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Original Article

Diagnostic value of gelatin particles aggregation less-sensitive method for HIV-1 antibody in human gingival crevicular fluid

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Abstract: This study detected the HIV-1 antibody in gingival crevicular fluid (GCF) using gelatin particles aggregation less-sensitive (PA-LS) method, which aimed to provide a foundation for non-invasive HIV infection test in dental clinic. GCF samples and serum samples were collected from three groups, including HIV-1 positive group, drug users group and general population group. Simultaneously the HIV-1 antibodies in GCF samples were detected by PA-LS, and the HIV-1 antibodies in serum samples were screened by ELISA and confirmed by western blot. According to the final results of western blot, it was evaluated about the sensitivity, specificity, omission diagnostic rate, mistake diagnostic rate, positive predictive value and negative predictive value of PA-LS method. For HIV-1 positive group, the sensitivity of PA-LS used to detect HIV-1 antibody in GCF samples was 100%, the omission diagnostic rate was 0. For drug users group, when used to detect the HIV-1 antibody, the sensitivity, specificity, omission diagnostic rate, mistake diagnostic rate, positive predictive value and negative predictive value of PA-LS were 100%, 99.10%, 0%, 0.90%, 95.57%, and 100%, respectively; the results of ELISA/WB and PA-LS had a high consistency (Kappa >0.8, $P<0.01$) and no significant difference ($P>0.05$). For general population, all GCF samples showed HIV-1 antibody negative results. In this study, PA-LS used to test HIV-1 antibody in GCF samples showed a good effect, and was non-invasive, simple, cost-effective compared to ELISA used to detect HIV-1 antibody in serum samples.

Keywords: Antibody detection, ELISA, gelatin particles aggregation less-sensitive method, gingival crevicular fluid, human immunodeficiency virus type-1, sensitivity

Introduction

Acquired Immune Deficiency Syndrome (AIDS) is an infectious disease caused by Human Immunodeficiency Virus (HIV). The principal symptoms of AIDS are a marked drop in $CD4^+$ T cell counts, opportunistic infections (virus, bacterium, fungus, protozoa) and malignant tumor (Kaposi's sarcoma, malignant lymphoma, cervical cancer) [1, 2]. HIV is transmitted by directly contacting with mucosal tissue (in oral cavity, genitals and anus, etc.) or HIV-contaminated blood, sperm and milk [3]. Medical staves in the department of stomatology are in a state of high occupational exposure during clinical diagnosis and treatment, and most complicated dental instruments are sharp; so there is a high risk of accidental injury for staves in the process of clinical diagnosis and treatment. The number of medical workers being exposed to HIV has an increase trend since the United States reported the first occupational HIV infection in 1983 [4, 5]. It can reduce the chance of HIV infection after occupational exposure if doing the non-invasive test of HIV antibody before oral outpatient treatment.

According to the serological reaction and nucleic acid sequence, HIV is classified into 2 types: HIV-1 and HIV-2, HIV-1 is the main type in China

Table 1. The basic information of three groups in study

	HIV-1-positive people	High risk group	Ordinary group
No.	50	666	400
Gender (male, %)	31 (62%)	633 (95%)	155 (39%)
Age (mean ± SD, year)	43.20±5.72	34.86±8.05	20.90±7.43
HAARI treatment	38	-	-
HIV positive diagnosis time (year)	0.25-5	-	-
History of transfusion	Unknown	Portion	None
HIV high-risk behavior	Unknown	All	None

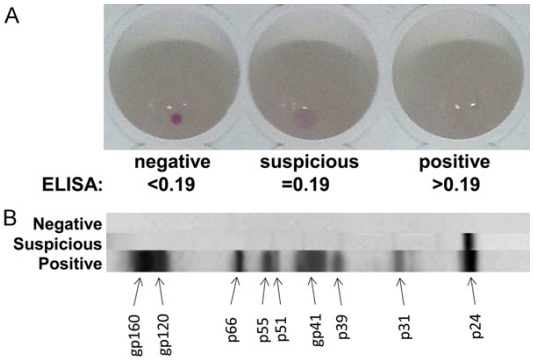

Figure 1. The results of HIV-1 antibody detection of GCF samples using PA-LS method (A) and the corresponding ELISA (A) and western blot (B) results. The critical ELISA value was 0.19, if the value was less than 0.19, the result was considered as negative, while the value greater than 0.19, the result would be consider as positive. Negative western blot showing no responses to key protein; Suspicious western blot showing antibodies to p24; Positive western blot showing responses to at least three key proteins.

[6]. Yunnan province is the area of serious HIV infection and AIDS epidemic in China, the statistics report about HIV exposure cases caused by occupational factors from 2007 to 2010 showed that: a total of 911 HIV occupational exposure cases occurred in four years and 74.6% (680) of which were doctors and nurses, who mainly infected by stabbing and cutting (74.4%), secondarily by skin and mucosa exposure 24.9% [7]. HIV antibody is the most common marker used for detection [8, 9], and in China, testing procedure of HIV antibody in blood sample mainly is early screened by ELISA and confirmed by WB (western blotting) [10]. But because of the wound, strict conditions and risk of cross infection, detection of blood sample is not suitable for oral outpatients to quickly screen the HIV antibody. Since Japanese scholar Yoshida et al developed gelatin particles aggregation (PA) method for detecting HIV [11], Li Hong and Constantine co-developed the gelatin particles aggregation less-sensitive (PA-LS) method for testing recently infected with HIV-1 [12], which improved the detection sensitivity and reduced the test cost, could detect the HIV antibody even if HIV positive serum were diluted to one over ten thousand.

A variety of body fluids from HIV infectors all contain HIV antibody, as urine, oral secretions, tear, sperm and vaginal fluids. Oral secretions are easy to obtain and no traumatic and non-invasive in the sampling process, so it may be applied to oral outpatients with HIV screening. And our previous study has showed the diagnostic accuracy of PA-LS method for HIV-1 antibody detection in oral mucosal transudate [13]. Therefore, to assess the feasibility of testing the HIV-1 antibody in gingival crevicular fluid (GCF) using PA-LS, this study detected the HIV-1 antibody in GCF from 1116 cases by PA-LS method, and the results were verified by the serum samples using ELISA and WB.

Materials and methods

Subjects

HIV-1-positive people: In total of 50 people living with HIV/AIDS were conformed seropositive for HIV-1 antibody by Yunnan provincial center for disease control and prevention, and the route of infection, symptoms and whether on immunosuppressive therapy were out of consideration.

High risk group: There were 666 drug addicts from Jiuxi district, the third compulsory isolated detoxification center of Yunnan province and addiction treatment center in Yingjiang county of Yunnan province. The duration of drug rehabilitation and the way of drug using were not distinguished.

Ordinary group: 400 people in this group were students from Kunming medical college, medical staff and outpatients at the stomatology

Table 2. Evaluation criteria of Kappa number in this study

Kappa value	The level of consistency
Kappa ≤0.2	Slight
0.2< Kappa ≤0.4	Fair
0.4< Kappa ≤0.6	Moderate
0.6< Kappa ≤0.8	Substantial
0.8< Kappa	Almost perfect

hospital of Yunnan province. They all denied the history of HIV high-risk infection behavior and blood transfusion.

All these subjects are Chinese, no restriction of gender, age and nation; and no absence of teeth or severe limited mouth opening; unknown about viral infection, oral diseases and systemic diseases.

Ethical approval was obtained from the Ethics Committees of the Kunming medical college, stomatology hospital of Yunnan province, Yunnan provincial center for disease control and prevention, the third compulsory isolated detoxification center and addiction treatment center in Yingjiang county of Yunnan province. This study has got the informed consent and information of each subject, and timely fed back the test results.

The basic information of three groups was shown in **Table 1**.

Sample collection and preservation

All 1116 subjects were no brushing teeth in one hour before sampling, then gargled with water and sat quietly for 5 minutes, wiped clean the facing with medical absorbent cotton ball. Inset the Whatman filter paper (2 mm×20 mm) in the crevicular of gingival keeping 30-60 s to wet 0.5-1 mm of the filter paper with GCF (abandoned samples with blood and sampling again), took it in a EP tube and saved at -70°C. In addition, serum was isolated from venous blood (3-5 mL) and saved at 2-8°C. GCF samples and serum samples were numbered uniformly.

Sample detection

The HIV-1 antibody in GCF sample was detected using gelatin particles aggregation less-sensitive (PA-LS) method. SERODIA®-HIV 1/2 (Fujirebio, Japan) was used to prepare sensitization particulate (SP) solution according to the instruction manual, and then diluted the SP solution with L (1:67). Mixed diluted SP solution (38 μL) with GCF sample (8 μL, dissolved in 100 μL M) in 96-well plates and shocked 30 s. Observing and recording the results in another day. Serum samples of high risk group were tested using ELISA at the same time and the negative or dubious results of GCF samples were confirmed by WB according to the National AIDS/HIV Test Technology Regulation (2009 edition, China). The negative or uncertain results in positive group would be rechecked using ELISA and WB as same as the positive or suspicious results in ordinary group.

Determination of result [14]: Negative reaction (-), gelatin particles agglutinate at the bottom of well with uniform smooth and macroscopic edge under light box or natural light; Dubious result (±), gelatin particles gather at the bottom of well to form a small ring with uniform and smooth edge; Positive reaction (+), gelatin particles form a big ring with non-uniform peripheral edge; Intense positive reaction (++), gelatin particles form a uniform agglutination and like a film in the bottom of well (**Figure 1**).

Evaluation index [15]

Sensitivity: true positive rate, the probability that patient is diagnosed with disease.

Sensitivity = true positives/(true positives + false negatives).

Specificity: true negative rate, the probability that healthy people is diagnosed as being not sick.

Specificity = true negatives/(true negatives + false positives).

Omission diagnostic rate: false negative rate, the probability that patient is diagnosed as being not sick.

Omission diagnostic rate = false negatives/(true positive + false negatives).

Mistake diagnostic rate: false positive rate, the probability that healthy people is diagnosed with disease.

Mistake diagnostic rate = false positives/(true negatives + false positives).

Table 3. HIV antibody testing in GCF from HIV positive group

	Total	Positive	Uncertain	Negative	Sensitivity	Omission diagnostic rate	Positive predictive value
WB (serum)	50	50	0	0	-	-	-
PA-LS (GCF)	50	50	0	0	-	-	-
Ratio	-	-	-	-	100%	0%	100%

Table 4. HIV antibody testing in GCF and serum from high risk group

PA-LS (GCF)	ELISA/WB (serum)			Total
	Positive	Uncertain	Negative	
Positive	108	0	1	109
Uncertain	0	0	4	4
Negative	0	0	553	553
Total	108	0	558	666

Positive predictive value: the proportion of patients in people diagnosed as sick.

Positive predictive value = true positive/(true positive + false positive).

Negative predictive value: the proportion of healthy people in person diagnosed as negatives.

Negative predictive value = true negatives/(true negatives + false negatives).

Statistical analysis

Statistical analysis of data was performed using SPSS17.0 software package, consistency check of diagnostic test was reflected by the value of Kappa. A evaluation criterion was shown in **Table 2** [16]. The serum samples were screened by ELISA and confirmed by WB as the gold standard of HIV test. SPSS17.0 software package were used to draw ROC curve and obtain area under the curve included 95% confidence interval. Using χ^2-test to estimate the difference between PA-LS and ELISA when screening HIV-1 antibody, and the difference was statistical significance when $P<0.05$.

Results

GCF samples test results of HIV-1 positive group and ordinary group

GCF samples from 50 HIV-1-positive people were tested the HIV-1 antibody using PA-LS method. The results showed that all GCF samples were positive (**Table 3**), and this was consistent with the results of serum samples using ELISA and WB, even if 38 of whom were accepted the highly active antiretroviral therapy (HAART) before being tested. So the sensitivity was 100% and omission diagnostic rate was 0%. 400 individuals were in ordinary group and were detected HIV-1 antibody in GCF samples using PA-LS too, and results showed 0 positive, 0 uncertain and 400 negative.

GCF samples test results of high risk group

Total of 666 drug users were detected the HIV-1 antibody in GCF samples by PA-LS. As shown in **Table 4**, in the results of GCF samples and serum samples testing, 108 samples were all positive and 553 samples were all negative; the rest of 5 were negative in serum testing, 1 positive and 4 suspicious in GCF testing. GCF sample by PA-LS using in HIV-1 antibody detection was analyzed using ROC curve to discriminate the group of patients who achieved HIV-1 antibody we found area under the curve [AUC (95% CI)] was 0.996 (0.991, 1.000) (**Figure 2**). The sensitivity, specificity, omission diagnostic rate, mistake diagnostic rate, positive predictive value and negative predictive value of PA-LS were 100%, 99.10%, 0%, 0.90%, 95.57%, and 100%, respectively (**Table 5**). Statistical analysis of detection results of high risk group between PA-LS (GCF) and ELISA/WB (serum) were shown in **Table 6**, and Kappa number between two results was 0.973 (Kappa >0.8, $P<0.01$), indicated the results of two methods had a high consistency; the difference between two results was not statistically significant ($P>0.05$).

Discussion

50 subjects in HIV-1 positive group had different disease duration and HIV-1 antibody confirmation times were ranged from 3 months to 5 years. In addition, 38 of them had accepted the highly active antiretroviral therapy before being tested. These didn't impact on the results of

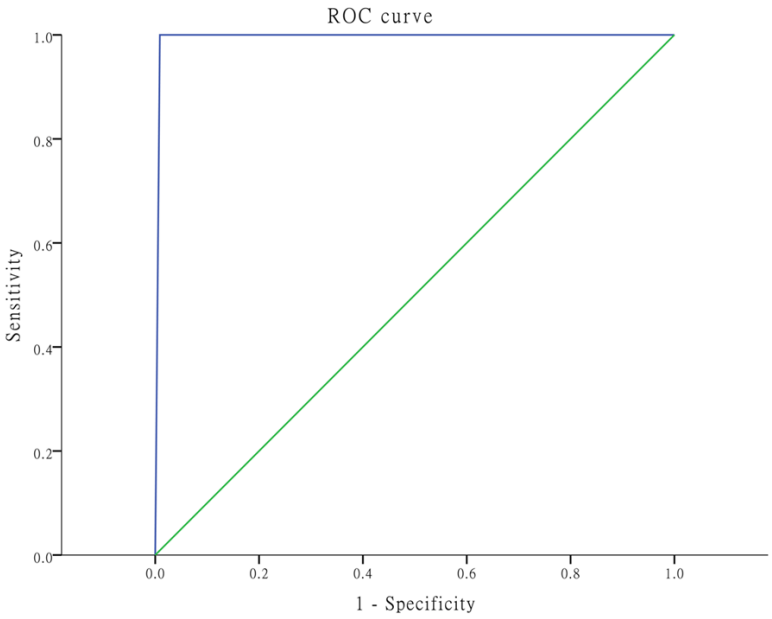

Figure 2. The ROC curve of PA-LS (GCF) using in HIV-1 detection.

GCF sample testing, perhaps because those subjects' conditions had not reach the terminal AIDS and had a high level of HIV-1 antibody. On the other hand, it also indicated the high sensitivity of PA-LS used for HIV-1 antibody detection.

Compared to our previous study, which was about detection of HIV-1 antibody in oral mucosal transudate (OMT) using PA-LS method [13], the results showed the sensitivity was 100% for all GCF samples as well as HIV-1 antibody detection in OMT samples, and for drug users, the detection of HIV-1 antibody in GCF samples seems to be better than that in oral mucosal transudate for the higher specificity (99.1% vs. 97.49%), lower misdiagnosis rate (0.90% vs. 2.51%) and higher positive predictive value (95.57% vs. 88.52%). These data indicate that GCF samples may be better than OMT samples for detection of HIV-1 antibody using PA-LS method.

In this study, we detected the HIV-1 antibody in GCF samples using PA-LS method, and the specificity in the results of high risk group was 99.10%, which was lower than that in the results of Li Hong's research (99.8%) [12]. The reasons might be the antibody concentration in GCF sample was lower than in serum and GCF samples were repeated freeze-thaw cycles during transport. 5 samples results were inconsistent between GCF testing (1 positive and 4 in doubt) and serum testing (all negative); this outcome has two possible reasons:

One possibility, subject was really infected with HIV-1 but the serum sample was failed to detect the HIV-1 antibody. This happened because the HIV-1 antibody was not completely generated in the early stage of HIV-1 infection (acute stage), and the negative or uncertain results of HIV-1 antibody testing was contributed to the low antibody concentration. But with the development of the disease course, HIV antibody levels will gradually rise, test results also can turn to be positive by negative or suspicious [17]. In Sandeep Ramalingam's study [18], 63 serum samples were weakly positive by PA and negative by ELISA, but they were all positive when tested again after three years. This suggested the sensitivity of PA for HIV antibody detection might be higher than that of ELISA when in the acute stage or low HIV antibody concentration. So to prevent the missing detection, those 5 subjects in this study should be rechecked the HIV-1 infection on a regular basis and end their addiction early under the supervision and management of staves in drug treatment center.

Another possibility, the results of GCF testing were false positive, that the subjects didn't infect with HIV-1. The reason might be that subjects were infected with other retrovirus when sampling, which had certain homology with HIV-1 and could induce antibody that had cross reaction with HIV-1, leaded to the abnormal antibodies-cross reaction. Besides, when people had certain autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus and Sjogren's syndrome), acute or chronic renal failure or malignant tumor and so on, their autoantibodies could occur abnormal immune responses with HIV [19]. Drug users in this study were at high risk of HIV-1 infection, the

Table 5. Evaluation of GCF testing result in accordance to the ELISA/WB results of high risk group

	True positive	True negative	False positive	False negative	Sensitivity	Specificity	Omission diagnostic rate	Mistake diagnostic rate	Positive predictive value	Negative predictive value
PA-LS (GCF)	108	553	5	0	100%	99.10%	0%	0.90%	95.57%	100%

Table 6. Statistical analysis of detection results of high risk group between PA-LS (GCF) and ELISA/WB (serum)

	Total	Positive	Uncertain	Negative	Kappa	Approx. Sig.*	χ^2	P
ELISA/WB (serum)	666	108	0	558	0.973	0.000	5.572	0.062
PA-LS (GCF)	666	109	4	553				

*corresponding to the *P* value.

risk of various infections was increased for the decrease of immune defenses, the influencing factors of HIV-1 testing results were also complex, so it should be noted when the results of several detection methods were inconsistent and suspicious people infected with HIV-1 should be tested the p24 antigen of HIV-1 [20, 21] or did qualitative analysis of HIV-1 nucleic acid [22].

For the positive or uncertain results of HIV-1 antibody in ordinary group, we should treated with re-inspection after communicating effectively with patients, and other auxiliary inspection of related diseases considered with the patients' own health should be done too. These things should be done to avoid panicking the patients caused by the misdiagnosis.

Compared with the other HIV detection methods, detection of GCF using PA-LS has several advantages: first for GCF sample, it does not transmit HIV and has a high level of biosecurity [23, 24], which effectively reduce the risk of cross infection between staff and patient; GCF sampling is non-invasive and no pain in the sampling process, this is more convenient for plenty of patients (children, hemophiliac, the crowd with less obvious superficial vein and so on) and has a better acceptability and compliance [25-28]; GCF samples can be preserved for a long time both at ordinary temperature or low temperature without centrifugal purification. Second for PA-LS, the detection process is easy and needs no special instruments and equipment; the testing results can be seen directly; test cost of each sample is only about RMB 1.00 [29], which is lower than ELISA (about RMB 10.00 for each sample) and OraQuik rapid detection (about $13.5 to $17.5 for each sample) [30].

Conclusions

This study detected the HIV-1 antibody in GCF sample and serum sample using PA-LS and ELISA, respectively. There was a good consistency between those two results showed by Kappa value (0.973) and no significant difference ($P>0.05$). The sensitivity, specificity, omission diagnostic rate, mistake diagnostic rate, positive predictive value and negative predictive value of PA-LS used for testing HIV-1 antibody in GCF sample were all good. Furthermore, PA-LS is a safe, simple, convenient and cost-effective method to detect HIV-1 antibody. So PA-LS would be available for HIV-1 antibody detection of GCF sample, and may be a method of non-invasive HIV-1 antibody testing for oral outpatients.

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Disclosure of conflict of interest

None.

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