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ORIGINAL ARTICLE

Accuracy of Human Immunodeficiency Virus Diagnostic Tests Simulating the CDC Algorithm

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ABSTRACT

Background: *The detection of HIV in Egypt is started by screening for HIV antibodies or antigen by EIA and confirming the reactive samples by Western blot test (WB). In many countries, a new algorithm has been proposed by Centers for Disease Control and Prevention that uses an HIV-1/HIV-2 antibody differentiation immunoassay instead of WB or immunofluoresence (IFA) for confirmation. The initially reactive specimens are followed by same day confirmation rapid assay that approved by FDA and if this test is negative they make a nucleic acid test (NAT) to confirm.* ***Objectives:*** *comparing different diagnostic tests results with WB result in HIV diagnosis and detecting the accuracy of each assay.* ***Methods:*** *This study was conducted from January 2016 to September 2017 on 100 people who were seeking for HIV diagnosis. Blood samples were tested by Fourth generation Enzyme linked immunosorbent assay (ELISA), Multispot rapid diagnostic test, and nested PCR.* ***Results:*** *The sensitivity was the best with ELISA. PCR was the most specific test, followed by Multispot, and finally ELISA.* ***Conclusion:*** *This algorithm provides accurate results in short time.*

INTRODUCTION

Two types of Human immunodeficiency viruses (HIV), type 1 and type 2, can infect cells of the immune system (IS) and destroy it or impair their function. After long time, the IS becomes very weak, and the infected individual can catch infections easily until reaching the AIDS stage. Approximately there were 36.7 million people with HIV at 2016. Although only 11% of world's population lives in Africa, roughly 25.6 million people had HIV in 2016 in Africa. The number of HIV cases remains low in Egypt, around 11,000 people according to the National AIDS Program in 2016 [1,2,3].

There is no cure for HIV infection, but antiretroviral drugs can restrict the virus and strengthen transmission prevention so HIV people can have productive lives. Accurate detection of HIV by laboratory tests is important to know persons who need therapy, to educate uninfected persons, and to decrease transmission of the virus [2,4].

Many assays were developed for HIV diagnosis. Centers for Disease Control and Prevention (CDC) provided a combination of tests with a certain order for the detection of acute and established HIV-1/2 infection which called algorithm [5]. Similarly, each place tried its own algorithm for accurate HIV diagnosis. In different countries, the standard algorithm for HIV detection has a multistep process. First step for screening is by using third or fourth generation enzyme immunoassay (EIA); the third-generation assay detects IgM and IgG against HIV, and the fourth -generation detects both immunoglobulins and P24 antigen. If EIA is reactive, it should be followed by any confirmatory assay as WB or IFA[6].

The 4th-generation EIAs can't differentiate between anti-HIV-1/HIV-2 antibodies, but have the advantage that it decreases the time between infection and the power to detect infection [7].

Western blot and IFA can't detect IgM antibody and sometimes may fail to differentiate between anti-HIV-1/HIV-2 antibodies as well. An algorithm has been created to improve HIV diagnosis and replaces the WB with an differentiation immune-assay and NAT as polymerase chain reaction (PCR)[8,9].

The Multispot is a differentiation immunoassay, which employs micro-particles coated with HIV-1 and HIV-2 antigens separately to detect HIV-1 and HIV-2 antibodies [10].

The Clinical and Laboratory Standards Institute suggested Multispot assay as a replacement to the WB assay in the second-stage of HIV algorithm after a reactive HIV EIA [11]. PCR also may be used in reactive EIA instead of a WB. However, since false negative results are possible the HIV PCR cannot replace the serological screening test. Also HIV PCR tests usually do not cover HIV-2 and so used to resolve positive immunoassay with negative differentiation test [12].

The present study is a trial to compare different diagnostic tests results with WB result in HIV diagnosis.

METHODOLOGY

Subjects:

This comparative study was conducted from January 2016 to September 2017. One hundred subjects were included after doing the fourth generation ELISA for HIV detection. Consent was taken from each subject in this study.

Samples:

Peripheral blood was collected and serum was separated and used for ELISA and the rest of the serum samples were collected in a screw caped sterilized plastic vial and stored at -20°C until use in Multispot test. For PCR, 4 ml of whole blood was aseptically transferred to screw-caped sterilized vials containing EDTA and stored at -70°C until use [13].

Fourth generation ELISA:

Two hundreds and fourteen persons, were seeking for HIV diagnosis, were screened for p24 antigen and specific antibodies against HIV (IgM, and IgG), by fourth generation ELISA (Genscreen™ULTRA HIV Ag-Ab, France), as recommended by manufacturer's instructions. One hundred cases enrolled in the current study and there serum samples results were as the following: 64 positive samples and 36 samples were negative. These selected samples were then sent for confirmation by WB test in Preventive Medicine Sector of Egypt Ministry of Health.

Multispot antibodies differentiation test:

Only fifty samples (40 positive and 10 negative by both ELISA & WB for HIV) were screened for HIV1/HIV2 antibodies by Multispot rapid test (Bio-Rad, USA), as recommended by manufacturer's instructions.

Extraction of integrated HIV DNA:

Proviral genome was extracted from 100 blood samples by using QIAamp DNA Blood Kits (QIAGEN, Hilden, Germany) as recommended by manufacturer's instructions.

DNA amplification and Nested PCR assay:

The following primers were used for the first round PCR (*TTAGYCCTATTGARACTGTACCAG*) and (*TGCCCTATYTCTAARTCAGATCC*) and *(GCCTGAAAATCCATYCAAYACTCC)* and *(AATATTGCYGGTGAYCCTTTCCATC)* for the nested PCR to amplify a target sequence of 325-bp within *pol* gene protected region of HIV-1 [14, 15].

Statistical analysis:

Data were analyzed using SPSS, version 16. Independent t-test and chi-squared tests were used to detect significant differences ($P<0.05$).

RESULTS

One hundred samples that were enrolled in this study after they had been tested by fourth generation ELISA (64 serum samples were positive and 36 were negative). These samples were sent for confirmation by WB test in Preventive Medicine Sector of Egypt Ministry of Health. Fifty two serum samples, out of 64 positive by ELISA, gave positive results and the remaining 12 gave negative results by WB. The entire negative samples by ELISA were negative by WB. In this study, HIV positive samples drawn from 28 male and 24 female patients, their age ranged from 24-70 years with a mean 42.34 ± 10.06 years and negative samples drawn from 27 male and 21 female patients, their age ranged from 23-69 years with a mean 40.30 ± 9.75 years. By comparing these two groups, there were no statistical significant differences found in the demographic data except that non-educated persons showed high statistically significant difference in HIV positive patients than in negatives (Table 1).

Table 1: Comparison between HIV patients according to WB results

Data	HIV positive (n=52)	HIV negative (n=48)	*P-value*
Age (year)	42.34 ± 10.06	40.30 ± 9.75	0.359
Gender (M/F)	28/24	27/21	0.654
Suspected routes of infection			
• **Transfusion**	31	18	0.433
• **Sexual**	5	8	0.342
• **Unknown**	16	22	0.260
Marital status			
• **Single**	13	16	0.359
• **Married**	39	32	
Education			
• **Educated**	9	31	<0.001*
• **No education or primary**	43	17	

*High significant differences

Thirty two samples out of forty were reactive for HIV-1 antibodies and 9 samples out of 10 samples were nonreactive by Multispot test. Forty six blood samples showed 325 bp DNA band and were reported positive for HIV-1 by nested PCR. If the WB is the gold standard test in HIV diagnosis, the results of ELISA, Multispot, and PCR will be as shown in table 2. Twelve and one false positive were reported by ELISA and Multispot test, respectively. Eight and six false negative samples were reported by Multispot test and PCR, respectively. Six of the false negative samples reported by the Multispot were positive by PCR and all reported false positive samples were negative by PCR.

Table 2: Comparison between the ELISA, Multispot, and PCR in HIV diagnostic results (If WB is considered as gold standard):

Assays	Positive	False negative	Negative	False positive
WB (100)	52	….	48	….
ELISA (100)	52	….	36	12
Multispot (50)	32	8	9	1
PCR (100)	46	6	48	….

The sensitivities were 100%, 88.5%, and 80% for ELISA, PCR, and Multispot, respectively. PCR was the most specific test (100%), followed by Multispot (90%), and finally ELISA (75%). The positive predictive value (PPV) was 100% in PCR followed by Multispot (97%) and the negative predictive value (NPV) was 100% for ELISA followed by PCR (89%). The accuracy was the best with PCR (94%) test followed by ELISA (88%) and finally Multispot (82%) (Fig. 1).

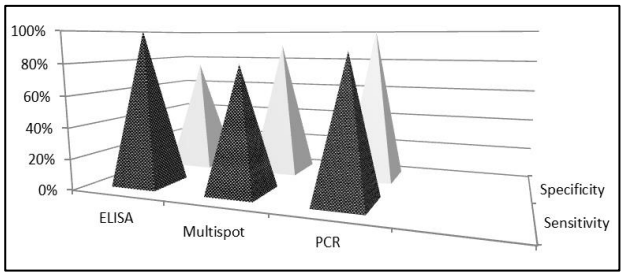

Fig. 1: Comparison between ELISA, Multispot, and PCR in HIV diagnosis

DISCUSSION

After decades of AIDS, there is still need to increase awareness of HIV and the importance of prevention and testing. According to the fact sheet of United Nations Program on HIV and AIDS (UNAIDS), approximately 1.8 million people infected with HIV in 2017, and the mortality of AIDS-related illnesses was nearly 940 000 [16]. For many years the Western blot assay was considered the gold standard test for HIV detection, however, WB is time-wasting, may produce indeterminate results and delay reporting, and may be miss-interpreted [17]. An alternative algorithm has been designed by the CDC to improve the speed and the accuracy of HIV diagnosis. In this algorithm, testing begins with a fourth generation screening assay followed by a rapid differentiating test that can detect both HIV-1 and HIV-2 in short time. Nucleic acid detection can be used for resolution of doubtful specimens by the rapid assay [18,19].

In the present study the fourth generation ELISA was the most sensitive assay but the least specific one. As regard the sensitivity this is in agreement with many studies results but disagree with them when come to specificity as they reported a higher percentages than what we did (98.5-100%) [20,21,22,23,24,25]. Another study reported lower sensitivities (70-91%) and a higher specificities (93-98%) for different fourth generation commercial assays[26]. Also Moon group in 2015 reported a lower sensitivity (95%) and a higher specificity (100%) than us [27].

Fourth-generation assays are the most sensitive; even ultrasensitive PCR technology can't detect low viral load in HIV-positive patients. Fourth-generation ELISA faces many challenges; one of them is Ag detection with HIV genetic variability and the other is combining two different test principles in one assay in which higher nonspecific reactivity might be expected. Previous studies reported a high frequency of false-positive results particularly during pregnancy and in infection with other viruses. Approximately 0.3% of blood donors at the time of donation have a reactive EIA, but the majority are negative by WB analysis [26,28].

In the present study the Multispot test were positive in 32/40 samples and reported 80% and 90% for sensitivity and specificity, respectively. These results were far away from many studies results which reported higher percentages of HIV diagnosis by Multispot test in positive samples and reported much higher sensitivities which in most of them were more than 94% and the specificities results were better (98-100%)[29,30,31,32,33,34]. Also many studies reported that the sensitivity of Multispot test is similar to WB test [35,36,37]. The fast time results from the Multispot (15 min) with a good sensitivity afford the opportunity to deliver same-day test results to HIV-infected persons who got reactive result by EIA [10, 19, 37].

Forty-six blood samples were positive by nested PCR test in the current study. There was no false positive result and all the reported false positive samples by ELISA or Multispot were negative by PCR; for this PCR was the most specific test (100%) and showed

sensitivity by 88.5%. These were approximated to some studies results [38, 39].

From these results we can understand that CDC selected the most sensitive assay at the beginning of the algorithm that will never miss any positive sample even doubtful one, followed in stage two by a rapid differentiating test which save time to confirm EIA and detect HIV-1 and HIV-2, and finally they finished by the most specific test which is PCR that clear any doubt for the final diagnosis report and exclude any false positive sample. This data was supported by Masciotra et al[40] who reported that the most important performance characteristic in multi-test algorithm is specificity.

In summary, the fourth-generation HIV EIAs offer the best sensitivities of any single tests which combined with still-high specificity; Multispot rapid test that improves the timeliness of HIV testing and confirm the former rapidly and considered as a second test; and finally the most specific test for accurate diagnosis is PCR testing which is necessary for the detection of true HIV-1 infections that may be missed by the second test but gives reactive result with the former.

Two limitations are in the findings of this study. First, no HIV-2 was diagnosed which limited the evaluation of its diagnosis by these assays. Second, only 50 samples were tested by Multispot test.

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