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EVALUATION OF DBS HCV RNA QUANTIFICATION AND GENOTYPING IN RESOURCE-LIMITED SETTINGS

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BACKGROUND

HCV is a major global health issue affecting several individuals around the world. High burden of HCV infection in people who inject drugs (PWID) in resource-limited settings (RLS) like India coupled with low access to HCV services necessitates an urgent need for evaluating less invasive DBS based HCV virologic assay for appropriately tailored therapy. Here, we present an HCV plasma viral load (PVL) and genotyping assays using dried blood spot (DBS) specimens to increase the scale-up of HCV diagnosis and monitoring.

METHODS

Thirty six paired plasma and DBS specimens [Whatman No. 903 filter paper (50µL/circle)] were collected and stored at -70°C. Two spots were excised and then lysed with 1.7mL of Abbott mLysis buffer. The lysate was then transferred and the RNA was extracted from plasma and DBS manually as per Abbott 0.5mL protocol and subjected to RT PCR and the DBS results was multiplied by dilution factor (Johansen *et al*., 2016). In addition, patients with > 3 $\log_{10}$ IU/mL DBS HCV RNA were further subjected to in-house one-step core/E1 RT-PCR followed by Sanger's gene sequencing. All the values were log transformed and analysis was performed on GraphPad Prism 5.0, A $p<0.05$ was considered significant.

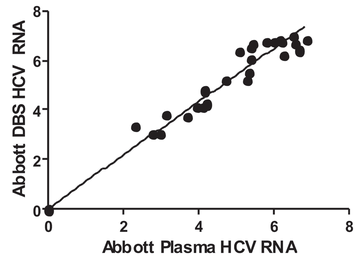

Figure 1: Linear regression comparing viral load results obtained from DBS and Plasma

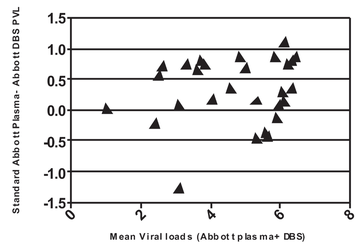

Figure 2: Bland-Altman plot for the average viral load values obtained from DBS and Plasma.

RESULTS

- Median PVL of standard plasma HCV and DBS HCV viral load by Abbott Real Time PCR were 4.45 (IQR 2.54-5.43) and 4.18 (IQR 2.24-5.74) $\log_{10}$ IU/mL, respectively. There was a good correlation between PVL values obtained by standard Abbott plasma and DBS assay (r = 0.97, r^2 = 0.94, $p<0.001$) (Figure 1).
- Bland-Altman analysis has shown that, the mean difference of HCV RNA between plasma and DBS was 0.25 $\log_{10}$ IU/mL and 95% limit of agreement was between -0.7321 to 1.23374 (Figure 2).
- Out of 36 samples, 21 (58%) [Median = 5.74, (IQR=4.96-5.91)] had > 3 $\log_{10}$ IU/mL HCV RNA in DBS, of which, DBS HCV genotyping was performed for 15 participants and the remaining 6 were not able to amplify. The result shows, majority of the infection was by genotype 3b (46.6%) followed by 6xa (33%) viruses (Figure 3).

Figure 3: Neighbor-Joining (NJ) phylogenetic tree of HCV Core/E1 sequences from DBS.

CONCLUSION

This study supports the use of DBS as an alternate to plasma specimen for the reliable quantification of HCV RNA and genotyping. Therefore, DBS could be a great interest for detecting and genotyping HCV viremic patients in resource-limited settings.

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