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Impact of chronic toxoplasmosis invasion on immunological parameters in HIV-infected people receiving antiretroviral therapy

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Abstract
The features of effectiveness of antiretroviral therapy are unknown in HIV-infected persons with chronic Toxoplasma gondii invasion. Under supervision for three years there were two cohorts of HIV-infected persons – 531 seropositive patients for Toxoplasma gondii and 345 – persons with seronegative status for Toxoplasma. The laboratory monitoring for number of CD4+T-lymphocytes and viral load of HIV RNA were conducted in all patients. Statistical data processing was carried out on the PC with the definition of Student's t-test. Found that the number of patients out seropositive for toxoplasmosis cohort who received antiretroviral therapy were by 12.38% less than in seronegative cohort (P<0,0001). This phenomen may be the result of exposure toxins Toxoplasma on neurotransmitter processes in the brain and cause inappropriate decisions regarding their own health. The viral load of HIV RNA in both cohorts are similar at the end of supervision. During three years the number of CD4 + T lymphocytes in seropositive cohort remained unchanged, unlike in seronegative individuals this indicator significantly increased from 400,93±13,34 to 473,03±14,95 cell/μL (P1<0,001) and it was by 48,82 cell/μL more than persons in seropositive cohort. Thus, HIV-positive people which infected by Toxoplasma gondii less frequently take ART then patients without Toxoplasma invasion and they have the insufficient immunological efficacy of ART.

Keywords: HIV-infection, Toxoplasmosis gondii invasion, immunological indicators, antiretroviral therapy

1. Introduction

In European countries, the prevalence of Toxoplasma gondii invasion is in the range of 10% - 60%, and in some regions is higher than 90% [1]. In most cases the asymptomatic form of invasion is occurred [1, 2, 3]. Approximately the 5% individuals have clinical manifestations of primary invasion and that normally associated with immunodeficiency [2, 3]. The toxoplasmosis invasion is the greatest danger for HIV-infected people with reduced level of CD4+T-lymphocytes <100 cells in 1 μl of blood. In patients with AIDS the toxoplasmosis is the serious opportunistic disease with damage the brain, lungs and other organs [4].

In literature there are evidences of a significant impact of chronic latent toxoplasmosis invasion on the health - mental disorders, epilepsy, cardiovascular disease, as well as various oncopathology [5, 6]. Some authors demonstrate that Toxoplasma gondii infection partially inhibits NO production by murine macrophages, suggesting that a deactivating macrophage mechanism may be used for better survival into phagocytic cells [7]. The obligate intracellular parasite Toxoplasma gondii infection to trigger apoptosis of bystanger host macrophages while parasitized cells became relatively resistant that is due to the secretion of NO and other soluble factors released by parasite-infected cells [8]. Also shown that the splenocytes of the infected mice produce increased levels of IL-10. In turn, the IL-10 downregulate the expression of IFN-γ mRNA in the splenocytes [9]. Thus, it is possible that chronic toxoplasmosis invasion may complicate the specific immunopathology of HIV-infected people and adversely affect on the results of antiretroviral therapy. However, to date there are no specific data about any possible consequences of such impact of toxoplasmosis invasion.

In Ukraine the Regional Centres for Prevention and Control AIDS carry out clinical supervision for HIV-infected persons according to approved protocol of antiretroviral therapy of the Ministry of Health of Ukraine [10]. This oversight solves various problems, chief among them: control the level of CD4 + T – lymphocytes, HIV viral load, diagnosis, treatment and prevention of opportunistic infections, carrying out of antiretroviral therapy and all necessary laboratory support.

During clinical supervision we can evaluate the effectiveness of ART, find out the patients with insufficient of immunological response and to plan of additional immunorehabilitation measures. So therefore we investigated the features of dynamics of CD4+ T-lymphocytes and viral load in HIV-infected individuals with chronic toxoplasmosis invasion under the influence of antiretroviral therapy.

2. Materials and Methods

The three-year longitudinal cohort study (2013-2015 years) was conducted. Under supervision there were 924 HIV-infected patients (men - 58.55%, women - 41.45%) with a mean age 35,54 ± 0,26 years (18-65 years) at the beginning of the observation. They were divided into two cohorts - the seropositive and seronegative for toxoplasmosis individuals according to the test results for antibodies to Toxoplasma IgM, IgG by ELISA (test system Diaprof-med anti-toxo IgM, and anti-toxo IgG, manufacturer Ukraine).

The criterion of inclusion of HIV-infected individuals in the study was their both positive and negative serological status

concerning Toxoplasma gondii confirmed by laboratory tests. The patients were conducted the clinical observation and laboratory monitoring number of CD4 + T-lymphocytes, the HIV viral load (VL). Statistical analysis of data was performed on a PC using the statistical package programs for Exceel with definition of Student's t-test, and 'P'-value was considered the statistically significant if it was less than 0.05.

3. Results

The seropositive cohort consisted of 531 individuals (61.63%-male and 38.37% female) with a mean age of 36.22 years at the beginning of observation, and the seronegative cohort consisted of 395 persons (54.43% - men and 45.57% - women) with a mean age of 34.3±0.41 years. In the seropositive cohort 91 persons (17,20%) had clinical stage I of HIV-infection, 101 (19.09%) - II, 80 (15.12%)- III and 257 person (48.58%) - IV. In the seronegative cohort the clinical stages of HIV-infection were distributed similarly: 51 people (12.91%) had clinical stage I, 84 people (21.26%) - II, 78 people (19.75%) – III and 182 (46.08%) – IV.

In the seropositive cohort the antiretroviral therapy were used by 362 patients (68,17±2,0%) that was by 12.38% less than in seronegative cohort (294 persons - 80,55±2,1%), P<0, 0001. If taking into account that appointment of antiretroviral therapy had the uniform approach for all patients, this difference could indicate on a certain inadequacy adoption the important decisions by some patients (in this case the decision concerned the initiation of therapy).

In both cohorts there were the patients who used and did not use ART, so to determine the impact of the therapy on immune parameters separately from another reason was difficult. Therefore, we estimated dynamics of CD4 + T lymphocytes during three-years only in those patients out both cohorts who was treated ART (362 patients out seropositive cohort and 294 - out seronegative). As seen from the data in Table 1, in the third year under the impact of ART in seronegative cohort the rate of CD4+ T-lymphocytes significantly increased from 408,36 ± 15,60 cell/μl of blood to 492,64 ± 17,40 (P1 <0.001), so, that it by 70.5 cell/μl exceeded the indicator in seropositive cohort (P2 <0.01). Instead, in seropositive patients the number of T-helpers was not changed and the rate of index was kept up at the same level for three years.

Table 1: Amount of CD4 + T lymphocytes in HIV-infected individuals during three years of observation in cohorts with seropositive and seronegative status regard toxoplasmosis who received ART

Observation period	CD4 + T lymphocytes (cell/μl of blood)	
	Seropositive patients N=362	Seronegative patients N=294
1-st year	434.95±16.88	408.36±15.60
2-nd year	413.44±14.93	446.93±16.28
3-d year	422.14±13.61	492.64±17.40 P_1<0.001 P_2<0.01

Notes: P_1- statistical significance of the difference between indicators at the 1-st year and at the end of third years of observation; P_2- statistical significance of the difference between indicators of two cohorts.

The more evident of effectiveness of ART is possible show through the assessment of changes in the immunological structure of patient in both cohorts (patient's grouped order to level of CD4 + T lymphocytes). As seen from the data presented in table 2, in the cohort of seronegative individuals receiving ART the number of persons without immunodeficiency (CD4 + T cells > 500 cell/μl of blood) increased from 32.65 ± 2.7% at the 1-st year to 45.24 ± 2,9% at the end of third years of observation (P_1<0.01) and was by 13.48% higher than in the similar group in seropositive cohort (P_2<0.001). At the same time, a group of people out seronegative cohort with moderate immunodeficiency (CD4 + T cells were 200-350 cell/μl of blood) decreased by 8,83% (P_1<0.01) and was by 8,04% (P_2<0.01) less than the similar group in seropositive cohort. Reducing the number of patients this group probably due to increased CD4 + T-lymphocytes, and therefore them moving to groups with higher indicators. In the similar group of seropositive patients the change was not occurred. Group of seronegative individuals with profound immunodeficiency (CD4 + T lymphocytes 50-99 cell/μl blood) decreased almost 5 times (P1<0.01), whereas in similar group of seropositive cohort there was no change. After three years in groups patients in those immunological categories with CD4 + T cells <50 cell/μl of blood and 100-199 cell/μl of blood out both cohorts the rates of CD4 + T cells was remained unchanged.

Table 2: Groups of HIV-infected patients which used ART by the level of CD4 + T cells

Indicators of CD4 + T lymphocytes in (cell/μl of blood)	Patients			
	Seropositive patients N=362		Seronegative patients N=294	
	2013 year Abs / %	2015 year Abs / %	2013 year Abs / %	2015 year Abs / %
>500	100 / 27.62±2.3	115 / 31.77±2.4	96 / 32.65±2.7	133 / 45.24±2/9 P_1<0.01 P_2<0.001
350-499	105 / 29.01±2.4	87 / 24.03±2.2	59 / 20.07±2.3 P<0,05	63 / 21.43±2.4
200-350	82 / 22.65±2.2	87 / 24.03±2.2	73 / 24.82±2.5	47 / 15.99±2.1 P_1<0.01 P_2<0,01
100-199	47 / 12.98±1.8	45 / 12.49±1.7	34 / 11.56±1.9	35 / 11.90±1.9
50-99	15 / 4.14±1.0	9 / 2.48±0.8	19 / 6.46±1.4	4 / 1.36±0.7 P_1<0,01
<50	13 / 3.59±1.0	19 / 5.24±1.2	13 / 4.42±1.2	12 / 4.08±1.2

Notes: P_1- statistical significance of the difference between indicators at the 1-st year and at the end of third years of observation; P_2- statistical significance of the difference between indicators of two cohorts.

The effectiveness of ART and immunorehabilitation of HIV-infected patients is dependent on suppressive ART impact on virus replication, resulting in reducing viral load and after 6 months from start of therapy it should not be determined. After 3 years observation the complete virological efficacy was observed not at all. According to the data presented in table 3, the stable undetectable VL (<20 HIV RNA copies in 1 ml of blood) was in 48.07% of seropositive patients and in 46.59% seronegative persons. Short increasing VL, so-called blip, up to 100 copies detected in 19.89% of seropositive and 18.37% of seronegative patients and blip up to 1000 copies reaveled in 11.05% and 12.24%, respectively. Inefficient therapy was in 76

patients (20.99%) out the seropositive cohort and in 67 (22.79%) out the seronegative cohort because they had VL more

Table 3: Virilogic indicators in the seropositive and seronegative of toxoplasmosis cohorts which received ART

Viral load of HIV (Copies RNA of HIV in 1 ml of blood)	Patients			
	Seropositive of toxoplasmosis N=362		Seronegative of toxoplasmosis N=294	
	Абс	%	Абс	%
<20	174	48.07±2.6	137	46.59±2.9
20-100	72	19.89±2.1	54	18.37±2.3
100-1000	40	11.05±1.6	36	12.24±1.9
1000-10000	17	4.70±1.1	24	8.16±1.6
10000-100000	40	11.04±1.6	26	8.84±1.7
>100000	19	5.25±1.2	17	5.78±1.4

than 1,000 copies in 1 mL of blood. Overall, virological indicators in both cohorts were similar (see tabl. 3), indicating on the equal virological efficacy of ART in patients from both cohorts.

4. Discussion

In the literature there are frequent evidences of Toxoplasma effect on neurotransmitter processes in the brain that leads to change a human behavior, and sometimes mental illness [1, 11, 12, 13].

In our study revealed one more behavioral feature of HIV-infected people who have chronic Toxoplasma invasion - inadequate attitude to their own health and the long term rejection of proposals to initiate ART. Above reasons may explain why in terms of a unified approach to the appointment of ART for HIV-infected patients the amount of seropositive for toxoplasmosis persons who used ART were by 12.38% less than in seronegative cohort. This feature puts additional tasks to clinical supervision for patients who are seropositive for toxoplasmosis because they require additional clarification and psychological counseling.

In patients ART should lead to decrease VL to undetectable level (<20 copies in 1 μl of blood) after 6 months of use [9]. Such virological efficacy of ART achieved only in 48.07% of seropositive and in 46.59% of seronegative patients. However, ART is recognized as effective in groups of people, which had both a stable undetectable VL and sometimes blips to 1000 copies. The percentage of such persons was 79.01% in the seropositive cohort and 77.21% - in seronegative. The significant number of patients in both cohorts had no viral suppression because their VL was > 1000 copies of HIV RNA (there are 19,39% such persons in seropositive cohort and 22.79% - in seronegative). The main reason of ineffectiveness of ART was a low compliance, dose omission, failure hold the mode of use and willful cessation the treatment by some patients. The second reason - the viral stability to antiretroviral drugs, or it is formed, it's probably occurs in people, which are have periodic blips [13]. There is another good reason of insufficient suppression of HIV, namely that ART was not administered for patients at the same time - some received it before the study, and some on the 1st, 2nd and 3rd year of study. Therefore, in both cohorts there was a contingent of people with high viral load (> 100,000 copies in 1 mL of blood). However, in general, at the end of three years of observation in both cohorts the virologic indicators were the same. Thus, the impact of ART on virological indicators are not dependent on the presence or absent of toxoplasmosis invasion in the body. However, despite the similar virological indicators the dynamics of immunological parameters in both cohorts was different. Thus, in seropositive patients there were no positive changes of amount of CD4 + T cells neither in the all cohort no in the immunological structure after three-year observation. Instead, in the seronegative cohort the positive immunological changes was recorded, that is the average value of CD4 + T cells was increased, and the number of persons with immunorehabilitation (CD4 + T cells> 500 in 1 microliter of blood) was elevated also. So, the infested persons by Toxoplasma gondii had lower immunological efficiency of ART than the seronegative patients for toxoplasmosis. This fact proves that the persists toxoplasma infestation causes the additional immunopathology in HIV-positive individuals, which is not eliminated by only appointment ART. Therefore, in condition of clinical supervision for seropositive for toxoplasmosis HIV-infected persons should provide the additional measures for immunological rehabilitation.

5. Conclusion

1. In the cohort of seropositive for toxoplasmosis HIV-infected patients the number of people receiving ART less than in seronegative cohort (68.17% and 80.55%, respectively, P<0,0001). Low compliance to initiation of therapy in patients with toxoplasmosis invasion possibly associated with a particular impact of the parasite toxins on neurotransmitter processes in the cerebral cortex and consequently on behavior.
2. The virological efficacy of ART in seropositive and seronegative for toxoplasma gondii cohorts did not differ. There was an effective ART at 79.01% of seropositive persons and 77.21% - of seronegative, however, stable undetectable viral load was only in 48.07% and 46.59%, respectively, and in other patients blips were registered.
3. In the cohort of seropositive for toxoplasmosis individuals the immunological efficacy of ART is lower than in the seronegative cohort. In the seropositive cohort increasing of CD4 + T-lymphocytes did not happen, and in the seronegative cohort the expressed positive trend was found for three years of observation, and the number of people with completely immunorehabilitation (CD + T-lymphocytes > 500 cells in 1 μl of blood) was increased by 12.35%.
4. The immunopathology in HIV-infected individuals who infested with Toxoplasma gondii is complicated and probably requires additional planning immunorehabilitation means.

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