IJBC 2021; 13(2): 44-47

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

ORIGINAL ARTICLE

Existence of Epstein-Barr Virus in the Umbilical Cord and Sera Samples of Healthy Pregnant Women

Ghaidaa Raheem Lateef Al-Awsi[1], Shireen Ali Obaid[2], Saade Abdalkareem Jasim[3], Fadyia Mahdi Alameedy[4*]

[1]College of Science, University of Al-Qadisiyah, Iraq
[2]Al-Diwaniyah Teaching Hospital, Diwaniyah, Iraq
[3]Al-maarif University College, Al-anbar, Iraq
[4]Department of Biology, Kufa, Najaf, Iraq

ARTICLE INFO

Article History:
Received: 03.02.2021
Accepted: 21.05.2021

Keywords:
Cord blood hematopoietic stem cell
Epstein Barr virus
Real time PCR
ELISA

**Corresponding author:*
Fadyia Mahdi Alameedy,
Department of Biology, Kufa, Najaf, Iraq
Email: bacteriology94@gmail.com

ABSTRACT

Background: Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disorder (EBV-PTLD) is a known and serious condition for the hematopoietic stem cell transplantation. This study evaluated the prevalence of EBV in umbilical cord samples of healthy pregnant women in Baghdad, Iraq.
Methods: 800 umbilical cord blood (UCB) samples were collected from healthy pregnant women. The enzyme linked immunosorbent assay (ELISA) test was conducted to detect IgM and IgG antibodies. The EBV deoxyribonucleic acid (DNA) was detected in the plasma and buffy coat contents using quantitative real-time polymerase chain reaction (RT-qPCR) and nested PCR techniques.
Results: Four (IgG-positive), five and six samples were positive in the ELISA, Nested PCR and RT-qPCR assays, respectively. The age of contaminated women included 26-34 years. Notably, in the ELISA method, all the plasma samples were IgM-negative.
Conclusion: The existence of EBV among healthy women is a concern and assessment of a larger sample size is necessary to determine large-scale presence of EBV infection. The RT-qPCR exhibited higher sensitivity than ELISA and nested PCR techniques which can be considered as a confirmatory test. For larger sample size investigation, the ELISA method is appropriate as a primary screening test for lower costs.

Please cite this article as: Al-Awsi GRL, Obaid SA, Alameedy FM. Existence of Epstein-Barr Virus in the Umbilical Cord and Sera Samples of Healthy Pregnant Women. IJBC 2021; 13(2): 44-47.

Introduction

The Epstein-Barr virus (EBV)-associated Post-transplant lymphoproliferative disorder (EBV-PTLD) can also occur following the transplantation of umbilical cord blood (UCB) which increases the mortality rate. Hence, the EBV detection is essential in this approach.[1-3]

EBV is a type 4 human herpes virus infecting >90% of world population and establishes a life-long latent infection. The virus is among important infectious diseases causing blood infection in Iraq.[4-6] In most cases, the virus enters the body through the pharynx, oral and lymphoid tissue epithelium causing malignancies in these tissues. The EBV binds to the C3d receptor of complement (CD21) on B cells and stimulates the DNA replication in these cells by expression of several antigens, such as viral capsid antigen (VCA), membrane antigen (MA), primary antigen (EA), both EA-D subgroups and EA-R, Ecthine Virus Nuclear Antigens (EBNA) and surface antigen, detectable by lymphocyte (LYDMA).[1] EBNA, EA and VCA viral proteins are used in serological screening.[7-9] Memory B cells act as EBV reservoir due to lack of viral antigens expression. Notably, the transmission of EBV during delivery is also possible through the UCB.[7] Patients who undergo hematopoietic stem cell transplantation are inevitably endured by conditions that can affect their immune system such as use of immunosuppressive drugs, chemotherapy regimens, and irradiation.[8, 10] The EBV play an important role in the development of PTLD mortality rate.[11-13] Moreover, the PTLD incidence ranges 8- 20% depending on the organ transplant.[14, 15] The aim of this

study was detection of EBV DNA in the umbilical cord buffy coat and sera samples from healthy women using RT-qPCR, nested PCR and ELISA techniques in Iraq.

Materials and Methods

Participants and Samples

Sampling was conducted from umbilical cord bank. In this method, according to a questionnaire that was designed, we tried to sample the umbilical cord that none of mothers had history of an infectious disease (hepatitis, AIDS, …) or genetic disease or diabetes and being completely healthy with a mean age of 25.2±3 years.

The UCB samples were collected from 250 neonates immediately after the birth using a sterile clamp. By inserting the needle into the umbilical vein, 10 mL of blood was collected into tubes containing ethylenediaminetetraacetic acid (EDTA). Also, 550 other samples were provided from the umbilical cord bank of Transfusion Organization. The were centrifuged at 2000 rpm (10 min) and the buffy coat layer was isolated and transferred into a new microtube and the plasma was kept to another tube and placed at -70 °C.

ELISA Test

The ELISA test was performed for the detection of IgG and IgM antibodies which bind to capsid antigens in plasma (Invitrogen) into 96-well plates. Their titers were measured using ELISA reader (Thermo Fisher Scientific) at optical density (OD) of 450 nm, where the Immune Status Ratio (ISR) value ≥1.10 was considered a positive result.

DNA Extraction

The DNA was extracted using the protocol provided by Roch (Germany). Briefly, 200 µL of buffy coat/plasma was taken and inoculated into 1.5 ml nucleases free tubes and then 5 µL Poly (A), 200 µl Binding Buffer, 5 µl of internal control and 50 µL Proteinase K were added. Then, all the reagents were mixed together with the sample and incubated for 20 s and then 10 min at 72 °C. After that, 100 µl of Binding Buffer was added and then mixed again. Using a filter tube and centrifugation the contents were precipitated onto the filter. The elution buffer (50 µl) was added onto the filters and centrifuged for a minute at 8000g. the obtained DNA were kept at -80 °C. The Brome Mosaic Virus Internal Control (BMV IC) was purchased from the Promega Company.

Quantitative Real-time PCR

For the detection of EBV genomic DNA, the RT-qPCR technique was performed on extracted DNA (Qiagen kit). The primers designed in house included forward: AAA CCT CAG GAC CTA CGC TG, Reverse: AGA CAC CGT CCT CAC CAC and Taq man probe: TAG AGG TTT TGC TAG GGA GGA GAC GTG TG. After the EBV DNA verification, the Nested PCR and RT-qPCR were implemented.

Nested PCR

The nested PCR was performed using two pairs of primers as followings: EBV 1: GCC AGA GGT AAG TGG ACT TT, EBV 2: TGG AGA GGT CAG GTT ACT TA, EBV 3: TTC TGC TAA GCC CAA CAC TC and EBV 4: CTG AAC GTG AAC CGC TTA amplifying a 192bp fragment.

The tube components included 10X PCR Buffer (2.5 µL), 1.25 µL (10 µM) of each primer, MgCl2 (3 µL, 2 µM), 0.5µL (100 µM) dNTP, Betain (2.5 µL, 2 µM), DNase/RNase free ddH2O (11.5 µL), 0.3 µL (1 U) of Taq polymerase and 2.5 µL of template DNA were mixed. The first and second stages of nested-PCR, reaction conditions included 94 °C (3min), followed by 25 cycles of 94 °C (15s), 55°C (1 min), 72 °C (1 min), 72 °C (10 min) and 4 °C (5 min).

Data Analysis

Data was analyzed using IBM SPSS version 20, where a $P<0.05$ was considered as a significant finding.

Ethical Approval

This study was approved by Baghdad University. The patients had the consent of participation in the study.

Results

Demographic Data of Participants

The women mean age included 25.2±3 years. There was not previous antiviral treatment among them. Also, none of them had underlying diseases such as diabetes or leukemia.

ELISA Test

In the ELISA assay, four (0.5% of) specimens were positive for anti-EBV IgG antibody. Additionally, none of the specimens were positive for the IgM, indicating lack of a primary intrauterine EBV infection. Moreover, 798 samples (99.75%) were negative for both antibodies.

Genomic Detection of EBV

Five (0.62% of) and six (0.75% of) samples were infected by the EBV DNA in the nested PCR and the RT-qPCR, respectively. These six participants aged 28-32 years (Table 1). None of them had used prior antiviral agent, but two had used antibiotic. Moreover, three (P=0.045) of them had the history of prior hospitalization which can

Table 1: The demographic data of infected healthy women

Participant No.	Age (year)	Antiviral use	Antibiotic use	Prior hospitalization
1	28	No	No	Yes
2	31	No	No	Yes
3	29	No	No	No
4	32	No	Yes	No
5	31	No	Yes	No
6	30	No	No	Yes

be considered as a risk factor for infection obtainment.

Discussion

EBV-PTLD includes serious and fatal complications following allogeneic hematopoietic stem cell transplantation of the UCB. This condition is developed due to the immunodeficiency B cell lymphocytes infected with the virus are transformed into lymphoblastic cells.[16, 17] The EBV remains latent in B cell lymphocytes and can be activated during the pregnancy.[16-20] Serologic evidence of EBV infection has been found in over 94% of the world's population without clinical manifestation in most of cases. However, if the infection occurs during puberty, the infection with the EBV can cause a benign lymphoproliferative disease, which is known as infectious mononucleosis.[1, 10, 21] Primary infection is rare during pregnancy and only 1.3 to 4.2% of pregnant women in industrialized countries are EBV seropositive.[10, 12] The anti-EA IgG has been detected among 55% of pregnant and 22-32% of non-pregnant women.[22] In another study, antibodies against the Bam HI region of the EBV was detected in 13% of pregnant and 3% of non-pregnant women.[23] EBV also causes several adverse conditions among offspring such as leukemia.[11]

In our study, of the800 umbilical cord samples, six and five cases were infected with the EBV using RT-qPCR and nested PCR, respectively, while four samples were positive in the ELISA assay. The age range of our patients was 25.2±3 years. The IgM titer has been detected among pregnant women with multiple sclerosis and age range similar to that from our study.[24-26] The frequency of EBV-infected cells in individuals varies from 23 to 625 cells per 10^7 B cells.[7, 27-29] Therefore, more samples and more sensitive tests are needed to determine the accurate level of infection of the UCB cells. Major limitations of this study included a narrow geographical area for the study and lack of gene expression analysis among positive cases.

Conclusion

In this study, a low number of healthy pregnant women were infected by the EBV which highlight the possibility of infection transmission through vertical way or UCB cells. The RT-qPCR and nested PCR had higher sensitivity than ELISA method; moreover, qPCR exhibited higher sensitivity than nested PCR technique which can be considered as a confirmatory test; particularly when larger sample size and volume be adopted. In addition, risk factors such as prior hospitalization and drug usage should be investigated in future studies.

Conflict of Interest: None declared.

References

1. Ferla V, Rossi FG, Goldaniga MC, Baldini L. Biological difference between Epstein–Barr virus positive and negative post-transplant lymphoproliferative disorders and their clinical impact. Front Oncol. 2020;10:506.
2. Tseng YJ, Ding WQ, Zhong L, Chen J, Luo ZG. Chronic active Epstein–Barr virus (CAEBV) enteritis. Inter J Infect Dis. 2019;82:15-7.
3. Salahuddin S, Fath EK, Biel N, Ray A, Moss CR, Patel A, et al. Epstein-Barr virus latent membrane protein-1 induces the expression of SUMO-1 and SUMO-2/3 in LMP1-positive lymphomas and cells. Sci Rep. 2019;9:1-3.
4. Barzinjy AA, Aziz KF, Hussen BM, Qader SS, Hamad SM, Qader AS, et al. Fear of COVID-19 as a precautionary measure to prevent the epidemic among the population of the Kurdistan Region/Iraq: based on a questionnaire survey. J Public Health. 2021; :1-8.
5. Al-Bdery ASJ, Mohammad GJ, Hussen B. Vancomycin and linezolid resistance among multidrug-resistant Staphylococcus aureus clinical isolates and interaction with neutrophils. Gen Rep. 2020;21:100804.
6. Nanakaly HT, Hussen BM. Seroprevalence of Cytomegalovirus Among Voluntary Blood Donors in Erbil Province, North Iraq. Zan J Pur Appl Sci. 2019;31:1-6.
7. Messick TE, Smith GR, Soldan SS, McDonnell ME, Deakyne JS, Malecka KA, et al. Structure-based design of small-molecule inhibitors of EBNA1 DNA binding blocks Epstein-Barr virus latent infection and tumor growth. Sci Translat Med 2019;11:eaau5612.
8. Cloudio G.Brustein, 'Marked increased risk of Epstein-Barr virus –related complication with the addition of anti thymocyte globulin to a nonmyeloablative conditioning prior to unrelated umblical cord blood transplantation', Blood J, 2013.
9. Vrzalikova K, Sunmonu T, Reynolds G, Murray P. Contribution of Epstein–Barr Virus Latent Proteins to the Pathogenesis of Classical Hodgkin Lymphoma. Pathogens 2018;7:59.
10. Kostadinova T, Ivanova L, Tsaneva D, Ermenlieva N, Stoykova Z, Tsankova G. Distribution of Epstein-Barr virus among women of reproductive age and children up to 1 year in the Varna region. J IMAB–Ann Proceed Sci Papers. 2019;25:2369-72.
11. Loutfy SA, Abo-Shadi MA, Fawzy M, El-Wakil M, Metwally SA, Moneer MM, et al. Epstein-Barr virus and cytomegalovirus infections and their clinical relevance in Egyptian leukemic pediatric patients. Virol J. 2017;14:1-1.
12. Maryan.H, Lansdor.P, 'Biology of Human Umbilical Cord Blood-Derived Hematopoietic Stem/Progenitor Cells, Oncological Research Unit, Oncology Hospital', National Medical Center, Mexico City, Mexico; Stem Cells 1998; 16:153-165.
13. Patrick. R Murray, Ken S. Rosenthal. Michaela Pf aller, 'Medical Microbiology', Seventh Edition, 2012, Chapter 53,517-518.
14. Kamel Khalili, Kuan-Teh Jeang. 'Viral Oncology: Basic Science and Clinical Applicationsby', 2010. Wlley-Blackwell
15. Fitzsimmons L, Boyce AJ, Wei W, Chang C, Croom-Carter D, Tierney RJ, et al. Coordinated repression of BIM and PUMA by Epstein–Barr virus latent genes maintains the survival of Burkitt lymphoma cells.

Cell Death Differ 2018;25:241-54.
16. Nkosi D, Howell LA, Cheerathodi MR, Hurwitz SN, Tremblay DC, Liu X. Transmembrane domains mediate intra-and extracellular trafficking of Epstein-Barr virus latent membrane protein 1. J Virol. 2018;92(17).
17. Jud A, Kotur M, Berger C, Gysin C, Nadal D, Lünemann A. Tonsillar CD56brightNKG2A+ NK cells restrict primary Epstein-Barr virus infection in B cells via IFN-γ. Oncotarget. 2017;8:6130.
18. Pattle SB, Farrell PJ. The role of Epstein–Barr virus in cancer Expert Opin Biol Ther 2006; 6:1193–205.
19. Hoebe EK, Wille C, Hopmans ES. Epstein–Barr virus transcription activator R upregulates BARF1 expression by direct binding to its promoter, independent of methylation. J Virol 2012; 86: 11322–32.
20. Hoffman BA, Wang Y, Feldman ER, Tibbetts SA. Epstein–Barr virus EBER1 and murine gammaherpesvirus TMER4 share conserved in vivo function to promote B cell egress and dissemination. Proceed the National Academy Sci 2019;116:25392-4.
21. Preikasaitis JK, Cockfield SM. Epstein–Barr virus infection and lymphoproliferative disease after hemopoietic stem cell or solid organ transplantation. In: Bowden RA, Ljungman P, Paya CV editors. Transplant Infections. 2nd Edition. Lippincott Williams & Wilkins; 2003. p. 326–49.
22. Pattle SB, Farrell PJ. The role of Epstein–Barr virus in cancer Expert Opin Biol Ther 2006; 6:1193–205.
23. Bu W, Joyce MG, Nguyen H, Banh DV, Aguilar F, Tariq Z, Yap ML, Tsujimura Y, Gillespie RA, Tsybovsky Y, Andrews SF. Immunization with components of the viral fusion apparatus elicits antibodies that neutralize Epstein-Barr virus in B cells and epithelial cells. Immunity. 2019;50:1305-16.
24. Pérez-Pérez S, Cuello JP, Martínez-Ginés M, Pardo-Rodríguez B, García-Domínguez JM, Domínguez-Mozo MI, et al. Evolution of antibody titres against Epstein–Barr virus and human herpesvirus 6A/B and expression of multiple sclerosis-associated retrovirus in the serum of pregnant multiple sclerosis patients. Sci Rep. 2021;11:1-5.
25. Izawa K, Martin E, Soudais C, Bruneau J, Boutboul D, Rodriguez R, et al. Inherited CD70 deficiency in humans reveals a critical role for the CD70–CD27 pathway in immunity to Epstein-Barr virus infection. The J Experiment Med. 2017;214:73-89.
26. Gottschalk S, Rooney CM, Heslop HE. Post-transplant lympholymphoproliferative disorders. Annu Rev Med 2005; 56:29–44.
27. Souza TA, Stollar BD, Sullivan JLLuzuriaga K, Thorley-Lawson DA Peripheral B cells latently infected withEpstein–Barr virus display molecular hallmarks of classical antigen-selected memory B cells. Proceedings of the National Acad Sci USA 2005; 102: 18093–18098.
28. Szymula A, Palermo RD, Bayoumy A, Groves IJ, Holder B, White RE. Epstein-Barr virus nuclear antigen EBNA-LP is essential for transforming naive B cells, and facilitates recruitment of transcription factors to the viral genome. PLoS Pathog. 2018;14:e1006890.
29. Frost TC, Gewurz BE. Epigenetic crossroads of the Epstein-Barr virus B-cell relationship. Curr Opin Virol. 2018;32:15-23.