Reviewer's report

Title: Type specific Real time PCR for detection of human herpes virus 6 in schizophrenia and bipolar patients: a case control study

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Reviewer: Duncan Sinclair

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The manuscript of Dr Yavarian and colleagues investigates a possible link between psychotic mental illness and HHV infection. While it is generally well written, there are a number of areas in which improvement is necessary to increase the rigorousness of the study and its reporting.

Major compulsory revisions

1) As mentioned by a previous reviewer, the low number of individuals in whom HHV-6A/B-positive PBMCs were detected makes interpretation of any disease association difficult. This does not necessarily mean the study should not be published. However, the issue should be discussed thoroughly. In particular:
   a) The number of positive individuals should be provided in the abstract, and the lack of statistical power to identify diagnostic differences mentioned in the discussion
   b) The rationale for detection of viral DNA in PBMCs rather than whole blood or plasma should be provided in the manuscript, rather than in responses to the previous reviewer. It should also be described more clearly. Does HHV-6 DNA in PBMCs reflect persistent, low level active infection in tissue, even in the absence of HHV-6 DNA in plasma (this appears to be what is suggested)? If so, references would be helpful, particularly given that the study by Nitsche at colleagues (ref. 24) suggests that HHV-6 DNA measurement in PBMCs is less sensitive than in plasma, especially for HHV-6A since HHV-6A was undetectable in PBMCs but detectable in plasma.
   c) In general, explanation of the methods for investigating HHV-6 infection should be provided, along with clarification of which methods are specific for active infection or viral particles, and discussion of the different incidences of HHV-6 prevalence by different methods.

2) Better citations across the manuscript are required. Without having checked all references in the manuscript, it appears some do not support the claims being made or are not peer-reviewed. For example:
   a) Neither of the claims in the sentence ‘Human herpes virus 6 infects most infants less than 12 months of age and about 90% of the population is seropositive’ are supported by the provided reference (ref. 6), which is entirely inappropriate. This sentence must be removed or appropriate references for both claims provided.
b) By my reading, ref. 24 is not cited accurately or faithfully- 4% of patients (not healthy controls) were HHV-6A positive in PBMCs, and it is not mentioned that 88% percent of patients were HHV-6B positive in PBMCs (inconsistent with this study)

c) References 14 and 22 are not peer-reviewed manuscripts, and for ref. 22 a link to a PDF of an abstract from an undisclosed conference is provided, which may not be appropriate

d) Numerous relevant papers, for example (Achour et al., 2007; Caserta et al., 2010; Levy et al., 1990; Okuno et al., 1989; Tanaka-Taya et al., 1996, see below) are not cited.

3) It would be helpful to include a figure with the assay standard curves, to indicate the reliability/sensitivity of the assays.


**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests