ HBV/HCV transmission, history of having STIs, etc.

Interviewed HIV positives were asked to be tested on Hepatitis B and C. Testing was done by ELISA method. For Hepatitis C was used following test: HCV3.0; Ortho, Ortho diagnostics, Germany; for HBsAg – HBsAg ELISA, 3.0, Biomerieux, France. HIV positives, which were screened positively by ELISA method on HCV, were considered as co-infected with Hepatitis C, and who had positive results on HBsAg - as co-infected with chronic form of hepatitis B.

All data were analyzed using statistical software SAS, Version 9.1. Descriptive statistics were carried out for the study variables. Associations were examined in bivariate and stratified analysis. Prevalence Ratios (PR) with respective 95% Confidence Interval (CI) were calculated.

Demographic characteristics: Mean age of participants was 36 (SD 8.29). Participants mostly were male (71.43%). Sixty six percent of HIV positive participants were married, 2.91 were widow, 3.49 were divorced and 27.33 were single.

Mode of HIV transmission and its associated risk factors: By mode of HIV transmission 53.71% were infected by drug injection (all male); 43.43% by hetero and 2.29% by homo sexual contacts. In one case (0.57%) route of transmission was unknown. None of homo or heterosexually infected HIV positive respondents reported history of drug use.

Mean age of first drug use was 21 year (min – 16; max - 26 year). Most frequently used drugs were Subutex (62%) and Heroine (28%). Sixty seven percent of IDUs reported usage of share needles or syringes and more then 43% of participants reported having more than 1 sex partner during last 2 years. Unprotected sex with non regular partners was reported among 45.3% of HIV positives (Figure 4).

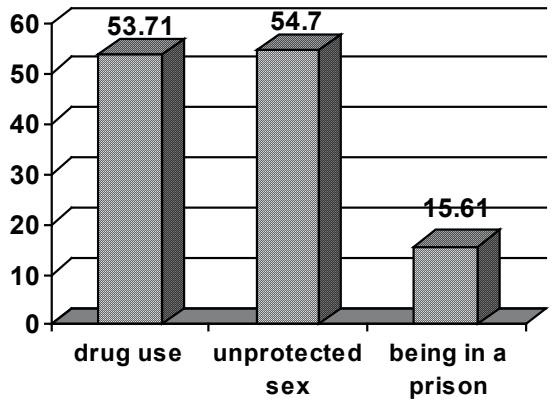

Figure 4. Percentage of High Risk Behavior among HIV Positives

Prevalence of HCV among HIV positives and its Associated Risk Factors. Prevalence of Hepatitis C among HIV positive patients still remains high and is 48.57%. Male were more likely to be co-infected with Hepatitis C compare to female HIV positives (60.80% and 18% accordingly). (Table 1).

Table 1. Prevalence of Hepatitis C among HIV positives (By Gender)

	HCV-	HCV+	Total
Male	49 (39.20%)	76 (60.80%)	125
Female	41 (82.00%)	9 (18.00%)	50
Total	90 (51.43%)	85 (48.57%)	175

Major risk factor of male co-infection was related to drug use (73.40%, 69/94 IDUs). Male IDUs had about 3 times more risk to be co-infected with Hepatitis C compare non IDU male participants (PR 3.25; 95%CI; CL – 1.89-5.26; p<0.01). Risk factor of HCV co-infection among HIV positives mostly was associated with drug use and shared needles or other injection equipment. Among 69 HIV/HCV positive IDUs 61 (88.40%) reported needle share, 64 (92.75%) share of injecting equipment. Sixty one HIV/HCV positive persons (88.40%) used both - shared needles and equipment. One HIV/HCV positive IDU (1.45%), had a history of blood transfusion, 2 (2.90%) undergo surgery and 6 (8.70%) had some kind of invasive medical manipulations. IDUs, which shared needles and/or injection equipment had 6.8 times more risk to be infected with Hepatitis C (PR= 6.8; 95% CI; 1.38-5.43; P=0.02) compare IDUs who had reported any kind of invasive medical manipulations (including surgery and blood transfusion). (Figure 5).

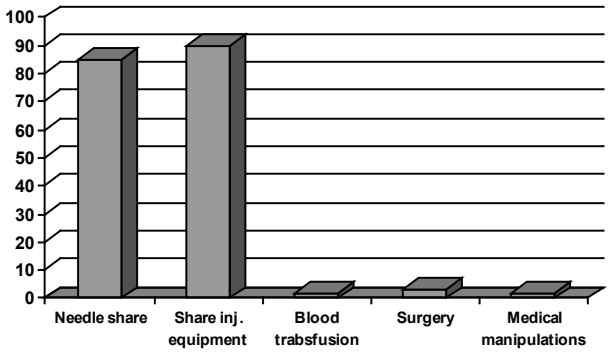

Figure 5. Percentage of High Risk Behavior/Factors of HCV among HIV Positive IDUs

HIV/HCV co-infection among non IDUs with HIV was determined in 16 (19.75%) cases. According to patient's response (by questionnaire) possible HCV transmission was related to blood transfusion and/or invasive medical manipulations (including surgery). Risk of getting HCV was 2.03 times higher among blood recipients (chi square 95% CI; 1.18-5.13; P=0.02) compare other medical manipulations and surgery. It is difficult to speak about significance of those results, due to following factors: 1. Small sample size (number of non IDUs who were screened on HCV was 81, and HCV positive were 16); 2. Informational bias - as it is known, drug users, as well as MSM are stigmatized and hidden population in Georgia, so, HIV positives, which use drugs could hide history of drug use during the interview, and reported the history of medical manipulations or blood transfusion as a reason of infection; 3. Unknown HCV status of regular sex partner/spouse.

Gender and age was not a statistically significant factor for HCV infection (PR 1.26; 95% CI; 0.92-4.02; p>0.1).

Prevalence of Chronic Hepatitis B (HBsAg) and its Associated Risk Factors. Prevalence of HBsAg and anti HBcor (total) was 6.85% and 43.42% accordingly among all 175 HIV positive respondents (Table 2). Like fir HCV, gender was not a significant factor for HBV infection (PR 2.00; 95% CI; 0.45-8.80. P>0.1).

Table 2. Prevalence of HBsAg among HIV Positives (Break Down by Risk Groups)

	HBsAg-	HBsAg+	Total
IDU	86 (91.49%)	8 (8.51%)	94
HETERO	72 (94.74%)	4 (5.26%)	76
HOMO	4 (100.00%)	0 (0.00%)	4
UNKNOWN	1 (100.00%)	0 (0.00%)	1
Total	163	12	175

Data stratification by IDU and non IDU participants showed, that IDUs were more likely to be co-infected with Hepatitis B compare non IDUs. Most common risk factors was needle and other injecting equipment sharing. Prevalence of anti HBcor among drug users was 66% and prevalence of HBsAg was 8.51% (Table 3).

Table 3. Prevalence of HBsAg among Male HIV Positives

	HBsAg-	HBsAg+	Total
IDU	86 (91.49%)	8 (8.51%)	94
Non IDU	29 (93.55%)	2 (6.45%)	31
Total	115	10	125

Age was on of the significant risk factors for HBV infection among IDUs. Drug users, older than 35, have more risk of being HBsAg positive compare IDUs less than 35 years old (PR 1.54, 95%CI; 0.93-7.23; P=0.02).

Unprotected sex and sexually transmitted infections (STIs) were other risk factors for Hepatitis B transmission. HIV/HBV co-infection among non IDU HIV positives was related to unprotected sex. Prevalence of Anti-HBc (total) among non IDUs was 17.28% (14/81) and prevalence of HBsAg was 4.94% (4/81). Blood transfusion and medical manipulations were not statistically significant variables for HBV co-infection.

Prevalence of HBsAg/HCV among HIV positive patients and its associated risk factors. Prevalence of co-infection with both HBsAg and HCV was 5.14% (9/175). Transmission of viral hepatitis B and C was strongly associated with unsafe drug injection (88.88%). In one case reason of Hepatitis transmission could be blood transfusion.

Results and their discussion. National response to HIV/AIDS in the county achieved a significant progress during last years. Development and use of different national guidelines for HIV and viral hepatitis co-infection treatment is one of the successes for appropriate care and treatment.

Realization of such important programs as HIV prevention of mother to child transmission (PMTCT) and methadone substitution, as well as development of outreach programs for IDUs is one of the major piece to achieve universal access on HIV prevention, care and treatment and support.

Besides prevention interventions, which are done in the country, prevalence of Hepatitis B and C, and HIV/hepatitis co-infection is high. Prevalence of Hepatitis C among HIV positive patients is 48.57%. Male were more likely to be co-infected with Hepatitis C compare female (60.80% and 18% accordingly). Drug users had 3.25% times more risk to be infected with Hepatitis C compare non IDUs. Mostly transmission of HIV was strongly related to HCV transmission and was associated to drug use.

Prevalence of chronic Hepatitis B was about 4 times higher among HIV positive persons compare to general population. Study results showed that prevalence of HBsAg among IDUs was 8.51% and 5.26% among non IDU HIV positives.

Risk of Hepatitis B and/or C transmission is mostly associated with drug use and high rate of sexually transmitted infections. Risk factors of co-infection with hepatitis among non drug users are mostly related to unprotected sex (for HBV) and blood transfusion and other medical manipulations (for HCV). As we mentioned above, it is difficult to speak about significance of HCV transmission through medical manipulations because of small sample size and possible informational bias. Further studies are needed to state in what percent HCV can be transmitted by nosocomial way in Georgian Health Care System.

Increase quality of universal precautions, routine counseling and testing of most at risk population (MARPS - IDUs, MSM, sex workers etc), increase number of VCT units, expand of: methadone substitution treatment, vaccination of vulnerable population on Hepatitis B, outreach work with hard to reach population will be a good prevention of HIV as well as hepatitis transmission.

Implementation of routine screening of MARPS on HIV and all HIV positive patients on Hepatitis B and C is one of the best strategies for identification of co-

infected patients, and for further effective treatment management. Implementation of those activities will prevent transmission of HIV and Viral Hepatitis and in a same time helps already co-infected patients for better treatment, care and support, which is one of the priorities of Georgian Health Care Sector.

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SUMMARY

PREVALENCE OF HEPATITIS B AND C AMONG HIV POSITIVE PATIENTS IN GEORGIA AND IT'S ASSOCIATED RISK FACTORS

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The aim of the study was to determine the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection among HIV positive patients, to identify most relevant risk factors of co-infection and develop preventive interventions.

Study participants were voluntary individuals 18 years of age or older recruited from AIDS Center VCT unit in Tbilisi, Georgia. Eligibility criteria of participants were: HIV positive result confirmed by western blot; age; and voluntary participation. Total 175 patients undergo interview with specially designed questionnaires. Most of the participants were male (71.4%), age range of HIV positives varied from 20 to 77 years old. Prevalence of HCV among HIV positive patients is high. Almost half (48.57%) HIV positive patients are co-infected with HCV. Men were more likely than women co-infected with HCV (60.80% and 18% accordingly). Major risk factor of male co-infection was related to drug use, needle and injection equipment sharing. Prevalence of HCV among injecting drug users was (73.40%). Drug users had 3.25 times more risk (PR 3.25; 95%CI; CL – 1.89-5.26; $p<0.01$) to be infected with HCV compare non IDUs.

Prevalence of being infected with HBV (Anti-HBc) among HIV positives was 43.42% (76/175) and the prevalence of Chronic HBV (HBsAg positive) was 6.86% (12/175). Prevalence rate of HBsAg among IDUs was 8.51% and among non IDU participants 5.26%. Triple infection (HIV, Hepatitis C and chronic form of Hepatitis B - HBsAg) was among 9 patients (5.14%). Infections were associated with injection drug use (88.88%) and mostly were related to share of needles/syringes and other injecting medical equipment. Transmission of HBV and HCV by sexual contact was not observed among those 9 participants. High risk behavior among HIV positive participants mostly related to drug use and unprotected sex with non regular partners. Other risk factors for Hepatitis transmission were associated with invasive medical manipulations, blood transfusion, surgery, abortions and etc. None of cases of HIV, or Hepatitis (B, C) transmission through medical manipulations can be documentary proved based on those research data.

Key words: hepatitis B virus and hepatitis C virus co-infection, HIV, HCV, HBV.

РЕЗЮМЕ

ПРЕВАЛЕНТНОСТЬ ГЕПАТИТА С И В СРЕДИ ВИЧ-ИНФИЦИРОВАННЫХ ПАЦИЕНТОВ И АССОЦИИРОВАННЫЕ ФАКТОРЫ РИСКА В ГРУЗИИ

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Целью исследования явилось определение распространения вирусного гепатита В и С среди ВИЧ-позитивных пациентов в Грузии, выявление наиболее важных риск-факторов, связанных с коинфекциями и разработка профилактических мероприятий.

Исследованы лица (n=191) в возрасте 18 лет и старше, которые добровольно проходили интервью с консультантом, заполняли специально для них составленный вопросник. Критериями для участия являлись ВИЧ-позитивный диагноз, подтвержденный методом иммуноблотинга, возраст и добровольное участие. По данным исследования распространенность гепатита С среди ВИЧ-позитивных пациентов составила 48,57%. Мужчины были более склонны (60,80%) к заболеванию, чем женщины (18%). Основной причиной заражения среди мужчин явилось употребление инъекционных наркотиков (73,40%). Лица, которые употребляли наркотики и пользовались т.н. общими иглами, шприцами и другими инъекционными материалами имели на 3,25% больше риска заражения гепатитом С по сравнению с теми, кто не употреблял наркотики (PR-3.25; 95% CI:1.89-5.26; P<0.01). Другими риск-факторами заражения гепатитом С (среди респондентов, которые не употребляли наркотики) были инвазивные медицинские манипуляции, переливание крови и продуктов крови, хирургические вмешательства и аборты. Распространенность гепатита В (anti HBc) среди ВИЧ-позитивных достигла 43,4%. Коинфекции ВИЧ и хронические формы гепатита В (HBsAg носители) были обнаружены у 6,86% (12/178) ВИЧ позитивных пациентов. Риск-факторами заражения гепатитом В как и при коинфекции ВИЧ/гепатита С, являются употребление наркотиков, а также незащищенные половые контакты с нерегулярными партнерами и высокое распространение болезней, передаваемых половым путем.

ORAL LESIONS IN HIV-POSITIVE PATIENTS IN GEORGIA

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HIV infection is a major global health problem affecting developing and developed countries alike. The oral manifestations are common in HIV infected patients, and often influence the debilitating general health status, worsens prognosis of the disease, and might be used as a diagnostic tool in the monitoring of the immune status of the patient [9,12]. Oral lesions in HIV infection have been well documented in developed countries, but there are fewer reports on oral lesions in HIV infected patients from developing countries.

Many HIV infected subjects are presented with at least one manifestation in the oral and perioral area [7,11]. In addition, the occurrence of these lesions indicate a greater susceptibility for opportunistic infections and a great possibility of rapid progression to AIDS [4].

The CD4+ count and viral load have been used lately as the most important laboratory parameters to evaluate the evolution of the disease. Several studies have been focused on the correlation between oral lesion prevalence

and the laboratory parameters such as CD4+ cell count and viral load in HIV/AIDS patients, showing a strong association between the oral lesions, low CD4+ count and high viral load, postulating that they might be used in monitoring of progression of the disease, as well as effectiveness of antiviral therapy [6,13].

The investigations have reported that the CD4+ count less than 200 cells/mm3 and viral load higher than 10.000 c/ml in association with other factors - tobacco consumption, poor oral hygiene and xerostomia, can facilitate the occurrence of oral lesions in HIV patients [1,2,5,8,10,13].

Materials and methods. The investigation was conducted at the Infectious Diseases, AIDS and Clinical Immunology research Center. Patients admitted for the first time from January 2006 till October 2008 were included in this study. 732 patients satisfied the entry criteria. 602 (82,2%) were men and 130 (17,8%) were women.

The HIV/AIDS patients were divided into three groups: 1. CD4 cell count <200/mm^3; 2. CD4 cell count between the 201/mm^3-500/mm^3; 3. CD4 ell count >501/mm^3. The possible correlation between CD4+ cell count and frequency and severity of mucous membrane diseases was assessed.

In all groups we studied the prevalence of the following mucous membrane diseases: oral candidiasis, HIV associated periodontal diseases, recurrent aphthous ulcerations, oral hairy leukoplakia, orolabial herpes simplex infection, human papillomavirus (warty like lesions) and Kaposi's sarcoma.

Testing on HIV/AIDS was based on identification of HIV antibodies by using Vironostica HIV Uni-Form II Ag/Ab, bioMerieux, The Netherlands by ELISA with further confirmation by Western Blot method using HIV BLOT 2.2 Western Blot Assay.

Each HIV positive patient underwent full clinical and standard laboratory examination.

Blood samples for laboratory examination for the assessment of the stage of HIV disease was obtained at the same day of patients' clinical evaluation.

CD4+ cell count was determined by the Becton-Dickinson FACSCalibur flow cytometer using the MultiTEST CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC in erythrocyte-lysed whole blood.

Participants were examined with a dental mirror using a portable halogen lamp. Oral manifestations were diagnosed according to presumptive criteria of EEC clearinghouse classification [3].

Oral candidiasis may present in several clinically distinct forms: erythematous, pseudomembranous, and angular cheilitis. Laboratory diagnose of oral candidiasis was based on the clinical appearance of the lesion. When the diagnosis was uncertain, culture on Sabouraud Dextrose Agar was used.

Recurrent aphthous like ulcerations were diagnosed by any recurrent interruption of the oral mucosa not attributable to trauma.

Oral (orolabial) herpes lesions caused by herpes simplex virus (HSV) were diagnosed by vesicular or ulcerative lesions +/- positive serology for HSV +/- swab for Tzanck smear.

Keposi's sarcoma was diagnosed by brown, red, blue or purple macule, papule or nodule. It has predilection for hard palate.

Oral hairy leucoplakia presents with vertically corrugated, slightly elevated white surface alteration of lateral or ventral tongue margin that cannot be scraped.

Periodontal disease -this chronic inflammatory process involving oral flora and affecting the tissues and bones supporting the teeth can occur in anyone regardless of HIV status, one particularly severe form (necrotizing ulcerative periodontitis) and a related condition (linear gingival erythema) appear to be unique to those with compromised immune systems.

Human papillomavirus -associated lesions frequently occur in the oral cavity, including the lips and sides of the tongue. They are usually raised, dull white and fleshy, smooth or rough, and may have a cauliflower-like appearance.

In case of unusual clinical presentation biopsies were performed for definite diagnosis.

Data collection was made using information from IDACIRC National HIV/AIDS Data Base for the period January 2006–October 2008.

Results and their discussion. The age ranges of HIV positive patients was 19-59 years. The most affected age group was – 31-40 years (42%). The most frequent route of HIV acquisition was injection drug use – 456 patients (62%). Other routes recorded were – heterosexual contacts – 232 patients (32 %), homosexual contacts – 26 patients (4%) and unknown rout of transmission 18 patients (2 %).

Table 1. Some Socio-demographic and epidemiological data of HIV patients

Variables	No of patients (%)
Age, year	
< 30	183 (25 %)
31 – 40	307 (42 %)
41-50	168 (23 %)
> 51	74 (10 %)
Unemployed	352 (48 %)
Employed	342 (47 %)
Student	38 (5 %)
Mode of HIV transmission	
Injection drug use	456 (62%)
Heterosexual contact	232 (32%)
Homosexual transmission	26 (4%)
Unknown	18 (2 %)

CD4+ cell count was lower than 200 cells/mm^3 in 307 (42 %) patients, from 201 to 500 cells/mm^3 in 293 patients (40 %) and above 500 cells/mm3 in 132 patients (18 %).

The occurrence of oral lesions in the evaluated subjects was 75 % (546 patients). 186 patients (25%) did not exhibit any oral lesions.

Furthermore in the present investigation, the prevalence of two or more simultaneously exhibited types of lesions were as following: three types of lesions were detected in 45 patients (6%) and two types of lesions were detected in 245 patients (33%).

Table 2. Relation between HIV patients CD4 cell count and number of simultaneously exhibited types of lesions

CD4+ cell count, cells/mm^3	No of patients (%)	No of simultaneously exhibited types of lesions per patient	No of patients (%)
0-200	307 (42%)	3	45 (15)
		2	145 (47)
		1	87 (28)
		0	30 (10)
201-500	293 (40%)	2	100 (34)
		1	84 (29)
		0	109 (37)
501 and more	132(18 %)	1	85 (64)
		0	47 (36)

According to the oral lesions identified in the present study, oral candidiasis constituted the most common form, representing a 64% (467 patients), followed by HIV associated periodontal diseases in 216 patients (30%), recurrent aphthous like ulcerations in 118 patients (16%), orolabial herpes simplex infection in 50 patients (7%), oral hairy leukoplakia presented in 58 patients (8%), Human papillomavirus(warty-like lesions) in 37 patients (5%) and Kaposi's sarcoma in 3 patients (0,4%).

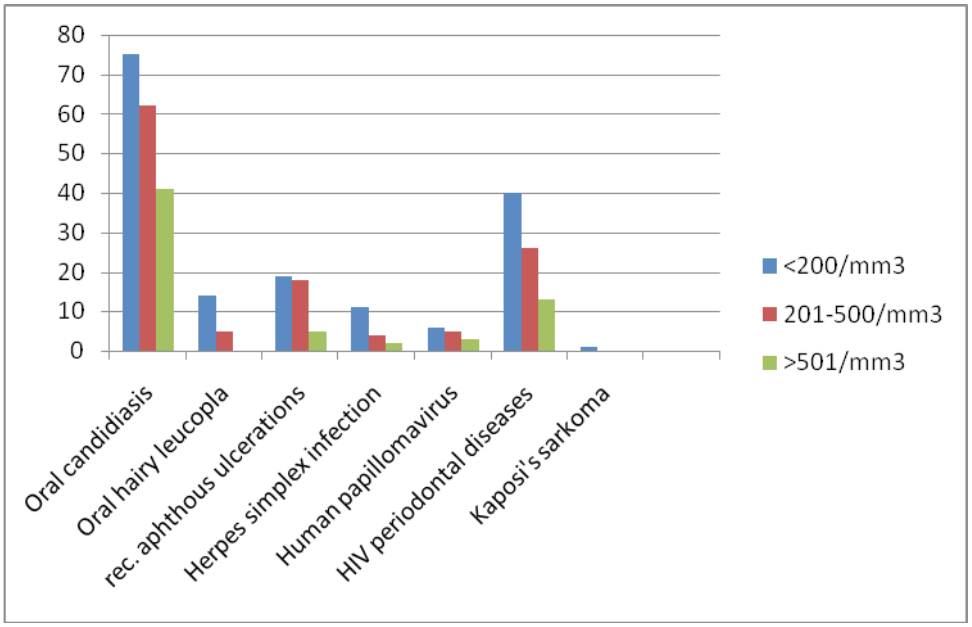

Fig. Types and distribution of oral lesions in HIV/AIDS patients in Georgia

Most cases of oral lesions appeared in patients with lower CD4+ count.

Table 3. Distribution of oral lesions according to patients' CD4 + cell count

CD4+ cell count, cells/mm3	No of patients (%)	Types of oral lesions	No of patients (%)
Less than 200 cells/mm^3	307 (42%)	Oral candidiasis	230 (75)
		HIV associated periodontal diseases	123(40)
		recurrent aphthous like ulcerations	59 (19)
		Oral hairy leucopla	44(14)
		Orolabial herpes simplex infection	34 (11)
		Human papillomavirus(warty-like lesions)	19 (6)
		Kaposi's sarcoma	3 (1)
201-500 cells/mm^3	256 (35%)	Oral candidiasis	183 (62)
		HIV associated periodontal diseases	76 (26)
		recurrent aphthous like ulcerations	52(18)
		Orolabial herpes simplex infection	13(4)
		Human papillomavirus(warty-like lesions)	14(5)
		Oral hairy leucopla	14(5)
More than 501 cells/mm^3	311(42%)	Oral candidiasis	54 (41)
		HIV associated periodontal diseases	17(13)
		recurrent aphthous like ulcerations	7 (5)
		Orolabial herpes simplex infection	3 (2)
		Human papillomavirus(warty-like lesions)	4 (3)
		Oral hairy leucopla	0(0)

Results of this study provide convincing evidence that mucous membrane disorders with HIV infection might serve as an indicator for advanced HIV infection, immunosuppression and decreased CD4 cell counts.

Mucosal manifestations of AIDS patients may appear atypical, they may be widespread, have prolonged course and the response to treatment may be poorer than expected.

The physicians who are taking care of HIV patients have to be familiar with HIV-associated mucocutaneous diseases, their diagnoses, and management.

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SUMMARY

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To study the prevalence of oral lesions in HIV infected patients and its relationship with CD4+ cell count in Georgia 732 HIV positive adult patients who were admitted to the Infectious Diseases, AIDS and Clinical Immunology Research Center (IDACIRC) since January, 2006 till October, 2008 were evaluated. Each patient underwent full clinical and standard laboratory examination. CD4+ cell count was determined by the Becton-Dickinson FACSCalibur flow cytometer (MultiTEST CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC Reagent). Socio-demographic data was obtained using a standard questionnaire at the epidemiology department of IDACIRC. Oral manifestations were diagnosed according to EEC clearinghouse classification (1993). Oral lesions were revealed in 546 patients (75%). 186 patients (25 %) did not exhibit any oral complications. The prevalence of two or more simultaneously exhibited types of lesions was as follows: three types of lesions were detected in 45 patients (6%) and two types of lesions were detected in 245 patients (33 %). The investigation revealed oral candidiasis constituted the most common form of oral lesions, representing a 64 % (467 patients), followed by HIV associated periodontal diseases in 216 patients (30 %), recurrent aphthous like ulcerations in 118 patients (16 %), oral hairy leukoplakia in 58 patients (8 %), orolabial herpes simplex infection in 50 patients (7 %), human papillomavirus (wart like lesions) in 37 patients (5%) and Kaposi's sarcoma in 3 patients (0,4%). Most of oral lesions cases were found in patients with low CD4+ cell count.

Results of this study provide evidence that mucous membrane disorders with HIV infection might serve as an indicator for advanced HIV infection, immunosuppression and decreased CD4 cell counts. The physicians who are taking care of HIV patients have to be familiar with HIV-associated mucocutaneous diseases, their diagnoses, and management.

Key words: oral lesions, oral candidiasis, HIV associated periodontal diseases, recurrent aphthous, oral hairy leukoplakia, orolabial herpes, Kaposi's sarcoma and HIV.

РЕЗЮМЕ

ЗАБОЛЕВАНИЯ СЛИЗИСТОЙ ОБОЛОЧКИ РТА СРЕДИ ВИЧ-ИНФИЦИРОВННЫХ ПАЦИЕНТОВ В ГРУЗИИ

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Целью данного исследования явилось определить корреляцию между ВИЧ-ассоциированными заболеваниями полости рта и абсолютным количеством CD4+ клеток.

В проспективном обзорном исследовании оценены 732 ВИЧ-положительных пациента, которые были зарегистрированы в Центре СПИда с января 2006 г. по октябрь 2008 г. Каждому пациенту проводилось полное клиническое и стандартное лабораторное обследование. Подсчет CD4 + клеток проводили с применением flow cytometry (MultiTEST CD3 FITC/ CD8 PE/ CD45 PeCP/ CD4 APC Reagent), а вирусная нагрузка - RT-PCR (The COBAS TaqMan HIV-1 test). Социально-демографические данные получены, используя стандартные вопросы эпидемиологов Научно-практического центра инфекционных заболеваний, СПИДа и клинической иммунологии. Поражение полости рта было диагностировано по классификации ECC clearinghouse (1993).

Пациенты были распределены по группам по количеству CD4+ клеток. Заболевания полости рта были выявлены у 546-и (75%) пациентов, у 186-и (25%) поражений слизистой полости рта не вывялены. Сочетанное проявление двух или более заболеваний полости рта распределилось следующим образом: три заболевания у 45-и (6%) пациентов, два – у 225-и (33%).

Самой частой причиной поражения слизистой полости рта являлся оральный кандидоз, зарегистрированный у 467-и (64%) пациентов, далее следуют ВИЧ-ассоциированные периодонтальные заболевания - 216 (30%), язвы полости рта - 118 (16%), «волосистая» лейкоплакия - 58 (8%) пациента, оролабиальный герпес (Herpes Simplex) - 50 (7%) и саркома Капоши – 3 (0,4%) пациента. В большинстве случаев у пациентов с поражением слизистой оболочки рта выявлено низкое количество CD4+ клеток.

Результаты данного исследования показали, что поражения слизистой оболочки рта могут служить индикатором иммунодефицита при ВИЧ-инфекции. Установлена корреляция между количеством CD4+клеток и поражением полости рта, что позволит врачам вовремя диагностировать возможные осложнения и правильно планировать лечение.

WORLD HEALTH ORGANIZATION'S HIV/AIDS POLICY AND GEORGIA

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WHO Country Office in Georgia

The World Health Organization (WHO) is the directing and coordinating authority on international health within the United Nations' system. It is responsible for providing leadership on global health matters, shaping the health research agenda, setting norms and standards, articulating evidence-based policy options, providing technical support to countries and monitoring and assessing health trends. WHO's objective, as set out in its Constitution, is the attainment by all peoples of the highest possible level of health. 2008 year marks the 60th anniversary of WHO as well as adoption of the Universal Declaration of Human Rights, both most important issues in Global Health agenda.

WHO takes the lead within the UN system in the global health sector response to HIV/AIDS. WHO's Mission on HIV/AIDS is to achieve the highest possible standard of health for all people by reducing the impact of HIV/AIDS on their lives, particularly reducing: HIV/AIDS incidence, HIV/AIDS-related morbidity, HIV/AIDS-related mortality, improving the quality of life of People leaving with HIV/AIDS (PLWHA). The HIV/AIDS Department provides evidence-based, technical support to WHO Member States to help them scale up treatment, care and prevention services as well as drugs and diagnostics supply to ensure a comprehensive and sustainable response to HIV/AIDS.

WHO HIV/AIDS Programme staff collaborate with other UN Agencies, Ministries of Health, development agencies, non-governmental organizations (NGOs), health services providers, health-care institutions, people living with HIV and other partners. The aim is to strengthen all aspects of the health sector in order to deliver much-needed HIV services. The WHO HIV/AIDS Department refers specifically to the unit dedicated to working with HIV/AIDS as opposed to the 'Programme' which refers to all WHO HIV-related work both at headquarters, regions and countries. In addition to the Department of HIV/AIDS, more than 30 departments at WHO carry out HIV-related functions, as part of WHO's global HIV/AIDS programme. These include sexual and reproductive health; tuberculosis; blood safety; child and adolescent health; essential drugs and medicines policy; disease surveillance; mental health; vaccine and microbicide development; gender and women's health; health education and substance dependence etc.

International mobilization to combat HIV
1981-1983 – Detection of the first case of HIV/AIDS and identification of provocative virus;
1986 – Launching of first WHO HIV/AIDS Global programme;
January 1, 1996 – Establishment of Joint United Nations Programme on HIV/AIDS (UNAIDS);
Millennium Development Goals (MDG) were established in 2000;
Declaration of Commitment on HIV/AIDS marked the beginning of a sea change in the response to AIDS in 2001 (UNGASS Declaration);
The Global Fund to Fight AIDS, Tuberculosis and Malaria was established in 2002;
The WHO-and-UNAIDS-led '3 by 5' initiative in 2003;
United Nations member States endorsed the universal access in 2005;
In 2005, G8 leaders announced their intention to "… work with WHO and UNAIDS and other international organizations to develop and implement a package of HIV prevention, treatment and care, with the aim of coming as close as possible to universal access to treatment for all those who need it by 2010;
June 2006 – UNGAS Summit – Evaluation of UNGASS 5 Years achievements;
7 In July 2008 at their Hokkaido Toyko Summit, G8 leaders reaffirmed their commitment to the universal access goal and also called for enhanced efforts to address gender inequalities and stigma and discrimination and to expand access to sexual and reproductive health services, especially for adolescents and most-at-risk populations

Universal access means establishing an environment in which prevention, treatment, care and support interventions are available, accessible and affordable to all who need them. It covers a wide range of interventions that are aimed at individuals, households, communities and nations.

«To me, this means that no one should die because they can't get drugs. It means that no one will miss being tested, diagnosed and treated because there aren't clinics. It means that HIV positive mothers will not unwittingly give a death sentence to their babies. Their parents will look after them instead of making them AIDS orphans.

There must be a relentless push to make sure that everyone who needs testing, counseling, treatment and care gets it. At the same time we will fully support every effort to make sure people know how to prevent HIV infection and are able to do so».
Dr LEE Jong-wook, former DG of WHO, 59th World Health Assembly, 2006

Recent estimates indicate that the health sector alone represents at least 55% of the resources required for the global response to HIV/AIDS. In order to better target much-needed interventions, the WHO HIV/AIDS Programme focuses on five strategic directions:
- Enable people to know their HIV status;
- Maximize the health sector's contribution to HIV prevention;
- Accelerate the scale-up of HIV treatment and care;
- Strengthen and expand health systems;
- Invest in strategic information to guide a more effective response
The Public health Approach is considered for the implementation of WHO strategies, particularly:
- Ensure the full and proactive involvement of governmental, non-governmental and private sector organizations and of civil society, especially people living with HIV including people with most-at-risk of infection;
- Tailor interventions to where the burden of the disease lies, taking into account the nature of the epidemic and the context (e.g., cultural traditions, social attitudes, political, legal and economic constraints) in specific settings;
- Create a supportive enabling environment by addressing stigma and discrimination, applying human rights principles and promoting gender equity, as well as by reforming laws and law enforcement to ensure that they adequately respond to the public health issues raised by HIV and AIDS;
- Offer a continuum of services from those that can be provided by home and community to those that require health facilities, all in conjunction with outreach to and consultation with community leaders and members and especially with people living with and affected by HIV.

The major operational elements of Public Health Approach are as follow:
- Identifying an essential package of integrated HIV prevention, treatment, care and support interventions to be delivered by the health sector
- Decentralization and integration of health services Standardization and simplification of protocols and procedures
- A «clinical team» approach to patient management, including task-shifting of the routine aspects of patient management
- Strengthening HIV prevention in health care settings
- Expansion of HIV testing through the routine recommendation of HIV testing in settings with high HIV prevalence
- Community mobilization to promote HIV testing, promote prevention, prepare communities for treatment and provide adherence support
- Population-based HIV drug resistance surveillance and pharmacovigilance
- Free ART at the point of service delivery
- The Model Essential Package of Integrated Health Sector Interventions for HIV Prevention, Treatment, Care and Support recommended by WHO is complex of:
- Health facility-based interventions
- Information and education on prevention of HIV transmission
- Prevention of mother-to-child transmission of HIV
- Prevention of sexual transmission
- Harm reduction for IDU
- Prevention of transmission in health care settings
- Prevention services for people living with HIV/AIDS
- Clinical management of people living with HIV/AIDS
- Community-based Interventions
- HIV testing and counseling
- Community-based prevention
- HIV/AIDS treatment and care
- Community treatment preparedness for both HIV and TB
- Interventions delivered through outreach to most at-risk populations (in partnership with other sectors)
- HIV testing and counseling, including HIV prevention outreach to most at-risk populations, including sex workers, drug users, men who have sex with men, young people and mobile populations
- HIV/AIDS treatment and care
- National measures needed to support service delivery
- Advocacy and leadership
- National strategic planning and programme man-

agement
- Procurement and supply management
- Laboratory services
- Human resources
- Sustained financing
- HIV and STI strategic information systems

WHO tools to support implementation of the Model Essential Package are Normative guidelines, Operational tools, Training and capacity-building materials, A core information system for facilities and districts and national programmes, Drug and diagnostic supply management, Policy guidance and evidence. WHO recommends the integration of HIV/AIDS into health services, particularly into Specific Programme or service areas Primary health care, STI programme, Maternal and child health, Sexual and reproductive health, Drug dependence treatment, Harm reduction services, Mental health, Palliative care, TB services, Prisons health, Blood transfusion services.

Key WHO partners in HIV/AIDS field are: United Nations agencies (UNAIDS, UNICEF, WB, UNFPA, UNODC, ILO, UNHCR, WFP, UNDP, UNESCO); Bilateral donor and development agencies (CIDA, OGAC, France/ESTHER, GTZ, Italian Cooperation, DFID, NORAD, SIDA, AusAID, USAID, European Commission); Private funds and foundations (GFATM, OPEC, The William J. Clinton Foundation, The Bill and Melinda Gates Foundation, Other Foundations); Partnership networks (AMDS, HIV Resnet, Stop Tuberculosis Partnership, IAVI); Community and faith-based organizations, WHO Collaborating Centers and other scientific and academic institutions.

The WHO European Region consists of 53 member states with the total population around 880 million, of which over 165 million live below the poverty line. The European Region is now experiencing the fastest rate of growth of HIV prevalence in any region of the word with the evidence of increasing transmission of HIV in several countries. Between 2000 and 2007, the annual rate of HIV infection has almost doubled, from 39 to 75 per million populations. The majority of new HIV infections are still occurring among injecting drug users and men who have sex with men, while the majority of new HIV infections due to heterosexual transmission are among immigrants from high-prevalence countries. Over 40% of all people living with HIV/AIDS in Europe are co-infected with Hepatitis C and over 20% with Hepatitis B.

In WHO European Region the regional tools and guidelines have been developed for HIV/AIDS treatment and care, including ART. In 2004, the Regional Office published HIV/AIDS treatment and care protocols for the Commonwealth of Independent States. In 2006, the protocols were revised and expanded to address the need for normative guidance in the entire European Region. Among the 13 new or revised protocols are two evidence-based protocols on HIV co-infection with hepatitis B and hepatitis C, treatment and care of patients co-infected with TB and HIV, and a protocol on sexual and reproductive health for people living with HIV/AIDS. A significant decrease in the price of antiretroviral drugs was achieved by end-2005. Grants from the Global Fund for AIDS, Tuberculosis and Malaria and World Bank loans for HIV/AIDS programmes are being implemented in 19 Member States, with considerable support from WHO country teams.

WHO/EURO played a key role in a number of Member States in: piloting the successful mobilization of resources to expand harm reduction activities; strengthening the involvement of people living with HIV/AIDS in decision-making and ART service delivery; supporting the adoption of standardized evidence-based European treatment regimens and training service-providers for ART and key prevention interventions. Pooling knowledge and expertise from the entire region to create sustained mechanisms for development of human resources for STI/HIV/AIDS prevention, treatment and care was the focus of the WHO/GTZ collaboration on capacity building for scaling up HIV/AIDS responses. Three Knowledge Hubs were established: (1) STI/HIV&AIDS surveillance, monitoring and evaluation, Andrija Stampar School of Public Health in Zagreb, Croatia, http://www.surveillancezagreb.org; (2) harm reduction for injecting drug users (Vilnius, Lithuania, http://www.ceehrn.org/hub; and (3) HIV/AIDS treatment and care (Kyiv, Ukraine, http://www.aidsknowledgehub.org.

In September 2002, the WHO EURO Regional Committee adopted a resolution on scaling up responses to the HIV/AIDS epidemic in Europe which provided the regional policy framework for the organization for the years to come. Since February 2004, the European Union has created significant political momentum in support of HIV/AIDS efforts in Europe, and has facilitated the development of a number of relevant political documents including the Dublin Declaration

on Partnership to Fight HIV/AIDS in Europe and Central Asia. Commitments in these declarations and various EU decisions reinforce the WHO EURO Regional Committee's Resolution of 2002, providing a binding framework for action in the EU and its neighbourhood. In 2008 WHO/EURO has summarised the progress on implementing the Dublin Declaration. The report draws on continuing efforts conducted by the United Nations, its agencies, the European Union and various national bodies in the Region, in accordance with global efforts to harmonize and streamline monitoring and evaluation activities. It comprises 15 thematic chapters followed by 9 country profiles.

Since Georgia joined WHO on 16 May 1992, the organization has played an important role in national health development agenda. WHO opened its first Liaison Office in Georgia in 1993; in 2005, the name was changed and its role strengthened when it became a fully Country Office (WHO CO GEO). The Office works in close collaboration with the Ministry of Labour, Health and Social Affairs of Georgia, UN agencies, governmental and nongovernmental organizations active in the health care sector on the BCAs (Biannual Collaborative Agreements) basis.

WHO assisted Georgia to elaborate and implement its first 1994-95 National Workplan on HIV/ AIDS. WHO/EURO activities in Georgia consist: The advocacy and promotion of HIV/AIDS-related WHO policies, strategies, recommendations, distribution of WHO publications, press releases, and reports; The technical assistance to MoLHSA, UN Theme Group on HIV/AIDS, GFATM, major national and international stakeholders involved in HIV/AIDS field in promotion, development and implementation of evidence based norms and standards. By the support of WHO seven guidelines and treatment protocols have been prepared and adopted on different topics of HIV/AIDS in Georgian language; The technical assistance in promotion and implementation of 3x5 and Universal Access initiatives in Georgia including needs' assessment, planning, management, coordination, monitoring and evaluation as well as support in related national capacity building. Support in development of consolidated programs, projects, plans and activities. WHO participated in the preparation of "Country Response Plans for HIV/AIDS ", "UN joint response plans for HIV/AIDS", "TB/HIV national plan", applications for GFATM proposals, UNGASS reports etc.; Support in promotion, planning and carring out HIV/AIDS related events (World AIDS Day, AIDS Memorial Day, etc). WHO CO has implemented training workshops for NGOs working in HIV/AIDS field in Georgia and published manual in Georgian language; Collection and analysis of the HIV/AIDS related epidemiological data, initiate, plan and implement epidemiological surveys, studies, other relevant activities and provide all partners with updated information; assistance in updating of HIV/AIDS country profiles on a regular basis. By the financial support of WHO/EURO in 2007-2008 was carried out the HIV/Hepatitis coinfection epidemiological study as well as in 2006 the socio-economic study for HIV/AIDS in Georgia; Contribution to the improvement of national HIV/AIDS surveillance system by active promotion of second generation HIV surveillance, support in development of relevant national normative materials and related national capacity building; Identification of the funding gaps for implementing HIV/AIDS programmes, developing proposals and perform intensive fund-raising efforts within donors and private sector at country level. By the initiative of WHO CO GEO in 2005 the special conference on "HIV/AIDS and Private Sector" was organized, which supported the motivation of business sector for the investment in HIV/AIDS field in Georgia; About 200 PHC physicians have been trained on HIV/AIDS early identification issues and appropriate guideline was published and currently this process continues; In different fields of HIV/AIDS (epidemiology, surveillance, diagnostic and treatment) around 20 National and Regional conferences and workshops have been organized in Georgia and about 400 Georgian specialists passed the training courses for capacity building in and outside country.

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SUMMARY

WORLD HEALTH ORGANIZATION'S HIV/AIDS POLICY AND GEORGIA

Gamkrelidze A.

WHO Country Office in Georgia

WHO takes the lead within the UN system in the global health sector response to HIV/AIDS. Recent estimates indicate that the health sector alone represents at least 55% of the resources required for the global response to HIV/AIDS. In order to better target much-needed interventions, the WHO HIV/AIDS Programme focuses on five strategic directions:
Enable people to know their HIV status;
Maximize the health sector's contribution to HIV prevention;
Accelerate the scale-up of HIV treatment and care;
Strengthen and expand health systems;
Invest in strategic information to guide a more effective response.

The European Region is now experiencing the fastest rate of growth of HIV prevalence in any region of the word with the evidence of increasing transmission of HIV in several countries. In WHO/EURO was developed 13 new or revised evidence-based protocols on HIV/AIDS prevention, treatment and care including co-infection with hepatitis B and hepatitis C, with TB, and a protocol on sexual and reproductive health for people living with HIV/AIDS.

WHO assisted Georgia to elaborate and implement its first 1994-95 National Workplan on HIV/ AIDS. WHO/EURO activities in Georgia consist: The advocacy and promotion of HIV/AIDS-related WHO policies, strategies, recommendations, distribution of WHO publications, press releases, and reports; The technical assistance to MoLHSA, UN Theme Group on HIV/AIDS, GFATM, major national and international stakeholders involved in HIV/AIDS field in promotion, development and implementation of evidence based norms and standards. By the support of WHO seven guidelines and treatment protocols have been prepared and adopted on different topics of HIV/AIDS in Georgian language; WHO participated in the preparation of "Country Response Plans for HIV/AIDS ", "UN joint response plans for HIV/AIDS", "TB/HIV national plan", applications for GFATM proposals, UNGASS reports etc.; Support in promotion, planning and caring out HIV/AIDS related events (World AIDS Day, AIDS Memorial Day, etc). By the financial support of WHO/EURO in 2007-2008 was carried out the HIV/Hepatitis coinfection epidemiological study as well as in 2006 the socio-economic study for HIV/AIDS in Georgia; In different fields of HIV/AIDS (epidemiology, surveillance, diagnostic and treatment) around 20 National and Regional conferences and workshops have been organized in Georgia and about 400 Georgian specialists passed the training courses for capacity building in and outside country.

Key words: HIV/ AIDS, WHO/EURO activities in Georgia.

РЕЗЮМЕ

ПОЛИТИКА ВСЕМИРНОЙ ОРГАНИЗАЦИИ ЗДРАВООХРАНЕНИЯ ПО ВИЧ/СПИДу И ГРУЗИЯ

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В качестве руководящего и координирующего органа в области международного здравоохранения Всемирная Организация Здравоохранения (ВОЗ) в рамках системы ООН возглавляет глобальные ответные меры сектора здравоохранения на ВИЧ/СПИД.

По современным оценкам только сектор здравоохранения представляет как минимум 55% ресурсов в глобальном ответе на ВИЧ/СПИД. С целью улучшения интервенций ВИЧ/СПИД программа ВОЗ фокусируется на пяти стратегических

направлениях:
- Предоставление возможности населению узнать свой ВИЧ статус;
- Максимилизация участия сектора здравоохранения в превенции ВИЧ;
- Ускорение расширения лечения и ухода за ВИЧ;
- Усиление и расширение систем здравоохранения;
- Инвестирование в стратегическую информацию с целью обеспечения лучшего ответа.

Европейский регион в настоящее время переживает быстрейший рост превалентности ВИЧ по сравнению с другими регионами мира. В Европейском региональном офисе ВОЗ были разработаны 13 новых, основанных на доказательствах, протоколов по превенции, лечению и уходу за ВИЧ/СПИД, включая коинфекции с туберлулезом и гепатитами, а также протокол по сексуальному и репродуктивному здоровью лиц с ВИЧ/СПИДом.

ВОЗ помог Грузии в разработке и внедрении (1994-1995 гг.) первого национального рабочего плана по ВИЧ/СПИДу. Деятельность ВОЗ в Грузии включает: адвокатирование и пропаганду политики, стратегии, рекомендации ВОЗ по ВИЧ/СПИДу, распространение ВОЗовских публикаций, пресс-релизов и докладов; техническую помощь министерству здравоохранения, тематической группе ООН по ВИЧ/СПИДу, Глобальному Фонду, основным национальным и международным организациям, работающим в сфере пропаганды, развития и внедрения основанных на доказательствах норм и стандартов по ВИЧ/СПИДу. При помощи ВОЗ семь методических пособий и лечебных протоколов были подготовлены и утверждены по различным вопросам ВИЧ/СПИД на грузинском языке; ВОЗ участвовала в подготовке «Планов странового ответа на ВИЧ/СПИД», «Объединенных планов ООН по ВИЧ/СПИДу», «ТБ/ВИЧ национального плана», в подготовке проектов для Глобального Фонда, докладов для специальной сессии ООН, а также в поддержке пропаганды, планировании и проведении мероприятий по ВИЧ/СПИДу (Всемирный день СПИДа, день памяти погибших от СПИДа, и т.д.). При финансовой и технической поддержке Европейского бюро ВОЗ в 2007-2008 гг. было проведено эпидемиологическое исследование распространенности ВИЧ/гепатитов коинфекций, а также в 2006 году социально-экономическое исследование бремени ВИЧ/СПИДа в Грузии. В различных сферах ВИЧ/СПИДа (эпидемиология, контроль, диагностика и лечение) было организовано около 20-и национальных и региональных конференций и семинаров в Грузии, 400 грузинских специалистов прошли курсы различных тренингов по повышению квалификации как в стране, так и за ее пределами.

PREVALENCE OF HCV AND GENOTIPES DISTRIBUTION IN GENERAL POPULATION OF GEORGIA

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Hepatitis C (HCV) is one of the major public health problems worldwide. By the recent estimations, there are about 180 million persons living with HCV worldwide, and this number is growing constantly [1,2,5].

Infection with the hepatitis C virus (HCV) remains chronic in 75-80% of infected individuals. In 20-40% cases of chronic hepatitis C infection leads to end-stage liver diseases: cirrhoses, hepatocellular

carcinoma and liver failure after 20-30 years of HCV infection.

HCV infection is the most common chronic blood borne infection in the US and some other countries. Almost 4 million Americans, or 1.8 percent of the US population, have antibody to HCV (anti-HCV) indicating ongoing or previous infection with the virus. Hepatitis C causes an estimated 8.000 to 10.000 deaths and for 1000 people undergoing liver transplantation annually in the United States. In the developing countries the situation is mash dramatic [1,2,5,7,8].

The great variation in prevalence of HCV infection occurs among persons with different risk factors for infection. Highest prevalence of infection is found among those with large or repeated direct percutaneous exposures to blood (injecting drug users, recipients of transfusions from HCV-positive donors, persons with hemophilia). Moderate or low prevalence is found in persons with smaller or unapparent percutaneous or mucosal exposure, such as hemodialysis patients, health care workers, persons with evidence of high-risk sexual practices [2,4,12,15]. There is an evidence of an association between exposure to multiple sex partners and acquiring hepatitis C. However, very low prevalence of HCV infection has been reported by studies of long-term monogamous partners of patients with chronic hepatitis C virus infection. HCV infection occurs among persons of all ages, but the highest incidence of acute hepatitis C is found among persons aged 20-39 years, predominantly males [9].

HCV isolates from around the world can be separated into at least 6 major genotypes, each with a number of subtypes. Complete genome sequences are now available for all six HCV genetic types and for several different subtypes. There is wide evidence that the HCV genotypes are an important predictor of response to interferon treatment. A poor response observed in individuals infected with genotypes 1 or 4 [2,3,6,13,14).

The prevalence of hepatitis C is not well-documented in many countries. Based on the statistics that are available, it is estimated that 3% of the world population is infected with the hepatitis C virus Most populations in the Americas, Europe, and South-East Asia have HCV prevalence rates of under 2.5%. In the Western Pacific regions and parts of South America, prevalence rates are higher - between 2.5-4.9%. As to Middle East and Africa, HCV prevalence has been shown to range from 1-12% [2,8,16].

Investigation of blood-born hepatitis showed that currently Hepatitis C is an emerging and expanding public health problem for Georgia as well. High prevalence of Hepatitis C has been found in persons who practice use of injecting drugs. Recent prevalence study in blood donors in Georgia showed also high seroprevalence of HCV. Widely disseminated injecting drug use and common needle sharing among IDUs, use of unsterile equipment in medical facilities and inadequate testing of donated blood rise risk of Hepatitis C dissemination in Georgia [10,11]. Due to these reasons Hepatitis C infection is considered by Ministry of Health of Georgia as a problem of top priority.

Therefore, there is an acute need for investigation of hepatitis C epidemiology, including a molecular epidemiology in Georgia for assessment of transmission patterns of this infection in the country and population groups that are at high risk of hepatitis C virus infection.

As hepatitis C is major public health problem worldwide and for Georgia as well, we decided to study the most interesting and significant issue regarding this infection: the prevalence of HCV and HCV genotypes distribution among general population of Georgian.

The aim of four years study was to determine the prevalence of HCV infection in the general population of Georgia and to assess HCV genotypes spread among them.

Materials and methods. For performing the planned investigation a cross-sectional study design was applied. 2000 persons from the general adult population of Tbilisi, Georgia were enrolled in the study. The multi-stage cluster sampling method was applied for selection of study subjects. Districts of Tbilisi were considered as sub-populations. At the first stage the number of study subjects was selected in each district of Tbilisi, capital of Georgia. The total number of districts in Tbilisi is ten.

At the next stage of sampling the primary sampling units (PSU) were chosen. As PSU primary health care units (PHCU) were used. Every PSU was chosen at random. Selection of PHCUs was performed by

systematic-random sampling with equal probability. For this purpose the following procedures were performed: A numbered list of primary sampling units ordered geographically by areas was developed.

The sampling interval was calculated by dividing the total number of PHCUs in the district by the number of PHCUs was selected from each district.

The random numbers were selected and PHCU on the numbered list corresponding to this number was addressed as the first sample unit.

Subsequent units were chosen by adding the sampling interval to the number identified in step c.

The next stage of sampling was a selection of respondents within primary sample units. The number of study subjects to be selected from each PSU was calculated by dividing total number of study subjects to be selected from given district by the number if the PSUs selected. Selection of the study subjects – individuals from clusters was done by simple random sampling from the list of individuals associated with each PHCU selected. The address of each selected study subject was obtained and a whole list of study subjects was developed.

At the next stage the fieldwork was organized. The fieldwork team has visited the households to interview the selected study subjects. The fieldwork team was included the interviewer and qualified person for blood drawing.

Individuals selected for the study were invited for voluntary participation in the investigation and if agreed, they signed consent forms along with blood drawing for testing on hepatitis C.

Collected blood samples were screened for HCV antibody. The presence of HCV antibodies was confirmed by confirmation method. Patients with confirmed HCV antibodies were included in the follow-up. All HCV (+) positive patients were tested for HCV RNA by qualitative PCR to confirm presence of active infection and than HCV genotyping was performed of all HCV RNA (+) positive patients (study designed fig.1)

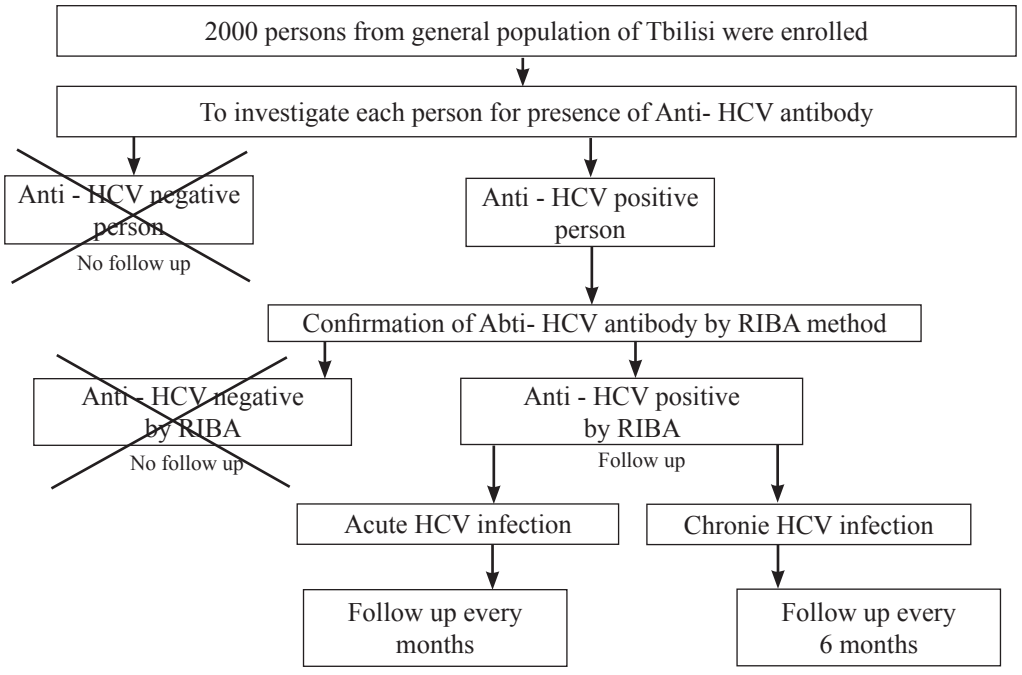

Fig.1 Study design

Diagnosis of HCV infection was made based on detection of antibodies against HCV in serum by enzyme-linked immunosorbent assay (ELISA) method using ORTO HCV 3.0 test and further confirmed by recombinant immunoblot assay (RIBA), using CHIRON RIBA HCV 3.0 SIA.

Detection of HCV RNA was performed by PCR method (qualitative) using AMPLICOR HCV RNA 2.0 test (Roche Diagnostics, Switzerland).

Detection of HCV RNA viral load was measured by Real time PCR technique using COBAS TaqMan HCV-2.0 Test.

HCV genotyping was performed by reverse hybridization line probe assay (Inno lipa) using VERSANT HCV Genotype kit 2.0 (Innogenetics, Belgium)

Results and their discussion. Population distribution by Tbilisi districts (ten districts) were calculated (Fig. 2).

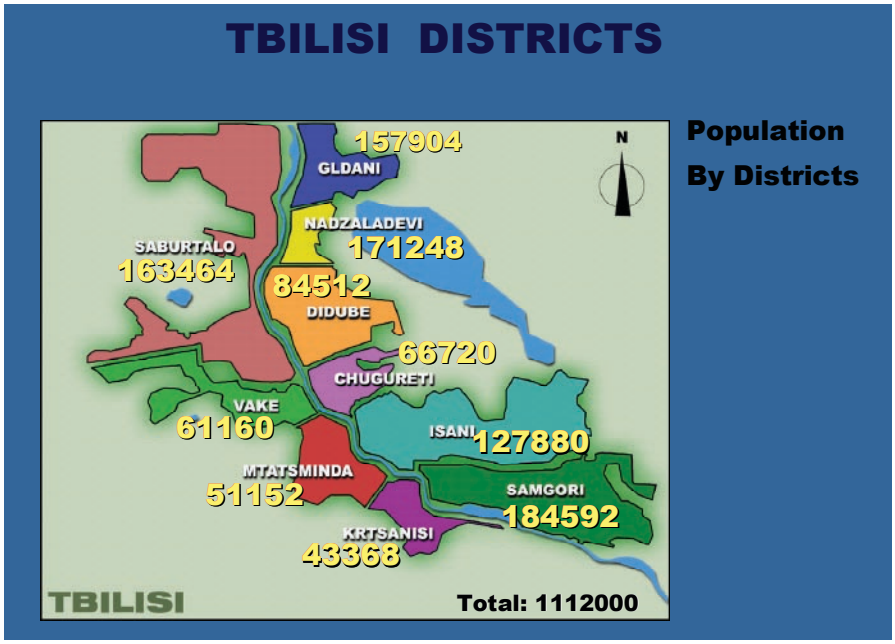

Fig. 2. Population distribution by districts

Number of study subjects by each districts were calculated proportionally population (Fig. 3).

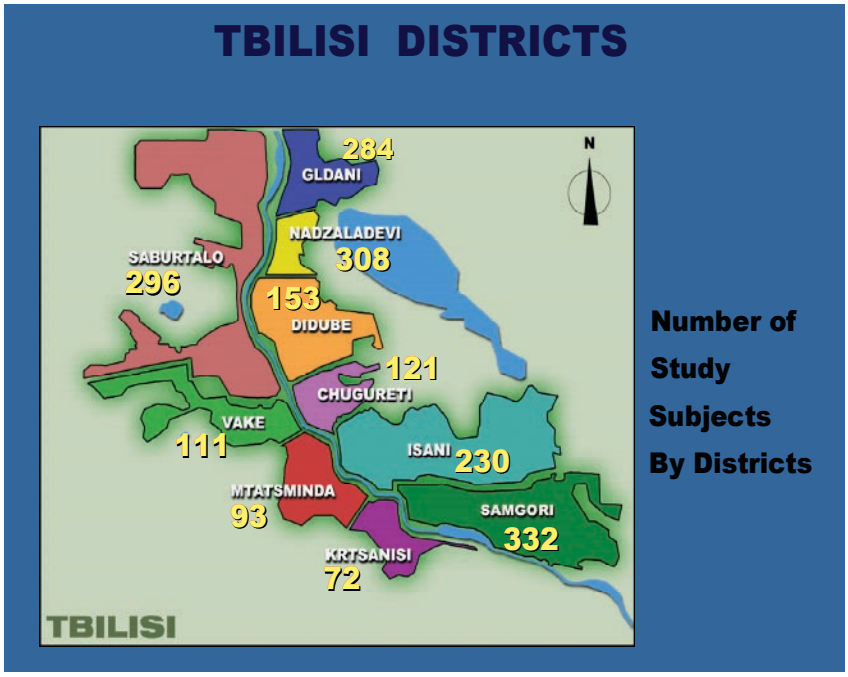

Fig. 3. Number of study subjects by districts

A list of study participants were obtained from primary sampling units – policlinics (Primary Health Care Units – PHCUs) in all ten districts of Tbilisi.

A fieldwork for interviewing of selected individuals - Approaching the study subjects in households, Face-to-face interview which included signing of the letter of consent by study subject and administering the questionnaires by an interviewer. In case of refusal or no-response (household was approached two times) another person from the initial list was addressed for the interview. Interviewing was stop when a positive response was received from 2000 persons. The blood of each study subject was drawn for testing on HCV antibodies.

It worth to mentioned that part of study subjects refused for participation in the survey. In totally Refusal rate was 19%.Taking into account these fact possible refusal from the study participation we selected a larger amount of persons (2470 instead 2000); this extra 470 was also distributed proportionally to population size among the Tbilisi districts.

In totally 2000 persons were investigated and accordingly 2000 blood samples were tested by ELISA for detection of HCV antibodies. 138 out of 2000 (6.9%) samples were found ELISA positive. 138 ELISA positive samples were tested with more specific Recombinant Immunoblot Assay (RIBA) for confirmation. 134 out of 138 (6.7%) of investigated samples were confirmed by RIBA as positives.

In this survey, we found that 134 (6.7%) of the 2,000 surveyed individuals were HCV seropositive.

According our study, which was based on very strict epidemiological design, we concluded that prevalence of HCV in General population of Georgia is 6.7%.

Out of 134 HCV seropositive persons 120 (89.5%) were found HCV RNA (+) positive, it confirmed active HCV infection.

We performed HCV genotyping of HCV RNA (+) positive patients.

Out of 120 HCV RNA (+) positive persons: 70 were with HCV genotype 1b, 32 persons were with HCV genotype 3a, 13 persons –with HCV genotype 2a/2c and 5 persons –with HCV genotype 1a.

So, HCV genotypes distribution among 120 HCV RNA (+) positive patients and accordingly in general population of Georgia were as follow: HCV genotype 1b- 59%, HCV genotype 3a- 27%, HCV genotype 2a/2c -11% and HCV genotype 1a -3%.

Our study found high prevalence of HCV among general population of Georgia. Besides, these surveys found an extensive spread of HCV 1b genotype.

The profile of HCV genotypes distribution in general population of Georgia was similar to that of USA and Russia and different compared to Asia, Africa and most of European countries. Unfortunately the HCV genotype 1b is less sensitive to current treatment regimens. The treatment effectiveness in patients with HCV genotype 1b is about 45% in comparison to 80% for non-1 genotypes.

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SUMMARY

PREVALENCE OF HCV AND GENOTIPES DISTRIBUTION IN GENERAL POPULATION OF GEORGIA

Sharvadze[1,2,4] L., Kenrad[3] E. Nelson, Imnadze[2] P., Karchava[1] M., Tsertsvadze[1,4] T.

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The aim of four years study was to determine the prevalence of HCV infection in the general population of Georgia and to assess HCV genotypes spread among them.

For performing the planned investigation a cross-sectional study design was applied.
Study subjects were Tbilisi residents selected through multiple clusters sampling technique application. Tbilisi is divided into ten districts.

2000 persons from the general adult population of Tbilisi, Georgia were enrolled in the study. The multi-stage cluster sampling method was applied for selection of study subjects. Districts of Tbilisi were considered as sub-populations. At the first stage the number of Population distribution by each districts were calculated. Number of study subjects by each districts were determined proportionally population. A list of study participants were obtained from primary sampling units – policlinics (Primary Health Care Units – PHCUs) in all ten districts of Tbilisi.

A fieldwork was arranged for interviewing of selected individuals - Approaching the study subjects in households, Face-to-face interview which included signing of the letter of consent by study subject and administering the questionnaires by an interviewer.

In totally 2000 persons were investigated and accordingly 2000 blood samples were tested by ELISA for detection of HCV antibodies. 138 out of 2000 (6.9%) samples were found ELISA positive. 138 ELISA positive samples were tested with more specific Recombinant Immunoblot Assay (RIBA) for confirmation. 134 out of 138 (6.7%) of investigated samples were confirmed by RIBA as positives.

In this survey, we found that 134 (6.7%) of the 2,000 surveyed individuals were HCV seropositive. According our study, which was based on very strict epidemiological design, we concluded that prevalence of HCV in General population of Georgia is 6.7%.

The part of our investigation was to assess HCV genotypes distribution among general population of Georgia. Based on our results the following distributions of HCV genotypes were found: HCV 1b - 59%, HCV 3a- 27%, HCV 2a/2c - 11%, HCV 1a - 3%.

Our study found high prevalence of HCV among general population of Georgia. Besides, these surveys found an extensive spread of HCV 1b genotype.

The profile of HCV genotypes distribution in general population of Georgia was similar to that of USA and Russia and different compared to Asia, Africa and most of European countries. Unfortunately the HCV genotype 1b is less sensitive to current treatment regimens. The treatment effectiveness in patients with HCV genotype 1b is about 45% in comparison to 80% for non-1 genotypes.

Key words: HCV infection, HCV genotypes, general population.

РЕЗЮМЕ

ПРЕВАЛЕНТНОСТЬ HCV И РАСПРОСТРАНЕНИЕ ГЕНОТИПОВ В ОБЩЕЙ ПОПУЛЯЦИИ ГРУЗИИ

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Целью четырехлетнего исследования явилось определение превалентности HCV инфекции в общей популяции Грузии и оценка распространения генотипов HCV. В ходе намеченных исследований был использован дизайн перекрестно-секционного исследования.

Исследование включило 2000 совершеннолетних лиц, представляющих общую популяцию г. Тбилиси. Участники были отобраны методом многокластерной выборки. Районы г. Тбилиси были рассмотрены как субпопуляции. На первом этапе было установлено распределение населения по каждому району. Число участников по каждому району было вычислено пропорционально населению. Список участников был получен из первичных участков выборки – поликлиник (первичного звена здравоохранения) во всех десяти районах г. Тбилиси.

Работа на местах была организована для опроса отобранных лиц – выезд на дом к участнику исследования, интервью «лицом к лицу», включая подписание информированного согласия и применение вопросника.

Обследованы 2000 лиц и соответственно 2000 образцов крови протестированы методом ELISA на наличие антител HCV. 138 (6,9%) из 2000 образцов были положительными. 138 положительных образцов были далее протестированы более специфическим методом рекомбинантного иммуноблотинга.

Нами выявлено, что 134 (6,7%) из 2000 обследованных лиц имели HCV серопозитивный статус. На основании нашего исследования, которое проведено с соблюдением строгого эпидемиологического дизайна сделано заключение, что превалентность HCV в общей популяции Грузии составляет 6,7%.

Частью нашего исследования явилась оценка распределения генотипов HCV в общей популяции Грузии. На основании наших результатов было получено следующее распределение: HCV 1b - 59%, HCV 3a- 27%, HCV 2a/2c - 11%, HCV 1a - 3%.

В данном исследовании нами обнаружена высокая превалентность HCV и генотипа 1 в общей популяции Грузии. К сожалению, HCV генотип 1 трудно поддается лечению, менее чувствителен к лечебным режимам, доступным на данный момент, требует более длительного применения антивирусных препаратов. Антивирусное лечение является дорогостоящим, соответственно, распространение HCV генотипа 1 в Грузии увеличит экономическое бремя этой инфекции.

EXPANSION OF CD3/CD16/CD56 POSITIVE NKT CELLS IN HIV/AIDS: THE PILOT STUDY

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NKT cells represent a subset of lymphocytes possessing features of NK cells and αβ T cells; they play a key role in the formation of innate immune response. CD1d receptor restriction and expression of semi-invariant TCR are hallmarks of these cells [1]. Upon stimulation, rapid production of large quantities of both Th type 1 and type 2 cytokines permits them to bridge the innate and adaptive immune responses by activating NK cells, T cells, B cells and dendritic cells [2,6].

It is commonly known that, one of the most important cellular targets of HIV is $CD4^+$ T helper lymphocytes, CD4 receptor in conjunction with CCR5 or CXCR4 co-receptor represent the important gateway for HIV entrance into these target cells. Subsequently, depletion of the $CD4^+$ lymphocyte subset is one of the important hallmarks of the disease and this change is mainly responsible for the development of the immunodeficiency condition. On the way of $CD4^+$ T cell investigation in HIV infected patients, it has become clear that $CD4^-$ and $CD4^+$ NKT cells also are implicated in the immunodeficiency, and new perspectives toward the understanding of and treatment strategies for HIV/AIDS have been disclosed.

NKT cells have been studied in the context of HIV-1 infection only recently. In 2002, Van der Vliet et al. demonstrated that the percentage of NKT cells declined progressively over 5 years of infection [8,9]. It has been shown that HIV-1 infects, and preferentially depletes, $CD4^+$ NKT cells in both humans and macaques [4,7]. It has been shown that $V\alpha24^+$, $CD161^+$ NKT cells are inversely proportional to HIV viral load [4], vasan et al. demonstrated that percentages of both $CD161^+$ NKT cells and $CD161^+$, $CD4^+$ NKT cells decline within the first few months after HIV-1 infection, but initiating therapy during the acute infection period can prevent a further decline in these NKT cell subsets during the first year [10]. Both $CD4^+$ and $CD4^-$ NKT cells can be restored in HIV^+ patients receiving a combination of IL-2 with HAART [3]. Sandberg et al. assessed NKT cell numbers in 57 children with vertically transmitted HIV-1 and found that $CD4^+$ NKT cells were selectively depleted in this patient cohort [7], Motsinger et al. found that $CD4^+$ NKT cells are indeed highly susceptible to R5-tropic, but less so to X4-tropic viruses, subsequently high susceptibility of NKT cells to R5-tropic HIV-1 was strictly dependent on cell surface levels of CCR5 [5].

The current classification of NKT cells, provided by Godfrey and colleagues, indicates emergence of new NKT cell subpopulations, negative on CD161 expression [1]. So, insights in NKT cell function is developing and represents the subject of future investigation.

Despite of scant but essential scientific data concerning the role of NKT lymphocytes in HIV infected individuals; more studies are important to provide a more definitive answer to the question of whether NKT cells fluctuate during the course of HIV infection and further investigations have to be conducted to clarify recently defined subsets of NKT cells and there role in the pathogenesis of AIDS.

The objective of this study was to investigate CD3+/CD16+/CD56+ NKT cell expansion in HIV/AIDS patients and explore its association with virologic and immunologic markers of HIV infection.

Materials and methods. A total of 30 HIV infected individuals were selected from the database of the laboratory of clinical immunology at the Infectious Diseases, AIDS & Clinical Immunology Research Center. Data on patient characteristics were obtained through chart review. Drawing and processing of blood, from patients had been complied with recommendations outlined in protocols for cell population phenotyping and viral load studies.

Phenotyping studies 5 ml of venous blood from patient was collected into K3 EDTA VACUTAINER blood collection tube. T lymphocyte phenotype characterization was performed on two laser FACScalibur flow cytometer (Becton Dickinson Inc.). Counting of $CD4^+$ and $CD3^+/CD16^+/CD56^+$ NKT cells was accomplished by two different MultiTEST kits: $CD3^{FITC}/CD8^{PE}/CD45^{PerCP}/CD4^{APC}$ and $CD3^{FITC}/CD16+56^{PE}/$

$CD45^{PerCP}/CD19^{APC}$ (Becton Dickinson Inc.). Further, Two "fluorochrome conjugated" monoclonal antibody panels were constructed aiming to analyze target cell populations: $CD3^{FITC}/CD8^{PE}/CD45^{PerCP}/CD4^{APC}$ $CD3^{FITC}/CD16+56^{PE}/CD45^{PerCP}/CD19^{APC}$
Data acquisition and first line statistical analyses went through the FACScalibur supportive "CellQuest Pro" software; gate was applied on $CD3^+/CD45^+$ lymphocyte region. For intra test statistical relevance, lymphocyte counting restriction was 4000 $CD3^+$ lymphocytes, per sample.

Plasma viral load studies. 500 µl of plasma per sample, taken from the patient venous blood, collected in K3 EDTA tubes was used for this study.

HIV Plasma viral load quantitative studies were performed by COBAS TaqMan HIV-1 test (Roche Inc.), which relies on real-time PCR amplification and detection technology, using the COBAS TaqMan or the COBAS TaqMan 48 analyzer, assay procedure complied with the manufacturer recommendations.

Statistical analysis was carried out using SAS 9.1 (SAS Institute, Cary, NC, USA). Covariate distributions were examined in univariate analysis. Associations were tested first in bivariate and then in multivariate analyses. Prevalence ratios (PR) with respective 95% Confidence Intervals (CI) are reported. For multivariate analysis modified Poisson regression with robust variance estimates was utilized. Two models were fitted, in Model 1 an outcome measure was viral load higher than 100 000, and in Model 2 – CD4 cell count less then 350 cells/mm^3. Predictive models were built with backward elimination technique. Significance level was set at 0.05.

Results and their discussion. A total 30 patients were selected for the analysis. Two patients had started antiretroviral therapy (ART) before measurement of NKT cells and were therefore excluded from analysis. Analysis was limited to 28 ART naïve patients. More then half of the subjects were women and nearly two thirds were over 30 years. Majority of them had category A disease (67.9%). Fifteen patients (53.6%) were co-infected with hepatitis C virus (HCV), and five patients (17.9%) had a history of active tuberculosis. As for the markers of HIV disease, approximately 36% had viral load higher than 100 000 copies per ml, and over the half (53.6%) had CD4 cell counts less than 350 cells/mm^3 (table 1). No association between the expansion of CD3/CD16/CD56 positive NKT cells and either viral load (table 2) or CD4 was found (table 3) in bivariate analysis.

Table 1. Patient characteristic (n=28)

Characteristic	N	%
Gender		
Male	13	46.4
Female	15	53.6
Age		
<30	10	35.7
≥30	18	64.3
HIV category		
A	19	67.9
B/C	9	32.1
HCV infection		
Positive	13	46.4
Negative	15	53.6
TB infection		
Positive	5	17.9
Negative	23	82.1
Viral load (copies/ml)		
<100 000	18	64.3
≥100 000	10	35.7
CD4 count (cells/mm^3)		
<350	15	53.6
≥350	13	46.4
NKT		
Positive	20	71.4
Negative	8	28.6

Table 2. Association of NKT cell expansion and viral load (n=28)

	Total N	Viral Load ≥100 000		Bivariate	Multivariate
		N	%	PR (95% CI)	PR (95% CI)
NKT					
Negative	8	3	37.5	1.1 (0.4, 3.1)	1.8 (0.6, 5.3)
Positive	20	7	35.0		
CD4					
<350	15	9	60.0	7.8 (1.1, 53.6)	--
≥350	13	1	7.7		
HCV					
Positive	13	7	53.9	2.7 (0.9, 8.3)	--
Negative	15	3	20.0		
TB					
Positive	5	4	80.0	3.1 (1.4, 6.9)	4.4 (1.5, 12.6)
Negative	23	6	26.1		
HIV category					
B/C	9	6	66.7	3.2 (1.2, 8.5)	--
A	19	4	21.1		
Age					
≥30	18	8	44.4	2.2 (0.6, 8.5)	--
<30	10	2	20.0		
Gender					
Male	13	8	61.5	4.6 (1.2, 17.9)	4.9 (1.6, 14.9)
Female	15	2	13.3		

PR = Prevalence Ratio; CI = Confidence Interval

Table 3. Association of NKT cell expansion and absolute CD4 cell count (n=28)

	Total N	CD4<350cells/µl		Bivariate	Multivariate
		N	%	PR (95% CI)	PR (95% CI)
NKT					
Negative	8	4	50.0	0.9 (0.4, 2.0)	0.5 (0.2, 1.0)
Positive	20	11	55.0		
Viral load					
≥100000	15	9	90.0	2.7 (1.4, 5.4)	2.6 (1.3, 5.1)
<100000	13	6	33.3		
HCV					
Positive	13	9	69.2	1.8 (0.8, 3.5)	--
Negative	15	6	40.0		
TB					
Positive	5	4	80.0	1.7 (0.9, 3.1)	0.4 (0.1, 0.8)
Negative	23	11	47.8		
HIV category					
B/C	9	8	88.9	2.4 (1.3, 4.5)	3.2 (1.6, 6.3)
A	19	7	36.8		
Age					
≥30	18	12	66.7	2.2 (0.8, 6.0)	--
<30	10	3	30.0		
Gender					
Male	13	9	69.2	1.7 (0.8, 3.5)	--
Female	15	6	40.0		

PR = Prevalence Ratio; CI = Confidence Interval

In multivariable analysis for Model 1 only TB and gender maintained statistical significance, while HCV status, CD4 cell count, HIV category and age were removed due to non-significance. In a final model there was increased risk (PR=1.8, 95% CI 0.6, 5.3) of higher plasma viral load associated with NKT expansion after controlling for TB and gender (table 2). However, it was not statistically significant. In a multivariable Model 2 Viral load, TB, and HIV category were statistically significant at all steps of model fitting and, therefore, were included in final model. Borderline significance (PR = 0.5, 95% CI 0.2, 1.0) between expansion of CD3/CD16/CD56 positive NKT cells and lower CD4 positive cell count was shown (table 3) after adjusting for viral load, TB and HIV category.

This study was one of the first attempts to explore the possible role of expansion of CD3/CD16/CD56 positive NKT cells in HIV/AIDS. Findings of this pilot study provided important preliminary data to foster further research in this area. Although study did not show strong associations of CD3/CD16/CD56 positive NKT cells with either viral load or CD4, borderline significance with lower CD4 counts deserves attention, which might be explained by compensatory expansion of CD3/CD16/CD56 positive NKT cells in response to T helper lymphocytes depletion, targeting to restore the emerging gap in T helper part of immune response caused by HIV intrusion. In both multivariate models history of active TB maintained statistical significance, which underscores importance of this infection in HIV infected individuals. In addition, all 5 TB co infected patients where NKT cell positive (fig.), which once again underlines the importance of TB, and its possible association with CD3/CD16/CD56 positive NKT cells in HIV/AIDS patients should be further investigated.

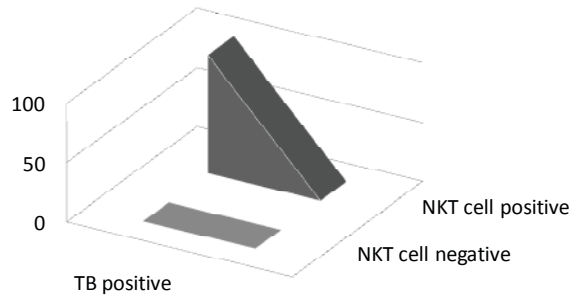

Fig. Percentage of HIV/TB positive patients, with or without NKT cell expansion

Although co-infection with HCV did not show statistical significance, its association with $CD3^+/CD16^+/CD56^+$ NKT cells could not be ultimately rejected because of small sample size. Other caveats should be also mentioned. Given the cross-sectional design of the study it was not possible to study dynamics of viral load and CD4 positive cell counts, as well as disease progression patterns. Longitudinal study with larger sample size is warranted to elucidate the role of $CD3^+/CD16^+/CD56^+$ NKT cells in the clinical course of HIV/AIDS.

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SUMMARY

EXPANSION OF CD3/CD16/CD56 POSITIVE NKT CELLS IN HIV/AIDS: THE PILOT STUDY

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NKT cells are a subset of lymphocytes possessing features of NK cells and αβ T cells; they play a key role in the formation of innate immune response. Upon stimulation, rapid production of large quantities of both T_h1 and T_h2 type cytokines permits them to bridge the innate and adaptive immune responses by activating NK cells, T cells, B cells and dendritic cells. Scientific knowledge has been collecting up to date toward the definition of the role of NKT lymphocytes in HIV/AIDS setting. This direction in HIV/AIDS immunopathogenesis is relatively new and quite concerning. The objective of this study was to investigate CD3+/CD16+/CD56+ NKT cell expansion in HIV/AIDS patients and explore its association with virologic and immunologic markers of HIV infection. Retrospective analysis of 30 HIV infected patients data, taken from the database of the laboratory of clinical immunology at the Infectious Diseases, AIDS & Clinical Immunology Research Center, was conducted. Results: there was slightly increased risk of higher plasma viral load related to lower NKT cell expansion. With regard to immunologic status, borderline significance between expansion of CD3/CD16/CD56 positive NKT cells and lower CD4 positive cell count was shown. However, study did not show strong associations of NKT cell expansion with either virologic or immunologic status, interestingly, all HIV/TB co infected patients where NKT cell positive, which underlines the possible role of TB in $CD3^+/CD16^+/CD56^+$ NKT cell expansion phenomena in HIV infected individuals. We think these new findings may serve as prerequisite for future, larger scale research in this direction.

Key words: HIV/AIDS, CD3/CD16/CD56 positive NKT cells, lymphocytes.

РЕЗЮМЕ

ЭКСПАНСИЯ CD3/CD16/CD56 ПОЗИТИВНЫХ NKT КЛЕТОК У БОЛЬНЫХ ВИЧ/СПИД-ом: ПИЛОТНЫЙ ПРОЕКТ

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Клетки NKT – субпопуляция лимфоцитов, обладают признаками NK-клеток и клеток αβ T, играют ключевую роль во врожденной иммунной реакции. При их стимуляции происходит быстрая выработка большого количества цитокинов как T_h1, так и T_h2 типов, которые дают возможность связывать врожденный и адаптивный иммунный ответы путем активации Т-клеток, В-клеток, а также дендровидных клеток. На сегодняшний день собрано большое количество научных данных, относительно роли NKT-лимфоцитов при ВИЧ-инфекции. Это направление в иммунопатогенезе ВИЧ-инфекции является относительно новым и весьма интригующим. Целью данного исследования явилось изучение роста клеток CD3+/CD16+/CD56+ NKT у больных ВИЧ/СПИД-ом и выявление взаимосвязи вирусологических и иммунологических маркеров при ВИЧ-инфекции. Материалы для ретроспективного анализа 30-и ВИЧ-инфицированных больных были взяты из базы данных лаборатории клинической иммунологии Научно-практического центра инфекционных заболеваний, СПИДа и клинической иммунологии. По данным исследования наблюдался слегка повышенный риск высшей плазменной вирусной нагрузки, связанный с более низким ростом NKT клеток. Что касается иммунологического статуса, то статистическая вероятность корреляции между ростом CD3/CD16/CD56 позитивных NKT лимфоцитов и снижением CD4 позитивных клеток находилась на грани статистической значимости. Однако, исследования не свидетельствуют о сильных ассоциациях между ростом NKT клеток с вирусологическим параметром или иммунологическим статусом. Интересно, что у всех паци-

ентов с коинфекцией ВИЧ/туберкулез наблюдался рост NKT клеток. Это подчеркивает возможную роль туберкулеза в феномене повышенного роста CD3+/CD16+/CD56+NKT клеток у ВИЧ-инфицированных пациентов. Мы полагаем, что эти новые выводы могут служить предпосылкой для будущих, более крупномасштабных научных исследований в этом направлении.

FIBROSCAN AND FIBROTEST/FIBROMAX TO ASSESS LIVER FIBROSIS/CIRRHOSIS IN PATIENTS WITH CHRONIC HBV AND HCV INFECTION IN GEORGIA

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Liver fibrosis (LF) is the principle feature of the injury caused by chronic liver disease (CLD) and determines the major clinical events, leading to liver-related deaths.

CLD is responsible for over 1.4 million deaths annually [13] and in the US is among the top ten disease related causes of death [10].

Statistical analysis estimated that about 1.700 billion people worldwide present with risk factor of developing LF: About 300 million with fibrosis risk due to viral hepatitis B, about 180 million with fibrosis risk due to viral hepatitis C, about 600 million with fibrosis risk due to metabolic disorders (ex: overweight, obesity, cholesterol, type II diabetes etc), about 600 million with fibrosis risk due to excessive alcohol consumption [6,9,13].

Thus, there is an alarming risk of fibrosis worldwide and it is clearly crucial to determine fibrosis stages.

LF is caused by the hystopathological changes due to the chronic liver diseases. As a result of chronic inflammation, excess amount of collagen is produced in the liver, which reduces hepatic elasticity without alteration of liver architectonic and functions [1].

Liver cirrhosis is a consequence of chronic liver disease, characterized by replacement of liver tissue by fibrous scar tissue as well as regenerative nodules and connective tissue septas; with altered hepatic architectonic and portal hemodynamic, development of intra – and extra hepatic portocaval anastomosis leading to progressive loss of liver sinthetic and desintoxication functions [1].

Although, liver biopsy (LB) is the gold standard in assessment of the degree of liver damage, the method has some limitations. The main limitation of the LB is that it represents a very small part of the liver (1/50000) and therefore, sampling error can occur. In addition, histological examination is prone to intra- and interobserver variation, which may occur even when widely validated systems are used to score liver damage. Finally, liver biopsy is an invasive procedure with different complications: pain occurs in 20% of patients and bleeding or hemobilia in 0.5%. For this reason, LB has a poor acceptance and assessment of liver damage using non-invasive methods is currently an important topic in hepatology [2,3]. Nowadays various noninvasive tools are used for evaluation of LF based on serum markers (AST/ALT ratio, APRI, Forns, Fibrotest, Fibromax, FIB-4, Hyaluronic acid, SHASTA, Hepascore, Fibrometer) or imaging techniques (ultrasonography, computed tomography scan, magnetic resonance imaging, transient elastography (TE)). Among them the most reliable methods are considered TE and FibroTest/FibroMax [2-5,7,12].

TE using Fibroscan (Echosens, Paris, France) is a new reproducible, painless and only non-invasive method for assessing LF. The device is based on pulsed elastography technology and evaluates LF by measurement of liver stiffness (LS). Fibroscan measures the stiffness of the right liver lobe by the intercostal approach. TE can be performed for the staging of LF independent from the underlying liver disease. LS is correlated with the quantity of fibrosis in patients suffering from the following CLD: viral hepatitis, chronic alcoholic liver disease, primary billiary cirrhosis, primary sclerosing cholangitis, etc. [11].

Fibrotest is a non-invasive blood test, which combines the quantitative results of six serum biochemical markers: alpha2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, gamma-glutamyltranspeptidase (GGT) and ALT, with a patient's age and gender in a patented artificial intelligence algorithm to generate a measure to fibrosis and necroinflamatory activity in the liver. It provides a numerical quantitative estimate of liver fibrosis ranging form 0.00 to 1,00 corresponding to the well-established Metavir scoring system of stages of F0 to F4. In edition the test provides a numerical quantitative estimate of necroinflamatory activity ranging from 0.00 to 1.00 corresponding to the Metavir scoring system of grades A0 to A3 [6,12].

FibroMax is a combination of five algorithm tests – FibroTest, ActiTest, SteatoTest, NashTest, and AshTest. FibroMax combines the quantitative results of the Fibrotest markers and in edition uses AST, fasting glucose, triglycerides, total cholesterol, weight and height data which, when entered into patented algorithms, accurately determines the level of liver disease without the need to undertake an invasive liver biopsy.

FibroMax results are directly corresponded to numerical standards, which are well correlated to fibrosis stages, necroinflammatory grades (A0-A3), steatosis (S0-S3), non-alcoholic steatohepatitis (N0-N2), and alcoholic steatohepatitis (ASH 0-3) grades by Metavir [6,12].

Due to the high prevalence of CLD worldwide, study of the prevalence of fibrosis stages in patients with chronic HCV and HBV infection in Georgia was considered reasonable.

The aim of the study was to evaluate liver fibrosis and cirrhosis using TE and FibroTest/FibroMax in patients with chronic HCV and HBV infection in Georgia and to compare Fibroscan and FibroTest/FibroMax results.

Material and methods. 252 patients were included in the study, among them 185 patients with chronic HCV infection and 67 – with chronic HBV infection. These patients were investigated at the Georgian-French Joint Hepatology Clinic "HEPA", from December 2007 to November 2008. All of them were investigated by TE and Fibrotest or Fibromax.

Diagnosis of HCV infection was made based on detection of antibodies against HCV in serum by Enzyme-Linked Immuno Sorbent Assay (ELISA) using ORTO HCV 3.0 test and further confirmed by Recombinant Immunoblot Assay (RIBA), using CHIRON RIBA HCV 3.0 SIA. Detection of HCV RNA was done by PCR method (qualitative) using AMPLICOR HCV RNA 2.0 test (Roche Diagnostics, Switzerland) and HCV RNA viral load was measured by- Real Time PCR technique using the COBAS TaqMan HCV-2.0 Test, respectively. HCV genotypes were identified among HCV RNA positive specimens by Reverse Hybridization Line Probe Assay (Inno Lipa) using VERSANT HCV Genotype kit 2.0 (Innogenetics, Belgium).

The diagnosis of chronic HBV infection was made based on detection of HBsAg by ELISA using ImmunoLISA HBsAg 2 step kit. HBV DNA viral load was measured by Real Time PCR technique using the Cobas TaqMan HBV Test (Roche Diagnostics, Switzerland).

TE was performed using the Fibroscan device. Mild amplitude and low-frequency vibrations (50Hz) were transmitted to the liver tissue, inducing an elastic shear wave that propagates through the underlying liver tissue. The median value of 10 successful acquisitions, expressed in kilopascal (kpa) with a success rate of at least 60% was kept as representative of the liver stiffness measurement (LSM). LS<5.5 kpa was considered as fibrosis stage F0-F1 by Metavir, 5.5 -8.0 kpa – fibrosis stage F2, 8.0-10.0 kpa – fibrosis stage F2-F3, 10.0-12.5 kpa – fibrosis stage F3, 12.5-14 kpa – fibrosis stage F3-F4 and LS>14.0 kpa – fibrosis stage F4 by Metavir.

Laboratory parameters for Fibrotest/FibroMax were performed at the Georgian-French Joint hepatology clinic "HEPA" and were calculated with special algorithm. TE and FibroTest/FibroMax were performed at one and the same day.

In case of necessity liver biopsy was performed.

Results and their discussion. Distribution of fibrosis stages by Metavir among 185 patients with chronic HCV infection using TE was the following: 68 (36.8%) patients had fibrosis stage F0-F1 (LS < 5.5 kpa), 48 (25.9%) patients had fibrosis stage F2 (LS – 5.5 – 8 kpa), 17 (9.2%) patients had fibrosis stage F2-F3 (LS – 8.0 – 10.0 kpa), 9 (4.9%) patients had fibrosis stage F3 (LS – 10.0 – 12.5 kpa), 4 (2.2%) patients had fibrosis stage F3-F4 (LS – 12.5 – 14.0 kpa) and 39 (21.0%) patients had liver fibrosis stage F4 (cirrhosis, LS>14 kpa).

Distribution of fibrosis stages by Metavir among 67 patients with chronic HBV infection using TE was the following: 41 (61.2%) patients had fibrosis stage F0-F1 (LS < 5.5 kpa), 14 (20.8%) patients had fibrosis stage F2 (LS – 5.5 – 8 kpa), 3 (4.5%) patients had fibrosis stage F2-F3 (LS – 8.0 – 10.0 kpa), 2 (3.0%) patients had fibrosis stage F3 (LS – 10.0 – 12.5 kpa), 2 (3.0%) patients had fibrosis stage F3-F4 (LS – 12.5 – 14.0 kpa) and 5 (7.5%) patients had liver fibrosis stage F4 (cirrhosis, LS>14 kpa).

Distribution of fibrosis stages by Metavir among 185 patients with chronic HCV infection using FibroTest/FibroMax was the following: 73 (39.5%) patients had fibrosis stage F0-F1, 41 (22.2%) patients had fibrosis stage F2, 15 (8.1%) patients had fibrosis stage F2-F3, 11 (5.9%) patients had fibrosis stage F3, 5 (2.7%) patients had fibrosis stage F3-F4 and 40 (21.6%) patients had liver fibrosis stage F4 (cirrhosis).

Distribution of fibrosis stages by Metavir among 67 patients with chronic HBV infection using FibroTest/FibroMax was the following: 42 (62.7%) patients had fibrosis stage F0-F1, 13 (19.4%) patients had fibrosis stage F2, 2 (3.0%) patient had fibrosis stage F2-F3, 2 (3.0%) patient had fibrosis stage F3, 2 (3.0%) patients had fibrosis stage F3-F4 and 6 (8.9%) patients had liver fibrosis stage F4 (cirrhosis).

In patients with chronic HCV or HBV infection fibrosis stages measured by Fibroscan and Fibrotest/FibroMax were coincided in 127 (68.6%) and 45 (67.2%) cases, respectively. Discordance in one degree of fibrosis stage was found in 36 (19.5%) patients with chronic HCV infection and in 14 (20.9%) patients with chronic HBV infection. Discordance in more then one degree of fibrosis stage was found in 22 (11.9%) and 8 (11.9%) cases.

Discordances were seen in those with elevated ALT and AST (4-5 times of the upper limit of the norm), in obese patients (BMI>25) and in those with Steatosis (S3-S4), as well as in cases of extrahepatic cholestasis or chronic hemolysis. LS values were significantly greater in patients with steatosis-S3-S4 by FibroMax. According to the several studies high degree of steatosis may increase liver stiffness and interfere with the estimation of liver fibrosis by FibroScan [9].

TE and FibroTest/FibroMax were characterized with an excellent accuracy. LF stages measured by abovementioned methods were well correlated with the clinical signs (spider angiomata, palmar erythema, hepatosplenomegaly, oesophageal varices, ascites, caput medusa, etc.) as well as with the results of laboratory and instrumental investigations (ALT, AST, leukocyte count, platelet count, prothrombin time, INR, albumin, Fibrotest, Fibromax, abdominal ultrasound, gastroscopy, etc).

In patients with Fibroscan and Fibrotest/FibroMax concordant results liver biopsy might be avoided. The latter is an invasive method and its frequent use for assessing liver disease progression is limited.

TE using Fibroscan and FibroTest/FibroMax are simple, non-invasive, reliable and easily reproducible methods for assessing liver fibrosis and cirrhosis in patients with chronic HCV and HBV infection.

They are characterized with an excellent accuracy. FibroScan and FibroTest/FibroMax results are well correlated with the clinical signs as well as with the results of laboratory and instrumental investigations.

Concordant results are obtained in high percentage of patients with chronic HCV and HBV infection using Fibroscan and Fibrotest/FibroMax and in this group of patients liver biopsy might be avoided.

Considering the high prevalence of fibrosis and cirrhosis among patients with chronic HCV and HBV infection, Fibroscan and Fibrotest/ FibroMax appear to be very valuable methods for detecting early stages of fibrosis allowing to avoid the progression of liver damage, as well as end-stage liver disease. These methods are easy to perform and therefore allows regular follow-up of the course of LF.

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SUMMARY

FIBROSCAN AND FIBROTEST/FIBROMAX TO ASSESS LIVER FIBROSIS/CIRRHOSIS IN PATIENTS WITH CHRONIC HBV AND HCV INFECTION IN GEORGIA

Dolmazashvili[1,2,3] E., Zhamutashvili[1,3] M., Svanidze[1] M., Nizharadze[1] N., Abutidze[1,3] A.

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Although, liver biopsy is the gold standard in assessment of the degree of liver damage, the method has some limitations. For this reason, assessment of liver damage using non-invasive methods is currently an important topic in hepatology.

The aim of the study was to evaluate liver fibrosis/cirrhosis using Transient Elastography and FibroTest/FibroMax in patients with chronic HCV and HBV infection in Georgia and to compare Fibroscan and FibroTest/FibroMax results.

252 patients were included in the study, among them 185 - with chronic HCV infection and 67 – with chronic HBV infection. These patients were investigated at the Georgian-French Joint Hepatology Clinic "HEPA", from December 2007 to November 2008.

In patients with chronic HCV or HBV infection Fibroscan and Fibrotest/FibroMax results were correlated in 127 (68.6%) and 45 (67.2%) cases, respectively. Discordance in one degree of fibrosis stage was found in 36 (19.5%) patients with chronic HCV infection and in 14 (20.9%) patients with chronic HBV infection. Discordance in more then one degree of fibrosis stage was found in 22 (11.9%) and 8 (11.9%) cases. In patients with Fibroscan and Fibrotest/FibroMax concordant results liver biopsy might be avoided. Fibroscan and Fibrotest/Max appear to be very valuable methods for detecting early stages of fibrosis among patients with chronic HCV and HBV infection, allowing to avoid the progression of liver damage, as well as end-stage liver disease. These methods are easy to perform and therefore allows regular follow-up of the course of LF.

Key words: liver fibrosis/cirrhosis, fibroscan, fibrotest, fibromax.

РЕЗЮМЕ

ЭФФЕКТИВНОСТЬ ФИБРОСКАН И ФИБРОТЕСТ В ОЦЕНКЕ ФИБРОЗА/ЦИРРОЗА ПЕЧЕНИ СРЕДИ ПАЦИЕНТОВ С ХРОНИЧЕСКОЙ HBV И HCV ИНФЕКЦИЯМИ В ГРУЗИИ

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Эластография печени с применением аппарата «ФиброСкан» является новым, безболезненным методом для оценки фиброза/цирроза и может проводиться при различных болезнях печени.

Целью исследования явилась оценка фиброза/цирроза печени у больных с хронической HBV или HCV инфекцией в Грузии, используя эластографию печени с применением аппарата “ФиброСкан” и фибротест (комплекс тестов под названием «Фибромакс»). В исследование были включены 252 пациента, среди них 185 - с хронической HCV инфекцией и 67 - с хронической HBV инфекцией. Эти пациенты были обследованы в грузино-французской совместной гепатологической клинике “Гепа” с ноября 2007 года до ноября 2008 года. Среди пациентов с хронической HCV или HBV инфекцией результаты эластографии печени с применением аппарата “ФиброСкан” и фибротеста совпали в 68,6% и 67,2% случаев, соответственно. Несовпадение в одной стадии фиброза было выявлено в 19,5% случаев с хронической HCV инфекцией и в 20,9% случаев с хронической HBV инфекцией. Несовпадение более чем в одной стадии фиброза было выявлено в 11,9% и 11,9% случаев, соответственно. Делается вывод, что при совпадении результатов эластографии печени с применением аппарата “ФиброСкан” и фибротеста биопсию печени можно избежать. Учитывая высокую распространенность фиброза и цирроза печени у больных с хронической HBV или HCV инфекцией, эластография печени с применением фибротеста и аппарата “ФиброСкан” является ценным методом выявления ранних стадий фиброза, позволяющими избежать осложнения цирроза печени. Эластография печени и фибротест легко выполнимы, что позволяет регулярно исследовать пациентов для оценки прогрессирования болезни.

OVERVIEW OF HIV EPIDEMIOLOGICAL SITUATION IN GEORGIA

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HIV/AIDS problem that was not known several decades ago, because of its devastating scale and impact not only for human health and rights, but also social and economical impact was resolute as one of the most formidable challenges to human life and dignity in the Declaration of Commitment on HIV/AIDS adopted on the United Nations General Assembly Special Session (UNGASS).

The Joint United Nations Program on AIDS (UNAIDS) and the World Health Organization (WHO) estimate that approximately 33.2 million people were

living with HIV worldwide in 2007, including 2.5 million children under 15. Estimated number of newly diagnosed HIV cases in 2007 was 2.7 million.

Georgia was one of the countries in the Newly Independent States (NIS) that immediately reacted to the epidemic in early 1990s. From the beginning of epidemic Georgia has identified AIDS as a priority, but the progress over the last 5 years is commendable. The new leadership of the Government is strongly committed to follow recommendations of G8, UNGASS, WHO and UNAIDS. Establishment of Country Coordinating Mechanism, One Agreed National Strategic Plan of Actions and development of one agreed Monitoring and Evaluation System (M&E), Law on HIV/AIDS Prevention are the key factors to meet Millennium Development Goals.

The most important achievements for the last years were implementation of Prevention of Mother to Child Transmission (PMTCT), Universal Access to HIV Treatment, Care and Support, implementation high technology methods such as genotyping and resistance testing on HIV/AIDS and opening of regional HIV Treatment Centers in West part of Georgia (Batumi, Zugdidi, Kutaisi).

Independence from Soviet Union, changes of economical situation from the low income to the middle income country, opening the borders and increased international contacts has have influence on HIV epidemiological situation.

Epidemiological Situation in Terms of HIV in Georgia. Georgia belongs to the countries, where notification of HIV infection is mandatory. By protocol screening on HIV is accompanied by pre and post test counseling (excluding blood donors). Counseling is carried out at Epidemiological Department of IDACIRC. Detection of HIV antibodies was performed by ELISA method using Vironostica HIV Uni-Form II Ag/Ab, BioMerieux, the Netherlands. By protocol, all reactive samples were confirmed by Western Blot method using HIV BLOT 2.2 Western Blot Assay. All HIV positive patients, who has willing to continue to have assistance with AIDS Center, are referred to the out and in-patient units for further examination, treatment and follow up.

Epidemiological Department collects information about HIV positive patients and their partners. Information collection includes socio demographic (age, sex, education, etc), risk behaviors/factors for HIV transmission and obtain possible ways of disease transmission. All information about HIV positive patients are entered into the specially designed data base.

First case of HIV infection in Georgia was reported in 1989. By December 1st, officially registered cases of HIV infection is 1825, with estimated number 3500-4000. Majority of the patients are male (75% of registered cases). Nearly 400 patients already died. Four hundred and sixty one patients are under the Antiretroviral (ARV) treatment, including 23 children.

By Epidemiological situation in terms of HIV/AIDS, Georgia still belongs to low prevalence HIV epidemic countries, with actual prevalence of 0.031 (31 per 100 000 population) and estimated prevalence 0.087 (87 per 100 000 population) in the country. Although by experts' estimation, urgent measures should be undertaken immediately to prevent rapid spread of HIV in the nearest future. Experts estimations are based on the numbers of injecting drug users in the country and their high risk behaviours as a needle, syringe and other injecting equipment share, high rate of sexually transmitted infections (STIs), high incidence and prevalence of Hepatitis B and C, and wide international contacts which leads to increased migration from Georgia to other countries and vice versa.

Due to above mentioned factors HIV incidence increases year by year. As it showed in the figure1, HIV incidence rate at the end of 2008 is 7.0 per 100 000 population.

First significant increase of HIV incidence rate was observed from 1999 to 2000 (2.24 times) and 2003 to 2004. From 2004 stabile, but slight increase of incidence rate is presented. Increased numbers of HIV patients are due to following factors: Situation from 2000 in Georgia was more stabile compared to 1997-1999 years and more people, especially most at risk population (MARPS) had possibility to get tested and knew their HIV test results. Injecting drug users (IDUs) who mostly became infected in different countries (Russia, Ukraine) did not know their HIV status and became the source of infection for their needle and sex partners. Increased number of HIV positives, especially those who did not undergo HIV testing was the major source of infection. Broad

campaigns on HIV prevention, active epidemiological surveillance, starting different behavioral surveillance surveys (BSS) among MARPS significantly increased the number of people testing on HIV and as a result increased the number of newly diagnosed patients in 2004. Country success on Global Fund II round proposal, which gave possibility to attain universal access to ARV treatment, was one of the strong motivations for high risk behavior groups to get tested on HIV.

Universal access to ARV also significantly increased prevalence rate of HIV infection in the country and prevalence rate from 2004 (when universal access was introduced in Georgia) jumped from 10.45 per 100 000 population to 31 in 2008.

In terms of HIV transmission, the major route of transmission is associated with drug use and needle share during drug injection. At the moment approximately 60% of all reported HIV cases are due to injection drug use and needle share practice. However over the last several years heterosexual route of transmission is gaining importance, and increased from 29.1% to 36.1% for last five years (fig. 2).

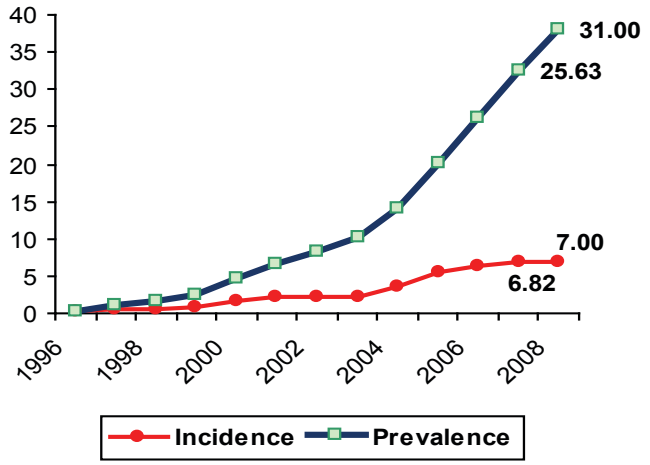

Fig. 1. Incidence and Prevalence of HIV Infection in Georgia (1996-2008)
Source: Infectious Diseases, AIDS and Clinical Immunology Research Center

Peculiarities of HIV by route of transmission are pretty the same if we compare Georgian data to Eastern European countries and the major proportion of most at risk population (MARPS) goes to IDUs. Like other Eastern and Western European Countries, where HIV is in concentrated epidemic stage, percentage of people who became infected through sexual contacts has been increased in Georgia.

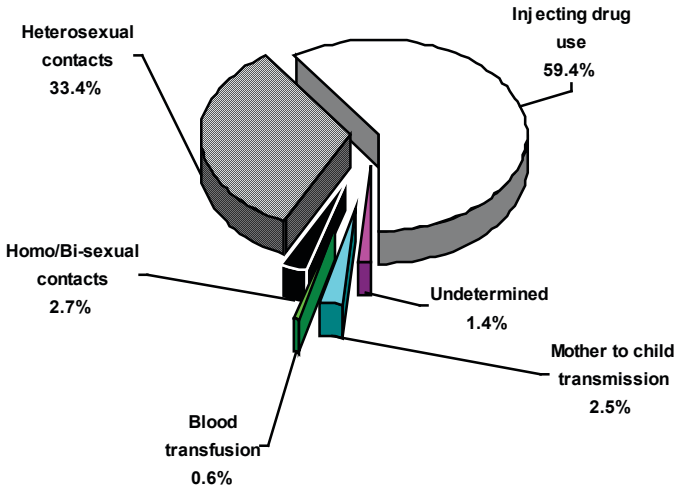

Fig. 2. Distribution of HIV registered cases by Route of transmission 2008
Source: Infectious Diseases, AIDS and Clinical Immunology Research Center

Patients who were possibly infected by heterosexual contact (n=598), 66% were female and 34% male (number of infected patients includes 25 foreigners who lived in a time of diagnoses in Georgia). For those 598 HIV positives, in 70% source of infection were HIV positive drug user sex partners. Most of them were infected in Georgia, and some - in other countries. Figure 3 illustrates percentage of HIV heterosexual transmission break down by gender and countries of infection transmission.

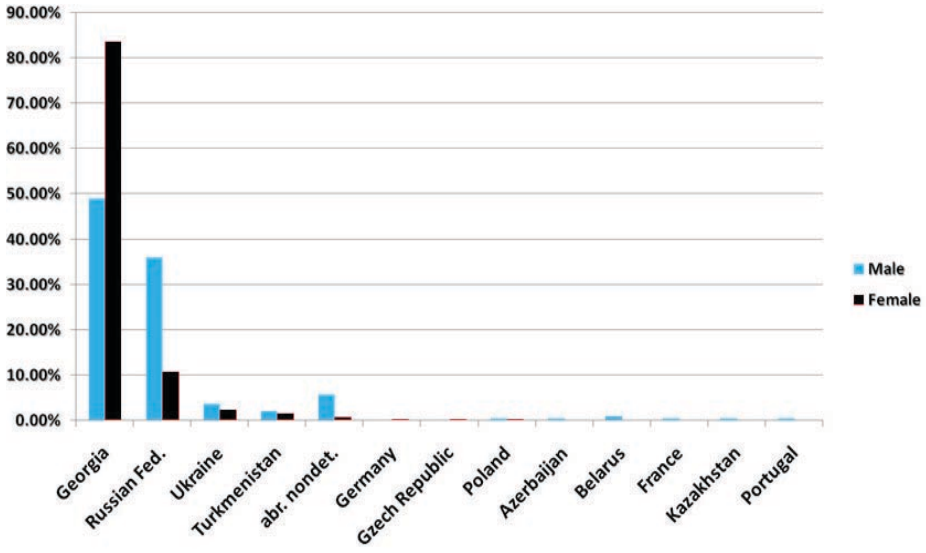

Fig. 3. Distribution of HIV Transmission through Heterosexual Contacts by Countries

As it is well known, women are more likely to be infected with HIV through heterosexual contacts compared to men. Based on this growing number of HIV positive women can be explained in Georgia (fig. 4).

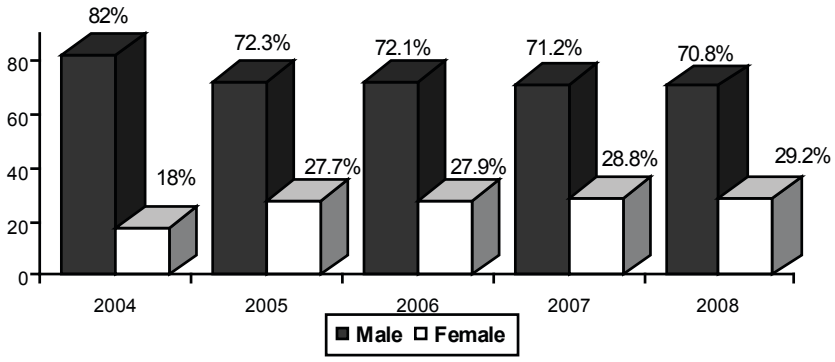

Fig. 4. Distribution of HIV Cases by Gender 1997-2008

Increased number of HIV positive women is also related to scale up of the screening among pregnant women. Since December 2004, all pregnant women have access to VCT at antenatal clinics and all HIV pregnant women have access to HIV prophylaxis treatment through PMTCT state program.

Age is one of the significant factors in terms of HIV transmission. Majority of our registered HIV positive patients are at a reproductive age at the moment of diagnoses. As it is showed in the figure 5, most of HIV positive patients are diagnosed at the age from 25 to 45 (fig. 5).

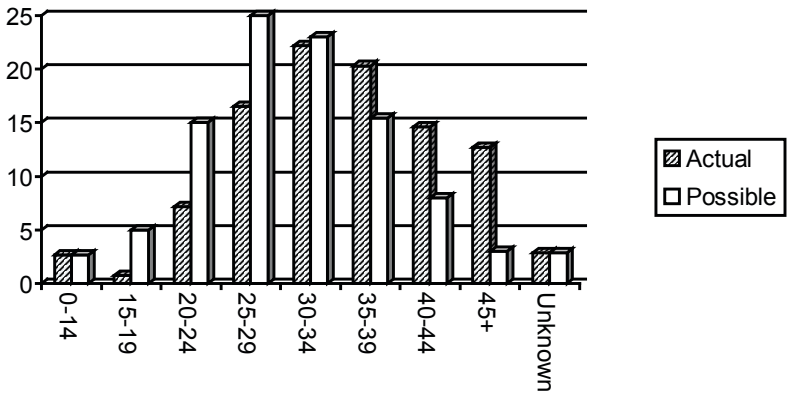

Fig. 5. Distribution of HIV Cases by Age at the Moment of Diagnoses

HIV prevalence in Georgia increases from 20-24 years and reaches pick at 30-39. Years of first drug injection in Georgia varies from 17 to 19 years old. In a same age of first sexual contacts take place. High risk behavior is mostly presented in these ages. MARPS undergo screening on HIV at least five years later after they became infected with HIV (when the signs and symptoms of AIDS are already presented). Accordingly the pick years of their HIV infectivity can be switched to 25-29 years. Besides, a high risk behavior (needle share, etc) among drug users depends on the drugs available in the country. People who did not share drugs for a several years may start to share needles due to the drug change and technology of its preparation.

Vertical transmission of HIV mostly took place before 2005. However these cases were revealed several years later after infection, when sings and symptoms of AIDS occurred. No case of mother to child transmission was detected from 2005, when the PMTCT program was introduced in the country. Within the frame of PMTCT all HIV positive pregnant women undergo comprehensive HIV prophylaxis treatment.

Distribution of HIV/AIDS cases by regions of Georgia seems to be quite remarkable. The highest HIV prevalence rates are found in Black Sea coast regions - Samegrelo and Adjara (with prevalence of 131.11 and 132.03 among adult HIV cases per 100 000 adult population). High HIV prevalence in these regions could be explained by their seaside and border location, high rate of migration to the neighboring countries (especially Ukraine and Russia having alarming HIV/AIDS situation), permanently increasing number of injecting drug users and big number of internally displaced population (fig. 6).

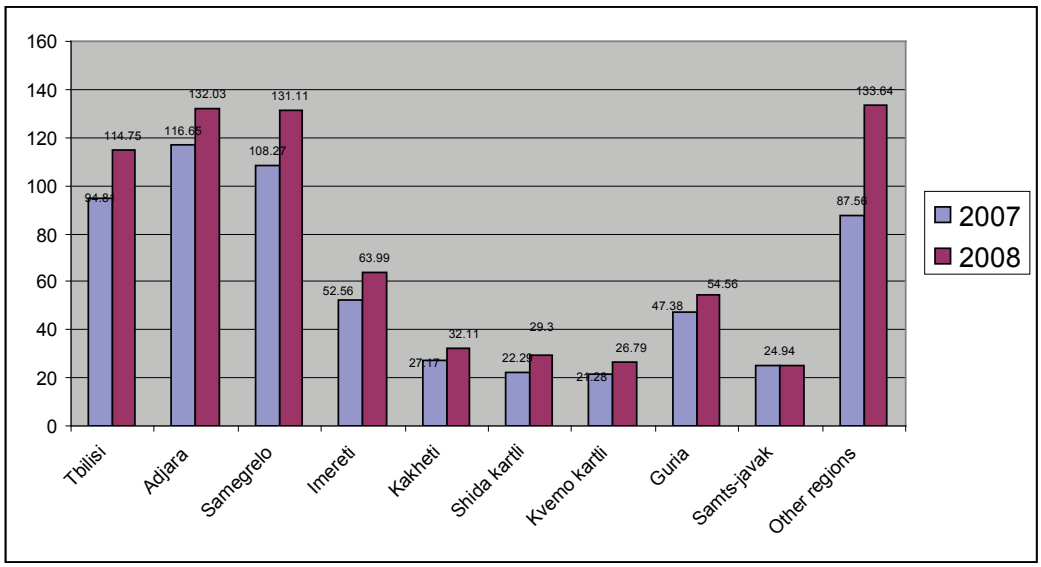

Fig. 6. Distribution of Adult HIV Registered Cases by Different Regions of Georgia Per 100 000 Population

Availability of HIV treatment Centers in West Georgia, increasing number of VCT units, conducting Second Generation Epidemiological Surveys in West Part of Georgia leads for better access to HIV testing and good registration.

HIV/AIDS is one of the leading Public Health problems worldwide. At the end of 2007, 33.2 million people are leaving with HIV/AIDS in the world. Forty one countries already have a generalized epidemic, and in 85 countries HIV infection is concentrated at MARPS. Georgia still belongs to the low prevalence countries, with estimated prevalence of 0.09.

Working on HIV in Georgia has been started in 1994. Georgian Government recognize HIV problem and pay attention to its prevention and treatment. Having One Country Coordinating Mechanism (CCM), which includes different ministries and governmental and non governmental institutions; National Strategic plan for 2007-2010; and One Agreed M&E System helps country for better coordination of HIV programs. Decentralization of HIV/AIDS treatment centers and development of National Guidelines on HIV treatment, care and prevention is one of the successes for advanced HIV treatment management. Universal access to HIV treatment and care increases the life expectancy of HIV positives and as a result increases HIV prevalence in the country. Implementation of PMTCT program prevents vertical transmission and helps HIV positive mothers to deliver healthy children. From 2005, all HIV pregnant women, who undergo ARV prophylaxis therapy, delivered healthy HIV negative babies.

Besides successes achieved by country, incidence rate of HIV is still increases among Georgian citizens. The fact that HIV is spread among MARPS who transmit HIV to their sexual partners is in face. In the other hand one of the reasons of increasing number of HIV cases (incidence rate) may be cause by better management and scaling up of VCT units and introduction of Second Generation Epidemiological Surveys.

For HIV transmission better prevention in the country it is necessary to enhance educational activities among MARPS and general population. Attention should be paid to teenagers. Courses for HIV prevention should be introduced as a basic subject of life skills at schools and Universities. Youth education will raise knowledge on HIV/AIDS prevention interventions, decrease stigma and its related discrimination towards people living with HIV/AIDS (PLHIV). Fewer stigma increase access to HIV screening and helps to detect HIV infection in early stage of infection. For early detection of HIV infection it is also important to improve knowledge of Medical personnel on HIV issues. Enlargement of VCT and implementation of Diagnosed Based Counseling and Testing (DCT) units, routine testing of MARPS on HIV improve detection of new HIV cases, helps to prevent transmission and increase life expectancy and quality of HIV positives. Because of high rate of needle share among IDUs and high prevalence of STIs, it is essential to expand harm reduction programs and increase access to condoms.

Good coordination of Governmental and non governmental institutions (NGOs), better financing of HIV programs and improvement of capacity building will help the country to keep HIV epidemic in a low prevalence and give country possibility to achieve "Universal Access to HIV Prevention, Treatment, Care and Support" for 2010 year

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SUMMARY

OVERVIEW OF HIV EPIDEMIOLOGICAL SITUATION IN GEORGIA

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Georgia still belongs to low HIV epidemic countries and by December 1st, 2008 there are 1825 HIV/AIDS cases registered at the IDACIRC with estimated number 3500 (estimated prevalence 0.09%). Majority of HIV/AIDS patients are male (75%). Four hundred and sixty one patients are under the Antiretroviral (ARV) treatment, including 23 children. Despite of HIV low prevalence, Georgia is considered to be at risk for an imminent epidemic spread of HIV mainly due to wide spread drug use with high risk practices (needle-sharing) and high rate of STIs. The major route of HIV transmission is associated with IDU. At the moment approximately 60% of all reported HIV cases are due to drug injection. However over the last several years heterosexual route of transmission is gaining importance, and increased from 29.1% to 36.1% for last five years. First significant increase of HIV incidence rate was observed from 1999 to 2000 (2.24 times) and 2003 to 2004. From 2004 stabile, but slight increase of incidence rate is presented. Most HIV positive patients are diagnosed at the age from 25 to 45. The highest HIV prevalence rates are found in Western Georgia, particularly Black Sea coast regions - Megrelia and Adjara (with prevalence of 131.11 and 132.03 among adult HIV cases per 100 000 adult population). Enlarging educational activities, prevention interventions, better financing of HIV programs and improvement of capacity building will help the country to keep HIV epidemic in a low prevalence and give country possibility to achieve "Universal Access to HIV Prevention, Treatment, Care and Support" for 2010 year.

Key words: HIV/AIDS, prevalence, incidence, in Georgia.

РЕЗЮМЕ

ОБЗОР ЭПИДЕМИОЛОГИЧЕСКОЙ СИТУАЦИИ ВИЧ В ГРУЗИИ

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Грузия по сей день принадлежит к странам с низкой эпидемией ВИЧ/СПИД. На 1 декабря 2008 года число регистрированных ВИЧ инфицированных в Научно-практическом центре инфекционных патологий, СПИДа и клинической иммунологии Грузии составляет 1825 случаев – оценочное число -3500 (предварительная распространенность 0,09%). Большинство ВИЧ/СПИД пациентов - мужчины (75%). 461 пациент находится на антиретровирусном (АРВ) лечении, включая 23 детей. Несмотря на низкую распространенность ВИЧ, Грузия находится под риском неизбежного роста эпидемии, в основном, ввиду широкого распространения наркомании с высоким риском поведения (использование общих шприцев) и высоким уровнем заболеваемости болезнями, передающимися половым путем в стране. Основной путь передачи ВИЧ связан с потреблением инъекционных наркотиков. Примерно 60% всех зарегистрированных больных заражены ВИЧ-инфекцией вследствие употребления инъекционных наркотиков. На сегодняшний день несколько лет наблюдается увеличение передачи инфекции гетеросексуальным путём (за последние пять лет заболеваемость увеличилась с 29,1% до 36,1%). Первое существенное увеличение числа ВИЧ-заболеваемости наблюдалось в период с 1999 по 2000 и с 2003 по 2004 гг. С 2004 года наблюдается незначительный рост выявления новых случаев. Большинство ВИЧ положительных пациентов диагностируются в возрасте от 25 до 45 лет. Наиболее высокие показатели распространенности ВИЧ обнаружены в Западной Грузии, особенно в регионах Черноморского побережья – Мегре-

лии и Аджарии (131,11 и 132,03 среди взрослых ВИЧ-инфицированных на 100 000 взрослого населения).

Расширение образовательных мероприятий, профилактических вмешательств, улучшение финансирования программ ВИЧ и информированности населения позволит сохранить ВИЧ-эпидемию на низком уровне распространения, и к 2010 году добиться «Универсального доступа» к профилактике, лечению, уходу и поддержке.

THE ROLE OF IMAGING STUDIES IN CNS INFECTIONS

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Recent advances in nervous system imaging techniques have greatly improved the diagnosis and treatment of Central Nervous System (CNS) infections. Nowadays, the most useful imaging modalities are harmless to the patient and widely available [23]. Whereas analysis of CSF, biopsy, and laboratory evaluation remain the gold standard to identify the infectious agent, neuroimaging is crucial in clearly depicting inflammatory lesions of brain and spine. Notably, in opportunistic disease neuroimaging has a pivotal role not only in diagnosis but also in monitoring therapeutic response [11].

Computed tomography (CT) remains the first line of imaging for the patient admitted to the clinic with fever and altered mental status. Firstly, it's considerably less expensive and secondly, compared to magnetic resonance imaging (MRI), CT scan takes less time which is important in acute conditions [23]. MRI offers superior soft tissues imaging of CNS infections and advanced techniques. MRI also is the standard of care for imaging spinal inflammatory diseases. With the advent of new MRI sequences such as T2-weighted fluid attenuated inversion recovery (FLAIR), diffusion-weighted imaging (DWI), and proton (1H) magnetic resonance spectroscopy (1H-MRS), it is possible to detect early and subtle abnormalities. FLAIR is particularly valuable in depicting complications like sub-/epidural empyema and vasculitis; DWI shows early spreading parenchymal complications of meningitis with more clarity and is of help in differentiation of pyogenic abscess (PA) from ring enchancing lesions of other etiology [13,22]. The development of 1H-MRS techniques has made it possible to detect different brain metabolites and their disease-related changes. It produces specific peak patterns in cases of brain abscess [5].

Below we discuss imaging characteristics of bacterial, viral, fungal and prion infections of the CNS.

Bacterial infections.

Meningitis: In cases of suspected bacterial meningitis with impaired consciousness, an immediate CT is recommended before lumbar puncture to rule out the herniation, caused by brain swelling [14]. In the early phase of meningitis, the CT findings are mostly normal. Contrast-enhanced CT may show beginning meningeal enhancement, which becomes more accentuated in later stages of disease [25]. CT venography is an excellent tool to diagnose complicating thrombosis of the transverse and sagittal sinus, necessitating therapeutic anticoagulation with intravenous heparin [4]. In later stages, persistent drowsiness and meningeal signs should be regarded as an indication for repeat CT to rule out a resorptive hydrocephalus. If external ventricular drainage is required, further

CT studies to check on ventricular size will help in timing of the drainage operation and later cessation of this measure [15].

Magnetic resonance imaging (MRI) is not routinely required in cases of uncomplicated bacterial meningitis. Although it can be of help in suspected TB meningitis, which is characterized by communicating hydrocephalus, basal cisterns enhancement and T2 hypointensity. Recently, magnetization transfer MRI has been proposed as a useful tool in the diagnosis of tuberculous meningitis [28].

In complicated cases with seizures and evolving focal signes, MRI is superior to CT in demonstrating parenchymal lesions due to meningoencephalitis or vaculitic complications on FLAIR (fluid-attenuated inversion recovery) sequences. In Lyme disease, multifocal nonenhancing patchy lesions are seen on T2 weighted imaging (T2 WI) [31].

Some pathogens have a predilection for brain stem involvement. It is readily visualized on MRI. Notably, the finding of rhombencephalitis points to listeria monocytogenes as the causative agent [29]. Neurobrucellosis is a relatively rare condition with a wide spectrum of imaging findings from normal to nonspecific signs of inflammation of CNS and nerve roots or vascular complications [2].

Vascular complications must be suspected in patients with rapid deterioration despite therapy. In these cases, the sensitivity of DWI is higher than that of conventional MRI. Pyogenic vasculitis is an uncommon but very severe intracranial infection requiring rapid diagnosis. MRI is more sensitive and shows periventricular high signal on FLAIR images. Extraaxial bacterial empyema is most reliably diagnosed by MRI. CT often leaves doubt as to the nature of the lesion and its exact location [9].

Pyogenic abscess: The diagnosis of pyogenic brain abscess remains challenging. It's a therapeutic dilemma mostly in those cases where a single ring-enhancing lesion with perifocal oedema has been identified on CT giving rise to the differential diagnosis of abscess versus necrotic tumor (e.g. glioblastoma) or metastasis. Gadolinium (Gd) enhanced MRI is of help to identify multiple small additional lesions indicating metastatic disease. In several studies, almost all pyogenic abscesses had markedly hyperintense signal on DWI [19]. In unclear cases, additional information can be gathered from 1H-MRS – it shows elevated lactate and lipid peaks and decreased N-acetylaspartate (NAA) [10] (Fig). This method is not routinely available but some authors have found the results promising.

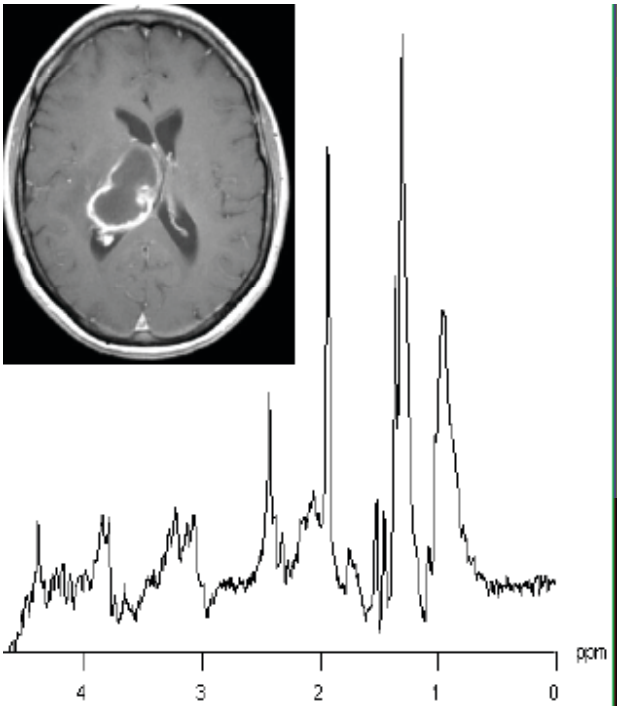

Fig. Brain abscess with corresponding spectrum. The markedly elevated lactate and lipid peaks are indicated with arrows

Viral Infections

Viral meningoencephalitis: Herpes simplex virus (HSV) I remains the most common cause of viral encephalitis. However, other viruses should be considered in acute encephalitis. This list includes recently observed epidemic of the West Nile virus [30], newly recognized viruses such as the Nipah virus [8], human herpes viruses 6 or 7 (HHV 6, HHV 7) [20] and enterovirus 71 [24]. Even in immunocompetent adults, HHV 6 can cause chronic encephalitis. In immunocompromised patients the spectrum of possible causative agents is even broader.

Herpes simplex encephalitis is characterized by T1 hypointense and T2 hyperintense signals in Limbic system with mass effect [12]. Detection of HSV DNA in the CSF by PCR remains the mainstay for the diagnosis of HSV encephalitis, although results of this laboratory test may be false negative or may arrive

belatedly. Thus, results of imaging studies are supportive to decide whether antiviral treatment has to be started in patients with suspected HSV encephalitis [18].

Enterovirus 71 (EV71), an enterovirus of the family Picornaviridae, may lead to a polio-like brainstem encephalitis and acute flaccid paralysis. MRI of EV71 typically shows hyperintense lesions on T2WI within the brainstem and dentate nuclei of the cerebellum . In some patients, lesions may expand to the spinal cord, thalamus, and putamen [15].

The West Nile Virus (WNV) has caused encephalitis outbreaks in Southern Europe, Russia, and the United States. Clinical, laboratory and neroimaging features were described in a recent study evaluating WNV seropositive patients [27]. T2WI and DWI revealed focal hyperintense lesions within the basal ganglia, thalamus and pons in only two of the eight encephalitic patients with acute flaccid paralysis. In some patients, the virus affects substantia nigra as suggested by hyperintensities on T2WI in this region. Similar to HSV and EV71 encephalitis, DWI seems to be more sensitive to detect signal abnormalities especially in the initial phase of WNV infection of the brain.

Infection of the CNS with the measles virus (MV) may result in acute postinfectious encephalitis, acute progressive encephalitis and subacute sclerosing panencephalitis (SSPE). Data about imaging findings in acute measles encephalitis are sparse. T2WI may reveal cortical edema and bilateral symmetric hyperintense lesions within the putamen and caudate nuclei as well as within the centrum semiovale. SSPE is a rare progressive CNS disease that usually occurs in childhood and early adolescence but may also be present in adults. A recent study compared MR spectroscopy and conventional MRI in children with early stage and with late stage SSPE. Conventional MRI revealed no abnormalities in early stage SSPE but detected widespread periventricular hyperintense changes on T2WI in late stage SSPE [17]. In contrat, MR spectroscopy demonstrated an increase in choline/creatinine ratios in the frontal and parieto-occipital white matter in all patients suggestive of inflammation also in early stages of SSPE. N-acetypaspartate/creatine ratios were normal in the early stage which reflects an absence of neuronal loss at this stage [1].

Progressive multifocal leukoencephalopathy (PML) develops almost exclusively in patients with severe immune deficiency and, subsequently, has become more frequent as a result of a high prevalence of the HIV infection. PML has also been reported in immunocompromised patients receiving prolonged treatment with methotrexate, cyclophosphamide, Natalizumab, etc [16]. This rare condition is caused by the reactivation of polyomavirus JC. Typical for PML is T1 hypo and T2 hyperintense lesions envolving subcortical white matter and cerebellum [32].

Parasitic infections

Toxoplasmosis: Toxoplasmosis is the most frequent opportunistic infection in immunosupressed patients with acquired immune deficiency syndrome (AIDS) or after bone marrow transplantation [7]. The lesions are typically multiple, with ring enhancement or homogenic. MRI delineates them most clearly, sometimes revealing hemorrhagic zones. Not infrequently, neuroimaging reveals toxoplasma lesions with mass effect and marked perifocal edema. If the lesions are unchanged or progressive in a week after the specific therapy, the diagnosis should be reconsidered [6]. In cases of severe immunosupression, the MR appearance can be completely atypical, misleading radiologist and clinician. Notably, in fulminate encephalitic variants of the disease, lesions are widespread on T2WI and are completely devoid of enhancement [13]. In these cases, antitoxoplasma therapy should be started until the diagnosis has been clarified. Spectroscopy is characterized by predominant lipid peak [5].

Fungal infections

Fungal infections of the CNS are very rare in the general population. They are most frequently encountered in immunocompromized patients such as those with AIDS or after organ transplantation [33]. Due to the lack of inflammatory response, neuroradiological findings are often nonspecific. Although almost any fungus may cause encephalitis, cryptococcal meningoencephalitis is most frequently seen, followed by aspergillosis and more rarely candidiasis. MRI shows punctuate or patchy signal hyperintensities on T2WI, gadolinium enhancement is frequently absent [15].

In cryptococcal meningoencephalitis, diffuse meningeal enhancement and also ventriculitis can be seen on MRI. Typical findings are multiple punctuate lesions, often in the basal ganglia. They are termed "soap bubble lesions" and allow the quick provisional diagnosis leading to rapid antifungal treatment. In nonimmunodeficient patients or patients with AIDS under highly active antiretroviral treatment, the lesions can become ring enhancing [21].

Prion Infections

Transmissible spongiform encephalopathies (TSE) or prion diseases are fatal neurodegenerative conditions of human and different animal species. This is a group of rare diseases, Creutzfeldt-Jakob disease (CJD) being the most common among them. Cortical atrophy and increased signal intensity is characteristic for all prion diseases. MRI is an important tool in differentiating sporadic and variant CJD: variant CJD is characterized by the pulvinar sign – a high T2 weighted magnetic resonance imaging signal in the posterior thalamus [26].

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SUMMARY

THE ROLE OF IMAGING STUDIES IN CNS INFECTIONS

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Recent advances in nervous system imaging techniques have greatly improved the diagnosis and treatment of central nervous system infections. Nowadays, the most useful imaging modalities are harmless to the patient and widely available. Whereas Analysis of CSF, biopsy, and laboratory analysis remain the gold standard to identify the infectious agent for instance in meningitis, neuroimaging is crucial in clearly depicting inflammatory lesions of brain and spine. Notably, in opportunistic disease neuroimaging has a pivotal role not only in diagnosis but also in monitoring therapeutic response. The present review discusses imaging characteristics of bacterial, viral, fungal and prion infections of the central nervous system.

Key words: MRI, CNS infections, CT.

РЕЗЮМЕ

РОЛЬ МЕТОДОВ ВИЗУАЛИЗАЦИЙ В ДИАГНОСТИКЕ ИНФЕКЦИЙ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ

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Благодаря усовершенствованию методов визуализации, значительно улучшились диагностика и лечение неврологических болезней. Практически все виды неировизуализации безвредны и широко доступны для пациента. Анализ церебромозговой жидкости, биопсия и лабораторный анализ остаются стандартом для идентификации инфекционного агента, но неировизуализация имеет решающее значение в четком описании воспалительных поражений головного и спинного мозга. В случае оппортунистических инфекций, нейровизуализация занимает значимую роль не только в диагностике, но и в наблюдении над лечебным процессом. В представленном обзоре обсуждаются особенности визуализации бактериальных, вирусных, грибковых и прионных инфекций центральной нервной системы.