

MONITORING THE HIGHS AND LOWS OF CONGENITAL CMV.

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BACKGROUND

- Human cytomegalovirus (CMV) is the most common congenital infection in humans.
- Symptomatic infection causes significant morbidity and mortality in the newborn period.
- Long-term disability such as sensorineural hearing loss (SNHL) and neurodevelopmental delay may be prevented by treatment with antiviral medication in those with central nervous system symptoms at birth.¹
- Higher viral load and the presence of CMV in the blood at birth have been associated with increased risk of long-term sequelae.^{2,3}
- Although clearance of virus from urine during treatment has been associated with normal neurological outcome⁴ there are no published data relating decline in blood viral load with clinical outcome.
- Saliva has been used qualitatively in many studies but the natural history of salivary viral load and how it relates to other body fluids has not been described.
- Measurement of CMV viral load during treatment is often used in clinical practice.
- Being able to correlate CMV viral load with subsequent clinical outcome may inform future treatment strategies.

AIMS

- To assess the validity of testing salivary swabs for monitoring treatment in congenital CMV (cCMV).
- To observe and compare changes in viral load in different body compartments during treatment for cCMV.

METHODS

Subjects

- 8 babies enrolled into a prospective study of CMV Viral load and Immune response in Congenital CMV (VICC study) (blood, urine and saliva viral load).
- 4 babies enrolled into a CMV treatment registry (blood and urine viral load).
- 3 babies whose samples were received in our labs for clinical management.

	Total Number of Samples	Number of Babies	Mean Samples per Baby
Blood	103	15	6.8
Urine	108	15	7.2
Saliva	49	8	7.0

DNA extraction and Real-Time polymerase chain reaction (PCR)

- Total nucleic acid was extracted from 110uL blood or urine or 1mL of VTM from salivary swabs using the semi-automated easyMag® (Bioerieux, UK) system into 110uL PBS.
- Quantitative viral load was measured using Real-Time TaqMan PCR detecting a target within the glycoprotein B gene of HCMV (limit of detection 1 copy per 5uL input = 200 copies/mL).
- Salivary viral load was adjusted according to a weight-based estimation that the volume of saliva on a saturated swab was ~ 27uL.

Methods – Salivary swabs

- Flocked swabs (Copan Italia - distributed by Sterilin® in UK) were taken at least one hour after a breast feed by placing in a baby's mouth until fully saturated (~15 seconds).
- Swabs were replaced into their sterile plastic holder and sent without further processing.
- On receipt in the laboratory swabs were vortexed in 1mL viral transport medium (VTM) and nucleic acid extracted (see above).
- Preliminary results using swabs dipped into a solution of AD169 infected fibroblasts diluted with saliva showed that viral load detected in swabs was around 0.5-1.0 log₁₀ less than the neat solution (Fig 1 and 2).
- Viral load was ~ 0.5-1.0 log₁₀ less in swabs left more than 1 day before extraction but 5 days was comparable to 8 days (Fig 1).



RESULTS – Salivary Swabs

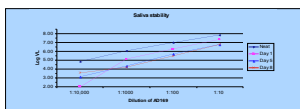


Figure 1 – Stability of salivary swabs over time
Swabs dipped into saliva spiked with AD169 infected fibroblasts and diluted to different concentrations. Swabs were left at room temperature and extracted either the following day (Day 1) or 5 and 8 days after initial preparation. For comparison the neat starting solution was also extracted and virus quantified.

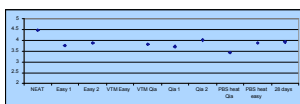
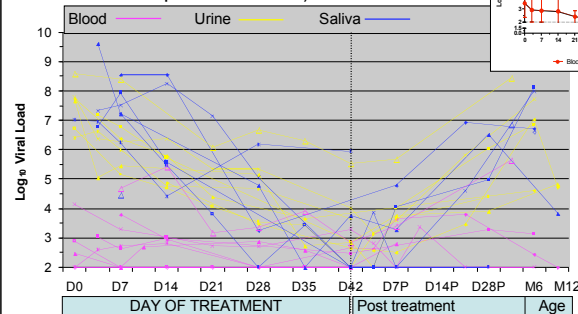


Figure 2
Viral load in 8 swabs analysed by Real-Time PCR following DNA extraction using varying different methods

Viral load is reproducible in swabs within ~ 0.5 log₁₀ but around 0.5 log₁₀ lower than starting solution.

RESULTS

Figure 3
Graph of viral load in blood, urine and saliva in 7 babies treated for congenital CMV. (Small box shows mean viral load and standard deviation at different time points in all 15 babies)

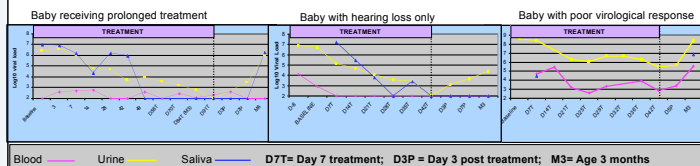


RESULTS

VIRAL LOAD	Day 0-7		Age 5-7 Months	
	Mean (SD)	No. of samples	Mean	No. of samples
Blood	3.62 (± 0.95)	21	2.52	3
Urine	7.49 (±1.01)	15	6.10	7
Saliva	7.28 (± 1.59)	7	7.52	5

- Blood viral load showed an initial rapid decrease with treatment but often then continued to be detectable at low levels until the end of treatment (Figure 3).
- Urine and salivary viral load decreased following a similar trend (Figure 3 and clinical examples below).
- At end of treatment virus was undetectable in- 4/12 (33%) blood samples available 2/10 (20%) urine samples available 3/5 (60%) saliva samples available
- At 5-7 months of age rebound in blood and urine was to a level ~1log₁₀ lower than starting viral load.
- Saliva rebounded to a similar level to baseline measurements.

CLINICAL EXAMPLES



CONCLUSIONS

- Despite a lower baseline, full suppression of viraemia at the end of a standard 42 day treatment course was seen in only 1/3 babies.
- Preliminary results show that saliva follows a similar trend to urine and may be a useful tool for monitoring viral response to treatment.
- Salivary swabs are easily taken by untrained staff or parents and potentially safer to send in the post than liquid specimens.
- The relevance of viral suppression in each of these body compartments and how they relate to VL in the central nervous system and clinical outcome are yet to be determined.
- Further data are needed in order to inform the development of evidence-based treatment regimes.

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