TRIANNIAL EXPERIENCE WITH HIV-I DETECTION USING IN-HOUSE RT-PCR WITH OTHER CORRELATES

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ABSTRACT
A total of 500 referred cases for HIV detection was screened using In-House Real-Time (RT) PCR for their viral load from extracted RNA in our laboratory. Only 136/500 detected positive. The data further revealed the minimum viral load (MVL) was 100 C/ml in our study cohort. The age groups of both sexes detected for HIV positivity, the viral load ranged from 49700 to 7337902 C/ml. Age groups i.e. 20-40 and 41-60 years were maximally affected with a gender ratio of 2.4:1 in male and females respectively. The CD4/CD8 ratio also remained higher in these age groups. The infection in these groups was correlated with their daily activities and other socio-economic status in males. Thus, in-house quantitative method with RT-PCR detection as one of the suitable techniques for HIV detection which is cost effective to HIV infection for anti-retroviral treatment (ARTS) in many diagnostic laboratories.


INTRODUCTION
Quantification of viral load (VL) of HIV-I in blood is very crucial for the disease monitoring and management. [1,2,3] One of the methods used in all diagnostic laboratories is RT-PCR using suitable kits. [4,5] The quantification of HIV is also supported by other cellular counts like CD4+ and CD8+ and their ratio as CD4+ cell is the target in the HIV in addition to immune based methods or serological assays. [6,7,8] The infection is dependent on many factors, like age, gender, economic status, education etc including STDS. The factors that induced HIV infection in relation to other factors is well documented by many researchers, recently. [9,10,11] Several laboratories now only on PCR based methods were used for quick detection of HIV in addition to other techniques. [12,13,14]

In our study we proposed PCR based method using Taqman probes for standardization and identification of HIV cases to monitor VL. Further, we also evaluated immune cells in relation to other socio-economic status of the patients in Gujarati population.

MATERIALS AND METHODS
Patients
Five hundred referral cases ranging in age from 0 to 60 above 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 is confirmed by the 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 kit of Automated Perkin Elmer Viral RNA/DNA or QIAamp® Viral RNA mini kit (Qiagen, Germany) as per manufacturer’s instructions for standardization and similarly, same method was used for patient samples.

Standard Curve
For initial standardization, in house plasmids with HIV-I infection were used (Concentration of 9.7×10^5 copies/ml). The HIV Taqman assay probe (Applied Biosystems, USA) was used for Real Time based PCR. It was prepared by amplification of 20µl mixture containing 1X Roche ROX Universal PCR Master Mix, 1ul of HIV Taqman assay probe, 2ul Express Super Script mix and 2.6ul of extracted RNA. As an internal control, we used the Taqman Exogenous Internal Positive Control Reagents kit with 1X IPC Mix (Primers and Taqman probe labeled with VIC) and 1X IPC DNA. All reagents were obtained from Applied Biosystems (Foster City, CA, USA) and cycled according to following instructions: 50°C for 15min, 95°C for 10min, followed by 45 two-step cycles of 95°C for 15sec and 60°C for 60 sec and a final extension at 37°C for 30sec. The HIV viral load was measured as the
copies/ml of RNA (ABI 7500 real-time PCR system). The graph was plotted with the help of HIV standards in duplicates and for HIV viral load detection with standard curve includes.

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

Cell Counts (CD4+/CD8+)
CD4+ and CD8+ lymphocyte counts were done using Flow Cytometry (FACSCAN). 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 \( \log_{10} \text{IU/ml} \) or C/ml. It was also monitored during last three years i.e. 2014 to 2016 with statistics.

RESULTS
Standard Curve
It indicated minimum viral load (MVL) of 100 c/ml with standard R value 0.993, Y-intercept 42.928 and slope -3.298 (Fig. 1). Reproducibility showed that the CV% obtained after the intra assay validation is very low around 3.31%, 1.08%, 1.67% and 1.93% and the similar way the inter assay validation CV% is 3.31%, 1.08%, 1.67% and 1.93% for dilution 1to 4 respectively.

The assay with in-house plasmids showed 100% specificity while did not cross react with any interfering pathogens.

Age groups and sex
The aged groups of 41-60years had maximum number of cases (84%) followed by 21-40year age group (43%). Sex ratio of male to female was 2.4:1 respectively (Table 1.).

CD4+ and CD8+ count
CD4+/CD8+ percent ratio was in 41-60yr.age group followed by 21-40 age groups. Where maximum number of case were detected (Table 1, Fig.4).

A total of 136/500 was found detected HIV positive (0.27%).

Time Vs Patients
Year wise distribution of patients revealed the HIV cases were higher in 41-60-year age groups in three years i.e. 2014-2016 (Fig.3).

Viral Load
The viral load ranged from 29235 to 4316412 c/ml. maximum cases (52) were within 10000 to 100000c/ml (Table 1, Fig. 2).

![Standard Curve](image)

Fig. 1: HIV viral load detection done with HIV standards in duplicate.
**Fig: 2** Detection of viral load by RT-PCR

![Viral Load Distribution Chart]

**Fig: 3** Year wise distribution of the HIV cases in age groups.

![Age Group Distribution Chart]

**Fig: 4** CD4/CD8 ratio Vs Age groups

![CD4/CD8 Ratio Pie Chart]
Table 1: Gender, Patient & Viral load and CD4/CD8 ratio in four age groups.

<table>
<thead>
<tr>
<th>Age group (Yrs)</th>
<th>Gender</th>
<th>Total</th>
<th>Viral load C/ml</th>
<th>CD4+ % (30-50)*</th>
<th>CD8+ % (10-35)*</th>
<th>CD4/CD8 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>2(3%)</td>
<td>1(1%)</td>
<td>3(2.2%)</td>
<td>29235</td>
<td>16</td>
<td>58</td>
</tr>
<tr>
<td>21-40</td>
<td>11(27.5%)</td>
<td>32 (33.3%)</td>
<td>43(31.61%)</td>
<td>1636347</td>
<td>13.91</td>
<td>53.58</td>
</tr>
<tr>
<td>41-60</td>
<td>24(60%)</td>
<td>60(62.5%)</td>
<td>84(61.76%)</td>
<td>1349019</td>
<td>14.75</td>
<td>55.89</td>
</tr>
<tr>
<td>&gt;60</td>
<td>3(7.5%)</td>
<td>3(3.12%)</td>
<td>6(4.41%)</td>
<td>2752163</td>
<td>13.16</td>
<td>56</td>
</tr>
<tr>
<td>TOTAL</td>
<td>40(29.4%)</td>
<td>96(70.6%)</td>
<td>136(100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Normal range: Abs CD4+:400-1500 CELL/CUMM Abs CD8+:200-1100 CELL/CUMM

*Percent ** Ratio percent

DISCUSSION

The aim of the study was to validate and standardize the in-House RT-PCR for assessment of VL in patients referred to our laboratory for ART in addition to CD+ counts, the age and gender ratio were also done. The in-House RT-PCR revealed minimum viral load detected was up to 100c/ml cases out of 500 cases only 136 were detected more than minimum level of viral load by our PCR which is less expensive and reliable. Most of these patients belong to 21-40, 41-60 years followed by other ages.

Similarly, other studies were done by[9,10] to support our studies. This could be related to their active participation in daily activities and their exposure to environmental factors in this cohort. Same results were reported by Erick et.al[10] who documented 26-55 years had higher infection than other age groups in Kantaka. In India Kavina et.al.[9] reported mid aged groups were more infected with HIV-1 to support our report. This is further supported by their decreased CD4+ counts and elevated CD4+/CD8+ percent ratio which were related to the severity of the infection and immunity reduction.

Thus, serology assays and CD4+/CD8+ percent ratio also related with viral load and age in support of our data (Kavina et.al.[9,11]) The gender ratio in our study was 2.4(M):1(F) to indicate that males are more affected as they expose to environment and free sex, as reported by other workers[16,17] who reported 3.45:1; 3.5 respectively between man and woman in India. High sex differences between male to female (5.1) were noted by Khandpur et.al.[18] Large number of cases in males in comparison to females is explained by the fact that in females the social stigma and discrimination prevent the woman to seek help of STD clinic facility. Further the high male preponderance is due to more freedom they enjoy in the society and also higher degree of promiscuity exists among them.[19,5]

The CD4+ and CD8+count ratio or CD4+ counts are further related to infection status of the HIV in addition to immunological basis were in use for monitoring HIV disease earlier. But VL is the one which is cost effective with low cost reagents with specific level of detection about 100c/ml in a patient with minimum resource limited settings using RT-PCR in our study. Though other RT-PCR based Taqman, Digital PCR etc are available with moderate cost and accurate upto zero viral load level.[14,20,21,22] Hence, we conclude that viral load upto 100c/ml using RT-PCR in most of the diagnostic laboratories with minimum cost is better than other new techniques adopted and well related to other correlates in our study cohort.

CONFLICTS OF INTEREST

No conflict of interest in expressed by the authors.

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REFERENCES