PO –06: HIV – FORENSICS: IDENTIFICATION OF HIV ANTIGENS AND ANTIBODIES ON BLOOD-STAINED CLOTHES

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to this study.

Abstract

Context: HIV is one of the sexually transmitted infections of viral origin that occurs by contact with or transfer of blood, pre-ejaculates, semen and vaginal fluids. It can be acquired through sexual assault incidents such as rape. Detection and confirmation of HIV in infected human blood traces and blood stains found on the blood-stained clothes of sexual assault survivors are very important in forensic analysis, particularly in rape cases involving suspected HIV positive perpetrator and a HIV negative victim. The genetic relatedness between the HIV strain in the survivor and that in the suspect can be used in criminal prosecutions as evidence of responsibility for HIV transmission to the survivor.

Aims: To determine the extent to which HIV antigens and antibodies can be detected on different blood stained clothes with a view towards developing a forensic diagnostic methodology for rape cases, and early intervention for Prophylaxis, particularly in a no-suspect case.

Materials and Methods: A case control clinic-based study of HIV positive patients (HIVPP) and HIV negative individuals (HIVNI) between the ages of 18 to 65 years was conducted. 10 HIVP samples spotted on clothing materials were tested consecutively after 1 month and after 4 months for the presence of HIV antibodies using Enzyme Linked Immunosorbent Assay (ELISA) and 10 HIVNI were tested using the same method as well. Sero-positive HIVP considered as “subjects” and 10 HIVNI Sero-negative as “controls. HIV-1 RNA PCR (viral load) was done on all the subjects and controls

Statistical analysis used: Data analysis was conducted with SPSS version 23 statistical software, and findings were presented as means, standard deviation and Student t Test to compare the Subject's and Control's where appropriate, p value of ≤ 0.05 was considered as significant.

Results: A total of 10 HIV positive samples from known adult HIV positive patients on antiretroviral (ART) drugs and 10 HIV negative individuals were studied. All the subject's HIV ELISA results were positive to HIV and their Plasma HIV-1 RNA PCR was detectable in different concentrations. While that of controls was negative and undetectable.

CONCLUSION: HIV antigens or antibodies are detectable using ELISA technique on 100% cotton, 50% cotton mixed with 50% polyester and 100% polyester clothing materials stained with HIV positive blood after four months found at room temperature in an open environment, but 100% cotton clothing material produced more accurate results as no much effect was noticed.

Key Words: HIV virus, Blood stains, ELISA and HIV-1 RNA PCR

INTRODUCTION

Human immunodeficiency virus (HIV) is a species of Lentivirus a subgroup of retroviruse, that infects human beings causing acquired immunodeficiency syndrome (AIDS) [1]. This virus causes failure of the immune system leading to life-threatening opportunistic infections and cancers in some cases[1, 2] . Human Immunodeficiency Virus is a sexually transmitted infection and transmission mostly occurs by sexual contact and exposure to or transfusion of blood, semen, vaginal fluids and pre-ejaculate fluid[3,4]

Research has shown that HIV is untransmissible if the HIV-positive person has a consistently undetectable Viral load[3,4]. The risk of HIV sexual transmission is highly variable and depends on the infectiousness of the source partner or in the case of rape, the assailant[5]. These include viral load, virulence of the strain, clinical stage of infection (with the recently infected and those at late stages being most infectious), mucosal damage and concomitant genital tract infections[5].

Study population

Ten (10) HIV positive patients on antiretroviral therapy and ten (10) HIV negative patients between the ages of 18-65years were recruited consecutively into this study. The HIV positive patients who are HIV sero-positive constitute the study "subjects" while those that were sero-negative were considered as the "control" group from Blood donors.

SAMPLE PREPARATION

- 2 Square centimeter of 100% cotton cloths, 50% cotton + polyester and 100% polyester were cut into 10 pieces to have 60 pieces of clothing materials
- Each piece of cloth was stained with 100microliter of HIV positive whole blood and allowed to air dry
- The dried blood-stained piece of cloth was transferred into a sterile plain bottle and left opened at room temperature
- 30 pieces of 100% cotton, 50% cotton + 50% polyester and 100% polyester blood-stained cloths were tested for HIV 1 &2 Ag-Ab after 4 weeks
- The remaining 30 pieces of 100% cotton, 50% cotton + 50% polyester and 100% polyester blood-stained cloths were tested for HIV 1 & 2 Ag-Ab after 16 weeks

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Table 1: socio-demographics and Drug characteristic of the participants

Sample No.	Age of participants (years)	Gender of participants	ART regimen	Date of ART initiation
1	50	Male	2d	1st October, 2005
2	50	Male	1b	7th June, 2006
3	42	Male	1b	23rd October, 2008
4	40	Male	1b	23rd January, 2009
5	46	Female	1b	17th June, 2010
6	44	Female	1b	1st September, 2010
7	48	Male	2c	7th March, 2011
8	37	Female	1b	1st April, 2011
9	38	Female	1b	11th June, 2012
10	54	Male	1b	18th February, 2020

Table 2: HIV-1 ELISA Assay (Ag/Ab) on plasma and on different blood stained fabrics of subjects after one month and RNA quantification

Samples	Plasma Viral load In copies/ml	Whole blood on 100% cotton		Whole blood on 50% cotton mixed with 50% polyester		Whole blood on 100% polyester		Plasma	
		Absorbance (Nm)	HIV Status	Absorbance (Nm)	HIV Status	Absorbance (Nm)	HIV Status	Absorbance (Nm)	HIV Status
1	2730	1.299	+	1.152	+	3.675	+	1.362	+
2	< 20	2.649	+	2.986	+	1.359	+	1.187	+
3	144	2.205	+	3.231	+	1.192	+	1.885	+
4	< 20	2.288	+	1.194	+	2.399	+	1.530	+
5	158,00	1.592	+	1.279	+	0.839	+	1.304	+
6	< 20	1.106	+	1.462	+	3.104	+	1.892	+
7	< 20	1.877	+	1.852	+	2.361	+	2.532	+
8	70800	1.613	+	0.962	+	3.763	+	2.466	+
9	< 20	1.286	+	1.698	+	1.932	+	1.202	+
10	1250	1.092	+	1.053	+	2.292	+	2.173	+

Table 3 HIV-1 ELISA Assay (Ag/Ab) on plasma and on different blood stained fabrics of subjects after four months

Samples	Absorbance of Whole bloodon100% cotton material (nm)		Absorbance of Whole blood on 50% cotton mixed with50%polyester (nm)		Absorbance of Whole blood on100%polyester (nm)	
	Absorbance	HIV Status	Absorbance	HIV Status	Absorbance	HIV status
1	0.380	+	3.667	+	3.430	+
2	3.812	+	0.920	+	3.337	+
3	2.383	+	3.993	+	4.000	+
4	0.932	+	3.529	+	3.998	+
5	3.602	+	3.770	+	0.890	+
6	3.954	+	3.230	+	3.700	+
7	3.610	+	0.659	+	3.749	+
8	1.484	+	3.498	+	3.841	+
9	0.371	+	3.895	+	3.476	+
10	3.742	+	3.760	+	3.581	+

Table 4: HIV-1 ELISA Assay (Ag/Ab) on plasma and on different blood stained fabrics for controls after one month

Samples	Whole blood on100% cotton material (nm)		Whole blood on 50% cotton mixed with50%polyester (nm)		Whole blood on100%polyester (nm)		Plasma (nm)	
	Absorbance	HIV Status	Absorbance	HIV Status	Absorbance	HIV Status	Absorbance	HIV status
1	0.087	-	0.055	-	0.077	-	0.021	-
2	0.089	-	0.035	-	0.057	-	0.033	-
3	0.073	-	0.067	-	0.059	-	0.076	-
4	0.076	-	0.078	-	0.061	-	0.053	-
5	0.052	-	0.061	-	0.041	-	0.045	-
6	0.090	-	0.054	-	0.055	-	0.066	-
7	0.021	-	0.081	-	0.045	-	0.062	-
8	0.039	-	0.043	-	0.088	-	0.056	-
9	0.056	-	0.033	-	0.099	-	0.037	-
10	0.077	-	0.031	-	0.031	-	0.022	-

Table 5: HIV-1 ELISA Assay (Ag/Ab) on plasma and on different blood stained fabrics for controls after four months

Samples	Whole bloodon100% cotton material (nm)		Whole blood on 50% cotton mixed with50%polyester (nm)		Whole blood on100%polyester (nm)		Plasma (nm)	
	Absorbance	HIV Status	Absorbance	HIV Status	Absorbance	HIV Status	Absorbance	HIV status
1	0.097	-	0.035	-	0.047	-	0.041	-
2	0.079	-	0.075	-	0.077	-	0.023	-
3	0.083	-	0.057	-	0.069	-	0.066	-
4	0.066	-	0.098	-	0.081	-	0.073	-
5	0.072	-	0.071	-	0.091	-	0.035	-
6	0.050	-	0.094	-	0.045	-	0.096	-
7	0.051	-	0.091	-	0.075	-	0.072	-
8	0.099	-	0.053	-	0.098	-	0.036	-
9	0.076	-	0.073	-	0.089	-	0.047	-
10	0.087	-	0.061	-	0.051	-	0.052	-

Table 6: Mean ±SD of whole Blood and plasma at one month after staining and that of plasma at zero days

	One Month		
	Mean ±SD	t-value	P-value
Plasma	1.75 ±0.51	0.175	0.756
Whole Blood on 100% Cotton material	1.70 ±0.54	0.197	0.846
Whole Blood on 50% cotton mixed with 50% polyester material	1.70 ±0.82	0.167	0.869
Whole Blood on 100% Polyester material	2.29 ±1.00	-1.529	0.144

Table 7 Median (IQR) t and p-values of whole Blood after four months

	Four Month		
	Median(IQR)	t-value*	P-value
Plasma	1.68(0.97)	23.01	0.06
Whole Blood on 100% cotton material	2.99(2.97)	38.00	0.364$^{\alpha}$
Whole blood on 50% cotton mixed with 50% polyester material	3.60(1.15)	20.00	0.023
100% Polyester material	3.64(0.47)	10.00	0.002

*Mann-Whitney U test, $^{\alpha}$Insignificant

Table 8 Median (IQR) t and p-values of whole Blood after one and four months

	One Month	Four Month		
	Median(IQR)	Median(IQR)	t	P-value*
Whole Blood on 100% Cotton material	1.60(0.99)	2.99(2.97)	-1.274	0.203$^{\alpha}$
Whole blood on 50% cotton mixed with 50%polyester material	1.37(1.01)	3.60(1.15)	-2.293	0.022
Whole Blood on 100% Polyester material	2.33(1.93)	3.64(0.47)	-2.191	0.028

*Wilcoxon sign rank, $^{\alpha}$Insignificant