Available online at http://www.ijcrls.com

International Journal of Current Research in Life Sciences
Vol. 07, No. 03, pp.1281-1284, March, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

MONITORING OF VIRAL LOAD WITH ROCHE COBAS TAQMAN RT-PCR AND ITS RELATION TO OTHER FACTORS IN HIV PATIENTS

[1]Hemangi Dixit, [1]Shiva V. Murarka, [1]Khushbu R Patel, [1]Divyang Upadhyay, [1]Bhavini Shah and [*,1,2]Mandava V. Rao

[1]Department of Molecular Genetics, Supratech Micropath Laboratory and Research Institute, Ahmedabad, India
[2]Ex-Director, School of Sciences, Gujarat University, Ahmadabad, India

Received 29th January, 2018; Accepted 22nd February, 2018; Published Online 30th March, 2018

ABSTRACT

A number of 381HIV-I patients referred to our diagnostic laboratory during 3 yearswere evaluated with respect to viral load using COBAS Taqman RT-PCR method. The viral load of these patients were correlated with CD4 count, CD4 and CD8 ratio, age and gender for their better understanding of severity of disease, viral resistance and antiretroviral therapy (ART). The data revealed that with this Taqman, the minimum viral load detected was <47 Copies/ml. However, our patients (298/381) viral load ranged from <47 to 1×107C/ml in 298 cases (78.21%). Among four age groups, 21-60 year aged cases were affected maximum with a dominance of males. Similarly, cell count also showed similar trend and could be correlated to active participation of males in daily activities like sex, life styles etc. The ratio of male to female was 2:1. Hence, our experience conclude that Taqman method is better than other older techniques with attractive cost and better understanding of the disease and its treatment in future with minimum viral load detection and also matched with other correlates.

Key words: COBAS RT-PCR, Viral Load, Sex, Age, Cell Counts, HIV-I Patients.

Citation: Hemangi Dixit, Shiva V Murarka, Khushbu R Patel, Divyang Upadhyay, Bhavini Shah, and Mandava V Rao, 2018. "Monitoring of viral load with roche cobas taqman Rt-PCR and its relation to other factors in HIV patients" *International Journal of Current Research in Life Sciences*, 7, (03), 1281-1284.

INTRODUCTION

Plasma RNA quantification of viral load (VL) assay of human immunodeficiency virus (HIV-I) is one of the parameters for HIV cases and understanding of HIV-I pathogenesis. Its levels and absolute CD4 – T lymphocytes are critical diagnostic tests for guiding patient care decisions in addition to immune methods (Gupta *et al.,* 2009; Hammer *et al.,* 2009; Hirsch *et al.,* 2008; Korenromp *et al.,* 2009).Viral load measurements of patients have been done using RT-PCR of different varieties to assess HIV severity(Church *et al.,* 2011;Patel *et al.,* 2017; Gatanaga *et al.,* 2009). On detection of HIV Type-I load by use of Roche COBASTaqman assay was reported with viral load previously undetectable by Roche COBASAmplicor Monitor. Thus, they found Roche COBASTaqman assay wasbetter than Amplicor Monitor having sensitivity of <47 copies/ml. Nkeze *et al.,* 2010 obtained that COBAS and Abbotts m2000 methods showed strong correlation in measuring VL within their testing dynamic range (Erali *et al.,* 1999; Perrin *et al.,* 2006). However, due to cost constraints, the VL is not frequently requested for patients monitoring in countries with limited resources.

***Corresponding author:** [1, 2]**Mandava V. Rao,**
[1]Department of Molecular Genetics, Supratech Micropath Laboratory and Research Institute, Ahmedabad, India.
[2]Ex-Director, School of Sciences, Gujarat University, Ahmadabad, India.

The recommendation of WHO advocated to such countries clinical data and CD+ counts (Erick *et al.,* 2014). Clinical evaluation of CD4 count alone is not sufficient to establish proper monitoring of patients for ART and it is one of the causes of poor evaluation of treatment failure and the calamitous treatment monitoring of patients in countries with limited resources(WHO,2006; Boyer *et al.,* 2013). However, we report here the use of Roche Taqman method for monitoring of viral load and its correlation with CD4+ counts and other factors to understand HIV condition in patients infected in Gujarat.

MATERIALS AND METHODS

Patient Selection: Three hundred and eighty one referral cases for HIV ranging in age from 0 to above 60 years were admitted in our study after filling their consent forms and formal information about HIV infection. The study duration was 3 years i.e. 2014 to 2016. The study protocol was confirmed by the Human Ethical Committee of Gujarat University (GUHEC/001/2015). Blood from these patients was drawn for this study.

Viral RNA Isolation: Isolation of viral RNA was done using the High Pure COBAS® TaqMan® HIV-1 Test, v2.0 as per

manufacturer's instructions for patient samples and similarly for controls which comes with the kit (Roche Molecular Diagnostics, USA).

Age groups: All patients referred to our clinic were divided into four groups i.e. 0-20, 21-40,41-60 and >60 years for both the sexes.

Cell Counts (CD4+/CD8+): CD4+ and CD8+ lymphocyte counts were done using Flow Cytometry (FACSCAN). Percent ratio of these cells were also calculated in all age groups (0-20 to >60 years).

Viral Load: Viral load in each age group was done and expressed as $\log_{10}$ IU/ml or Copies/ml. It was also monitored during last three years i.e. 2014 to 2016.Roche COBASTaqman method was applied using Taqman kits on COBAS Taqman -48 v2.0.

Statistics: Data were analyzed for statistical analysis using ANOVA and percentage.

RESULTS

Age groups and sex: The age group of 41-60 years had maximum number of cases (159/298; 53%) followed by 21-40 age group (108/298;36%) and others (9% and 2% for above 60 years and 0-20 years). The gender ratio from male to female was 2:1(Table-1)

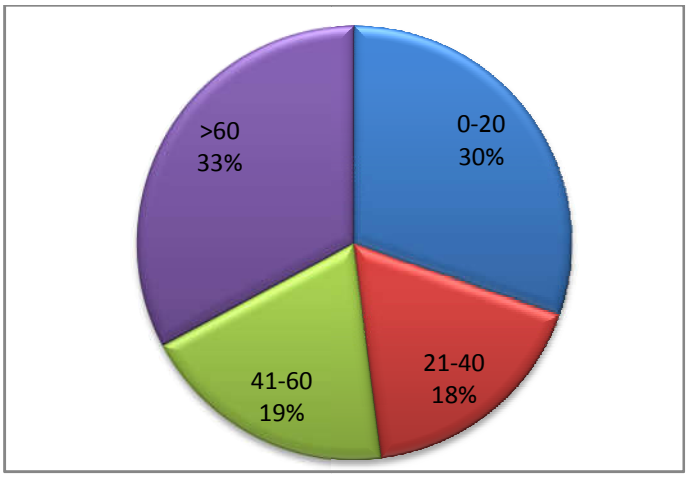

Fig. 1. CD4/CD8 ratio % with age groups

Duration vs Patients: Maximum number of cases has registered in age of 41-60 years (53%) and 21-40 years (37%) followed by old age and young groups 9% and 2% respectively (Table- 1). In all 3 years, higher number of cases was obtained in 2015 in all age groups comparatively (Fig.3).

Viral load: The viral load was higher in 41-60 age groups (215092 copies/ml) comparatively ranging from 19474 to 215092 C/ml in all age groups. Out of 298, VL of <47 copies/ml was in 136 (45.6%)and 10^4-10^5 C/ml in 53 (17.78%) cases followed by 35 (11.74%) with viral load of 10^5-10^6 C/ml and 24 (8.0%) with 10^3-10^4 C/ml(Fig.2).

Table 1. Gender, Patient & Viral load and CD4/CD8 ratio in four age groups

Age group (Yrs)	Gender		Total (%)	Viral load	CD4+	CD8+	CD4/CD8 RATIO % (0.6-1.5)
	Female (F)	Male (M)		C/ml	Count (%) (30-50)	Count (%) (10-35)	
0-20	6	0	6 (2%)	19474	32.33	44.66	0.76
21-40	50	58	108 (36%)	142328	20.89	51.88	0.44
41-60	43	116	159 (53%)	215092	19.92	47.72	0.48
>60	7	18	25 (9%)	182746	19.56	44.53	0.82
TOTAL	106	192	298 (100%)	-	-	-	-

M: F: 2:1: Numbers in parenthesis indicate percent.; **Referral cases:** 381; **Detected cases:** 298; **Percent:** 298/381=78.2%

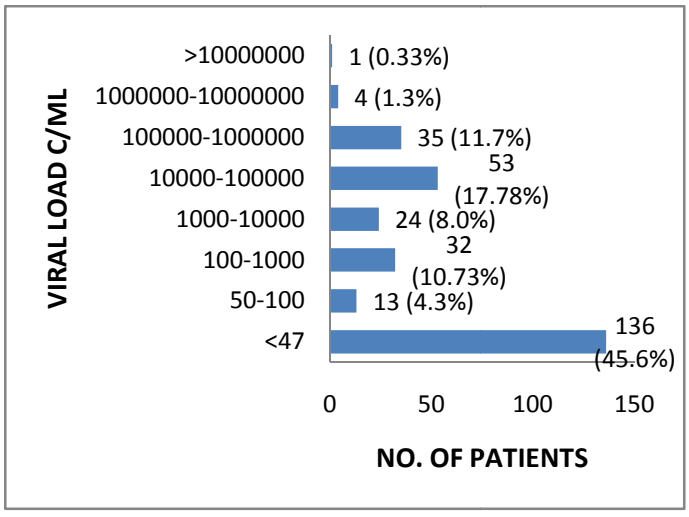

Fig. 2. Viral load vs Patients: Figs. in parentheses indicate percent (%)

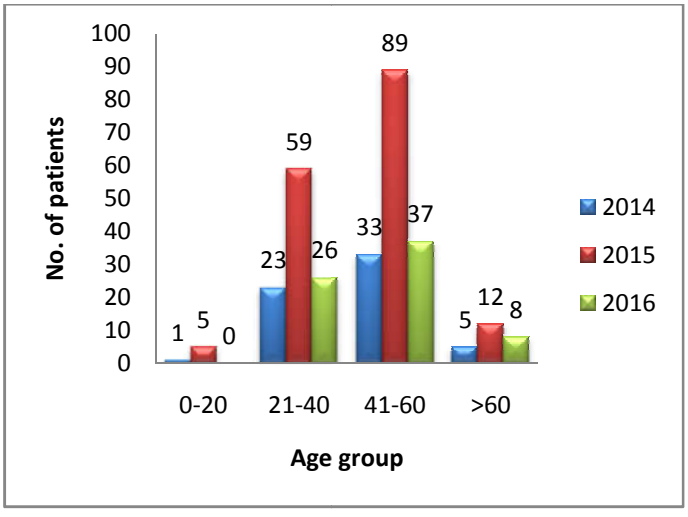

Fig. 3. Year wise distribution of patients with age groups

CD4+ and CD8+ counts: CD4+ and CD8+ ratio and the percent ratio was lower in 21-40; 41-60 age groups and as their CD+ percent was decreased in same age groups. Contrarily other groups had higher values (Table 1; Fig1)

Higher viral load (1× 10^7 C/ml) had one patient and 10^6-10^7 C/ml with 4 cases. The COBAS Taqman kit thus possessed MVL of <47 C/ml with higher viral load of >1× 10^7 C/ml (Fig.2).

DISCUSSION

The aim of this study was to investigate minimum viral load of HIV-I in attending our diagnostic laboratory since 3 years for ART and status of HIV-I in Gujarat state. The severity of this disease was also attempted to overall comparison with CD4+ counts, age, gender and other STDs in addition to other clinical indices (WHO, Geneva, 2010). Our study contained referral cases of 381 from which 298 detected positive for HIV-I infection (78.2%). Age is one of the factors that affected the seventy of the disease. It is evident from our data that the 41-60 year age group had higher viral load followed by 21-40 and above 60 years group. The infected patients in our study also maintained same pattern. Such studies were reported by other workers (Mehta *et al.,* 2009;Erick *et al.,* 2014;Kamangu *et al.,* 2015;Patel *et al.,* 2017) using COBAS Taqman and In- house RT-PCR methods. Similarly gender is one of the factors that increases the HIV-I infection as noticed in our study. We found males are maximally affected by it, where the ratio was near to 2:1 in our study. Same way, the gender ratio obtained by Patel *et al.,* (2017) from males to females was 2.4:1 in their cohort using RT-PCR method as they exposed to free environment and free sex. This was further supported by other investigations in India who observed high sex difference in their study cohort (Kamangu *et al.,* 2015; Mendiratta *et al.,* 2004; Gharami *et al.,* 1999; Khandpur *et al.,* 2001).

The higher sex difference between male to female is explained by the fact that in female, the social stigma and discrimination prevent the woman to seek help of STD clinic facility. Further, the higher male preponderance is due to more freedom, they enjoy in the society and also higher degree of promiscuity exists among them (WHO, 2006; 2010; Kavina *et al.,* 2005; Brown *et al.,* 2005) along with other sexually transmitted diseases (STDs) like Herpes, Gonorrhea etc. Further, CD4 cells are the targets of HIV-I. In our study cohort the percent CD4+ cells were declined in all age groups being the highest in 41-60 year age group comparatively. This gives a clue that in this cohort CD4+ ratio are correlated with loss of immune functions of T_H cells. This is further supported by decline in percent ratio of CD4+/CD8+cells in our report. Such patients were suitable for ARTS (Ngo-giang-huong *et al.,* 2008; WHO, 2010; Boyer *et al.,* 2013). In our cohort, we followed Roche COBAS Taqman method with RT PCR version-1which gives better results than In-House RT PCR with a minimum viral load limit of <47C/ml and low cost effectiveness in comparison to other methods like Abbott m2000 and others. Hence, this Roche COBAS technique is better for sensitivity and precision with moderate cost but definitely economical to Abbott m2000 and other methodologies.

Conclusion

We conclude that Roche COBAS Taqman method is minimum as cost is concerned and for better results, though many diagnostic laboratories follow In-House RT-PCR. The viral load data, so obtained were correlated with other factors to support disease severity and use for anti-retroviral treatment (ART).

Acknowledgment: We acknowledge, for support and assistance rendered from all Clinicians and non-Clinical staff of Supratech labs, Ahmadabad, India.

Conflict of interest: No conflict of interest is expressed by authors.

REFERENCES

Boyer S, March L, Kouanfack C, et al. 2013. Monitoring of HIV viral load, CD4 cell count, and clinical assessment versus clinical monitoring alone for antiretroviral therapy in low-resource settings (Stratall ANRS 12110/ESTHER): a cost-effectiveness analysis. *Lancet Infect Dis.,* 13(7):577-576.

Brown BK, Darden JM, Tovanabutra S, et al. 2005. Biologic and genetic characterization of a panel of 60 human immunodeficiency virus type 1 isolates, representing clades, A, B, C, D, CRF01_AE, and CRF02_AG, for the development and assessment of candidate vaccines. *J Virol.,* 79(10):6089-6091.

Church D, Gregson D, Lloyd T, Klein M, Beckthold B, Laupland K, et al. 2011.Comparison of the RealTime HIV-1, COBAS TaqMan 48 v1.0, Easy Q v1.2, and Versant v3.0 assays for Determination of HIV-1 Viral Loads in a Cohort of Canadian Patients with Diverse HIV Subtype Infections, Amersoc microbial; 1;118-124

Erali M, Hillyard DR, 1999. Evaluation of the ultrasensitive Roche Amplicor HIV-1 Monitor assay for quantitation of human immunodeficiency virus type 1 RNA, *J Clin Microbiol.,* 37:792-795.

Erick Ntambwe Kamangu, Adawaye Chatte, Raphael Boreux, Richard Lunganza Kalala, et al.2014. Implementation of an In-House Quantitative Real-Time PCR for Determination of HIV Viral Load in Kinshasa. Open access lib J.1: e 855.1-5.

Gatanaga H, Tsukada K, Honda H, Tanuma J, Yazaki H, Watanabe T, *et al.,* 2009. Detection of HIV Type 1 Load by the Roche CobasTaqMan Assay in Patients with Viral Loads Previously Undetectable by the Roche Cobas Amplicor Monitor, CID 48 (15) 260-61 https://doi. org/10.1086/595707.

Gharami, R.C., Garg, B.R., Thappa, D.M., Hemanth, R.H. et al.1999. Profile of sexually transmitted diseases in HIV seropositive patients. *Indian J Sex Transm Dis.,* 20(2): 12-17.

Gupta RK, Hill A, Sawyer AW, Cozzi-Lepri A, von Wyl V et al.2009. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. *Lancet In-fect. Dis.,* 9:409-417.

Hammer SM, Eron JJ Jr, Reiss P, Schooley RT, Thompson MA, Walmsley S., et al.2009. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel; *JAMA,* 300:555-570.

Hirsch MS, Gunthard HF., Schapiro JM., Brun-Vezinet F., Clotet B., Hammer SM., et al. 2008. Antiretroviral Drug Resistance Testing in Adult HIV-1 Infection: 2008 Recommendations of an International AIDS Society–USA Panel. Top. HIV Med., 16:266-285.

Kamangu, E N., Chatte A., Boreux R., Susin F., Kalala RL., Mvumbi G L., et al. 2015. Comparison of an In-House Quantitative Real-Time PCR and COBAS AmpliPrep/TaqMan Roche for Determination of Viral Load for HIV Type 1 non-B. *Open Access Journal of Urology*. 2. e1402 http://dx.doi.org/10.4236/oalib.1101402. 2015

Kavina B.K, Billimoria F.E., Rao M.V, 2005. The pattern of STDs & HIV seropositivity in young adults attending STD clinic of civil hospital ahmedabad. *Indian J Sex Transm Dis.,* 26 (1): 60-63.

Khandpur, S., Agrawal, S., Kumar, S, Sharma, V.K. Reddy, BSN et al.2001.Clinico epidemiological profile and HIV seropositivity of STD patients. *Indian J Sex Transm Dis.,* 22(2): 62-65.

Korenromp E.L., Williams BG, Schmid GP, and Dye C, 2009. Clinical prognostic value of RNA viral load and CD4 cell counts during untreated HIV-1 infection--a quantitative review; PLoS One 4:e5950.

Mehta N, Trzmielina S, Nonyane BA, et al. 2009. Low-cost HIV-1 diagnosis and quantification in dried blood spots by real time PCR. PLoS ONE ;4(6): e5819,1-10.

Mendiratta, V., Koranne, R.V. and Bhawna H.,2004. Profile of Sexually transmitted infections in HIV Positive Patients - A retrospective study. *Indian J Sex Transm Dis.,* 25 (1): 18-21.

Mitui M, Leos NK, Lacey D, Doern C, Rogers BB, Park JY, 2013. Development and validation of a quantitative real time PCR assay for BK virus. *Mol Cell Probes,* 27 (5-6):230-236.

Ngo-giang-huong N, Khamduang W, Leurent B, et al. 2008. Early HIV-1 diagnosis using in-house real-time PCR amplification on dried blood spots for infants in remote and resource-limited settings. *J Acquir Immune Defic Syndr.,* 49(5):465-471.

Nkeze J, Liang D, Adkins H, Zhao RY.2010. Comparison of HIV-1 Viral Load between Abbott m2000 and Roche COBAS TaqMan Methods. *J Antivir Antiretrovir,* 2(3):42-45.

Patel KR, Murarka SV, Dixit H, Shah BS, Rao MV, 2017.Triannial experience with HIV-I viral load detection using In-House RT-PCR with other correlates. EJBPS; Dec. Issue in process.

Perrin L, Pawlotsky J M, Alias M, Sarrazin C, Zeuzem S, Colucci G. Multicenter,2006. Performance Evaluation of a New TaqMan PCR Assay for Monitoring Human Immunodeficiency Virus RNA Load, *J. Clini. Microbiol.,* 44, 4371-4375.

Steegen, K., Luchters, S.S., De Cabooter, N., Reynaerts, J., Mandaliya, K., Plum, J., Jaoko, W., Verhofstede, C. and Temmerman, M, 2007. Evaluation of two commercially available alternatives for HIV-1 viral load testing in resource-limited setting. *Journal of Virological Methods,* 146, 8-187. http://dx.doi.org/10.1016/j.jviromet.2007.06.019.

World Health Organization (WHO) Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach (2010 Revision). World Health Organization, Geneva. www.who.int/publications/2010/9789241599764 _ eng.pdf. 2010.

World Health Organization (WHO) Towards Universal Access by 2010. Hosw WHO Is Working with Countries to Scale-Up HIV Prevention, Treatment, Care and Support. World Health Organization, Geneva. www.who.int/hiv/ universalaccess. 2006.

****