



ORIGINAL ARTICLE



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Three-year analysis of confirmatory testing outcomes in individuals with positive anti-HIV screening results

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Abstract

Despite the increase in preventive health measures and awareness-raising efforts against HIV worldwide, it continues to be a significant global public health problem today. This study aims to determine anti-HIV seropositivity, trends in false-positive rates, and demographic characteristics of patients with reactive anti-HIV test results. In this retrospective study, anti-HIV test results of a total of 55,763 patients between January 2021 and December 2023 were analyzed. Samples were studied with anti-HIV fourth-generation ELISA test (Roche Diagnostics, Mannheim, Germany). Reactive samples were sent to the National HIV-AIDS Confirmation and Viral Hepatitis Reference Laboratory for confirmation. Of the 55,763 samples evaluated, 169 (0.30%) were found to be reactive with the anti-HIV fourth-generation ELISA test (Cobas e 601, Roche, Mannheim, Germany). The confirmation test result of 75 patients was positive and 94 patients were negative. Patients with positive confirmation test results constituted 0.13% of all patients examined. Of the patients with positive confirmation results, 68 (90.7%) were male and 7 (9.3%) were female. When we examined the age groups, the HIV positivity rates were found to be 59% in the <35 age group and 57.8% in the 35-55 age group. As a result of this retrospective study, the results of HIV seropositivity in gender and age groups were found to be consistent with both our country's data and other studies conducted in our country. The high rate of false positive results in screening tests showed the importance of confirmatory tests.

Keywords: Anti-HIV, confirmatory test, enzyme-linked immuno sorbent assay

Introduction

Human Immunodeficiency Virus (HIV) causes immune suppression by settling in the host cell DNA and is known to cause opportunistic infections and malignancies that can progress from acute retroviral syndrome to AIDS (Acquired immunodeficiency syndrome) [1]. The process, which started with the reporting of the first HIV-positive case in our country in 1985, continues to increase in importance day by day [2]. According to the 2023 report published by the United Nations HIV/AIDS Joint Program (UNAIDS); 39 million people worldwide are infected with HIV and continue to live, and approximately 4000 people are diagnosed with HIV infection per day [3].

Despite the increase in preventive health measures and awareness-raising efforts against HIV worldwide, it continues to be a significant global public health problem today. It is known that

UNAIDS has 95-95-95 Global Goals to stop the spread of HIV by 2030. These goals are to have 95% of those living with HIV diagnosed, 95% of those diagnosed access treatment, and 95% of those receiving treatment have the virus reduced to undetectable levels. For this reason, rapid diagnosis of HIV infection and verification with effective testing methods are very important. In this way, early initiation of treatment and prevention of HIV transmission are ensured.

Accurate diagnosis of HIV infection is vital for effective patient management and public health interventions. Typically, the diagnostic process should first involve initial screening with enzyme immunoassay (EIA) or rapid tests to detect anti-HIV antibodies, followed by confirmation of reactive results with confirmatory tests. Confirmatory tests such as Western blot (WB), Immunofluorescence Assay (IFA) or more recently nucleic acid tests (NAT) and fourth-generation tests are used to distinguish

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true positive cases from false positives, minimizing diagnostic errors and enabling follow-up.

We conducted a retrospective study to evaluate the outcomes of anti-HIV seropositivity and confirmatory testing in patients who visited our hospital from January 2021 to December 2023, with the objective of identifying trends in false-positive rates and changes in the demographics of individuals with reactive anti-HIV test results.

Material and Methods

In this retrospective study, the anti-HIV test results of a total of 55,763 patients who applied to Ordu University Faculty of Medicine Training and Research Hospital between January 2021 and December 2023 were analyzed. Samples sent to our laboratory were centrifuged at 4000 rpm for 20 minutes, serum was obtained, and then examined with anti-HIV fourth-generation ELISA test (Elecys® HIV combi PT, cobas 6000 e 601, Roche Diagnostics, Mannheim, Germany). The kit used is a fourth-generation kit that detects both HIV p24 antigen and HIV type 1 and HIV type-2 antibodies, with a signal/"cut off" value in the range of 0-0.89. The age and gender information of the patients was recorded from the hospital automation system.

If the anti-HIV tests for the patients showed reactive results, a new sample was requested and the test was repeated. Samples in which even a single test was detected as reactive were sent to the national laboratory specializing in HIV-AIDS confirmation and viral hepatitis of the General Directorate of Public Health of the Republic of Turkey Ministry of Health, Microbiology Reference Laboratories and Biological Products Department for confirmation tests. The study samples were re-studied here using another 4th generation ELISA test, Vidas HIV duo ultra (bioMerieux, France). Reactive samples were also evaluated with Geenius HIV-1/2 Supplemental Assay (Bio-Rad, France), a rapid HIV confirmation test that detects antibodies. Positive samples were evaluated as HIV infection. HIV RNA was studied using the artus HIV virus-1 QS RGQ RT-PCR (Qiagen, Germany) kit to detect the presence of acute HIV infection in samples that were detected as reactive by ELISA test and whose confirmation result was negative. This study was approved by Ordu University Clinical Studies Ethics Committee (Date: 2023-11-24, Number: 2024/302).

Statistical analyses were performed using the MedCalc (version 20.009; Ostend, Belgium) statistical package program. Number, frequency, percentage, and arithmetic mean values were used for statistical definition of the data. Numerical data were evaluated by determining whether the study groups were found to align with normal distribution based on the Kolmogorov-Smirnov test. The Mann-Whitney U test facilitated the pairwise comparisons of the groups. The Chi-Square test was used to evaluate categorical data. The groups were graphically shown as percentages in a stacked column. In order to evaluate the HIV test diagnostically, sensitivity, specificity, positive and negative predictive values, and AUC (Area Under Curve) values were calculated. In the interpretation of the results, the significance level was taken as $p<0.05$.

Results

Out of the 55,763 samples analyzed, 169 (0.30%) were reactive and were sent to the National HIV-AIDS and Viral Hepatitis Verification Laboratory under the Republic of Turkey Ministry of Health's General Directorate of Public Health, Department of Microbiology Reference Laboratories and Biological Products. Of these 169 samples, 117 (69.2%) were male and 52 (30.8%) were female. Of the 169 samples sent for verification, 75 (44.4%) were found to be positive. Of the 75 samples found to be positive as a result of verification, 68 (90.7%) were male and 7 (9.3%) were female (Figure 1). The positivity rate was statistically analyzed in terms of gender and the positivity rate in males was found to be significant ($p<0.05$). According to the confirmation test result, the average age of male patients who were positive was 44.3 ± 14.6 , while the average age of female patients was 42.8 ± 10.4 , and no statistically significant difference was detected ($p>0.05$). HIV positivity rates were found to be 59% (23/39), 57.8% (37/64) and 22.7% (15/66) in the <35 , $35-55$ and >55 age groups, respectively (Figure 2). When the age distribution of the patients was examined, it was determined that HIV infection was less common in the >55 age group ($p<0.001$). Table 1 shows the relationship between HIV confirmation result and gender, years and age group.

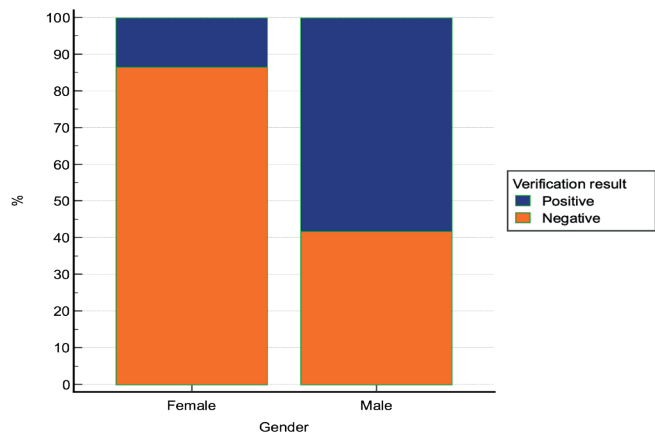


Figure 1. Relationship between gender and HIV confirmation result

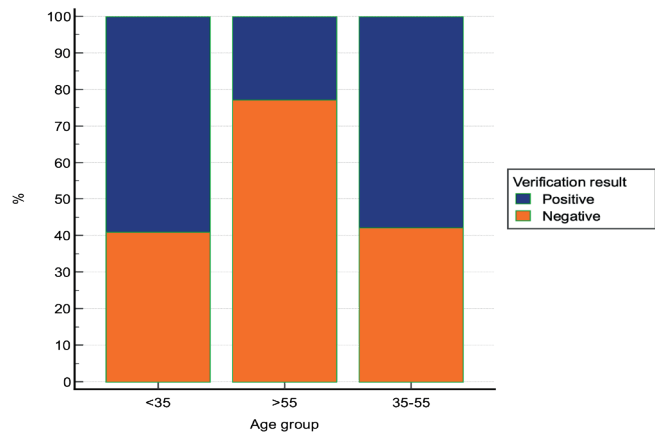


Figure 2. Relationship between age group and HIV confirmation result

Table 1. Relationship between HIV confirmation result and gender, date and age group

		Confirmation result				p-value
		Negative		Positive		
		n		n		
Gender	Male	49	41.90%	68	58.10%	<0.0001*
	Female	45	86.50%	7	13.50%	
Year	2021	16	47.10%	18	52.90%	0.019*
	2022	33	73.30%	12	26.70%	
	2023	45	50.00%	45	50.00%	
Age group	<35	16	41.00%	23	59.00%	<0.0001*
	35-55	27	42.20%	37	57.80%	
	>55	51	77.30%	15	22.70%	

* Significant difference at <0.05 level according to chi-square test

In our study, 94 (55.6%) patients with negative confirmation results were accepted as false positive. When these results are examined, it is seen that the false positivity rate in the ELISA test is quite high. When the results of the ELISA test were examined according to the confirmation test results, the lowest true reactive anti-HIV value was determined as 11 s/co and the highest false reactive anti-HIV value was determined as 43.49 s/co.

HIV-RNA was studied for the presence of acute HIV infection in patients who were detected as reactive by ELISA but whose confirmation result was negative. However, there were no cases of acute HIV infection.

When the confirmation results were examined by years, 53% (18/34) positivity was detected in 2021, 27% (12/45) in 2022 and 50% (45/90) in 2023 (Figure 3). While all of the confirmation results in 2021 and 2022 were male, 84.4% of the patients were detected as male in 2023. When the relationship between confirmed HIV-positive patients was investigated by years, the increase in cases by years was not found to be statistically significant (p>0.01).

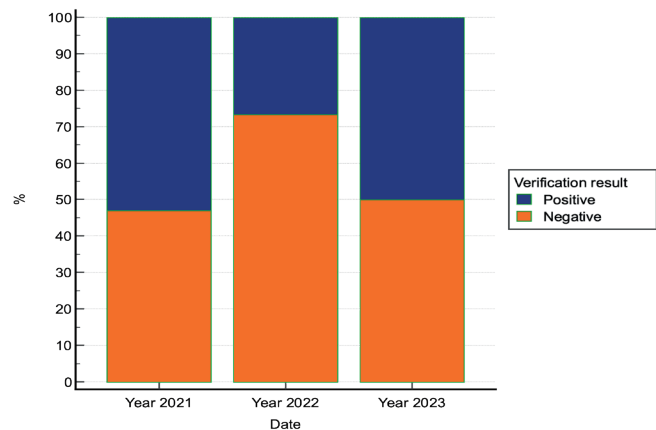


Figure 3. Relationship between years and HIV confirmation result

The sensitivity, specificity, positive and negative predictive values of our ELISA test were found to be 98.7%, 100%, 100%, 98.9%, respectively. With ROC analysis, when the S/CO value was 43.49, the area under the ROC curve was AUC: 0.99 (95% CI: 0.99-1.00) and was found to be statistically significant (p<0.001) (Figure 4).

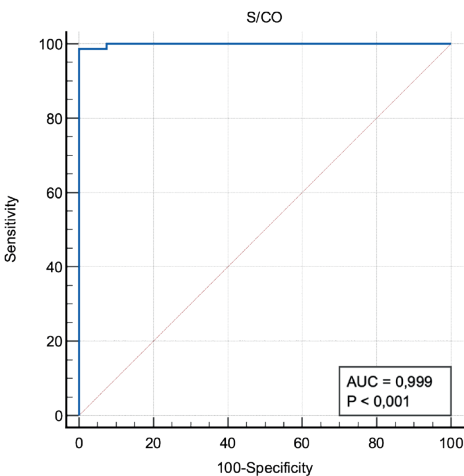


Figure 4. ROC analysis of HIV confirmatory test

Discussion

AIDS, caused by the HIV virus, continues to be one of the most important public health problems of our day despite all the measures taken. As in the rest of the world, there is a rapid increase in the number of cases in our country. In our study, we aimed to compare the distribution in demographic data by examining the verification results of cases detected as reactive with anti-HIV/1-2 test and to determine the appropriate threshold value to prevent false positive results.

When we evaluated our findings, our HIV seropositivity was detected as 0.13% with the 4th generation ELISA test Elecsys

HIV combi PT. It was noted that 90.7% of the confirmation test positive samples were male; however, while no positivity was observed in the female population in 2021 and 2022, positivity was also observed in the female population (15.6%) in 2023. It was determined that 49.3% of the confirmation test positive patients were between the ages of 35-55.

In a study conducted in Rize, HIV seropositivity was found to be 0.04%, and in a study conducted in Eskişehir, it was found to be 0.11% [3,4]. Dinç et al. found the HIV prevalence (0.11%) in the 2019-2022 period to be similar to the study conducted in the same center in the 2015-2018 period [5,6]. In the study conducted in China, the HIV prevalence was analyzed as 0.054% [7]. Unlike other studies, our study obtained higher results (0.13%) than other provinces. Although this study is the first to evaluate HIV seropositivity in Ordu province, it should be supported by data from other hospitals to determine the patient profile of Ordu province.

In the studies in the current literature, it has been found that the confirmation test positive samples were highly positive in males and in the 21-30 age range [1,8,9]. In the study conducted by Toptan et al., it was seen that 89% of the patients with a positive confirmation test were male, but most of the positive patients were between the ages of 26-35 [10]. In our study, consistent with both the data of the Ministry of Health and similar studies in the literature, a significant HIV positivity was found in males, but unlike other studies, our positivity was detected more in the 35-55 age range. Again, strikingly, 86.5% of female patients were detected as false positives. This can be explained by the fact that the reasons causing false positivity (such as pregnancy, autoimmune diseases, rheumatoid factor positivity) are frequently seen in women. Yaman et al. They also found the false positive rate as 71.1% in women ($p < 0.001$) [11].

False positives in screening tests continue in societies with low HIV prevalence. Determining the cut-off index value in test kits is very important for predicting real HIV patients. A high cut-off index value or S/CO ratio is associated with true positivity. Toptan et al. found the lowest true reactive value as 5.29 s/co and the highest false reactive value as 13.3 s/co [10]. Bahçeci and Yıldız found the lowest true reactive value as 9.36 s/co and the highest false reactive anti-HIV value as 173.92 s/co [3]. In our study, the lowest true reactive value was 11 s/co and the highest false reactive anti-HIV value was 43.49 s/co. When Xia et al. calculated the optimum cut-off index threshold as 9.87, they eliminated 196 false-positive patients with 100% sensitivity and 99.99% specificity [7]. When Yaman et al. calculated the s/co value as 7.20, they found the sensitivity and specificity as 100% and 95%, respectively [11]. In our study, when the anti-HIV threshold value was taken as >43.49 s/co, sensitivity and specificity were determined as 100%.

Conclusion

Surveillance studies are extremely important in controlling the infection. The study is the first to analyze patients in terms of HIV infection in Ordu province and is largely consistent with the results of other studies conducted throughout our country. There is a high probability of false reactivity with low reactive results in S/CO values. Determining the threshold values in each study center's own screening tests will prevent false reactivity results. However, it should not be forgotten that acute HIV infections may be missed in this case.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical Approval

This study was approved by Ordu University Clinical Studies Ethics Committee (Date: 2023-11-24, Number: 2024/302).

References

1. Türkoğlu E, Öner SZ. 4. Evaluation of fourth-generation ELISA assay cut-off values in the context of confirmation test results. *Abant Med J.* 2021;10:1-6.
2. Aslan FG, Altındış M. Current diagnostic algorithm of HIV and emerging prevention methods. *Türk Mikrobiyol Cem Derg.* 2017;47:47-60.
3. Bahçeci İ, Yıldız İE. HIV seropositivity in patients admitted to our hospital: Six year evaluation. *Duzce Med J.* 2021;23:25-9.
4. Doğaner İ, Tabak FA, Us T. The incidence rate of confirmed real HIV positivity in Eskişehir region. *T Tıp Öğr Arş D.* 2019;1:73-7.
5. Dinç HÖ, Keskin E, Kocak BT, Kocazeybek B. Cerrahpaşa Medical Faculty Hospital HIV-1/-2 serological test data: 2019-2022 retrospective evaluation. *Experimed.* 2022;12:215-8.
6. Özbey D, Kocazeybek BS, Sirekbasan S, Dinç HÖ. Seroepidemiologic evaluation of HIV scanning and verification datas during the period of 2015-2018. *ANKEM Derg.* 2019;33:89-94.
7. Xia X, Zhang X, Zhou J, Zhang M. An analysis of predictive sample-to-cutoff index for HIV infection confirmation using elecsys® HIV combi PT assay. *Int J Clin Pract.* 2022;2022:5097189.
8. Öner P, Aytaç Ö, Şenol FF, et al. Analysis of the nine year HIV/AIDS results in Elazığ province. *Türk Mikrobiyoloji Cemiy Derg.* 2020;50:100-7.
9. Varışlı AN, Demir T, Tekin S. Confirmatory test results in patients with a reactive anti-HIV test result: four-year data. *Klimik Derg.* 2020;33:24-8.
10. Toptan H, Aslan FG, Karakeçe E, et al. Evaluation of patients with anti-HIV ½ reactivity with confirmation test results. *Journal of BSHR.* 2019;3:27-32.
11. Yaman M, Bakır A, Karabulut N, et al. Evaluation of signal cut-off ratio with fourth generation HIV Ag/Ab screening test for the diagnosis of HIV infection. *Smyrna Tıp Dergisi.* 2023;13:1-7.