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CO INFECTIOUS PARTICIPATION OF LEISHMANIASIS PARASITE IN PATIENTS WITH HIV / AIDS

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ABSTRACT

Summary

During the 1980s the first cases of leishmaniasis/HIV co-infection have been registered in the world. In co-infection it is registered the negative effect of the participants: patients infected with HIV are particularly sensitive to leishmaniasis, and leishmaniasis itself accelerates replication of HIV and its progression into AIDS. In Montenegro, co-infection visceral leishmaniasis VL/HIV was diagnosed for the first time during 1914/15 in 5 cases (within a total population of 640,000 inhabitants). For the purpose of examinations, there were used epidemiological, clinical, hematological, pathological and serological methods (IIF, ELISA), PCR, Ultrasound and x-ray diagnostics.

In patients with co-infections VL / HIV, clinical characteristics were presented difficult-generalized infective syndrome in all the patients − 100%, with enlarged spleen in 87%, with pancitopenia in 55%, icterus in 37%. Respiratory syndrome – pneumonia in all the cases, and in 2 cases neurological syndromes were registered.

An analysis of HIV infection in patients with co-infection has shown the reduction of the number of CD4 T-lymphocytes to the level below <200 mm^3 in 3 cases and in 2 cases <90 mm^3.

Analysis of the effects of classical therapy (Glucantime, Miltefosine) in combination with HART has shown its failure because recidives VL was registered in all 5 treated cases. The further treatment of this cases with HART and Amfotericine B (AMb), present good results. After a year of their monitoring, there were not registered recidives or disease progression, non-resistance to the applied medicine. It is though necessary to emphasize that a long-term monitoring of these patients is required in order to obtain valid conclusions.

KEYWORDS: *Leishmaniasis /HIV Co-Infections, Diagnosis, Therapy, Prognosis*

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INTRODUCTION

The human immunodeficiency virus (HIV) is the cause of an infectious disease that destroys the immune system. AIDS (Acquired immune deficiency syndrome) is the terminal phase of the infection. Immunodeficiency is the consequence of the infection of CD4+T lymphocytes and monocyte-macrophage cell lines with HIV, which results in the elimination of entire clones of CD4 + T lymphocytes, especially the memoryphenotype (CD45RO). Holes are created

within the memory of the immune system along with the reduction of immune response to HIV, as well as other exogenous and endogenous infections, with the consequent occurrence of opportunistic infections, tumors, and co-infections. (1-3)

Studies conducted across the world have shown the frequency of HIV involvement in coinfections with numerous infectious agents: hepatitis (HBV, HCB) (4, 5), CMV, EBV, Kaposi's sarcoma virus (KSHV) and other herpes viruses (6 – 8), tuberculosis (9, 10, 11). The list of potential participants in coinfections continuously increases, including the entire spectrum of exogenously introduced and endogenously reactive infectious agents. (12 – 16)

Leishmaniasis is the name for a group of infectious diseases, which cause parasitic variants of the Leishmania species. (spp.).(Table 1).(17, 18). Infectedphlebotomusare the primary parasitic vectors and participants in designing various forms of the disease. Coinfections between Leishmania parasite and HIV have emerged as a completely new form (16 – 17) and are considered to be dangerous infectious diseases. (18, 19)

Table 1: Etiological Causes of Leishmaniasis

Visceral Form (Kala-azar)	L.donovani, L.infantum, L. chagasi (depending on the geographical area)
Cutaneous (oriental ulcer, wet ulcer, dry ulcer)	L.mexicana, L.amsonensis, L.tropica, L.major, L.etiopica
Mucocutaneous (nasal – oral form or spp.)	L.Vianabrasiliensis, L.VianaGuanensis, LV panamensis, LV peruviana

In the endemic areas of visceral leishmaniasis, within the majority of the infected population, the infections were mostly sub-clinical (asymptomatic). (20, 21) Modern researches warn that severe clinical manifestations are registered more frequently in endemic foci, especially in VL/HIV co-infection. (22, 23)

In regards to the ever so rapid worldwide spread of the HIV infection, severe clinical manifestations of the visceral form of leishmaniasis are occurring in the old expanding geographic areas as well as the new ones. (24 – 29) In Southern Europe, it is considered that up to 70%of VL cases in adults are associated with coinfection. (30) In cases of leishmaniasis in urban areas, the conditions often support the explosiveness of an epidemic, transforming the disease from sporadic into an epidemic threat, which in return dangerously modifies the epidemiology, making it difficult to diagnose, treat by therapy, or even give disease prognosis.

The epidemiological importance of asymptomatic carriers of the Leishmania parasite in HIV-infected is gaining significance (32, 33), seeing how the disease is rapidly activated in development due to the co-infections amongst the asymptomatic carriers of the parasite. In HIV infected people, leishmaniasis, due to processes like cumulative immune suppression and stimulation of virus replication, accelerates the beginning phase of AIDS. On the other hand, the HIV infection itself increases the risk of developing an active form of Visceral Leishmaniasis (VL) by 100 to 2320 times. (34, 35) Of the 1700 cases of VL / HIV co infection registered by the World Health Organization (WHO) until 1998, from 33 countries around the world, 1440 cases were from Southwestern Europe (30): Spain (36), Italy (37), France 38) and Portugal (39). Of the 956 cases that were retrospectively analyzed, 83.2% were men, 85.7% were younger adults (20-40 years old) and 71.1% were a group of intravenous drug addicts (IDUs). Sharing needles with intravenous drug users contributes to the spread of leishmaniasis and HIV in Europe (40, 41).

The HIV infection, with over 40 million people infected and over 10 million people suffering from it, shows signs of a pandemic spread across the world, as well as among the Mediterranean countries where the leishmaniasis is endemic and very common, which also applies to the HIV infection as a prerequisite for co-infection. (42 – 45) In the United States,

most HIV/Leishmaniasis co-infections were reported in Brazil where the incidence of HIV infection increased from 0.8 cases / 100,000 inhabitants in 1986 to 10.5 cases / 100,000 inhabitants in 1997, with a continuously high incidence and prevalence of leishmaniasis. (46)

Although the geographical distribution of the Leishmania species as well as their phlebotomus carriers (Phl) is limited to endemic areas, the results of modern trials warn of the rapid expansion of those same endemic areas suitable for leishmaniasis; also they warn of a possibility of modifying anthroponotic patterns of transposition of these parasites, which is in line with the drastic changes happening within our ecosystem. (42 – 45)

For the time being, other (alternative) pathways of transmission of this parasite are relatively rarely described by using common intravenous drugs in drug addicts (40, 47), blood transfusions (48), sexual and congenital pathways, and laboratory infections. (49)

In endemic areas, the Leishmania spp. has many hosts: small rodents, mammals, wild and domestic canine. The expansion of endemic areas as well as the increased number of patients is due to a number of reasons such as ecological disturbances, new parasite properties, increased vector density, numerous causative agents.

Leishmaniasis is a widespread parasitic disease that plagues the world. It is considered endemic in 88 countries of the world, of which 72 are developing countries, and 13 are underdeveloped countries. According to the WHO, around 350 million people around the world are affected by leishmaniasis, while around 12 million people are infected. It is estimated that 1.5 to 2 million cases annually end up with patients seeking out medical aid, and only about 600,000 officially applying.

Large-scale changes that occurred in our ecosystem have contributed to the new discoveries in the respective fields of epidemiology, pathogenesis, and immune pathogenesis in regards to leishmaniasis, which in turn led to the disease being classified as an “emerging infectious disease”. Montenegro is an endemic area for VL, along with having high incidence and prevalence of HIV / AIDS cases in relation to the total population (640000 thousand) (50-52). For Montenegro, as well as our surroundings, leishmaniasis represents a globe-like and still insufficiently perceived the public-health problem.

MATERIAL AND METHODOLOGY

The first cases of leishmaniasis in Montenegro were registered in 1924/1925, on the Montenegrin seashore, on the peninsula Lustica (Baosici). The spreading of the infection south, along with the Adriatic coast, resulted in the establishment of a new endemic focus in the southern part of the Montenegrin seashore, between Bar and Ulcinj. During subsequent years most of the patients have been registered from this geographical area. After 1996, the endemic focus kept expanding, capturing the entire coastal part of the Adriatic Sea, from Ulcinj to Herceg Novi, the Skadar Basin, including Podgorica and Cetinje, continuing its expansion towards the northern regions of Montenegro.

In Montenegro, inthe period from 1992 to 2018, 116 cases of visceral leishmaniasis (VL 1 cases of cutaneous leishmaniasis (CL)) were diagnosed. The percentage of children with the disease throughout 2002 was 3% compared to the total population of 640,000 inhabitants.

The first case of HIV infection in our country was diagnosed in 1989. In the period from 1989 to 2018, a total of 254 cases with HIV were registered by standard diagnostic methods (49% with AIDS, 51% with asymptomatic or

symptomatic non-AIDS phase of HIV infection), and in 21% of cases, there was a lethal outcome of the disease. In the period from 2005 to 2018, 186 patients were tested for HIV/Leishmaniasis coinfection, of which in 5 cases, coinfection VL/HIV was found/proven. The study was conducted by Elisa screening and the IHA method for confirming specific antibody (At) on leishmanias, which isn't sufficient to gain insight into the true condition, since serological tests limit the diagnostic evaluation, in other words, over 40% of individuals with HIV/Leishmaniasis co-infection, there's no increase of specific detectable At towards Leishmania.

In coinfection studies, other methods were also used: epidemiological, clinical, laboratory (IIF, ELISA, agglutination test), PCR, Ultrasound, X-Ray. In addition to immunobiochemical methods, for the etiological confirmation of the diagnosis, the Golden Standard Diagnosis of Leishmaniasis - Histopathological Diagnostics (bone marrow biopsy and the analysis of preparations colored according to Romanovsky or Giemsa) was used.

RESULTS

According to the results of the epidemiological studies in Montenegro, in the period from 1996 to 2018, the endemic VL focus area spread to the southern part of the Montenegrin seashore and an increase in the number of patients with clinically manifested forms of Leishmaniasis. The presence of 2 types of Leishmania parasites has been confirmed in humans and canides. With the predominantly represented Leishmania infantum (L.I), there was also a highly represented Leishmania donovani (LD). Veterinary studies have shown a high level of infected dogs with Leishmania at 83% in a sample of 1500 stray dogs from an asylum.

Vectric examination, by analyzing 4770 phlebotomal samples (Phl), showed the presence of 5 species. Throughout 1993, for the first time in Montenegro, Phl.Kandelaki was identified, as well as the vector of L.I, the cause of VL.

In the period from 2005 to 2018, out of the overall number of 254 HIV/AIDS infected patients, IgG antibodies (At) of LD were verified in 32% of HIV infected. Clinically presented leishmaniasis was registered at 5.58%. The diagnosis of leishmaniasis in all 5 patients with HIV infection was confirmed by bone marrow biopsy and the analysis of preparates colored according to Romanovsky (Picture 1).

All patients were male, and the average age of the infected was 34 years +/- 3.

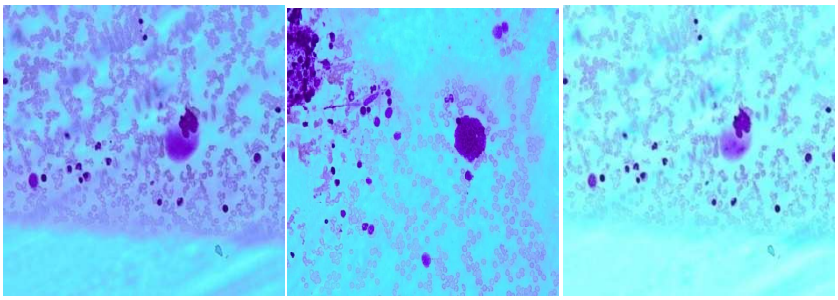

Figure 1: By Bone Marrow Biopsy and the Analysis of Preparates (Colored According to Romanovsky) Amastigotic Forms of Leishmania Parasites in the Cells and Extracellular Organs were Found from the Original Photo Documentation by Prof. Bogdanka Andric and Prof. Dr. Miletagolubovic, 2015

Earlier observations that Leishmaniasis mainly flows subclinically in endemic areas, and often remains undiagnosed, weren't confirmed during our studies. A significantly higher number of clinically manifested infections with Leishmania are registered. According to literature data, most coinfections of HI/LD have classical characteristics of

Leishmaniasis, but may also have a significantly more serious clinical presentation, as confirmed by our studies. Table 1 shows the clinical characteristics of patients with co-infective HIV/VL (5 cases).

In all 5 diagnosed cases of HIV/LD coinfection, the presence of a severe parasitic infection with marked symptoms of the general infectious syndrome and other severe clinical manifestations was registered, which is expected with HIV/AIDS patients with deeply endangered immunity.

The practice has shown that these severely clinically manifested syndromes are largely complemented by numerous opportunistic infections. Finding out about the survival of living Leishmania in the organism after primary infection, and the possibility of their reactivation in the event of immunodeficiency, places them in significant opportunistic agents.

CD + cells are a critical marker of relapse of VL and the coefficient of HIV/VL co-infection. In all of our 5 cases, the average number of CD4+T lymphocytes was about 200 cells /mm^3 in 3 cases and 90 cells in mm^3 in the other two.

In our series, all co-infectious cases of VL/HIV had clinical manifestations of the disease. In Table 1, the results of the clinical characteristics of patients with coinfecting HIV/VL were presented (5 cases).

Table 2 Clinical Presentation of Visceral Leishmaniasis in our Study N/5 (2015)

Symptoms and Signs	Number of Cases	Percentage of Frequency
General infectious syndromes: high temperature, fever,exhaustion, pain in bones, muscles, joints	5	100
Enlarged spleen	5	100
Enlarged liver	5	100
AST/ALT	5	100
Anemia	5	100
Pancytopenia	5	100
Icterus	4	
Bronchopneumonia (Pneumocystis carinii)	5	100
TBC meningoencephalytis	2	
Glucantim (resistance)	5	100
Miltefosine (resistance)	3	100
Amfotericin B (AmB) (without resistance)	5	100
CD4 + T lymphocytes (<200 in mm3) CD-4 + T lymphocytes (<90 in mm3)	3 2	

Recurrences of VL were recorded in all 5 treated co-infectious VL/HIV cases. An analysis of the therapeutic effect of antimony drugs (glucantime) administered by standard therapeutic schemes in combination with HART therapy, showed therapeutic inefficiency in all 5 treated co-infected cases. Satisfactory effects weren't achieved not even by giving miltefosine in 3 recurrent cases.

After unsuccessful usual therapy with glucantim and miltefosine, amphotericin B (AmBisome) (AmB), was used by all 5 patients with co-infection with VL/HIV, in parallel with HART in the treatment of relapse. A favorable therapeutic effect was achieved, as relapses ofleishmaniasis were no longer reported, nor was the resistance to the administered drug registered during the follow-up period of the patient for one year.

DISCUSSIONS

Coinfections VL/HIV appear as serious new and increasingly frequent diseases in the extended endemic foci of VL (17 – 19). On the basis of data of the WHO, out of the total 1700 cases registered in 33 countries worldwide until 1998, 1440 cases were from southwest Europe: Spain (835), Italy (229), France (269),Portugal (117). (31, 36, 37, 39,53).

Concerning the sex, age, and intravenous drug misuse – due to the sharing of needles among users, there were 956 cases included in a retrospective analysis. In the group the number of men was dominant 83,2%, young adults (age 20-40) in 85,7% and 71,1% were intravenous drug addicts (IDU) (31)

VL/HIV co-infections represent significant epidemiological, clinical, diagnostic and prognostic problem (19, 20, 22). Mutual effect of confections' agents can be seen in the fact that HIV infected patients are very sensitive to VL, while patients with VL accelerate HIV replication and its progression into AIDS. (54, 55, 56).

Apart from diagnostic problems, therapy also represents a huge problem. (57, 58, 59). In all our examined cases, there were registered relapses of leishmaniasis - in 2-3 cases, in response to the commonly used therapy with antimony salts (glucantime) and miltefosine. After an application of AmB and HART, relapse of leishmaniasis were repeated during one-year long monitoring of the patient.

Until recently, it was thought that VL can mainly be found worldwide in HIV negative persons and in pediatric patients. A connection of this infection with HIV infection brought significant changes considering VL characteristics in relation to age of patients that are at risk (60,16). In South East Europe 75% of seronegative and 80-83% HIV positive patients in which VL were identified were men (17, 18, 57).

In our examinations of coinfections HIV/VL, the clinical forms of leishmaniasis were registered in all the patients, and a number of CD4+T lymphocytes in our patients was 200 cells in mm^3 in 3 cases, and in 2 cases about 90 cells in mm^3 and it was indicating a serious threat to the immune system.

Through examinations performed worldwide, it has been proved that the incubation period is variable and it can be connected with the age of the patients (45, 51, and 63).

The following opportunistic infections, most of all pneumocystis carini pneumonia, according to the global data are diagnosed in 42 – 68% of HIV positive patients. (46, 47, 48). In our examinations, pneumocystis carini pneumonia has been diagnosed in all 354 (100%) HIV/AIDS patients as the first registered opportunistic infection, including all 5 cases with coinfection HIV/LD. In 2 cases of coinfection, there were developed neurological syndromes with comatose state and clinically diagnosed tuberculosis meningoencephalytis.

Majority of VL/HIV coinfection cases manifest classical VL characteristics but can have other characteristics, i.e. atypical localizations and manifestations. It depends on several factors. In cases of atypical localizations it is of a particular importance the reduction of immune reaction activating phagocytes, i.e. cell immune response (CIO) (49, 52).

Clinical presentation depends on coinfection agents, but very frequently manifestations and complications of associate opportunistic infections are present. In these coinfections the cases of high temperature of unknown origin (PUO) were described, pulmonal tuberculosis with haemoptysis (15, 16, 20) with epistaxis, extensive X-ray report, followed by TBC meningoencephalytis, which has been registered in our examinations in 2 cases, in spite of respecting standard therapy and care and its application.

Examinations in the world show that co infections HIV/VL lead to intensified immunological disturbances. Both infections predominantly infect immunological relation from Th-1 to Th-2 immune response through complex cytokines mechanisms, which lead predominantly to humoral immune response (49, 52). It is registered defect in the lytic capacity of macrophages that are not able to eliminate intercellular amastigotes forms of Leishmania through nitrogen oxide reactions (41, 49, and 52).

According to global data, the diagnosis is very serious because only 40-50% of confections VL / HIV have positive leishmaniasis serology (65). This percentage is inversely proportional to the reduced number of CD4.+T lymphocytes. Anti leishmaniasis At in HIV positive patients are up to 50 times lesser than in HIV negative patients, therefore, false negative test results are frequent.

On the other side, it is known that the results of immunological diagnostics can show sensitivity to 70% and specificity up to 73% but it must be taken into account the existence of an important non-specific cross-reactivity (53).

In our examinations for the selection of the cases with coinfections there were used Elisa screening and IHA test for detection of specific At for Leishmania, which does not allow an insight into the real situation, i.e. into the real number of coinfections considering the fact that in over 40% of cases of coinfections HIV / Leishmaniasis there is no increase of specific detectable At for leishmaniasis. In seropositive cases of coinfections HIV / Leishmaniasis, in further etiological verification, it was used the golden standard of diagnostics for leishmaniasis, i.e.direct methods of checking the presence of amastigotes in the biopsy sample of bone marrow stained according to Romanovski.

Today in the world there are tendencies of introducing routine diagnostics, numerous non-invasive and highly sensitive tests. Detection of the antigen of leishmaniasis is performed with the help of Western Blood (WB) test from the urine sample, as well as the use of rk39 tapes, that have sensitivity and specificity almost 95% (62, 63).

PCR technique is rather complicated but with the high sensitivity of 95% in the peripheral blood and with 100% in the bone marrow and in the spleen aspirates (53, 54, 55).

By using the method of long-term monitoring of VL patients with HIV / AIDS, researchers were trying to find answers weather the secondary episodes of VL can be assigned to reactivation or to the new infection, and weather the long-term AmB treatment has, as a consequence, creation of the parasite resistance.

Studies have confirmed that the reactivation of parasites and repeated infection can even be the reason of secondary episodes ofVL in coinfectious forms VL/HIV, warning that the reactivation of parasites is far more frequent. (50, 51). In the studies all patients, despite HAART therapy had a small number of cells CD4+, and less, representing a strong predictable factor for reactivation of VL.(49) It was the case with our patients too.

By examinations that were performed in the world, it has been concluded too that in 2 out of 4 IDU patients with HIV / VL co infections, secondary episode VL was in connection with isolates that were different from isolates from the initial episode, indicating re infection rather than reactivation. Possible explanations are different: starting from the theory that patients were infected with a heterogenic population of parasites to the fact that during the examination process there were isolated their different clones. One of the possibilities is the change of parasite features in the infected body (34).

In the study of Morales who used analysis of the independent is o enzymes it was not proved that re-infections were in question. However, by using the PCR method, the received data were about the rate of re infections which was 7,5%. Since PFGE analysis has proven to be a good method for differentiating isolates of Leishmania types (36), it enables

detection of more cases of re infections. Re infections were registered in 5 out of 6 non – IDU and in 2 out of 4 IDU cases with HIV / AIDS co-infection. The patient has been infected with the same parasite during the period longer than 10 years, despite the treatment with AmB in a period longer than 76 months (34).

In the world it has been discovered through examinations that the parasite isolates from primary and secondary episodes VL in patients with HIV / VL coinfection, were different in Karyotype for only 1 rank per each PFGE profile, proving that the same sample was in question and, that in the patients the reactivation of disease took place, leading to the conclusion that reactivation happens more frequently than reinfection. (51, 56, 58)

From the therapy aspect, it is important that inhibitory concentration IC50 for AmB for all primary isolates varies slightly between samples with cariotypes that have the value IC50 varying from 0,2 – 0,6 ng/ml. Similar values were noticed in promastigotes and amastigotes, which additionally support the opinion that the parasites phase has no effect on the sensitivity to AmB. A relatively non-specific pattern of activity of AmB on the level of cell membrane can be responsible for a smaller resistance to AmB. Therefore, the failure of the therapy for VL can probably be assigned to other factors. (56, 58)

On mouse models VL was treated with AmB (63-64), with T cells, CD4+, INF-gama and it were shown that for the prevention of recidives of the disease, after the treatment with AmB, TNF was necessary (40).

These experiments on animal models have confirmed that CD4+ cells present a critical marker of recidive of LV, despite adequate therapy and secondary prophylaxis in HIV / VL (58). The absence of the resistance of the parasites to AmBhas great importance and confirms that AmB will remain the proper therapy pattern, even after repeated treatments and the use in prophylactic purposes.

CONCLUSIONS

Leishmaniasis represents dangerous parasite disease transmitted by phlebotominaeworldwide. In our country, there is an endemic area with the tendency of enlargement of endemic foci. In a pandemic eraof HIV / AIDS and considering new information regarding possible coinfections between these different etiologic agents, one comes up with conclusions that due to heavy immunologic disturbances, coinfections HIV / LD have unfavorable implifications regarding the course of the disease, its level, and prognosis. During 2014/2015 there were registered first cases of HIV / LD coinfections in Montenegro.

Earlier considerations were that the majority of infections caused by the parasite LD in endemic areas tend to appear asymptomatic like “silent disease”.

In recent times and particularly in coinfections HIV / LD these latterconsiderations were declared invalid. Significant ecological changes in coinfections too, deep disturbances of immunity condition more frequent participation of clinically evident and very serious forms of disease followed by symptoms of opportunistic infections, additionally complicating diagnosis, therapy, and prognosis of the disease. Despite the fact that the clinical presentation is not necessarily the measure of the disease, compromitting of the immunological defense, decrease of CD4+ values in coinfections HIV / LD creates conditions for heavier manifestations supported by the effect of numerous opportunistic agents.

The choice of therapy for the treatment of leishmaniasis simple and co infectious forms is AmB, most of all because of the proven absence of the development of the resistance of leishmaniasis towards the therapy. From aforementioned, it can be concluded that the problem of leishmaniasis ought to be seen through multidisciplinary work and through numerous segments of the health care system in order to establish corresponding measures of the strategy and prevention of the society.

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