ELISA FALSE POSITIVITY IN RELATION TO HIV -1 PREV ALENCE IN ETHIOPIA

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Bekele Shonko ABSTRACT: A close investigation of data accumulated over several years at the National Referral Laboratory for AIDS (NRLA), revealed a certain pattern in the number of false positive results that occur in population groups with a given prevalence of HIV -1 infection. To provide more accurate information regarding this observation 8850 serum samples from females with multi-partner sexual contact (MPSC) residing in different regions of Ethiopia with varying HIV -1 prevalence rates, were collected and tested using 1st and 2nd generation enzyme immuno-assays (EIA) and a western blot. The results obtained from this study suggest that the rate of the occurrence of false positive enzyme linked imrnuno-sorbent assay (ELISA) results during laboratory testing for HIV -1 antibody is (regardless of the quality of antigen used) inversely proportional to the prevalence of HIV-l in a given population group.

INTRODUCTION

ELISA false positivity has been a major problem for most HIV-1 screening laboratories for a number of years. In particular, first generation enzyme immuno-assays which utilize whole virus as antigen source have been giving false positivity rates as high as 40% (as observed from data collected at the National Referral Laboratory for AIDS (NRLA), Ethiopia). With the introduction of recombinant technology, i.e. the emergence r of second generation enzyme immuno-assays, a dramatic decrease by 66% in the rate of false positivity was observed (figures 1 and 2).

However, false positive results do Occur even with recombinant technology-based enzyme immuno-assays and what is more interesting is that the number of false positive results seems to correlate inversely with the prevalence of HIV-1 infection within a given population group. It was the purpose of this study to validate and provide an explanation for this observed relationship between HIV prevalence and false positive results.

MATERIALS AND METHODS

Serum samples from 6234 MPSC females in 23 major towns allover Ethiopia and 2616 MPSC females from 25 sectors in the capital city, Addis Ababa, were collected in 1988 and 1989. The screening assays for samples from these two groups of MPSC females were carried out using 1st generation Wellcozyme anti-HTLV III antibody test kits (for the 6234 samples), and 2nd generation Wellcozyme HIV Recombinant and Abbott HIV Recombinant test kits (for the latter 2616 sera). Repeatedly reactive sera were further confirmed by western blot. (Bio-Rad Novapath lmmunoblot Assay).

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The criteria for reactivity on the western blot assay was the presence of specific antibodies to at least two of the following three groups of viral proteins: p24, gp41, and

gp120/160. A sample was considered to be non-reactive if no viral-specific antibodies were observed. Any other combinations of antibodies were taken as indeterminate results. A result was said to be false-positive if a sample was twice reactive by ELISA but non- reactive by western blot.

After completion of laboratory testing of all samples, the 23 major towns were categorized into seven groups based on the prevalence of HIV infection in each area. The
number of false positive results in each prevalence group was then identified. The same was done for the 25 sectors in the capital city. The results were then analyzed for any relationship between HIV prevalence and false positivity rates.

RESULTS

In the first group of MPSC females from the 23 towns in Ethiopia, 1050 samples (out of 1489 sera identified to be reactive by the 1st generation enzyme immuno-assay) were found to be positive by western blot giving a false positivity rate (FPR) of 29.5 % .In the
second group of MPSC females from the 25 sectors, out of a total of 720 ELISA positive (2nd generation Wellcozyme assay) sera, 647 were further confirmed by western blot, resulting in a 10.1% false positivity rate. The detailed figures are shown in tables 1 and 2.

The relationship between HIV-1 prevalence and false positivity rates is more clearly seen in figures 1 and 2. The correlation coefficients for the data from the two groups of MPSC females are -0.75 and -0.71.

DISCUSSION

Some of the actual data obtained in this study fall a little above or below the ideally expected values, however it is not difficult to observe a general trend or pattern, namely the fact that in both groups of MPSC females (i.e. those tested using 1st and 2nd generation EIAs), the occurrence of false positive results is inversely related to the prevalence of HIV-1 infection. This is also clearly indicated by the negative correlation coefficient values. A reasonable explanation for this would be the relative competition for binding sites (antigen) between 'true' antibodies for HIV-l and non-specific antibodies which are the major cause for the occurrence of false positive results. In a population group with a low prevalence of HIV -I infection, non-specific antibodies would, facing no challenge from 'true' HIV-I antibodies, have a chance to react with the readily available reaction sites and consequently lead to false positive results. However in the case of a population group with high HIV -I prevalence rate, the effect of non-specific antibodies would be masked by the competing 'true' HIV-I antibodies which occupy most of the re-action sites. Hence, even though false positive results can occurhere, they will not be as significant as they could be in population groups with low prevalence of HIV-I infection.

This simple fact has an important role to play in the designing of test strategies, i.e., depending on the type of population group being tested, one can choose the appropriate test kits. In population groups with low HIV-I prevalence, it would be useful to use assays having high specificities rather than sensitivities. On the other hand, assays with high sensitivities become important when the population group under investigation is one where the prevalence of HIV-I is high. A by-product of this study is the observed fact that the use of paired ELISA screening assays (Wellcozyme and

Abbott HIV Recombinant kits) is chiefly responsible for the 66% reduction in the false positivity rate.

This is also in line with reports by Day and Mortimer (1988) and Downie et al (1989) which suggest that sera which are repeatedly positive by two ELISA screening assays (one indirect and the other competitive) have a 97-98% chance of being confirmed for HIV -I antibody by western blot. Also, the use of recombinant technology based EIAs, rather than lst generation assays, had its own contribution with respect to the observed decrease in the false positivity rate ,in all prevalence groups.

Table 1. Laboratory test results and false positivity rates in MPSC females
fr~ 23 major towns in Ethiopia using a 1st generation assay
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Prevalence Group (%)	Town NUItIer*	Total Tested	FPR E2(+)	Average lIB(+) (%)	Prevalence (%)
1-5	1,2,3	1065	97	22	77.3 2.1-
5.10	4,5,6	826	166	65	60.8 7.9
10.15	7,8,9	906	230	122	46.9 13.5
15-20	10,11,12,13, 14,15,16	1731	379	305	19.5 17.6
20-25	17,18	483	127	116	8.7 24.0
30-35	19,20,21	587	191	185	3.1 32.5
35+	22,23	636	299	235	21.4 36.9
Total/Average		6234	1489	1050	29.5 16.8

.Reference HIV-1 infection and related risk factors anKlng female sex workers urban areas of Ethiopia.

Table 2. Laboratory test results and false positivity rates in MPSC females from 25 sectors in Addis Ababa using a 2nd generation assay

Prevalence Group (%)	Sector NUItIer	Total Tested	FPR E2(+)	Average lIB(+)	Prevalence (%)
(Kefitegna) 1.5	12,25,20,18 16,10,8	283	17	9	47.1 3.2
5-10	6,9,17,19 24,11	395	45	32	28.9 8.1
10-15	3,13,22	253	32	29	9.4 11.5
15-20	1,4,7	239	51	44	13.7 18.4
20-25	15,21,23	457	118	106	10.2 23.2
25.30	2	313	91	79	13.2 25.2
35+	5,14	676	366	348	4.9 51.5
Total/Average		2616	720	647	10.1 24.7

E2(+) = Twice ELISA reactive sarr.,les.
WB(+) = lIestern Blot reactive sarr.,les.
FPR = False positivity rate.

FIGURE 1. RATE OF FALSE POSITIVITY FOR THE THREE POPULATION GROUPS TESTED. THE KIT USED WAS WELLCOZYME ANTI-HTLV-111

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250

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p O **150 S I T I V 100**

E ~

50

0

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HRG

HP POPULATION GROUP

-TRUE POSITIVES

FALSE POSITIVES

FIGURE 2. FALSE POSITIVITY RATE FOR THE THREE DIFFERENT POPULATION GROUPS WHEN USING WELLCOZYME RECOMBINANT KIT.

5001

E **400**

L I S A
300

! 200 f
E
S 100

ol

HRG HP SW **POPULATION GROUP**

-TRUE POSITIVES -FALSE POSITIVES

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