

ORIGINAL ARTICLE

The seroprevalence and salivary shedding of herpesviruses in Behçet's syndrome and recurrent aphthous stomatitis

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Background: Behçet's syndrome (BS) is one of the multisystemic diseases that presents with oral ulceration and several other systemic manifestations including genital ulceration, folliculitis, erythema nodosum-like lesions, uveitis, and arthropathy. Ocular manifestation, central nervous system involvement, and gastrointestinal manifestation account for most of the complications of this disease, whereas orogenital ulceration and dermatological involvement affects the quality of life. The cause of the disease is not fully elucidated; however, herpesviruses have long been thought to play a pivotal role in the disease pathogenesis.

Objective: To investigate the seroprevalence and salivary shedding of herpesviruses in BS.

Method: The levels of specific immunoglobulin G in six different herpesviruses in serum samples collected from 54 BS, 28 healthy controls (HC), and 7 recurrent aphthous stomatitis (RAS) patients were investigated. Salivary viral load was also quantified for these viruses in matched saliva samples using quantitative real-time polymerase chain reaction.

Results: The BS had lower cytomegalovirus (CMV) IgG level in comparison to HC ($p = 0.0226$) and RAS ($p = 0.0450$). There was statistically significant higher salivary shedding of Epstein-Barr virus (EBV) in BS in comparison to HC ($p = 0.0052$), but not RAS ($p = 0.3318$).

Conclusions: A high EBV shedding was observed in both BS and RAS and a lower level of CMV IgG was observed in BS only. The reason for the observed lower level of CMV IgG in BS is not clear. However, one explanation might be a defect in the cross-talk between innate and adaptive immune responses which was suggested by a previously described defect in the toll-like receptor 1 and 2 heterodimer formation and function, this being the initial receptor sensing of CMV.

Keywords: *Behçet's syndrome; herpes; oral mucosa; saliva*

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Behçet's syndrome (BS) is a multisystemic, immune-related disease with complex aetiopathogenesis. The major clinical manifestations of BS are recurrent oral ulceration affecting 97–99% that classically precede any systemic features (1).

Risk of visual loss reaches 25% after 10 years of the BS ocular disease. Furthermore, severe central nervous system and pulmonary involvements, catastrophic bleeding from large vessels, bowel perforation, and complication of immunosuppressive therapy has been reported (2).

The aetiology of BS has not been completely elucidated. An infectious aetiology was postulated more than

50 years ago by Hulusi Behçet who suggested a viral cause for BS. Later, Denman et al. and then Eglin et al. have shown by *in situ* DNA–DNA hybridisation that at least part of the herpes simplex type 1 (HSV-1) genome is transcribed in mononuclear cells of some patients with BS (3–5). However, administration of high doses of acyclovir in association with plasma exchanges failed to produce positive treatment results and treatment with acyclovir alone failed to alleviate the frequency and severity of orogenital ulceration or other BS features in a randomised, double-blind, placebo-controlled crossover trial (6).

In other studies, the level of immunoglobulin G (IgG) antibody against HSV-1 was found to be significantly increased in patients who had BS but there was no HSV-1 DNA found in peripheral blood leukocytes and oral smears from the same cohort (7). Also, there was no statistically significant increase in HSV-1 DNA observed in the saliva of BS patients in comparison to healthy controls (HC) (8).

In addition to HSV-1, human cytomegalovirus (CMV) has been studied in relation to BS. CMV DNA was detected in biopsy specimens from the oral mucosa of BS patients (9). In another study, the mean titre of IgG and IgA antibodies to CMV were found to be significantly lower in BS patients than in HC. Interestingly, the number of patients having IgM antibody against CMV was similar between BS patients and HC (10). These studies examined BS cohorts from Taiwan and Korea; however, there is no study investigating the prevalence of CMV infection in BS patients from Western European countries.

Epstein–Barr virus (EBV) was also suggested as a potential cause of BS. EBV DNA was observed in the pre-ulcerative oral ulceration of patients with BS and recurrent aphthous stomatitis (RAS) in a very small cohort (four BS, five HC, and nine RAS) (11).

To the best of our knowledge, this study is the first statistically powered case-control study investigating the seroprevalence of almost all human herpesviruses in BS from a Western country. We also investigated the salivary viral shedding in the same cohort of patients. A small cohort of RAS was also investigated as a disease control as these patients experience recurrent oral ulceration similar to BS but they do not experience the other complex systemic manifestation of BS.

Materials and methods

The patient cohort was recruited from the outpatient departments at the Royal London Hospital and St. Thomas Hospital after ethical approval and informed written consent were obtained. Eighty-nine matched serum and saliva samples were collected from 54 BS (F/M: 35/19, mean age: 44 ± 11), 28 HC (F/M: 15/13, mean age: 34 ± 10.5), and 7 RAS (F/M: 4/3, mean age: 49.7 ± 8). All BS patients were diagnosed according to the international study group criteria (12). Fourteen BS (14/54, 26%) and two RAS (2/7, 29%) had oral ulcers at the time of collecting the samples. HC were recruited from the Oral Medicine clinic in the Royal London Hospital and from the Queen Mary University staff. RAS were patients having recurrent oral ulceration without any systemic manifestation of BS and were also recruited from the Oral Medicine clinic in the Royal London Hospital. The specific IgG response to the different herpesviruses was measured by quantitative immunoassay in the serum samples. Viral nucleic acid titres in saliva samples were

quantified using quantitative real-time polymerase chain reaction (qPCR) with specific primers.

Immunoassays

HSV-1 and 2 immunoglobulin G (IgG) ELISA

A 100- μ l volume of the 1/100 diluted serum samples (as recommended by the manufacturers) was added to enzyme-linked immunosorbent assay (ELISA) plates pre-coated with either recombinant-gG1 (HSV-1) or gG2 (HSV-2) antigen (Biokit, Barcelona, Spain). The antibodies in the test samples were incubated for 1 h at 37°C to allow binding to the specific antigens. The wells were then washed to remove unbound material and enzyme-labelled detection antibodies specific for human IgG (conjugate) were added and incubated for 30 min at 37°C. After washing to eliminate the unbound material, the enzyme substrate solution containing a chromogen was added and incubated for 30 min at room temperature, 20–25°C. The reaction was stopped with 100 μ l of stop solution and the colour was read in a spectrophotometer at 620–630 nm reference filters. The intensity of the colour reaction was directly proportional to the amount of the antibodies in the specimens.

The samples were tested in duplicate and seven control samples were tested simultaneously to validate the assay and extract the quantifiable data. The mean absorbance of the low positive control was calculated and considered to be the cut-off value, followed by dividing the sample absorbance by the cut-off value to obtain a ratio absorbance/cut-off, which is proportional to the concentration of the HSV-1 and HSV-2 IgG antibodies in each specimen.

Varicella-zoster virus immunoglobulin G ELISA

Varicella-zoster virus (VZV) IgG was detected and quantified using a similar method to the HSV-1 IgG ELISA. However, the wells of the VZV IgG ELISA kit were coated with VZV antigen from partially purified extract of human fibroblast infected with VZV, strain ELLEN (ATCC) (Diamedix, FL.). To determine the ELISA Unit (EU)/ml, the following formula was used following the manufacturer's instructions (Absorbance of calibrator \times Absorbance of sample = EU/ml of sample).

Cytomegalovirus immunoglobulin G ELFA

A CMV IgG was quantified by an automated quantitative two-step enzyme immunoassay sandwich method with a final fluorescent detection, enzyme-linked fluorescent assay (ELFA). All of the assay steps were performed automatically by Vitek Immuno-Diagnostic Assay System instrument according to the manufacturer's instructions (bioMerieux, Basingstoke, UK). The solid phase receptacle was coated internally by the CMV antigen (strain AD169) and also acted as pipetting device for the assay. Fluorescence was measured twice for each sample tested; the first reading is a background reading of the serum and

the second one was taken after the incubation period with the antigen. The calibration curves were stored, automatically calculated, and the results were expressed as AU/ml. The samples were tested in duplicate, along with calibrator and control samples to validate the assay.

Epstein–Barr viral capsid antigens specific immunoglobulin G chemiluminiscent immunoassay

EBV viral capsid antigens IgG was quantified in the investigated samples by the LIAISON analyser, following the manufacturer's instructions (DiaSorin, Dartford, UK). The samples were tested in duplicates, along with calibrator and control samples, to validate the assay. The fully automated LIAISON immunoassay was used with its magnetic microparticle technology, chemiluminescence with flashlight kinetics, and an isoluminol derivative as labels. Of each sample, 30 µL were used for this analysis. All reagents required for the assay (magnetic particles, luminescence-labelled tracer, two calibrators, diluent, and assay buffer) were provided ready to use and assembled in one integrated reagent cartridge identifiable by a bar-coded label providing information such as lot number, expiry date, and calibration data. The analyser automatically calculated the antibody concentrations and expressed it as U/ml.

Human herpes virus 8 immunoglobulin G indirect fluorescent assay

An IFA method was used to semi-quantify the HHV-8 IgG in the serum samples (Advanced Biotechnologies, MD). The 1:40 diluted serum samples were incubated with HHV-8-infected cells on a slide, allowing the specific antibody in the serum sample, if present, to bind with the antigen expressed on the infected cells, forming an immunological complex. Incubation was done in a moist chamber for 30 min at 37°C. After the wash step (5–10 min in wash buffer), the anti-human IgG conjugated to the dye fluorescein isothiocyanate was added to each well on the slide and allowed to bind to the antibody–antigen complexes for 30 min at 37°C. The slides were then washed (5–10 min in wash buffer), mounted, and blindly evaluated under a fluorescent microscope by two different investigators. Each slide contained 10 tests; positive and negative controls along with the wash buffer sample were tested simultaneously with each of the seven patients' samples to validate the assay. The positive control was given a semi-quantitative score equal to 4 and the negative control was given a score of 0. Each patient sample was scored relative to the positive and negative controls. The results were checked for consistency and re-evaluated if there were any discrepant results between the two investigators.

Validation of the immunoassay

All samples were analysed in duplicate. Negative control, positive control, and calibrator samples were tested each time, along with the investigated samples. To validate the

results, the value of these control samples was checked against the validation range suggested by the manufacturer's instructions.

Nucleic acid purification from saliva samples

Nucleic acid in 300 µl of all saliva samples was simultaneously purified along with six water samples as negative controls. All samples were processed by the BioRobotMDx workstation using the QIAamp DNA extraction kit, following the manufacturer's protocol (Qiagen, Manchester, UK). This method used the selective binding properties of a silica-based membrane to purify DNA samples. Purified DNA samples were then eluted in 10 mM Tris Cl, 0.5 mM EDTA, pH 9 (elution buffer: AE) and were free from protein, nucleases, and other contaminants or inhibitors. All samples were subsequently spiked with phocine herpesvirus (PHV) DNA to act as internal control during the qPCR assay.

Real-time polymerase chain reaction

HSV-1, HSV-2, and VZV QPCR

Multiplex real-time amplification of HSV-1, HSV-2, and VZV DNA using glycoprotein-B-specific primers was performed (Table 1). The 143 bp, 140 bp, and 97 bp products were detected in qPCR by the use of TaqMan probes that are labelled at the 5' end with FAM, JOE, and Cy5, respectively, and at the 3' end with the appropriate black hole quenchers (Table 1). The composition of the qPCR was as follows: 2.5 µl of 1 × PCR buffer contained 1.5 mmol/L MgCl₂ (Qiagen), 5 µl of MgCl₂ (25 mmol/L), 7.5 µl of dNTPs (6.25 mmol/L each nucleotides), 1 µl of the forward and reverse primers (15 pmol/µl), 1 µl of TaqMan probe (5 pmol/L), and 0.25 µl of HotStarTaq polymerase (0.25 IU/µl; ABI) made up to a final volume of 20 µl with sterile water (Applied Biosystems, Paisley, UK). Of extracted DNA, 5 µl was added to each reaction. The PCR cycling conditions were 2 min at 50°C, 10 min at 95°C, and 60 cycles of 15 s each at 95°C and at 60°C. All samples were analysed in duplicate and the average load was calculated by using the sequence detection system software available on the ABI 7700 platform (Applied Biosystems).

QPCR for CMV and EBV

Quantitative real-time amplification of CMV and EBV DNA using glycoprotein-B-specific primers was performed (Table 1). The 150 bp product of CMV was detected in qPCR by using a TaqMan probe which is labelled at the 5' end with FAM and at the 3' end with the quencher tetramethylrhodamine (TAMRA). The 96 bp product of EBV was detected with the TaqMan probe labelled at the 5' end with JOE and at the 3' end with BHQ1 (Table 1). The composition of the PCR and cycling conditions were as in a previously published method (13).

Table 1. Primers used in the qPCR for detecting the salivary viral load of HSV-1, HSV-2, VZV, CMV, EBV, HHV-8, and PHV

Target		Primers & probes	Product size/bp
HSV-1	Fwd	TATTGGTGGCGATGGCGACAC	143
	Rev	CTTTCCGCATGTGGGCTCTC	
	Probe	FAM-CCCCGCCCATACCCCTACCCGC-BHQ1	
HSV-2	Fwd	AGCATCCCGATCACTGTGTACTA	140
	Rev	GCGATGGTCAGGTTGTACGT	
	Probe	JOE-CAGTGCTGGAACGTGCCTGCCGC-BHQ1	
VZV	Fwd	GCCCGTCTATTCCATTGAGCAA	97
	Rev	CCCGCAAACCTGTAGAACTGTTG	
	Probe	Cy5-CACACGACGCCTCCGCGCAG-BHQ3	
CMV	Fwd	GAGGACAACGAAATCCTGTTGGGCA	150
	Rev	TCGACGGTGGAGATACTGCTGAGG	
	Probe	FAM-CAATCATGCGTTTGAAGAGGTAGTCCACG-BHQ1	
EBV	Fwd	GGCCAGAGGTAAGTGGACTTTAAT	96
	Rev	GGGGACCCTGAGACGGG	
	Probe	JOE-CCCAACACTCCACCACCCAGGC-BHQ1	
HHV-8	Fwd	TCGGTGGCGATGCTTTAGAC	97
	Rev	TGAAGCAGACGATGCTTTGC	
	Probe	FAM-TCGTAACCCCGTCTACTTTCCCGG-TAMRA	
PHV	Fwd	CGTTCCAACAACACAACCTACTG	101
	Rev	CTCTCATATCATCATTCAACTCAGTGT	
	Probe	JOE-ACCACCAATTACTCCTAGTCCACCACCG-BHQ1	

HSV, herpes simplex virus; VZV, varicella-zoster virus; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HHV-8, human herpes virus 8; PHV, phocine herpesvirus.

HHV-8 QPCR

Primers, probes for the detection of HHV-8 were chosen according to the previously published method (14). Probes were labelled at the 5' and 3' end with FAM and TAMRA, respectively. TaqMan probes were synthesised with a 3' phosphate group to block extension by Taq polymerase (Applied Biosystems) (Table 1). The qPCR conditions were as described above in the CMV/EBV qPCR experiment.

PHV QPCR and assay validation

Prior to DNA extraction, all clinical samples and negative control samples were spiked with PHV DNA as an internal control. Real-time amplification of PHV DNA using glycoprotein-B-specific primers was performed as internal control. The 101 bp product was detected in qPCR by the use of a TaqMan probe which is labelled at the 5' end with FAM and at the 3' end with the quencher BHQ1 (Table 1). The composition of the PCR and the PCR cycling conditions were as described above in the CMV qPCR experiment. All samples were analysed in duplicate. They were positive for PHV, which excluded the presence of PCR inhibitors in the reaction.

Statistical analysis

The statistical power calculation was carried out using StatMate 2 software (GraphPad, CA) to determine the

minimum number of samples required in each group. The statistical power calculation was based on previously published information regarding the seroprevalence of HSV-1 in London in people aged 30 years or above (54%) (15).

Mean, median, range, minimum, maximum, standard deviation (SD), standard error of means (SEM), and percentage were used in the descriptive statistics. The seroprevalence of human herpesviruses was measured in the form of the percentage of positive subjects for the virus-specific IgG divided by the total number of subjects studied in each group. The absolute quantity of asymptomatic herpesvirus shedding in saliva was expressed as means of the $\log \pm$ SD. The data obtained were analysed by the Mann–Whitney *U*-test when comparing quantitative data from two groups, and non-parametric ANOVA (Kruskal–Wallis test) when comparing quantitative data from more than two groups. Differences in the rate were analysed using Fisher Exact test. The GraphPad Prism® statistical package was used (GraphPad).

Results

HSV-1 and HSV-2 seroprevalence and salivary viral load

The HSV-1 IgG level between BS, HC, and RAS showed no statistical difference ($p = 0.6697$). There was also no

difference in BS patients with or without oral ulcers at the time of sampling ($p=0.0779$). Mean and SD expressed as the ratio absorbance/cut-off were as follows: BS (0.87 ± 0.92), HC (0.96 ± 0.98), and RAS (0.75 ± 0.89). There was also no statistically significant difference in the seroprevalence of HSV-1 infection between the three groups as measured by percentage of positive samples for HSV-1 IgG, with positivity rate ranges between 46 and 57% ($p=0.2892$) (Table 2). Only one BS patient's saliva was positive for HSV-1 by qPCR indicating asymptomatic shedding of the virus in the saliva.

Similarly, there was no statistically significant difference in the HSV-2 IgG level between BS, HC, and RAS ($p=0.9297$). There was also no difference observed between BS patients with or without oral ulcers at the time of sampling ($p=0.2659$). Mean and SD expressed as the ratio absorbance/cut-off were as follows: BS (0.16 ± 0.36), HC (0.12 ± 0.22), and RAS (0.21 ± 0.45). There was also no statistically significant difference in the seroprevalence of HSV-2 infection between BS and HC ($p=0.2507$) and BS compared to RAS ($p=0.2507$), with positivity rate ranges between 4 and 14% (Table 2). Salivary HSV-2 viral load was negative for all patients in each of the three groups.

VZV seroprevalence and salivary viral load

There were no statistically significant difference in the VZV IgG level of expression between BS, HC, and RAS ($p=0.6054$). There was also no difference observed between BS patients with or without oral ulcers at the time of sampling ($p=0.3224$). Mean and SD were as follows: BS (98.66 ± 35.1 EU/ml), HC (106.8 ± 38.5 EU/ml), and RAS (91.94 ± 50.6 EU/ml). There was also no statistically significant difference in the seroprevalence of VZV infection between the three investigated groups, with positivity rate ranging between 86 and 100% ($p=0.6978$) (Table 2). Salivary VZV viral load was negative for all patients in each of the three groups.

Table 2. Seroprevalence of herpes virus infections in BS patients, RAS patients, and HC

	BS (n = 54)	RAS (n = 7)	HC (n = 28)
HSV-1	(29/54) 53.7%	(3/7) 42.9%	(14/28) 50%
HSV-2	(5/54) 9%	(1/7) 14%	(1/28) 4%
CMV	(27/54) 50%	(5/7) 71%	(19/28) 64%
EBV	(48/54) 89%	(6/7) 85.7%	(27/28) 96.4%
VZV	(54/54) 100%	(6/7) 85.7%	(28/28) 100%
HHV-8	(14/44) 32%	(3/7) 42%	(13/27) 48%

CMV, cytomegalovirus; EBV, Epstein–Barr virus; HHV-8, human herpes virus 8; HSV-1, herpes simplex virus 1; HSV-2, herpes simplex virus 2; VZV, varicella-zoster virus.

CMV IgG level is low in BS

There were statistically significant lower CMV IgG levels in BS patients' samples compared to those of HC ($p=0.0266$) and RAS ($p=0.0450$). There was no difference observed between BS patients with or without oral ulcers at the time of sampling ($p=0.1755$). Mean and SD were as follows: BS (30.67 ± 35.2 AU/ml), HC (48.32 ± 37.9 AU/ml), and RAS (55.6 ± 46.8 AU/ml) (Fig. 1). Despite the fact that a lower seropositivity was observed in BS, there was no statistically significant difference between the three investigated groups, with the following positivity rates: BS (50%), HC (64%), and RAS (71%) ($p=0.2206$) (Table 2). The salivary CMV viral load was negative for all patients in each of the investigated groups.

EBV salivary viral load is high in BS

There was no statistically significant difference in the EBV IgG level of expression between BS, HC, and RAS ($p=0.9742$). Mean and SD were as follows: BS (412.3 ± 285.0 A/ml), HC (403.2 ± 255.5 A/ml), and RAS (399.4 ± 345.8 A/ml). There was also no statistically significant difference in the seroprevalence of EBV between the three groups, with positivity rates ranging between 86 and 96% ($p=0.1181$) (Table 2).

However, there was a significantly higher salivary shedding of EBV in the saliva of BS compared to HC ($p=0.0057$). There was no statistically significant difference between BS and RAS ($p=0.3318$). It was observed that RAS salivary shedding of EBV was lower than in BS but higher than in HC. However, there was no statistically significant difference between RAS and HC in EBV salivary shedding ($p=0.4837$). Mean and SD of the absolute shedding of the virus were as follows: BS (2.3 ± 2.3 log), HC (0.89 ± 1.7 log), and RAS (1.4 ± 2.1 log). There was also statistically significant higher positivity rate in BS (55%) compared to HC (25%) ($p < 0.0001$), but not

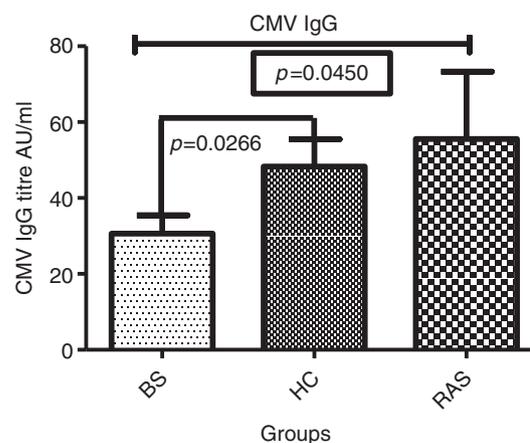


Fig. 1. CMV IgG level of expression.

RAS (43%) ($p = 0.0926$). There was statistically significant higher positivity rate in RAS (43%) in comparison to HC (25%) ($p = 0.0109$) (Fig. 2).

Comparing EBV salivary shedding in BS patients who had oral ulcers ($n = 14$) with BS patients who did not have oral ulcers at the time of sampling ($n = 40$) ($p = 0.135$) showed no statistically significant difference. Mean and SD of the absolute shedding of the virus were as follows: BS with oral ulcers (1.8 ± 2.4 log) and BS without oral ulcers (2.8 ± 2.4 log). There was also no statistically significant difference in the positivity rate in BS with oral ulcers (43%) compared to BS without oral ulcers (65%) ($p = 0.1348$).

HHV-8 seroprevalence and salivary viral load

There was no statistically significant difference in HHV-8 IgG level of expression between BS, HC, and RAS ($p = 0.3150$). The HHV-8 immunoassay used in this study is a semi-quantitative assay. The six BS patients' samples and that of one HC which had high levels of non-specific binding (identified by the total absence of any negative cells in the whole slide) were excluded from the analysis (Fig. 3a). Positive and negative controls are illustrated in Fig. 3b and c. The prevalence of the HHV-8 is reported in Table 2. HHV-8 qPCR was negative for all patients in each of the three investigated groups.

Discussion

There was no statistically significant difference in the seroprevalence of HSV-1, HSV-2, VZV, and HHV-8 in BS in comparison to HC and RAS. Moreover, the salivary viral load of these viruses was negative in the three investigated groups, apart from only one BS patient who was positive for HSV-1. These results contradict some of the earlier studies, especially regarding HSV-1 prevalence but the validated methodology used in this study along with the larger cohort substantiate those results (4, 5, 7,

8, 16). In addition, our results are supported by the fact that BS was not successfully treated by the administration of high doses of acyclovir in association with plasma exchanges or treatment with acyclovir alone (6).

There was a statistically significant lower level of detectable CMV specific IgG in the serum samples of BS. This result is in concordance with the previously published study by Lee et al. (10). Interestingly, the seroprevalence of CMV in our BS was only 50%, whereas a much higher seroprevalence of the CMV infection was reported from countries along the 'Silk Route' reaching 97% in the ages between 17 and 40 years (17). Initial sensing of CMV is suggested to be through toll-like receptor (TLR) 1/2 heterodimer (18). Given the known influence of TLR on the adaptive immune response modulation, it is very possible (while not the only possible explanation) that the observed decrease in the CMV IgG is a consequence of the previous observation of our group regarding the potential dysfunction of the TLR1/2 heterodimer in BS (19, 20). Another possible explanation is the lack of viral exposure; however, one would expect similar viral exposure between HC and BS who reside in the same place. Moreover, the BS group showed similar seroprevalence and specific IgG titre to HC for the other herpesviruses.

The seroprevalence of EBV in BS was comparable to that of HC and RAS. However, in concordance with the results of Sun and colleagues (11), the EBV salivary viral load of BS was significantly higher compared to HC but similar to RAS. The dynamic of EBV salivary shedding was previously studied and variation in the rate of shedding was observed in the same individual over time (21). Furthermore, EBV replication was reported in the salivary glands of other oral diseases such as Sjogren's syndrome (22). Systemic infection by microorganisms can also lead to the reactivation and higher shedding of EBV (23). Interestingly, the initial sensing of EBV is suggested to be through TLR2 independent of TLR1 or TLR6 (24). It is also documented that the released EBV-encoded dUTPase from infected cells into the extracellular environment acts as a ligand for TLR2 resulting in the classical signalling cascade of TLRs activating nuclear factor kappa B ($\text{NF}\kappa\text{B}$) and inducing the production of pro-inflammatory cytokines such as interleukin 6 (IL6) and tumour necrosis factor alpha ($\text{TNF}\alpha$) (25). IL6 in turn supports the latent infection and persistence of EBV through its potent activation effect on signal transducer and activator of transcription 3 gene signalling that regulate Epstein-Barr nuclear antigen 1 transcription which plays a crucial role in the maintenance of the EBV episome in infected cells (26). Interestingly, an enhanced inflammatory reaction is a recognised pathological feature of BS with a well-recorded, high serum pro-inflammatory cytokine levels such as $\text{TNF}\alpha$ and IL6 (27).

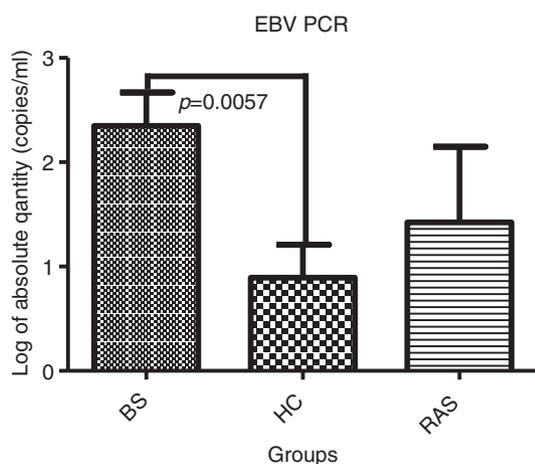


Fig. 2. The salivary shedding of EBV.

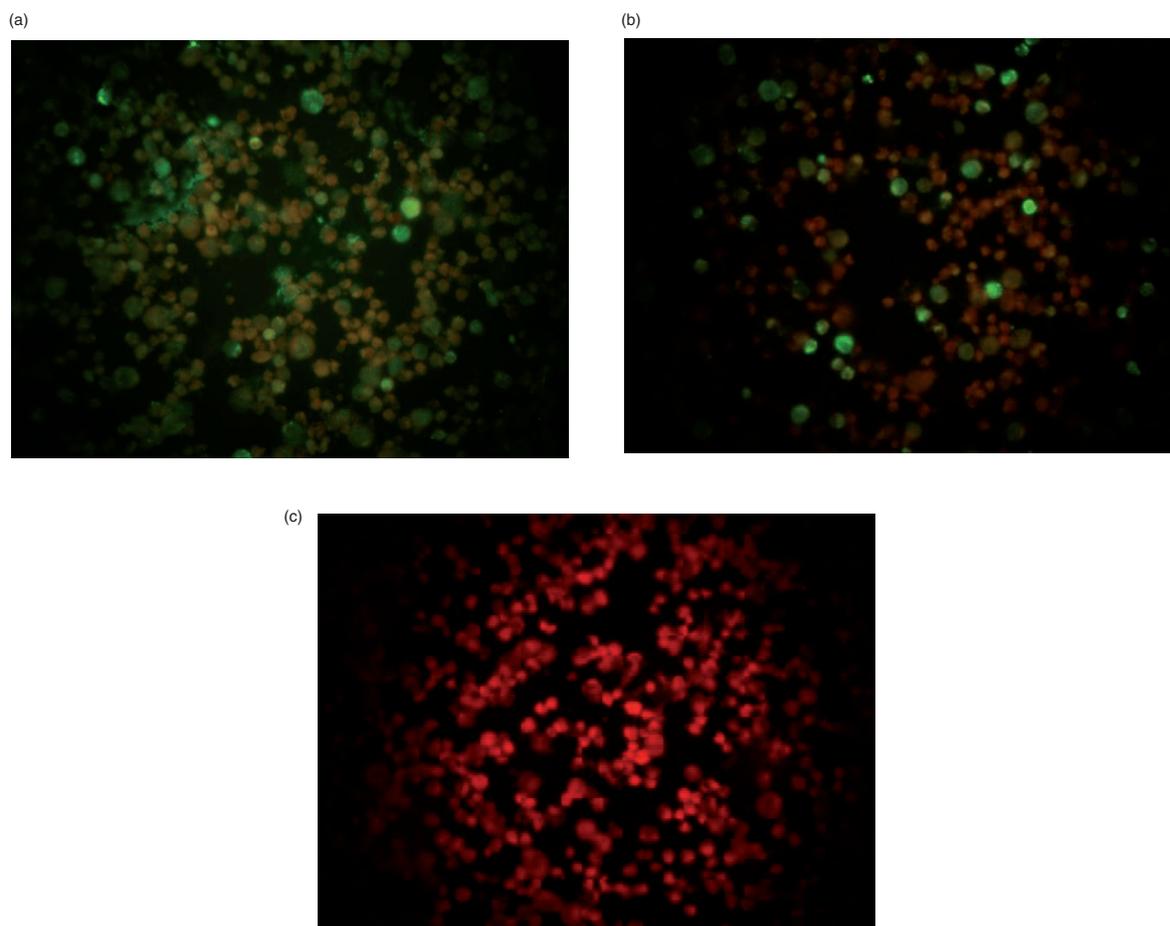


Fig. 3. HHV-8 indirect fluorescent assay (IFA). (a) Non-specific binding of HHV-8 infected cells by immunoglobulins in the serum samples of a BS patient. (b) Positive sample for HHV-8. (c) Negative sample of HHV-8.

In conclusion, despite the uncertainty whether the reported difference in the EBV salivary viral load in BS is of a causative or reactive nature, it may help unravel some of the questions in relation to the disease pathogenesis. Furthermore, the lower levels of CMV IgG may explain the previously investigated defect in the TLR1/2 heterodimer function that act as the initial receptor sensing CMV (19).

Key message

1. There was statistically significant low level of expression of CMV IgG in the serum samples of BS. As this result is consistent with the findings of Lee et al. (10), we postulate that this difference can play an important role in understanding the complex nature of the pathogenesis of this disease.
2. The defect in the adaptive immune response to CMV in BS may be explained by the previously reported defect in the TLR 1/2 heterodimer function, the initial receptor sensing CMV.
3. The EBV salivary viral load of BS was significantly higher compared to HC but similar to RAS.

Limitation of the study

The number of samples in the RAS group was small; however, the study was powered. The statistical power was calculated based on the seroprevalence of HSV-1 in London, UK, in people aged 30 years or above (54%) using the StatMate 2 program (GraphPad).

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Conflict of interest and funding

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