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Epstein - Barr Virus Salivary Shedding in Patients with Acute Infectious Diseases: A Pilot Study

Izlučivanje Epstein-Barrova virusa u slini bolesnika s akutnim infektivnim bolestima: pilot-studija

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Abstract
Epstein-Barr virus (EBV) is a widely disseminated herpesvirus for which antibodies have been demonstrated in over 90% of adults worldwide. After subclinical primary EBV infections, as well as after infectious mononucleosis, the virus can be shed in saliva for a prolonged period of time. **Aim:** Diseases and disorders that can induce EBV salivary shedding include mental disorders and sex, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, malaria and HIV infection. Since the occurrence of EBV in saliva during acute infectious diseases has not yet been systematically researched, we aimed to investigate the possible relationship between acute infectious diseases and salivary shedding of EBV. **Material and methods:** This pilot cross-sectional study included consenting adults hospitalized for acute infectious conditions and their peers free of acute infectious diseases. A total of 40 patients with acute infectious diseases were enrolled, along with 41 adults free of acute infections. Peripheral venous blood samples for serodiagnosis and saliva samples for EBV PCR testing were collected from both groups. We fitted logit and general linear models to proportions and to ln (viral copy counts) to generate adjusted proportions and geometric mean values in the two groups of subjects. We used SAS for Windows 9.4. **Results:** The most common acute infectious disease was COVID-19 pneumonia, followed by hemorrhagic fever with renal syndrome. Crude proportions of people with positive serological test results and those with saliva viral shedding were similar in the two groups. **Conclusions:** The presented preliminary data do not indicate acute infectious conditions as a marked "contributor" in increasing salivary EBV shedding.

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Introduction:

Epstein-Barr virus (EBV) is a widely disseminated herpesvirus that is spread by intimate contact between asymptomatic EBV shedders and susceptible persons. Antibodies to EBV have been demonstrated in over 90 percent of adults world-

Uvod

Epstein-Barrov virus (EBV) široko je rasprostranjeni herpesvirus koji se prenosi bliskim kontaktom između simptomatskih i asimptomatskih osoba koje izlučuju EBV i neimunih osoba. Protutijela na EBV dokazana su kod više od 90 % odra-

wide (1, 2). Most primary EBV infections that occur during childhood are subclinical, but infections in adolescents and adults frequently result in infectious mononucleosis (IM) which is the most common clinical presentation of EBV infection (1,3). A typical clinical presentation of IM includes fever, pharyngitis, adenopathy, fatigue, and atypical lymphocytosis (1, 3, 4).

EBV has primary tropism for the type B lymphocytes. The hallmark of B lymphocyte EBV infections is the establishment of latency that results in a lifelong infection which cannot be cleared by the immune system (1, 3).

Following IM, the virus can be shed in saliva at high levels for a prolonged period of time, (5, 6). It is very important to emphasize the fact that the virus may be intermittently shed in the oropharynx for decades (5, 7) and can be present in saliva and throat washings from healthy people (8).

EBV reactivates under conditions of cellular immune response impairment, which is important in the long-term control and suppression of the replication of persistent and asymptomatic EBV in a healthy person (9). EBV reactivations documented by serological tests can be caused by psychological stress (10) such as student examination stress (11, 12), marital stress (13), attachment anxiety (14), loneliness (15), autoimmune diseases (16), chronic fatigue syndrome (17) and COVID-19 (18, 19).

EBV reactivation identified by PCR testing in plasma has also been documented in patients in intensive care units (20, 21), in patients with COVID-19 (22-24), long COVID-19 (19), malaria (25) and HIV (26, 27).

Diseases and disorders that can induce shedding of EBV in saliva are also being investigated, but not extensively. EBV salivary shedding is connected to psychological stress (10), mental disorders and sex (28), connective tissue diseases (29), multiple sclerosis (30), systemic lupus erythematosus (31), malaria (25) and HIV infection (26,27,32-35).

As the occurrence of EBV in saliva during acute infectious diseases (with the exception of HIV and malaria) has not yet been systematically researched, this pilot cross-sectional study aimed to investigate possible relationships between acute infectious diseases and salivary shedding of EBV. The study was performed as an initial part of a larger study that will include measurements of EBV in saliva and plasma, along with EBV serology in different stages of acute infectious diseases. In addition, easily collectable saliva assays are a growing area of research which can provide additional information in the investigation of diseases and disorders. Assessing the diagnostic value of saliva in the context of infectious diseases, which was the goal of our present and future studies, can be valuable and potentially useful in everyday practice (36-38).

Patients and methods

General design and ethics

This pilot cross-sectional study was conducted at a tertiary care university-affiliated teaching hospital between September 2020 and December 2021, and was approved by the

slih u svijetu (1, 2). Većina primarnih EBV infekcija tijekom djetinjstva supkliničke su, a zaraze kod adolescenata i odraslih često se klinički prezentiraju kao infektivna mononukleoza (IM) koja je ujedno i najčešća klinička manifestacija EBV infekcije (1, 3). Tipična klinička slika IM-a uključuje vrućicu, faringitis, limfadenopatiju, umor i atipičnu limfocitozu (1, 3, 4).

EBV ima primarno tropizam prema B-limfocitima. Obilježje infekcije B-limfocita EBV-om jest uspostavljanje latencije s posljedičnom doživotnom infekcijom koju imunosni sustav ne može eliminirati (1, 3).

Nakon IM-a virus se može izlučivati u slini u većem broju tijekom duljeg razdoblja (5, 6). Vrlo je važno istaknuti činjenicu da se virus može desetljećima povremeno izlučivati u orofarinksu (5, 7), a može ga se naći u slini i u ispirku grla zdravih ljudi (8).

EBV se reaktivira u uvjetima poremećaja stanične imunosti, što je važno u dugoročnoj kontroli i supresiji replikacije perzistentnog i asimptomatskog EBV-a kod zdrave osobe (9). Reaktivacije potvrđene serološkim testovima mogu biti prouzročene psihološkim stresom (10) kao što je stres tijekom studentskih ispita (11, 12), u problematičnim brakovima (13), može biti izazvan strahom od napuštanja i odbacivanja (14) te usamljenošću (15), zatim pojavljuje se u autoimunim bolestima (16), sindromu kroničnog umora (17) i bolesti COVID-19 (18, 19).

Reaktivacija EBV infekcije dokazane prisutnošću virusa u plazmi PCR metodom također je opisana kod bolesnika u jedinicama intenzivne skrbi (20, 21), kod onih s bolešću COVID-19 (22 – 24) te dugim COVID-om 19 (19), ali i tijekom malarije (25) i infekcije HIV-om (26, 27).

Bolesti i poremećaji koji mogu prouzročiti izlučivanje EBV-a u slini također se istražuju, ali ne tako rašireno. Izlučivanje EBV-a u slini do sada je povezano sa psihičkim stresom (10), mentalnim poremećajima i spolom (28), bolestima vezivnoga tkiva (29), multiplom sklerozom (30), sistemskim eritemskim lupusom (31), malarijom (25) i infekcijom HIV-om (26, 27, 32 – 35).

Kako pojava EBV-a u slini tijekom akutnih zaraznih bolesti (s iznimkom HIV-a i malarije) do sada nije sustavno istraživana, ova pilot-studija imala je za cilj istražiti potencijalnu povezanost između akutnih infektivnih bolesti i izlučivanja EBV-a u slini. Provedena je kao inicijalni dio veće studije kojom će se obuhvatiti određivanje EBV-a u slini i plazmi te EBV serologija u različitim stadijima akutnih infektivnih bolesti. Uz to, testovi za koje se koristi slina kao lako dostupan uzorak sve su više u središtu istraživanja i njihova je vrijednost u pružanju dodatnih informacija pri proučavanju raznih bolesti i poremećaja. Procjena dijagnostičke vrijednosti sline u kontekstu infektivnih bolesti, što je svrha našega sadašnjeg i budućeg istraživanja, može biti dragocjena i potencijalno korisna u svakodnevnoj praksi (36 – 38).

Ispitanici i metode

Opći dizajn i etika

Ovo presječno pilot-istraživanje provedeno je u sveučilišnoj kliničkoj bolnici tercijarne zdravstvene zaštite između rujna 2020. i prosinca 2021., a odobrilo ga je Etičko povje-

institutional Ethics Committee. Consenting adults hospitalized for acute infectious conditions and their peers free of acute infectious diseases provided blood and saliva samples for serological and virological tests related to EBV.

Subjects

Patients hospitalized for acute infectious diseases were eligible for inclusion during the first three days of hospitalization while febrile. All participants were 18 years of age or older at the time of the study and had provided a written informed consent. Patients with infectious mononucleosis, HIV infection, suspected but not confirmed infectious diseases and patients with fever of unknown origin or of non-infectious genesis were not included. Their peers free of acute infectious conditions were recruited on a voluntary basis among Hospital staff and students and were eligible if they were adults, free of any acute infectious condition within 3 months prior to being included in the study and had provided a written informed consent.

Hospitalized patients were managed in line with standard procedures regarding their respective diagnoses, except for the provision of additional blood and saliva samples. The same was obtained from "healthy" control subjects, together with detailed medical histories.

Peripheral venous blood samples (5-10 mL) for serodiagnosis were collected into Vacutainer tubes. Sera were stored at -20°C until testing.

Unstimulated whole saliva samples (3 ml) for PCR testing were collected into sterile plastic containers before, or two hours after a meal. Samples were frozen at -72°C until assaying.

EBV serological diagnosis

EBV serostatus was defined by the presence of IgM and IgG antibodies against EBV viral capsid antigen (VCA), IgG against early antigen-diffuse (EA-(D)) and IgG against EBV nuclear antigen (EBNA) which were simultaneously tested using chemiluminescent immunoassay ((CLIA), DiaSorin, Saluggia, Italy). The tests were performed according to the manufacturer's instructions.

Acute infection was characterized by IgM anti-VCA and anti-EA (D) IgG without antibodies against EBNA. The presence of anti-EBNA IgG and anti-VCA IgG antibodies was interpreted as past infection. EBV reactivation was assumed when the level of anti-EA (D) IgG was high, and anti-VCA and anti-EBNA IgG were positive, and viremia was performed for confirmation.

EBV DNA quantification

DNA was extracted from 200 µl of saliva using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Quantification of EBV DNA was performed by using RealStar® EBV PCR Kit 2.0 (CE-IVD, Altona Diagnostics, Hamburg, Germany) following the standard manufacturer's instructions (reaction volume 30 µl) by using LightCycler®480 System PCR Instrument (Roche Diagnostics, Mannheim, Germany). The kit contains reagents required for the PCR set up, internal control and four quantification standards (ranging from 10^1

renstvo ustanove. Odraslim bolesnicima hospitaliziranima zbog akutnih infektivnih bolesti koji su dali informirani pristanak te zdravim kontrolama uzeti su uzorci sline i krvi za serološke i virološke pretrage vezane za EBV.

Ispitanici

Za sudjelovanje u istraživanju bili su izabrani bolesnici hospitalizirani zbog akutnih infektivnih bolesti i to tijekom prva tri dana boravka u bolnici dok su još bili febrilni. Svi su imali 18 godina ili više i potpisali su informirani pristanak. Nisu uključeni bolesnici s infektivnom mononukleozom, infekcijom HIV-om, suspektnim, ali nepotvrđenim infektivnim bolestima te bolesnici s vrućicom nepoznatog podrijetla ili vrućicom neinfektivne geneze. Zdrave kontrole izabrane su na dobrovoljnoj osnovi među bolničkim osobljem i studentima i to ako su ispunjavali sljedeće uvjete: odrasla dob, odsutnost akutnih infektivnih bolesti unutar tri mjeseca prije uključivanja u studiju i potpisivanje informiranog pristanaka.

Hospitalizirani bolesnici zbrinuti su u skladu s postavljenom dijagnozom, osim što su im dodatno uzeti uzorci krvi i sline. Jednaki postupak primijenjen je i za zdrave kontrole.

Uzorci periferne venske krvi (5 – 10 mL) za serodijagnostiku skupljeni su u epruvete Vacutainer. Serumi su do testiranja bili pohranjeni na -20 °C.

Nestimulirani uzorci sline (3 mL) za PCR testiranje skupljeni su u sterilne plastične posude prije obroka ili dva sata poslije. Uzorci su do ispitivanja bili zamrznuti na -72 °C.

EBV – serološka dijagnostika

Serološki status za EBV definiran je prema prisutnim protutijelima IgM i IgG na virusni kapsidni antigen (engl. *viral capsid antigen* – VCA), IgG na rani difuzni antigen (engl. *early antigen-diffuse* – EA-(D) i IgG na nuklearni antigen EBV-a (engl. *nuclear antigen* – EBNA) koji su istodobno testirani kemiluminiscentnim imunotestom (engl. *chemiluminescent immunoassay* – CLIA; DiaSorin, Saluggia, Italija). Testovi su rađeni prema uputama proizvođača.

Akutnu infekciju karakterizirala su protutijela IgM anti-VCA i IgG anti-EA(D), uz izostanak protutijela na EBNA-u. Prisutnost IgG protutijela anti-EBNA i anti-VCA interpretirala se kao prošla infekcija. Reaktivacija EBV-a pretpostavila se kada je uz pozitivna IgG protutijela anti-VCA i anti-EBNA, razina IgG anti-EA(D) bila visoka, a za potvrdu je određena viremija.

Kvantifikacija EBV DNK

Za izolaciju DNK iz 200 µL uzoraka sline korišten je standardizirani komplet reagensa QIAamp DNA Mini Kit (Qiagen, Hilden, Njemačka). Broj kopija EBV DNK određen je primjenom PCR testa u stvarnom vremenu RealStar® EBV PCR Kit 2.0 (CE-IVD, Altona Diagnostics, Hamburg, Njemačka) prema preporukama proizvođača (reakcijski volumen 30 µL) na instrumentu LightCycler®480 System PCR Instrument (Roche Diagnostics, Mannheim, Njemačka). Komplet reagensa RealStar® EBV PCR Kit 2.0 sadržava sve reagenci-

to 10^4 IU/µl) that were calibrated against the 1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques (NAT) (NIBSC code: 09/260). The assay utilizes a highly conserved (non-EBNA-2) primer binding site. The probes specific for EBV DNA are labelled with a fluorophore FAM whereas the probe specific for the internal control is labelled with the fluorophore JOE. The temperature time profile of the real-time PCR reaction includes a denaturation (stage hold, 1 cycle repeat, 90°C, 10 min) and amplification [(stage cycling, a total of 45 cycles, no acquisition (95°C, 15s) and acquisition (58°C, 1 min.)]. Analytical sensitivity of RealStar®EBV PCR Kit 2.0 is 1.59 IU/µl eluate [95% confidence interval (CI): 1.04 IU/µl to 3.37 IU/µl] with a linear range of 1.00E+08 IU/µl to 1.00E+01 IU/µl.

Data analysis

Considering the preliminary (pilot) nature of the present report, we planned no formal statistical tests, but reasoned that 35-40 infected and 35-40 control patients would suffice to indicate numerical trends suggestive of a marked difference between them in indicators of EBV salivary shedding, had it existed. We therefore provide summarized data for patients with acute infectious diseases and "healthy" controls. Serological test results are summarized as proportions of subjects with positive findings. Virological test results are summarized as proportions of subjects with viral presence in saliva and as numbers of viral copies in those with positive findings. We fitted the logit and general linear models to proportions and to ln (viral copy counts) to generate proportions and geometric mean values in the two groups of subjects, adjusted for age, sex and body mass index. For the number of viral copies, we report differences between infected and control patients (as geometric means ratios, GMRs, with 95% CIs) considering those with identified viral copies, and also considering all patients based on a zero-inflated Gaussian model (estimated difference subsumes both probability of a non-zero value and measured values in subjects with non-zero viral copies). We used SAS for Windows 9.4 (SAS Inc., Cary, NJ).

Results

A total of 40 patients with acute infectious diseases and 41 adults free of acute infections were enrolled in the study (Table 1). The former were somewhat older than the latter, while the prevalence of men, body mass index, the prevalence of mild or heavy smokers and alcohol consumption habits (only sporadic) were similar in the two groups (Table 1). Hospitalized patients were somewhat more commonly diabetic and more commonly suffered from hypertension and other cardiovascular morbidity than the control subjects, while other background comorbidities were comparably rare (Table 1). The most common acute infectious disease was COVID-19 pneumonia (10/40 patients), followed by hemorrhagic fever with renal syndrome (6/40 patients) (Table 1).

je potrebne za PCR reakciju, interne kontrole te četiri kvantifikacijska standarda (u rasponu od 101 do 104 IU/µL) koji su kalibrirani prema standardnom pripravku 1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques (NAT) (NIBSC code: 09/260). U ovom molekularnom testu kao ciljane strukture početnica koriste se konzervirane regije genoma virusa (non-EBNA-2). Početnice specifične za EBV obilježene su fluoroforom FAM, a početnice specifične za internu kontrolu reakcije obilježene su fluoroforom JOE. Uvjeti PCR reakcije koji se primjenjuju u ovom testu jesu denaturacija (90 °C, 10 min.), amplifikacija (45 ciklusa, 95 °C, 15 s) i prikupljanje podataka (58 °C, 1 min.). Analitička osjetljivost testa RealStar®EBV PCR Kit 2.0 iznosi 1.59 IU/µL eluata [95 % interval pouzdanosti 1.04 IU/µL do 3.37 IU/µL] s linearnim rasponom kvantifikacije od 1.00E+08 IU/µL do 1.00E+01 IU/µL.

Analiza podataka

S obzirom na preliminarni (pilot) karakter ovog istraživanja, nisu korišteni formalni statistički testovi. Zaključeno je da bi od 35 do 40 bolesnika s infektivnim bolestima i od 35 do 40 u kontrolnoj skupini bilo dostatno da bi se zamijetila eventualna značajna razlika u izlučivanju EBV-a u slini među tim skupinama ispitanika.

Zato su prikazani samo sažeti podatci za bolesnike s akutnim infektivnim bolestima i za zdrave kontrole. Rezultati seroloških testova prikazani su kao proporcije ispitanika s pozitivnim nalazima. Rezultati viroloških testova prikazani su kao proporcije ispitanika s prisutnošću virusa u slini i kao broj virusnih kopija kod onih s pozitivnim nalazima.

Prilagođeni su logit i opći linearni modeli prema proporcijama i ln (broj virusnih kopija) u svrhu generiranja proporcija i geometrijskih srednjih vrijednosti u dvjema skupinama ispitanika, prema dobi, spolu i indeksu tjelesne mase. Za broj virusnih kopija prikazane su razlike između bolesnika s infektivnim bolestima i kontrolne skupine (kao omjeri geometrijskih srednjih vrijednosti, GMR-ovi, s 95 % CI) uzimajući u obzir one s identificiranim virusnim kopijama, sve bolesnike na temelju Gaussova modela s nultom inflacijom (procijenjena razlika uključuje i vjerojatnost nulte vrijednosti i izmjerene vrijednosti kod ispitanika s nenultim virusnim kopijama). Koristili smo se SAS-om za Windows 9.4 (SAS Inc., Cary, NJ).

Rezultati

U ispitivanje je bilo uključeno ukupno 40 bolesnika s akutnim infektivnim bolestima i 41 odrasla osoba bez akutnih infekcija (tablica 1.). Ispitanici u prvoj skupini bili su nešto stariji od onih u drugoj, dok su prevalencija muškaraca, indeks tjelesne mase, prevalencija blagih ili teških pušača i navika konzumacije alkohola (samo povremeno) bile slične u objema skupinama (tablica 1). Hospitalizirani bolesnici nešto su češće bili dijabetičari te su češće patili od hipertenzije i ostalih kardiovaskularnih bolesti nego kontrolni ispitanici. Ostali komorbiditeti bili su jednako rijetki u objema skupinama (tablica 1.). Najčešća akutna infektivna bolest bila je pneumonija COVID-19 (10/40 bolesnika), a zatim hemoragijska vrućica s bubrežnim sindromom (6/40 bolesni-

Table 1 Summary of subject characteristics: subjects with acute infectious conditions requiring hospitalization and subjects free of acute infections over the past 3 months. Data are mean±SD (range), count (%) and geometric mean (range) for the number of viral copies.
Tablica 1. Sažetak karakteristika ispitanika: osobe s akutnim infektivnim bolestima koje zahtijevaju hospitalizaciju te osobe bez akutnih infekcija u posljednja tri mjeseca. Podatci su: srednja vrijednost ± standardna devijacija (raspon), broj (%) i geometrijski prosjek (raspon) za broj virusnih kopija.

	Acute infection • Akutna infekcija	No infection • Bez infekcije
N	40	41
Age (years) • Dob (godine)	56±16 (20-86)	42±16 (19-75)
Men • Muškarci	16 (40.0)	16 (39.0)
BMI (kg/m^2) • BMI (kg/m^2)	27.4±4.7 (19-41)	24.9±4.4 (18-37.1)
Non-smokers[1] • Nepušači[1]	29 (72.5)	27 (65.9)
Mild smokers[1] • Blagi pušači[1]	7 (17.5)	9 (22.0)
Heavy smokers[1] • Teški pušači[1]	4 (10.0)	5 (12.1)
Alcohol consumption[2] • Konzumacija alkohola[2]	7 (17.5)	11 (26.8)
Diabetic • Dijabetičari	9 (22.5)	1 (2.4)
Hypertension • Arterijska hipertenzija	18 (45.0)	6 (14.6)
Other cardiovascular morbidity[3] • Druge kardiovaskularne bolesti	7 (17.5)	0
COPD/asthma • KOBP/astma	3 (7.5)	2 (4.9)
Gastrointestinal[4] • Gastrointestinalne bolesti[4]	3 (7.5)	3 (7.3)
Immunocompromised[5] • Imunokompromitirani[5]	4 (10.0)	3 (7.3)
Acute infectious diseases • Akutne infektivne bolesti		
COVID-19 pneumonia (bilateral) • COVID- 19 pneumonija (obostrana)	10 (25.0)	---
Haemorrhagic fever with renal syndrome • Hemoragijska vrućica s renalnim sindromom	6 (15.0)	---
Legionella pneumonia • *Legionella* pneumonija	5 (12.5)	---
Pyelonephritis (*E. coli*) • Pijelonefritis *(E. coli)*	4 (10.0)	---
Herpes zoster • Herpes zoster	3 (7.5)	---
Gram-negative sepsis (unknown infection site) • Gram-negativna sepsa (nepoznato ishodište)	2 (5.0)	---
Meningitis (*Listeria*) • Meningitis *(Listeria)*	2 (5.0)	---
Other pneumonia • Druge pneumonije	2 (5.0)	---
Salmonellosis • Salmoneloza	2 (5.0)	---
Various other (one case each) • Ostalo	4 (10.0)	---
Septic • Sepsa	6 (15.0)	---
VCA IgM positive • VCA IgM pozitivan	3 (7.5)	0
VCA IgG positive • VCA IgG pozitivan	38 (95.0)	36 (87.8)
EA IgG positive • EA IgG pozitivan	4 (10.0)	2 (4.9)
EBNA IgG positive • EBNA IgG pozitivan	36 (90.0)	34 (82.9)
Meet criteria of virus reactivation • Ispunjava kriterije za reaktivaciju virusa	4 (10.0)	2 (4.9)
Virus in saliva • Virus u slini	12 (30.0)	10 (24.4)
Number of viral copies (if positive) x10^3/ml • Broj virusnih kopija (ako je pozitivno) x 10^3/mL	85.3 (2.3-4610)	16.3 (1-217)

[1] Mild smokers – < 20 cigarettes a day; heavy smokers - ≥ 20 cigarettes a day • [1] Blagi pušači – < 20 cigareta na dan; teški pušači – ≥ 20 cigareta na dan
[2] All declared only sporadic/occasional consumption of alcoholic beverages • [2]Svi su prijavili samo sporadičnu/povremenu konzumaciju alkoholnih pića
[3] Includes cardiac arrhythmia history of occlusive cardio- or cerebrovascular incidents • [3]Uključuje anamnezu srčanih aritmija i okluzivnih kardio ili cerebrovaskularnih incidenata
[4] Practically exclusively peptic disease • [4]Praktički samo peptička bolest
[5] Suffered malignant or chronic autoimmune diseases or were substance addicted • [5]Bolovali su od malignih ili kroničnih autoimunih bolesti ili su bili ovisnici o drogama
COPD – chronic obstructive pulmonary disease; EA – early antigen; EBNA – Epstein Barr nuclear antigen, VCA – viral capsid antigen • KOBP – kronična opstruktivna bolest pluća; EA – rani antigen; EBNA – Epstein Barrov nuklearni antigen; VCA – virusni kapsidni antigen

Crude proportions of people with positive serological test results and those with saliva viral shedding were similar in the two groups (Table 1). Age, sex and body mass index-adjusted proportions were also closely comparable in patients with acute infectious diseases and their peers free of acute infections (Table 2). Considering patients with identified salivary virus, the (adjusted) number of viral copies appeared higher in infected patients than in controls (Table 2), however the estimated difference (GMR=2.55, 95%CI 0.18-36.0) was

ka) (tablica 1.). Proporcije ispitanika s pozitivnim serološkim testovima i onih s izlučivanjem virusa u slini bile su slične u objema skupinama (tablica 1.). Proporcije prilagođene dobi, spolu i indeksu tjelesne mase također su bile slične kod bolesnika s akutnim infektivnim bolestima i zdravim kontrolama (tablica 2.). Uzimajući u obzir bolesnike s virusom identificiranim u slini, (prilagođeni) broj virusnih kopija čini se većim kod bolesnika s infektivnim bolestima nego kod zdravih kontrola (tablica 2.). Međutim, procijenjena je razlika (GMR =

Table 2 Adjusted (for age, sex and body mass index) proportions of subjects with positive serological tests/viral presence in saliva and geometric means (95%CI) for the number of viral copies in patients with a positive viral detection in saliva, for patients with acute infection requiring hospitalization and people without acute infectious conditions.
Tablica 2. Prilagođene (za dob, spol i indeks tjelesne mase) proporcije ispitanika s pozitivnim serološkim testovima/prisutnošću virusa u slini i geometrijski prosjeci (95 % CI) za broj virusnih kopija kod bolesnika s pozitivnim virusom u slini, za bolesnike s akutnom infekcijom koja zahtijeva hospitalizaciju i osobe bez akutnih infektivnih bolesti.

	Acute Infection Akutna infekcija	No infection Bez infekcije
N	40	41
VCA IgM positive • VCA IgM pozitivan	0	0
VCA IgG positive • VCA IgG pozitivan	98.2%	99.1%
EA IgG positive • EA IgG pozitivan	1.7%	0.8%
EBNA IgG positive • EBNA IgG pozitivan	91.0%	93.8%
Meet criteria of virus reactivation • Ispunjava kriterije za reaktivaciju virusa	1.7%	0.8%
Virus in saliva • Virus u slini	29.1%	26.3%
Copies (if positive) x10^3/ml • Kopije (ako je pozitivno) x 10^3/mL	62.5 (12.4-315)	24.5 (4.0-152)

EA – early antigen; EBNA – Epstein Barr nuclear antigen, VCA – viral capsid antigen
EA – rani antigen; EBNA – Epstein Barrov nuklearni antigen, VCA – virusni kapsidni antigen

very imprecise, likely due to a limited number of subjects with identified viral copies and high variability (wide CIs) of observed copy numbers in both subject groups (Table 2). The estimate based on a zero-inflated model (subsumes both the probability of a non-zero finding and the copy numbers in those with non-zero values) indicated no major difference between infected and control subjects: GMR= 0.87 (95%CI 0.30-2.54).

Discussion

EBV infection is very common in humans, which is why the virus has been extensively researched (1, 2). It is known that it can occasionally appear in the blood during some diseases and conditions such as psychological stress (10), in patients in intensive care units (20, 21), in patients with COVID-19 (22-24) and in patients with long COVID-19 (19). In contrast, the occurrence of EBV in saliva has been less investigated. For this reason, it seems of interest to try to identify the diseases and conditions that lead to EBV shedding in saliva. Such findings would only help increase our understanding of the virus and its interaction with the human body.

Certain infections such as malaria (25) and HIV infection (26, 27, 32-35) have been associated with EBV occurrence in saliva.

The study of Miller et al. (35) reported higher prevalence of EBV in saliva of HIV positive (90%) than in HIV negative group (48%) with significantly higher EBV viral loads in HIV- seropositive patients than in HIV-seronegative persons.

Scaggiante et al. (26) reported that the incidence of EBV in saliva in HIV-positive MSM with successful HIV viremia control was comparable to that in patients with unsuccessful HIV viremia control. However, patients with active plasma HIV replication had a significantly higher frequency of high viral load of EBV in saliva. When comparing EBV salivary shedding in HIV-positive MSM with controlled and those with uncontrolled plasma HIV viremia in the study of Basso et al. (32), EBV was proven to be present in both groups with a higher viral EBV load in HIV-viremia patients.

2,55, 95 % CI 0,18 – 36,0) neprecizna, vjerojatno zbog ograničenog broja ispitanika s identificiranim virusnim kopijama i visoke varijabilnosti (široki CI) promatranih brojeva kopija u objema skupinama ispitanika (tablica 2.). Procjena temeljena na modelu s nultom inflacijom (uključuje i vjerojatnost nenultog nalaza i broj kopija kod onih s nenultim vrijednostima) ne upućuje na veću razliku između oboljelih i kontrolnih ispitanika: GMR = 0,87 (95 % CI 0,30 – 2,54).

Rasprava

Infekcija EBV-om vrlo je česta kod ljudi, zbog čega je virus ekstenzivno istražen (1, 2). Poznato je da se povremeno može pojaviti u krvi tijekom nekih bolesti i stanja kao što su psihički stres (10), kod bolesnika na odjelima intenzivne njege (20, 21), kod onih s bolešću COVID-19 (22 – 24) te s dugim COVID-om 19 (19). Nasuprot tomu, pojava EBV-a u slini manje je istražena. Iz tog razloga čini se izazovnim pokušati identificirati bolesti i stanja koja rezultiraju izlučivanjem EBV-a u slini. Rezultati takvog istraživanja pomogli bi boljem razumijevanju virusa i njegove interakcije u ljudskom tijelu.

Određene infekcije poput malarije (25) i infekcije HIV-om (26, 27, 32 – 35) povezane su s pojavom EBV-a u slini.

Millera i suradnici (35) u svojoj su studiji izvijestili o većoj prevalenciji EBV-a u slini HIV-pozitivnih (90 %) nego kod HIV-negativnih osoba (48 %) sa značajno većim brojem EBV kopija kod HIV-pozitivnih bolesnika nego HIV-negativnih osoba.

Scaggiante i suradnici (26) izvijestili su da je učestalost EBV-a u slini HIV-pozitivnih bolesnika iz MSM populacije kod kojih je HIV viremija uspješno kontrolirana jednaka kao i kod bolesnika s neuspješnom kontrolom HIV viremije. No, bolesnici s aktivnom replikacijom HIV-a u plazmi imali su značajno veći broj kopija EBV-a u slini.

Kada su Bassoa i suradnici (32) uspoređivali izlučivanje EBV-a u slini HIV-pozitivnih MSM bolesnika kod kojih je viremija dobro kontrolirana s onima kod kojih HIV viremija nije dobro kontrolirana, dokazali su da je EBV prisutan u objema skupina, ali s većim brojem kopija EBV-a kod bolesnika s HIV-om s prisutnom HIV viremijom.

A similar observation was published by Byrne et al. (23). In this study, HIV-infected patients had an increased risk of EBV presence in saliva and higher viral loads when compared with people who were not infected with HIV.

Agudelo-Hernandez et al. (27) investigated herpesvirus shedding in HIV-infected men in blood, semen, throat washings, urine and stool in comparison with HIV seronegative MSM. HIV-positive patients had significantly higher EBV shedding rates, and EBV was detected in the throat washes of all HIV-positive patients. Among all tested body compartments, the highest number of EBV shedding episodes was detected in throat wash.

An interesting observation was published by Donati et al. (25) concerning EBV DNA loads in the plasma and saliva of Ugandan children with acute malaria before and after antimalaria treatment. Plasma levels were higher in children with malaria than in those without malaria, but there were no significant differences in EBV DNA in saliva between these two groups. In the majority of cases, antimalaria treatment led to the clearance of plasma EBV DNA, but it did not affect the levels in saliva.

Considering these facts, it could be expected that other acute infectious diseases might also be associated with the occurrence of EBV in saliva. However, since infectious diseases are diverse in their etiology (viruses, bacteria, parasites, fungi), they stimulate different types of immune responses in the human body, resulting in clinical presentations of varying severity.

Therefore, we did not initially select one infectious disease or group of diseases to investigate, rather we wanted to include patients with varying acute infectious conditions in order to "screen" the possibility that acute infections with moderate-severe clinical presentation might be associated with increased salivary EBV shedding. The present preliminary data are limited by their cross-sectional nature (and, hence, no insight into the possible dynamics of viral shedding during and after resolution of acute infections) and a rather limited sample size. Also, we did not screen the subjects for their level of stress at the time of sample collection although it may be a factor that could affect EBV shedding in saliva. Nevertheless, it is reasonable to assume that a certain amount of stress is unavoidable in patients suffering from acute conditions, especially in hospitalized ones, regardless of how benign it might be. It seems almost impossible to separate the element of stress caused by the entire condition from the stress caused by an acute infectious disease.

Taking all limitations into account, it seems fair to state that data do not indicate acute infectious conditions (in general) as a marked "contributor" to increased salivary EBV shedding.

Conclusions

The aim of this study was to correlate the occurrence of Epstein-Barr virus copies in the saliva of patients with acute infectious diseases. Our preliminary results suggest that acute infectious diseases do not appear to increase the frequency of EBV copies in saliva. However, it should be taken into ac-

Slično zapažanje objavili su Byrne i suradnici (23). U njihovoj studiji bolesnici zaraženi HIV-om imali su veći rizik od prisutnosti EBV-a u slini i veći broj virusnih kopija u usporedbi s osobama nezaraženima HIV-om.

Agudelo-Hernandez i suradnici (27) istraživali su izlučivanje herpesvirusa kod HIV-pozitivnih muškaraca u krvi, sjemenoj tekućini, ispirku grla, urinu i stolici te su ih uspoređivali s HIV-negativnim osobama iz MSM populacije. HIV-pozitivne osobe imale su značajno veću učestalost izlučivanja EBV-a, a EBV je otkriven u ispirku grla svih HIV-pozitivnih bolesnika. Među svim testiranim uzorcima, EBV je najčešće detektiran u ispirku grla.

Zanimljivo zapažanje objavili su Donati i suradnici (25), a odnosi se na pojavu EBV DNK u plazmi i slini djece iz Ugande prije i poslije liječenja akutne malarije. Razina virusa u plazmi bila je viša kod djece s malarijom nego kod one bez malarije, ali između tih dviju skupina nije bilo značajnih razlika u EBV DNK-u u slini. U većini slučajeva liječenje malarije omogućilo je nestanak EBV DNK iz plazme, ali nije utjecalo na razinu u slini.

S obzirom na navedene činjenice, moglo bi se očekivati da i druge akutne infektivne bolesti mogu utjecati na pojavu EBV-a u slini. Međutim, infektivne bolesti različite su u svojoj etiologiji (virusi, bakterije, paraziti, gljive) te potiču i različite vrste imunosnih odgovora u ljudskom tijelu, a kao posljedica pojavljuje se određena klinička slika bolesti.

Zato na početku ovog istraživanja nismo odabrali jednu infektivnu bolest ili skupinu bolesti, nego smo željeli uključiti bolesnike s različitim akutnim zaraznim bolestima kako bismo testirali tezu da akutne infekcije s umjereno teškom kliničkom slikom mogu biti povezane s povećanim izlučivanjem EBV-a u slini. Preliminarni rezultati ograničeni su vrstom istraživanja koje je presječno (i stoga bez uvida u moguću dinamiku izlučivanja virusa tijekom i razrješenja akutnih infekcija) i ograničenom veličinom uzorka. Također nismo testirali ispitanike na prisutnu razinu stresa u vrijeme prikupljanja uzorka, iako je to čimbenik koji bi mogao utjecati na izlučivanje EBV-a u slini. No, može se pretpostaviti da je određena razina stresa neizbježna kod akutno oboljelih, osobito ako su hospitalizirani, bez obzira na povoljan tijek bolesti. Gotovo je nemoguće odvojiti element stresa prouzročen cjelokupnim stanjem od stresa izazvanog akutnom infektivnom bolešću.

Uzimajući u obzir sva ograničenja, ipak se može zaključiti da dobiveni podatci pokazuju da akutne infektivne bolesti (općenito) ne pridonose značajno povećanom izlučivanju EBV-a u slini.

Zaključak

Cilj ovog istraživanja bio je ispitati povezanost između izlučivanja Epstein-Barrova virusa u slini bolesnika s akutnim infektivnim bolestima. Naši preliminarni rezultati pokazuju da akutne infektivne bolesti ne utječu značajno na pojavu EBV kopija u slini. No, treba uzeti u obzir da se te bolesti

count that infectious diseases vary based on their etiology and can therefore stimulate the immune system in many different ways. Therefore, we believe that further studies on individual diseases and their causative agents are needed to either confirm or deny their effect on the occurrence of EBV in saliva.

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razlikuju u etiologiji i da mogu stimulirati imunosni sustav na različite načine. Zato će biti potrebna daljnja istraživanja koja će obuhvatiti različite infektivne bolesti i mnogobrojne uzročnike kako bi se stvarno potvrdio njihov utjecaj na pojavu EBV-a u slini.

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Saţetak

Epstein-Barrov virus (EBV) široko je rasprostranjen herpesvirus pa 90 % odrasle svjetske populacije ima protutijela. Nakon supkliničkih primarnih infekcija EBV-om i poslije infektivne mononukleoze, virus se može dulje izlučivati u slini. Bolesti i stanja koja se pritom pojavljuju obuhvaćaju mentalne poremećaje, bolesti vezivnoga tkiva, multiplu sklerozu, sistemski eritemski lupus, malariju i infekciju HIV-om. **Cilj:** Kako pojava EBV-a u slini tijekom akutnih infektivnih bolesti do sada nije sustavno istražena, cilj je bio istražiti moguću vezu između akutnih zaraznih bolesti i izlučivanja EBV-a u slini. **Materijal i metode:** U ovo presječno pilot-istraživanje uključeni su odraslih bolesnici hospitalizirani zbog akutnih infektivnih bolesti te kontrolna skupina s osobama bez akutnih zaraznih bolesti. Odabrano je ukupno 40 bolesnika s akutnim infektivnim bolestima i kao kontrola 41 zdrava osoba. Od ispitanika iz obje skupine uzeti su uzorci periferne venske krvi za serodijagnostiku i uzorci sline za EBV PCR testiranje. Prilagođeni su logit i opći linearni modeli prema proporcijama te ln (broj virusnih kopija) u svrhu generiranja prilagođenih proporcija i geometrijskih srednjih vrijednosti u dvjema skupinama ispitanika. Upotrijebljen je SAS za Windows 9.4. **Rezultati:** Najčešća akutna infektivna bolest bila je pneumonija COVID-19, a slijedi hemoragijska vrućica s bubrežnim sindromom. Proporcije ispitanika s pozitivnim serološkim testovima i onih s izlučivanjem virusa u slini bile su slične u objema skupinama. **Zaključak:** Naši preliminarni rezultati pokazuju da akutne infektivne bolesti ne utječu bitno na pojavu EBV kopija u slini.

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