

Relationship of plasma viral load, CD4+ lymphocyte count and clinical manifestations in HIV infected patients

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ABSTRACT

Objectives: To assess the relationship between the viral load (level of Human Immunodeficiency virus type – 1 (HIV - 1) RNA in plasma), CD4 count and clinical manifestations in Georgian HIV infected patients. **Materials and Methods:** a total of 82 adult HIV infected antiretroviral naive patients were studied into the prospective cross-sectional study. Patients were divided into the three groups by the viral load level: 1st group – 22 patients with low level of viral load (<10,000 copies/ml), 2nd group – 17 patients with medium level of viral load (between 10,000 – 100,000 copies/ml) and 3rd group 42 patients with high level of viral load (>100,000 copies/ml). Viral load was tested by Polymerase Chain Reaction (PCR) and CD4+ lymphocyte count was detected by Immunofluorescent technique. **Results:** 1st group: mean value of viral load - 3835 copies/ml (range undetectable - 9983), mean value of CD4 count - 572 cells/ μ l (range 378 - 889). Among this group asymptomatic patients and patients with Persistent Generalized Lymphadenopathy (PGL) predominated (category A by CDC classification of 1993). 2nd group: mean value of viral - 40765 copies/ml (range 12789 - 98437), mean value of CD4 count - 451 cells/ μ l (range 231 - 721). Among the patients of this group 10 patients were asymptomatic or presented with PGL (category A) and the other 7 were symptomatic (category B or C by CDC classification of 1993). 3rd group: mean value of viral load - 547801 copies/ml (range 106734 - 1754342), mean value of CD4 count - 150 cells/ μ l (range 23 - 457). Majority of this group patients were presented with advanced stage of HIV disease (category B or C). **Conclusions:** There was a significant correlation between the increased viral load, decreased CD4 count and presence and severity of clinical manifestation of HIV infection.

KEYWORDS: HIV, plasma HIV RNA, CD4+ lymphocyte count, viral load, clinical manifestations

The typical course of HIV infection includes three phases: primary infection, clinical latency and clinically apparent disease. However, duration of HIV infection is very individual. Depending on the duration of infection and kinetics of virologic and immunologic events three dominant patterns of HIV disease have been described: 1) 80-90% of HIV patients are "typical progressors" with median survival time of approximately 10 years; 2) 5-10% of HIV infected persons are "rapid progressors" and experience unusually rapid (3 to 4 years) course of disease; 3) about 5% of HIV infected persons, so called "long-term non-progressors" do not experience disease progression for an extended period of time (at least 7-10 years).

Prognosis of course of HIV infection has a significant value for prediction of disease outcome, as well as for evaluation when to start therapy [1,2]. CD4+ lymphocyte count has been considered as the predictor of the risk of developing AIDS-related complications [3,4]. The risk of developing AIDS or dying over the next 24 months is less than 5% among individuals with CD4 count above 500 cells/ μ l and more than 70% among those having fewer than 50 cells/ μ l [3, 4]. The most importantly is that on the given CD4 count development of certain opportunistic infections (OI) is anticipated [5].

Despite of the value of CD4 count as a general marker of disease stage, it alone is inadequate as a means of measuring prognosis and response to antiretroviral therapy. Decrease in CD4 count is a result of viral replication and represents a clinical endpoint rather than a "surrogate marker" of disease activity. Therefore, value indicating the rate of viral replication such as viral load would be better predictor of disease progression (since concentration of HIV RNA in plasma directly reflects the titer of HIV virus particles) [3,4].

Besides, it is suggested that changes in plasma HIV RNA levels and CD4+ lymphocyte counts predict both response to antiretroviral therapy and therapeutic failure [6,7].

We decided to study correlation of clinical manifestation of HIV disease with plasma viral load and CD4 count in Georgian HIV infected patients.

MATERIALS AND METHODS

A total of 82 randomly selected HIV infected patients were involved. HIV infection was diagnosed by ELISA and/or rapid/simple method and was confirmed by Western blot and qualitative PCR methods. Among them 62 were male and 22 female aged 19 – 65. All patients were antiretroviral naive.

Clinical evaluation, measurement of viral load and CD4 count was performed for the study purposes.

Detection of HIV-1 RNA (viral load). 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.

HIV-1 RNA was extracted following the standard specimen preparation procedure: viral RNA was isolated directly from 200 μ l plasma by lysis of virus particles with a chaotropic agent followed by alcohol precipitation. A known number of quantitation standard RNA molecules was introduced into each specimen with the lysis reagent. 155-nucleotide sequence within the highly conserved region of HIV-1 gag gene was amplified by the thermostable recombinant enzyme rTth. GeneAmp PCR System 9700 thermal cycler programmed according to the AMPLICOR HIV-1 MONITOR Test, version 1.5 was used for PCR amplification. Following PCR amplification Hybridization and Detection Reactions were performed. The amounts of HIV-1 RNA and QS were calculated after stopping the reaction and measuring optical density (OD450) of the microwells.

HIV-1 RNA levels were expressed as number of HIV-1 RNA copies in 1 ml plasma. Lower detection limit was considered to be 400 copies/ml.

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

Diagnosis of opportunistic infections was made by clinical, laboratory (Culture, ELISA, PCR) and instrumental investigations. Diagnosis of nervous system manifestation of HIV disease was performed by clinical and laboratory investigations and supported by neuropsychologic assessment and neuroimaging study. Staging of HIV disease was based on the review of medical history, physical examination results and blood CD4+ lymphocyte counts according to the CDC classification system for HIV infection of 1993 [8].

Clinical evaluation, quantification of HIV RNA in plasma and measurement of CD4+ count was performed within one week period.

Statistical analysis: Data were processed with use of SPSS software, v.11.0

Design: Cross-sectional study has been conducted.

RESULTS

Viral load was detectable in 91,5% of cases. Remaining 9,5% (7 patients) had undetectable viral load – number of HIV-1 RNA copies in 1ml plasma was less than 400. Patients were divided into the three groups by the level of viral load: 1st group included patients with viral load less than 10000 copies/ml, 2nd group patients had viral load between 10000 and 100000 copies/ml and 3rd group patients – higher than 100000 copies/ml.

Following results were obtained across the groups:

1st group: 22 patients met the criteria for the 1st group. Mean value of viral load was 3835 copies/ml, ranged from undetectable to 9983 copies/ml, mean value of CD4+ count was 572 cells/ μ l, ranged from 378 to 889 cells/ μ l. Among the patients of this group 17 patients were asymptomatic and 4 presented with Persistent Generalized Lymphadenopathy (category A by CDC classification of 1993), and the other 3 were presented with mild symptoms (all of them category B by CDC classification of 1993). Among the symptomatic patients 1 had the oral candidiasis (thrush), 1 was presented with cutaneous Herpes Zoster, and 1 had the peripheral neuropathy and recurrent herpes simplex genitalis.

2nd group: 17 patients with medium level of viral load (between 10,000 – 100,000 copies/ml). Mean value of viral load was 40765 copies/ml, ranged from 12789 to 98437 copies/ml, mean value of CD4+ count was 451 cells/ μ l, ranged from 231 to 721 cells/ μ l. Among the patients of this group 10 patients were asymptomatic or presented with Persistent Generalized Lymphadenopathy (category A by CDC classification of 1993) and the other 7 were symptomatic (category B or C by CDC classification of 1993). Among the symptomatic patients 2 had recurrent cutaneous herpes simplex, 2 had AIDS Dementia complex, 1 had oropharyngeal candidiasis, 1 - constitutional symptoms, 1 had a pulmonary TB (Tuberculosis).

3rd group: 43 patients with high level of viral load (>100,000 copies/ml). Mean value of viral load was 547801 copies/ml, ranged from 106734 to 1754342 copies/ml, mean value of CD4+ count was 150 cells/ μ l, ranged from 23 to 457 cells/ μ l. Among the patients of this group 6 patients were asymptomatic or presented with Persistent Generalized Lymphadenopathy (category A by CDC classification of 1993), other 6 patients had clinical manifestations with category B of HIV infections by CDC classification of 1993 (constitutional symptoms, oral candidiasis, Herpes Zoster) and the rest 31 patients had category C disease. Among the symptomatic patients 8 had M.tuberculosis infection (Pulmonary TB, TB meningitis or other extrapulmonary disease), 6 had a PCP, 3 had AIDS dementia complex, 2 had Toxoplasmosis, 2 had Kaposi's sarcoma, 2 - wasting syndrome, 1 – Lymphoma, 1 – CMV retinitis and 1 - CMV encephalitis., 1 – cryptococcosis with cutaneous and CNS infection.

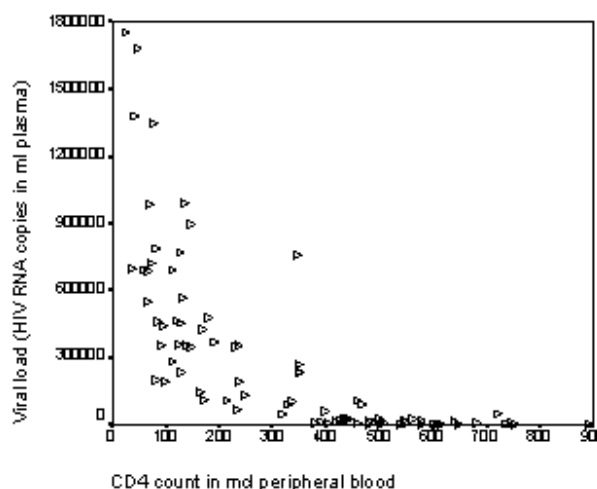


Fig.1 Relationship between plasma viral load and CD4 count.

There was strong correlation between the plasma viral load and CD4 count in peripheral blood ($p < 0,01$ by Spearman's correlation) (see Fig.1). Likewise, plasma viral load and clinical manifestations showed significant correlation, as well as CD4 count and plasma viral load ($p < 0,01$)

CONCLUSIONS

We found that almost all cases of clinically apparent HIV infection was associated with increased viral load and decreased CD4 count.

Higher HIV RNA level correlated with lower CD4 count.

Increase of viral load and decrease of CD4 count not always was accompanied by the clinical manifestations. In some cases of asymptomatic HIV infection increased viral load alone or increased viral load and decreased CD4 count was observed. These data suggests that increase of viral load precede decrease of CD4 count [2]. And increase of viral load and decrease of CD4 count occurs prior to clinical manifestations. However, to conclude long-term follow-up on these patients is warranted.

In summary, the correlation between the increased level of viral load, decreased CD4 count and frequency of various clinical manifestations has been observed.

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Взаимосвязь между вирусной нагрузкой в плазме, количеством CD4+ лимфоцитов и клиническими проявлениями ВИЧ инфекции

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Р Е З Ю М Е

Целью работы являлось оценка взаимосвязи между вирусной нагрузкой (уровень ВИЧ РНК в плазме), CD4+ лимфоцитов и клиническими проявлениями у ВИЧ-инфицированных больных в Грузии. В проспективные исследования были включены 82 нелеченных ВИЧ-инфицированных больных. Больных подразделили на 3 группы с учётом уровня вирусной нагрузки: I группа - 22 больных с вирусной нагрузкой <10000 копий/мл, II группа - 17 больных с вирусной нагрузкой между 10000 и 100000 копий/мл и III группа - 42 больных с вирусной нагрузкой >100000 копий/мл. Число клеток CD4+ измерялось методом непрямо́й иммунофлуоресценции, а вирусная нагрузка (ВН) – методом ПЦР. Установлено, что в I группе: средний уровень вирусной нагрузки составляет 3835 копий/мл (между неопределяемой и 9983), среднее число CD4 лимфоцитов - 572 клетки/μл (между 378-889); в этой группе преобладали бессимптомные больные и больные с персистентной генерализованной лимфаденопатией (ПГЛ) (категория А по классификации CDC 1993 года). Во II группе средний уровень вирусной нагрузки составлял 40765 копий/мл (между 12789-98437); среднее число CD4+ лимфоцитов - 451 клетки/μл (между 231-721); среди больных этой группы у 10 отмечалась А категория заболевания, и только у 7 категория В или С. III группе пациентов: средний уровень вирусной нагрузки оказался равным 547801 копий/мл (между 106734-1754342), среднее число CD4+ лимфоцитов - 150 клетки/μл (между 23-457). У большинства из них отмечалась продвину́тая стадия заболевания (категория В или С). Таким образом, установлена значительная корреляция между повышенной вирусной нагрузкой в плазме, пониженным числом CD4+ лимфоцитов, наличием и тяжестью клинических проявлений ВИЧ-заболевания.

Ключевые слова: ВИЧ, ВИЧ РНК в плазме, CD4+ лимфоциты, вирусная нагрузка

International committee of medical journal editors. Uniform requirements for manuscripts submitted to biomedical journals. *Ann Intern Med* 1997;126:36-47.

The Role of the Correspondence Column

All biomedical journals should have a section carrying comments, questions, or criticisms about articles they have published and where the original authors can respond. Usually, but not necessarily, this may take the form of a correspondence column. The lack of such a section denies readers the possibility of responding to articles in the same journal that published the original work.

Competing Manuscripts Based on the Same Study

Editors may receive manuscripts from different authors offering competing interpretations of the same study. They have to decide whether to review competing manuscripts submitted to them more or less simultaneously by different groups or authors, or they may be asked to consider one such manuscript while a competing manuscript has been or will be submitted to another journal. Setting aside the unresolved question of ownership of data, we discuss here what editors ought to do when confronted with the submission of competing manuscripts based on the same study.

Two kinds of multiple submissions are considered: submissions by coworkers who disagree on the analysis and interpretation of their study, and submissions by coworkers who disagree on what the facts are and which data should be reported.