

Original Article

HIV Indeterminate Western Blot Results in Blood Donors in Northeast of Iran, 2009-2014

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Abstract

Background and Aims: HIV is spreading rapidly among people world wide. Infection with this virus leads to immune suppression and finally acquired immune deficiency syndrome (AIDS). Early HIV detection is depended on antibody screening against virus by Enzyme Linked Immunosorbent Assay (ELISA). Some confirmatory tests such as; Western Blot and Recombinant Immunoblotting Assay (RIBA), are used to verify viral infection. Many of confirmatory tests results are indeterminate. The aim of this study is comparing the frequency and patterns of indeterminate results in two groups; blood donors and patients with high risk behaviors, in northeast of Iran.

Materials and Methods: From October 2009 to March 2014 total number of 1055 serum samples with previous positive HIV ELISA test history, were tested in our laboratory. Some by RIBA and some by western blot method.

Results: Most of indeterminate results belonged to blood donors that were tested by Western Blot analysis and were positive. The most reacting band was P24 in both methods and groups.

Conclusion: RIBA assay is more sensitive and reliable than western Blot; but it's necessary to use other supplementary tests with less indistinctive results.

Keywords: Western blot; RIBA; HIV; Indeterminate

Introduction

HIV is spreading rapidly among people world wide (1). Most of infections are due to HIV1, which belongs to retrovirus family (2), and infects CCR5⁺, CD4⁺ lymphocytes (3, 4). It was first reported in United States of America in 1981 (5).

Infection with this virus leads to immune suppression and finally acquired immune deficiency syndrome (AIDS) (6). Early HIV detection is depended on antibody screening

tests against the virus by Enzyme Linked Immunosorbent Assay (ELISA) method (7). Whenever first serodiagnostic test is positive, confirmatory tests such as: Nucleotide Amplification Test (NAT) (8, 9), Recombinant ImmunoBlotting Assay (RIBA) (10), western Blot (WB) (1, 9, 11-13) and recently Polymerase Chain Reaction (PCR) are used to verify viral infection(12, 14).

The most confirmatory practical test, actually the gold standard method is WB (1, 14). Results of this test are very important in diagnosis of the disease but a large number of them are indeterminate. These results are very controversial and judging them is not easy (11). For as much as HIV infection awareness is very important for patients, therefore, it is

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necessary to use a reliable tests to clearly show the infections otherwise the false results will cause unnecessary stress and complications in patients (15).

The reasons of indeterminate results can be cited: HTLV1 or other retroviruses infectious; due to infection with HTLV1 and other retrovirus which contain a homologous region on surface virus glycoprotein (16), some medical conditions such as: leprosy, autoimmune disease and multiple blood transfusion and polyclonal gammopathy and hemodialysis (17, 18). Human contact with coprine arthritis encephalitis virus (CAEV) (19); due to cross reaction with P24 and other abnormal immune reactions (20, 21).

It has been reported, false positive results can occur in some certain conditions such as leishmania (22) and after influenza vaccination. It can be said, molecular protein semblance of HIV1 envelope and influenza is responsible for these false positive results (8).

In this study we compare the frequency of indeterminate results of two supplementary tests; RIBA and WB, in two groups; blood donors that are known as low risk group and patients with high risk behaviors.

Methods

From October 2009 to March 2014 a total number of 1055 serum samples with history of previous positive HIV Elisa test, were tested in our laboratory (the only laboratory which performs WB and RIBA test in North East of Iran). 395 of these samples belong to blood donors, and 610 to high risk group; who had referred to health centers. Testing pattern implemented is shown in figure 2.

Elisa for Anti HIV antibody

The vironostika HIV Ag/Ab kit (biomerieux Sa-France), that is based on one step sandwich principle was used according to the manufacture's instruction.

Western blot analysis

Western blot is a quantitative enzyme immunoassay for in vitro detection of antibodies against HIV. It was used as a supplemental test for samples found repeatedly positive ELISA by test (23). The HIV BLOT

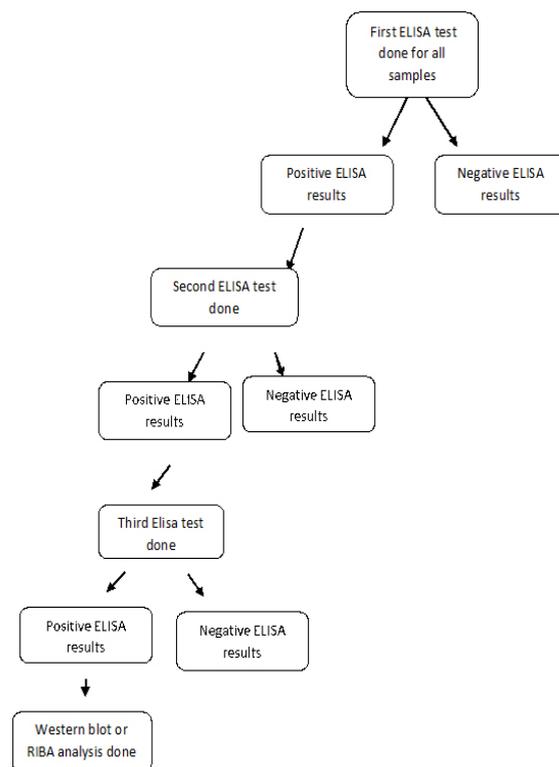


Fig. 1. Schematic diagram of HIV tests on samples.

2.2 WB assay (MP biomedical Asia pacific company) was used according to the manufacture's instruction. Interpretation the results were done according to the manufacture's instruction too. HIV infection was confirmed, when positive results were obtained from WB assay.

RIBA analysis

The INNO-LIA HIVI/II SCORE (INNOGENETICS N.V_ Belgium) was used according to the manufacturer's instructions. Recombinant proteins and synthetic peptides from HIV-1 and HIV-2, and a synthetic peptide from HIV-1 group O were coated as discrete lines on a nylon strip with plastic backing. Five HIV-1 antigens were applied: sgp120 and gp41, which detect specific antibodies to HIV-1, and p31, p24, and p17, which may also cross-react with antibodies to HIV-2. HIV-1 group O. peptides are present in the HIV-1 sgp120 band. The antigens gp36 and sgp 10⁵ were applied to detect antibodies to HIV-2. Interpretation the results were done according to the manufacturers instruction (24).

Table 1. Frequency distributions of results

| | Western blot | | RIBA | | |
|------------------------|--------------|------------|------|-----|-----|
| | Pos | Neg IND | Pos | Neg | IND |
| Blood donors | 0 | 155 142 | 5 | 78 | 5 |
| High risk group | 166 | 209 88 | 99 | 96 | 12 |

Pos= positive Neg= negative IND= indeterminate

Samples with negative Elisa result were excluded from experimental procedure.

Among 1055 samples with positive ELISA result, 395 samples belonged to blood donors. Infection was determined in 95 cases of RIBA test and 297 others by western blot assay. The rest of the samples, which belong to high risk group, were examined by WB and RIBA methods, 463 and 207 cases respectively.

Results

Frequency distribution of the results of the two methods is shown in table 1. The indeterminate results of WB and RIBA divided in to glycoprotein reactivity are shown in comparison in both groups in table 2 and 3, respectively.

In this cross sectional study, which was conducted from October 2009 to March 2014, 1.26% of blood donors and 39.55% of high risk group, who referred to health centers, were HIV infected, in north east of Iran.

P24 antigens were reactive in most cases. Their bands appeared in 76.17 % of WB analysis and 64.28% of RIBA tests.

Discussion

In this study we evaluate the frequency of HIV indeterminate results and glycoprotein reactivity in two groups, by two supplementary tests. As its obvious; frequency of indeterminate results are varied in different

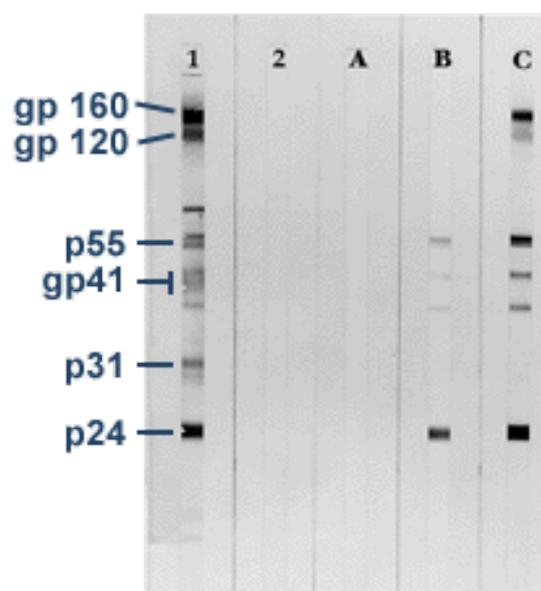


Fig. 2. HIV glycoproteins reaction in Western blo.

groups and different manners (14). It's very important to approve or disprove HIV infection in suspected patient with indeterminate results of supplementary test; as false positive results may have unwanted consequences (11).

Comparing redundancy of confirmatory trials exerts, low plenty in RIBA assay, just as Mas A *et al.* expressed, decrease in the number of indeterminate conclusion; In addition, he indicated high sensitivity and specificity for RIBA outcomes (10).

p24 band was the most frequently appeared band in both confirmatory tests. As it has been discovered by Carneiro-Proietti *et al.* study in 1999 (25).

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In Huang *et al.* study in the field of serologic tests for indeterminate results of western blot

assay, 22.5% of results were indeterminate;

Table 2. HIV glycoprotein reactivity in indeterminate result of WB.

| High risk group | Blood donors | HIV glycoprotein |
|-----------------|--------------|------------------|
| 65.51 | 86.84 | P24 |
| 4.59 | 5.26 | P17 |
| 2.29 | 3.86 | gp120 |
| 3.44 | 0.00 | P31 |
| 0.00 | 0.00 | P39 |
| 3.44 | 0.00 | P41 |
| 3.44 | 0.00 | P51 |
| 1.14 | 0.00 | P55 |
| 8.05 | 0.00 | P66 |
| 8.05 | 3.86 | gp160 |

Table 3. HIV glycoprotein reactivity in indeterminate result of RIBA analysis.

| High risk group | Blood donors | HIV glycoprotein |
|-----------------|--------------|------------------|
| 28.27 | 100 | P24 |
| 21.42 | 0.00 | P17 |
| 14.28 | 0.00 | gp120 |
| 7.14 | 0.00 | P31 |
| 28.57 | 0.00 | P41 |
| 0 | 0.00 | gp105 |
| 0 | 0.00 | P36 |

this value is very similar to the obtained data in this study (7). Just as Dodd and associates reported, frequency of indistinctive results of WB assay is 6-60% (15).

Comparison the results of confirmatory tests in both groups, revealed high frequency of indeterminate results in blood donors; who are known as low risk group. This finding has been reported by the study of Cremonesi *et al.* about prevalence of indeterminate results in Brazil in 2005 (1), and Dodd in 2000 (15).

As discovered by Guan, WB indeterminate patterns occurred more commonly for core antigens; such as: P24, P17 and P55. P24 band was the most abundant bands, which appeared in indistinctive patterns of WB and RIBA

assay. it's necessary to know that P24 is a viral protein, which is very impermanent and will disclose at advanced stage of disease (11). Interaction with P17 antibody bands, the most frequent appeared band after P24, was 21.42% in high risk group. No appears were seen in blood donors. Indeed, it's evident that incidences of different bands in both confirmatory tests are depended on the stage of disease.

Tend to positive results can say that; in WB analysis, reactivity with all bands was dominant pattern. Reactivity with all bands is reported by Sudha *et al.* in 92.91% of WB assays. P31 was the most missing band in their positive templates report, while it didn't appear

in most of indeterminate results of this study (26).

Conclusion

According to high incidence of indeterminate results, it seems requisite to use other supplementary tests with less indistinctive results. Most diagnostic laboratories use complex tests to ensure infectious presence. It's necessary to pay attention to HIV glycoprotein reactivity in some methods such as; WB and RIBA as a criterion to determine the stage of disease. Further studies suggested, in the field of follow up patients with indeterminate patterns to determine that which templates are more possible to get positive.

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