Quantification of human immunodeficiency virus using quantitative polymerase chain reaction (PCR) compared to ELISA in early diagnosis of infection

Seyedeh Tahereh Aghamirzaei, Amin Hemati and Selvam Arjunan*

Department of Biotechnology, Indian Academy Degree College, Hennur cross, Hennur Main Road, Kalyan Nagar, Bangalore, India
_____

ABSTRACT

In the era of modern medicine, owing to an increasing incidence of HIV infection, many indirect tests including ELISA are readily available for the detection of HIV infection. However, they are not useful in early diagnosis of HIV infection. As early diagnosis is vital to treat HIV infection promptly, direct test like PCR is used for the earlier diagnosis and treatment of the infection. As a result, in the current study, we compared the efficacy of quantitative polymerase chain reaction (qPCR) with the Enzyme linked Immunosorbent assay (ELISA). Sera collected from HIV patients were analyzed using the qPCR, which detect the targeted gene of HIV and the results were compared with ELISA test, which reacted with antibodies against HIV antigens in the same sera.

Keywords: HIV, ELISA, QPCR, Genomic DNA
_____

INTRODUCTION

HIV, which is considered to be a foremost global public health issue constantly, having asserted greater than 36 million lives to date. According to the World Health Organization (WHO), about 35.3 million people globally are living with HIV infection [1]. It has also been found that approximately 94% of those infected people are from Sub-Saharan Africa, which accounts for 67%, Eastern Europe, South and Southeast Asia, Latin America, and Central Asia. In India, about 2 to 3.1 million people are living with HIV [2].

In an attempt to reduce the increasing mortality due to HIV infection, WHO has intensified the access to antiretroviral treatment (ART), which is capable to efficiently suppress HIV-1 replication and impede disease, in developing countries. However, owing to one of the prime reason of the lack of cost-effective ART monitoring, the goal of universal access to ART treatment remains challenging [3].

Two currently available methods to monitor HIV ART monitoring are HIV-1 viral load in plasma which specifies viral replication in infected individuals and CD4+ T lymphocyte count which reveals the functionality of the host immune system [4]. WHO recommendation to combine CD4+ cell count with a disease staging system to commence ART in countries with limited resource settings often resulted in delayed recognition of ART failure [5]. Besides, this may lead to accretion of drug-resistant strains and decrease the effectiveness of second-line drugs. Consequently, there is a persistent need for viral load monitoring to manage AIDS patients on ART guidelines [6]. In an attempt to improve the early detection and treatment of HIV infection, we compared the sensitivity of the

64

_____

quantitative PCR (qPCR), which is used to quantitate HIV load, with ELISA which is used routinely for the detection of antibodies against HIV infection [7, 8, 9].

MATERIALS AND METHODS

MATERIALS
HIV-IgG ELISA Kit, Maxisorp ELISA plate (NUNC®) were purchased from Statens Serum Institute Denmark and eBioscience, Inc respectively. The lyophilized HIV antigen were purchased from S. Serum Institute Denmark. Human standard antiserum and Rabbit-Anti-Human IgG HRP were from MP Biomedicals Pvt Ltd, India. Fluorescent dyes and Fluorescent probes were purchased from Life Technologies, India.

ELISA (Enzyme Linked Immunosorbent Assay):
Citrate or EDTA treated HIV infected serum or plasma samples can be stored up to 3 days at 2-8°C or at least 6 months below -20°C. Sample should not be repeatedly frozen and thawed. The sera were thawed on the day of analyzing the tests and tested for HIVAg using enzyme linked Immunosorbent assay kits as per the manufacturer's instructions. All serum samples were tested in triplicate.

Sample Preparation: a) Serum: Blood   was drawn using   standard vein puncture techniques and were allowed to clot for 1hr at RT and then centrifuged for 10 minutes at 4°C and serum was extracted. b)   Plasma: In order to prepare the plasma, blood sample was drawn using standard venipuncture techniques and plasma was collected using sodium citrate, EDTA, or heparin were used as an anticoagulant. Plasma was quickly separated with less than 30 minutes on ice to ensure optimal recovery and minimal platelet contamination. Centrifugation was done for10 minutes at 4°C to remove any particulate. This HIVsAg ELISA Kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, such as sodium citrate, EDTA or heparin. Serums and plasma samples were always stored at -20°C to avoid loss of bioactivity and contamination.

ELISA was done on blood using the kit which was obtained from all patients by Erba Lisa assay method. The test was performed to detect antibodies against HIV antigen using commercially available kits according to manufacturer's instructions. Serum dilution was done of 1:100 in the assay. Positive and negative control sera were also included along with test sera for the ELISA analysis. In order to determine the level of antibodies in the serum, the curve was created by plotting the OD values of different reference sera. Thereby, the concentration of specific antibodies in test serum sample was studied by extrapolating the OD value of serum.

HIV Real-TM Quantification:
DNA was extracted from plasma using the phenol, chloroform, and isoamyl alcohol (25:24:1) extraction method as described by the manufacture instruction guide (Life technologies, India). For the quantitative detection of HIV from the patient's samples, commercial Real-TM Quant kit was used. HIV DNA is extracted from plasma amplified the specific region of HIV using real time amplification using Forward primers 5′ ACA GGA CAC AGC AAT CAG GTC 3′ and Reverse Primer 3′ GCT CTG GTC TGC TCT GAA GAA 5′ was performed based on the manufactured guidance. Internal Control (IC) serves as an extraction and an amplification control for each individually processed sample and to identify possible inhibition. The assay of fluorescence intensities during Real Time process which allows the detection and quantification of the accumulating product without having to re-open the reaction tube after the real time amplification.

Statistical Analysis
All statistical analyses of each experiment were performed using the student's paired t test or fisher exact probability test. The descriptive statistics which including the frequency, mean value, median, and the standard deviation (SD) were determined.

RESULTS

We made a comparative study of among the 35 sera from the patient samples were studied the sensitivity of the quantitative PCR (qPCR) with the ELISA method used routinely for the detection of HIV infected patient samples. Various age group of people from 15 to 55 were analyzed the sensitivity. Interestingly, as showed in figure.1, all

_____

those with ELISA reactivity were positively confirmed by qPCR. Thus, we conclude qPCR is more sensitive and specific than the ELISA method.
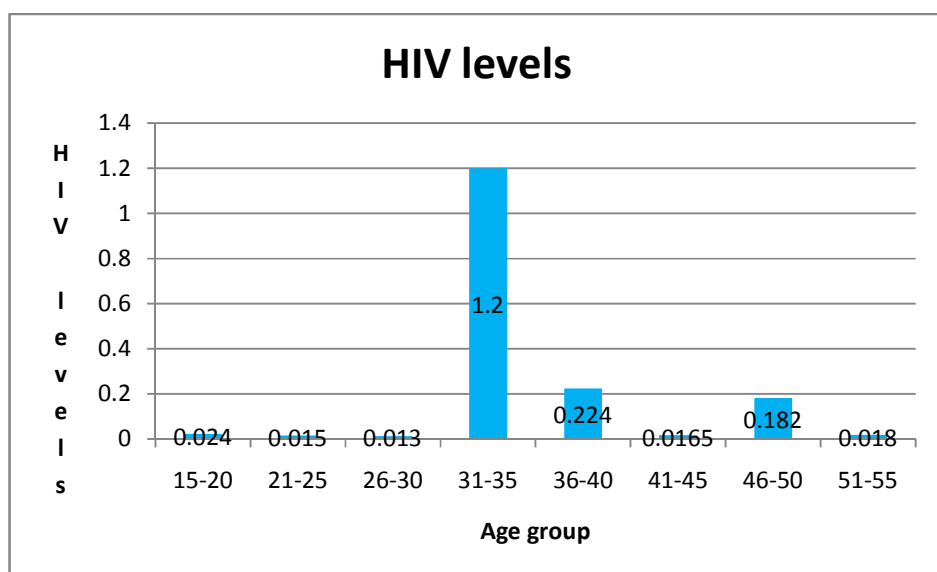

Figure.1: Graph showing the HIV values of different age groups. All the values are means of the triplicates

Healthy people in our community were tested both quantitatively and qualitatively. Our result shows that the significant ELISA values are in the age group of 31- 35 as shown in the figure 1, the Real time PCR confirms of the values of the ELISA, at the same time the Ct value of the gene amplified showed significant values in the age group 31-35, which confirms the readings of the ELISA.

Table.1: Table showing comparative values of the ELISA readings and Real time PCR Ct values of the HIV levels

Age group	HIV value	Ct value	Copies
15-20	0.024	Nil	
21-25	0.015	38	868
26-30	0.011	Nil	
31-35	1.2	28	112868
36-40	0.224	37	4638
41-45	0.022	Nil	
46-50	0.182	36	7535
51-55	0.018	Nil	

As shown in the table 1, there was significant amplification at 28th cycle in which the gene copies reaching up to approximately 112868 with the age group between 31-35. The HIV gene also showed amplification in the age groups 21-25, 36-40 and 46-50 where the ct values confirm the gene amplification and shows that the experimental samples were positive for HIV infection. These are the confirmatory readings for the ELISA.

DISCUSSION

The majority of the people with acute HIV infection are asymptomatic and about 80% of them develop chronic infection [10, 11]. Of them, about 15 to 20% of patients develop at a variable rate to cirrhosis, with a 1 to 4% annual risk of developing hepatocellular carcinoma [12, 13, 14]. The finding of HIV genome in 1989 led to the development of serological and molecular assays for viral HIV [15]. ELISA was associated with 50% to70% of false positive results among low-risk blood donors and in the presence of hyperglobulinemia [16]. However, the reverse-transcriptase polymerase chain reaction (RT-PCR), which detects HIV RNA directly, is considered the gold standard for the diagnosis of HIV infection.

_____

Thus, the current study, which studied the effectiveness of RT qPCR compared to ELISA, found that RT PCR results confirm the values of the ELISA which showed a significant value at the age group of 31- 35. There was amplification at 28th cycle with the gene copies reaching up to approximately 112868. The HIV gene also showed amplification in the age groups 21-25, 36-40 and 46-50. The ct value confirms the gene amplification and shows that the experimental samples were positive for HIV infection. These are the confirmatory readings for the ELISA.

CONCLUSION

The current study demonstrated a good sensitivity and specificity of RT qPCR compared to the ELISA test for the detection of HIV virus. As these techniques have been performed in a small number of samples for the early detection of HIV infection, the study recommends further studies involving large number of samples to confirm the sensitivity of qPCR i.e. probe based qPCR when compared to ELISA.

REFERENCES

[1] WHO **2013**. http://www.who.int/mediacentre/factsheets/fs360/en/
[2] WHO **2007**. http://www.who.int/mediacentre/news/releases/2007/pr37/en/
[3] World Health Organization. Scaling up priority HIV/AIDS interventions in the health sector-Progress report, **2009.**
[4] Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, et al. *Ann Intern Med***1997**; 126:946–54.
[5] van Oosterhout JJG, Brown L, Weigel R, Kumwenda JJ, Mzinganjira D, Saukila N, et al. *Trop Med Int Health* **2009**;14:856–61.
[6] Usdin M, Guillerm M, Calmy A. *J Infect Dis* **2010**;201 (Suppl 1):S73–7.
[7] Penezina O, Krueger NX, Rodriguez-Chavez IR, Busch MP, Hural J, Kim JH, O'Connell RJ, Hunter E, Aboud S, Higgins K, Kovalenko V, Clapham D, Crane D, Levin AE. *Clin Vaccine Immunol*, **2014** Mar; 21(3):391-8
[8] Bagheri R, Rabbani B, Mahdieh N, Khanahmad H, Abachi M, Asgari S. *Bull Soc Pathol Exot,* **2013** Oct;106(4):258-63.
[10] Janssen RS, Satten GA, Stramer SL, et al. *JAMA*, **1998**;280: 42–8.
[11] Joint United Nations Programme on HIV/AIDS (UNAIDS). Global Summary of the HIV/AIDS Epidemic, December **2003**.
[12] Wyndham-Thomas C, Delforge M, Mulkay JP, De Wit S. *Acta Clin Belg*, **2013** Sep-Oct;68(5):349-55.
[13] Montes Ramírez ML, Miró JM, Quereda C, Jou A, von Wichmann MÁ, Berenguer J, González-García JJ, Hernando A, Ortega E, Sanz J, Arribas JR. *J Acquir Immune Defic Syndr*, **2014** Jan 1;65(1):82-6.
[14] Di Benedetto N, Peralta M, Alvarez E, Schroder MT, Estepo C, Paz S, Fainboim H. *Ann Hepatol*, **2013** Jan-2014 Feb;13(1):38-44.
[15] Bartlett JG. Serologic tests for the diagnosis of HIV infection. Accessed February 1, **2003**.
[16] Uko GP, Griffiths M, Dawkins RL, Cobain T, Mohammed I, Hedo C, Okafor G, Umotong AB. *Afr J Med Med Sci,* **1994** Dec;23(4):385-9.