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Research article

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The relationship of antibodies detected in the Western Blot test with clinical and immunological stages in HIV-infected patients

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Ethics Committee Approval
The approval for this study was granted by the Ethics Committee of Bakirkoy Sadi Konuk Training and Research Hospital, and the National Ethics Committee (Number: 04.10.2021-2021/459).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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Abstract

Background/Aim: The laboratory diagnosis of human immunodeficiency virus (HIV) infection is based on detection of anti-HIV antibodies in an initial ELISA (enzyme-linked immunosorbent assay) test and its confirmation with the Western Blot (WB) procedure. Even though the specificity of WB is high for the detection of antibodies against various viral proteins, there are important differences in the timing of the appearance of antibody bands and intensities in different stages of HIV infection. The aim of this study was to evaluate antibodies detected in WB testing and the relationship to clinical and immunological stages in HIV-infected patients.
Methods: Newly diagnosed 78 patients with HIV/AIDS (Acquired Immunodeficiency Syndrome) in our outpatient department between April 2009 and September 2012 were included in the study as a retrospective cohort. Age, gender, complaints, clinical signs, CD4+ T lymphocyte counts and HIV RNA level at diagnosis were collected retrospectively from medical records. WB band patterns obtained from the reference laboratory of the Istanbul Public Health Center were examined retrospectively.
Results: Of the 78 HIV/AIDS cases, 68 (87.2%) were male. Mean age was 38.87(13.09) years (range, 17-83 years). Median CD4+ T lymphocyte count at diagnosis was 410.2 /mm³ (range, 3-1114). Mean HIV RNA level at diagnosis was 592.894 copies/ml. Rare band profiles were seen in 29.4% (23/78). According to World Health Organization (WHO) clinical staging, 59 (75.6%) patients were at stage I, 4 at stage II, 10 at stage III and 5 at stage IV. Gp120, gp160 and gp41, known as envelope glycoproteins in WB band antibodies, were seen in all patients. There was determined to be a decrease in the p17, p51, p55 and p39 bands in WB tests of the advanced grade (Grade IV) of HIV infection.
Conclusions: Reduction of p17, p51, p55, p39 antibodies in advanced stages were related with the progression of HIV infection. This shows that WB test is an important parameter not only in the diagnosis of HIV infection, but also in the follow-up of clinical progression in the absence of these antibodies.

Keywords: Western Blot, ELISA, HIV/AIDS

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Introduction

In the laboratory identification of human immunodeficiency virus (HIV), 4th generation ELISA (enzyme-linked immunosorbent assay) method is used based on the determination of anti-HIV antibodies, and positive samples are confirmed with Western Blot (WB), Line Immunoassay (LIA), or the HIV-1/2 antibody differentiation rapid confirmation test [1, 2]. HIV-RNA examined for viral load in patients diagnosed with HIV/AIDS can be determined 12 days after HIV infection is encountered. The first serological marker detected during acute infection is p24 antigen of the virus [3].

When seroconversion occurs, antibodies emerge against both gp120 and p24 antigens. At this stage, p24 antigen disappears. While p24 antibodies are lost, gp 120 antibodies are determined in the AIDS-related complex (ARC) cycle, which is seen as fever together with generalized lymphadenopathy, diarrhea, weight loss and opportunistic infections. In this period, the lost p24 antigen re-emerges. When it comes to the stage of AIDS, anti-p24 is not determined in serum, while p24 antigen is detected at a high level [3]. Opportunistic infections (bacteria, viruses, fungi and protozoa) are defined as more severe and more frequent infections due to immunosuppression [4]. As HIV-related immunosuppression improves with early antiretroviral therapy, opportunistic infections are prevented [5].

Despite the high specificity of WB for the determination of antibodies which develop against various viral proteins, there are significant differences in the timing at which antibody bands are seen and their severity at different stages of HIV infection. The aim of the current study was to evaluate the relationship of antibody profiles which develop in the WB test of HIV infected individuals, according to clinical and immunological stages.

Materials and methods

Study design and participants

The study included 78 cases who presented at the Infectious Diseases and Clinical Microbiology Department with HIV/AIDS diagnosis between April 2009 and September 2012.

Approval for this retrospective study was granted by the Ethics Committee of Bakirkoy Sadi Konuk Training and Research Hospital, and the National Ethics Committee (Number: 04.10.2021-2021/459). Retrospective data were collected from outpatient records and the age, gender, reason for presentation, clinical findings, CD4+ T cell count and HIV RNA levels at diagnosis, were evaluated. The WB test results were obtained from the Ministry of Health, which is the reference laboratory of the Istanbul Public Health Directorate, and the WB band patterns were retrospectively examined. The laboratory methods listed below were used in the study:

1- ELISA: Genscreen ULTRA HIV Ag-Ab method
2- Quantitative methods used in the determination of HIV RNA in serum: RT-PCR method Cobas Taqman 48 (Roche), <47 copies/ml - >10,000,000 copies/ml
3- Methods used in the determination of serum CD4+ T cell count: CD4 FITC monoclonal antibodies, (Becton Dickinson Immunocytometry Systems, San Jose, CA).
4- Western Blot method: HIV BLOT 2.2 kit was used (MP Biomedicals Asia Pasific Pte Ltd.). The gp160, gp120, p66, p55, p51, p39, gp41, p31, p24, p17 band proteins found in this test were evaluated.

Within the scope of this study, patients with CD4+ T lymphocyte count <350 cell/mm^3 were defined as infected with late-diagnosed HIV [6].

Clinical grading of patients was applied according to the World Health Organization (WHO) classification [7].

Statistical analysis

SPSS version 22 (Version 22.0. Armonk, NY: IBM Corp.) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, ratio, minimum, maximum) were used when evaluating the study data. The relationships between the CD4+ T lymphocyte count, WHO clinical grading at diagnosis and WB band patterns were evaluated using the Pearson Chi-square test. A value of $P<0.05$ was accepted as statistically significant.

Results

78 cases, who were admitted at the Infectious Diseases and Clinical Microbiology Department and were newly diagnosed with HIV/AIDS between April 2009 and September 2012, comprised 68 (87.2%) males and 10 (12.8%) females with a mean age of 38.87 (13.09) years (range, 17-83 years). At diagnosis, the mean CD4+ T lymphocyte count was 410.2 cells/mm^3 (range, 3-1114) and the mean HIV RNA level was measured as 592,894 copies/ml.

All patients who presented at the outpatient clinic were determined to be infected with HIV-1 and all WB band proteins were found to be positive in 70.51% (55/78). Rare band profiles were observed in the remaining 29.48% (23/78). The envelope glycoproteins, gp120, gp160, and gp41, were observed to be present in the band profiles of all the cases.

WB band patterns of the 78 patients were examined according to the WHO grading.

Grade I: This grade was determined in 59 patients. The envelope proteins of gp160, gp120 and gp41 were positive in all patients, and p24 in 57 (96.6%). The presence of p17, p31, p39, p51, p55 and p66 proteins were determined in 53 (89.8%), 52 (88.1%), 47 (79.7%), 48 (81.4%), 52 (88.1%), and 51 (86.4%) patients, respectively.

Grade II: In all 4 (5.1%) patients at this grade, gp160, gp120, gp41 and p24 and p17 bands were detected. From the other proteins, p66, p55, p51, p39, and p31 proteins were present in 3 (75%) patients.

Grade III: In all 10 (12.8%) patients at this grade, gp160, gp120, gp41, and p55, p51 proteins were present. From the other proteins, p66, p31, p24, and p17 were present in 9 (90%) patients, and p39 protein was present in 8 (80%).

Grade IV: In all 5 (6.4%) patients at this grade, gp160, gp120, gp41 and p31 and p24 bands were present. From the other proteins, p66 was present in 4 (80%) patients, and p55, p51, p39 and p17 in 2 (40%) patients. There was determined to be a decrease in the p17, p51, p55 and p39 bands of the WB test in the advanced grade (Grade IV) of HIV infection. This decrease was evaluated statistically significant in p17, p51, p55, p39 bands ($P=0.001$, $P=0.002$, $P=0.016$, and $P=0.042$; respectively) (Table 1).

Table 1: Distribution of WB band patterns according to the WHO grading system

Band proteins	Grade I-II-III(73)	Grade IV(5)	*P*-value
p17	66	2	0.001
p55	65	2	0.002
p51	61	2	0.016
p39	58	2	0.042

Pearson Chi-square test

While CD4+ T lymphocyte counts were <350 cells/mm^3 in 33 patients, CD4+ T lymphocyte counts were ≥350 cells/mm^3 in 45 patients, which does not represent any statistical difference (P>0.05) (Table 2).

Table 2: Distribution of WB antibody bands according to immunological grading

WB antibody bands	<350 cells/mm^3 n(%)	≥350 cells/mm^3 n(%)	*P*-value
p66	30(90.9)	37(82.2)	0.276
p55	29(87.9)	38(84.4)	0.667
p51	29(87.9)	34(75.6)	0.172
p39	27(81.8)	33(73.3)	0.380
p31	31(93.9)	38(84.4)	0.195
p24	31(93.9)	44(97.8)	0.384
p17	27(81.8)	41(91.1)	0.225

Pearson Chi-square test

Discussion

Despite the high sensitivity and specificity of the WB test in the identification of viral proteins, there are variations in the time of formation of antibody bands and their severity at different stages of HIV infection [8]. After infected with HIV, the earliest antibodies determined in the WB test are p24 and its precursor, p55, and at advanced stages of the disease, their detection frequencies are decreased [9]. In the current study, p24 antibody was detected at the same rates in both the early and advanced stages of HIV infection. This was contrary to studies from Europe and North America which have explained that the loss of p24 is a marker of advanced HIV/AIDS infection [10, 11]. However, studies conducted in Africa and India have similarly found positive rates of p24 antibody at both early and advanced stages of HIV infection [8, 12]. Moreover, it has been reported that envelope precursor protein gp160 and envelope proteins gp120 and gp41 can be seen in all HIV infected cases, independent from clinical stage [9]. Similarly, in the current study, gp160, gp120, and gp41 antibodies were detected in the WB test, independent from clinical stage in all patients.

In 67 (85.9%) cases, p55 band was detected. The p55 reactive group was found in 89% of grade I-II-II patients and in 40% of the grade IV patients. In other studies, p55 antibody usually cannot be determined at advanced stages [13-15]. By fragmenting the precursor protein p55, which is a gag gene product, protease enzyme forms p17 and p24 which are in mature capsid. The p55 antibody band emerges in WB when there are very high levels of p24 or p17 antibody proteins in the samples.

In a study by Sivakumar et al. [12], antibodies developing against p17 were determined in 64% of WHO grade I patients and in only 33% of grade IV patients. In this study, the p17 antibody band was determined in only 40% at advanced grade. With grade progression of HIV infection, the positivity of p17 and p55 antibody bands was found statistically significantly decreased. The decrease in antibody level and increase in p17 antigen are in the matrix between the virion nucleocapsid and membrane, and play a role in the fusion of the infection virion and the target cell [16]. Consistent with the current study findings, previous studies have found a relationship between antibodies against p17 and slower progression of HIV disease [17].

In the current study, the p51 band, which affects reverse transcription, was found to be reactive at a rate of 83.6% in grade I-II-III, and 40% in grade IV. The decrease in positivity of p51 antibody band together with disease progression was found to be statistically significant. This finding was similar to the study by Srikanth et al. [18] in India. In studies by Fiebig et al. [19] and Hecht et al. [20], p31 band was determined to be the last antibody of the WB band proteins formed (2-4 months) after infection with HIV. Srikanth et al. [18] reported a correlation between AIDS grade and the non-determination of p31 band. Sudha et al. [8] reported that p31 was the antibody most frequently not determined at both initial and advanced stages. In the current study, p31 protein was detected in 88.1% at grade I and in 100% at grade IV.

The presence of p39 band, which is a fragment of p55, is in %79.5 of early stages HIV/AIDS infection and in 40% at an advanced stage, which shows statistical significance. Sivakumar et al. [12] observed p39 band in 35% at the early stage and in 44% at advanced stages, both similar to the current study. In a study of antiretroviral-naive pregnant HIV-infected patients, Duri et al. [21] emphasized the absence of p39 band in the WB test and reported that advanced stage HIV infection was correlated with a high viral load.

p66, which plays a role in reverse transcription, was determined by Sivakumar et al. [12] and Sudha et al. [8] at the rates of 81% and 97.8%, respectively, in the early stage, and at 100% and 88.9% at advanced stages, respectively. Similar findings were obtained in the current study with the determination of p66 at 86% in the early stage and at 80% at the advanced stage.

Positivity was observed in all the WB band proteins in 70.51% (55/78) of the current study, and rare band profiles were seen in 29.48% (23/78). Sudha et al. [8] determined rare band profiles at a rate of 7.09%. This rate seems to be extremely low compared to the current study results.

Limitations of the current study were low number of patients and the design in a single hospital.

Although studies in different countries have shown different band parameters in WB tests, when the relationship with the clinical status of the patient is examined, similar results to those of the current study have been obtained. This is the first national study in Turkey investigated the relationship between WB antibody bands and the clinical grade of HIV infection.

Conclusion

Although a statistically significant relationship was determined between clinically advanced stages of HIV infection and the absence of p17, p51, p55 and p39 antibodies in WB test, significance was not found immunologically. HIV patients are offered treatment as soon as they are diagnosed. It should be kept in mind that opportunistic infections may be seen more frequently in advanced clinical stage due to antibody deficiency. In treatment, diagnosis and treatment of opportunistic infections should be considered with or before antiretroviral therapy. This can be shown that the WB test is an important parameter not only in the diagnosis of HIV infection but also in the follow-up of clinical progression, even in the absence of these antibodies.

References

1. Ministry of Health Directorate of Public Health. HIV/AIDS Diagnosis Treatment Guide - 2019. Ministry of Health Publications. https://hsgm.saglik.gov.tr/depo/birimler/Bulasici-hastaliklar-db/hastaliklar/HIV-ADS/Tani-Tedavi_Rehberi/HIV_AIDS_Tani_Tedavi_Rehberi_Yeni.pdf. Access Date: September 16,2021
2. United Nations Programme on HIV/AIDS. UNAIDS DATA 2019. Documents (2019) Available from: https://www.unaids.org/en/resources/documents/2019/2019-UNAIDS-data. Access Date: August 26, 2021
3. Dyck EV, Meheus AZ, Piot P. Human Immunodeficiency Virus. In: Laboratory Diagnosis of Sexually Transmitted Diseases. World Health Organization, Geneva 1999: 85-98.
4. Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-opportunistic-infection/whats-new-guidelines (Access Date: April 26, 2020).
5. Brooks JT, Kaplan JE, Holmes KK, Benson C, Pau A, Masur H. HIV-associated opportunistic infections--going, going, but not gone: the continued need for prevention and treatment guidelines. Clin Infect Dis. 2009 Mar 1;48(5):609-11. doi: 10.1086/596756.
6. Antinori A, Coenen T, Costagiola D, Dedes N, Ellefson M, Gatell J, et al. European Late Presenter Consensus Working Group. Late presentation of HIV infection: a consensus definition. HIV Med. 2011 Jan;12(1):61-4. doi: 10.1111/j.1468-1293.2010.00857.x.
7. Antiretroviral Therapy for HIV Infection in Infants and Children: Towards Universal Access: Recommendations for a Public Health Approach: 2010 Revision. Geneva: World Health Organization; 2010. PMID: 23741772.
8. Sudha T, Lakshmi V, Teja VD. Western blot profile in HIV infection. Indian J Dermatol Venereol Leprol. 2006 Sep-Oct;72(5):357-60. doi: 10.4103/0378-6323.27752.
9. From the Centers for Disease Control. Interpretation and Use of the Western Blot Assay for Serodiagnosis of Human Immunodeficiency Virus Type 1 Infections. 1989 Jul 21;38(Suppl7):1-7.
10. Biggar RJ, Melbye M, Ebbesen P, Alexander S, Nielsen JO, Sarin P, et al. Variation in human T lymphotropic virus III (HTLV-III) antibodies in homosexual men: decline before onset of illness related to acquired immune deficiency syndrome (AIDS). Br Med J (Clin Res Ed). 1985 Oct 12;291(6501):997-8. doi: 10.1136/bmj.291.6501.997.
11. Lange JM, Coutinho RA, Krone WJ, Verdonck LF, Danner SA, van der Noordaa J et al. Distinct IgG recognition patterns during progression of subclinical and clinical infection with lymphadenopathy associated virus/human T lymphotropic virus. Br Med J (Clin Res Ed). 1986 Jan 25;292(6515):228-30. doi: 10.1136/bmj.292.6515.228.
12. Sivakumar MR, Sanath Kumar J, Viswanath R, Thatchinamoorthy G, Mini Jacob, Samuel NM. Western blot pattern in HIV positive individuals in Namakkal, South India. The Internet Journal of Infectious Diseases. 2008 Volume 6 Number 2.
13. Chattopadhya D, Aggarwal RK, Kumari S. Profile of antigen-specific antibody response detectable by western blot in relation to diagnostic criteria for human immunodeficiency virus type-1 (HIV-1) infection. Clin Diagn Virol. 1996 Oct;7(1):35-42. doi: 10.1016/s0928-0197(96)00251-6.
14. Garland FC, Garland CF, Gorham ED, Brodine SK. Western blot banding patterns of HIV rapid progressors in the U.S. Navy Seropositive Cohort: implications for vaccine development. Navy Retroviral Working Group. Ann Epidemiol. 1996 Jul;6(4):341-7. doi: 10.1016/s1047-2797(96)00053-1.
15. Huang J, Wang M, Huang C, Liang B, Jiang J, Ning C, et al. Western Blot-Based Logistic Regression Model for the Identification of Recent HIV-1 Infection: A Promising HIV-1 Surveillance Approach for Resource-Limited Regions. Biomed Res Int. 2018 Jan 14;2018:4390318. doi: 10.1155/2018/4390318.
16. Fiorentini S, Marini E, Caracciolo S, Caruso A. Functions of the HIV-1 matrix protein p17. New Microbiol. 2006 Jan;29(1):1-10.
17. Fiorentini S, Giagulli C, Caccuri F, Magiera AK, Caruso A. HIV-1 matrix protein p17: a candidate antigen for therapeutic vaccines against AIDS. Pharmacol Ther. 2010 Dec;128(3):433-44.
18. Srikanth P, George Babu P, Sridharan G, John TJ, Mathai D. Immunoblot reactivity in relation to Human Immunodeficiency Virus disease progression. Indian Journal of Medical Microbiology, 1998 Jan;16(3):118-20.
19. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implication for diagnosis and staging of primary infection. AIDS 2003, 17: 1871-9.
20. Hecht FM, Holte S, Busch MP. Absence of p31 band identifies persons with recent HIV seroconversion. Abstract presented at XIVth International AIDS Conference. Barcelona, Spain, 7–12 July 2002 [Abstract TuPeC4885].
21. Duri K, Müller F, Gumbo FZ, Kurewa NE, Rusakaniko S, Chirenje MZ, et al. Human Immunodeficiency Virus (HIV) types Western blot (WB) band profiles as potential surrogate markers of HIV disease progression and predictors of vertical transmission in a cohort of infected but antiretroviral therapy naïve pregnant women in Harare, Zimbabwe. BMC Infect Dis. 2011 Jan 6;11:7. doi: 10.1186/1471-2334-11-7.

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