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Epstein-Barr virus infection and cervical cancer risk: a meta-analysis

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Summary

Purpose of investigation: Cervical cancer is the fourth most common malignancy in women and the third leading cause of cancer death among females in less developed countries. Epstein-Barr virus (EBV), as an ubiquitous oncogenic herpes virus has been suggested functioning in several cancers, but its role in cervical cancer as a possible viral cofactor remains a controversial topic. *Materials and Methods:* The authors searched all English reports on studies for the association between EBV infection and cervical cancer using PubMed, Embase, Web of Science, and all Chinese reports were identified manually and online using China National Knowledge Infrastructure (CNKI). The strict selection criteria and exclusion criteria were determined. An unconditional logistic regression model was used to analyze potential parameters related to the EBV prevalence. Odd ratios (ORs) with 95% confidence intervals (CIs) were pooled to assess the strength of association between EBV infection and risk of cervical cancer. Publication bias was estimated using funnel plots. *Results:* A total of 21 studies with 1,260 patients were included in the present analysis.The overall prevalence of EBV infection in cervical cancer was 17.00% with 95% CI of 16.00-19.00%. In the statistical analysis, EBV infection has a stronger association with risk of cervical cancer (OR = 2.94, 95% CI = 1.65-5.22). *Conclusion:* The EBV infection has a tighter link with increased risk of cervical cancer.

Key words: Epstein-Barr virus; Prevalence; Cancer risk; Cervical cancer.

Introduction

Cervical cancer is the fourth most common malignancy in women despite advances in the detection, prevention, and treatment, with an estimated 485,000 new cervical cancer cases and 236,000 deaths worldwide in 2013 [1]. It is the third leading cause of cancer death among females in less developed countries where insufficient screening programs resulted in an increase of incidence and mortality [2].

It is appreciated that virtually all cases of cervical cancer are caused by persistent infection of human papillomavirus (HPV), which has more than 100 genotypes and can be divided into low risk HPV (lrHV) and high risk HPV (hrHPV) based on their capacity to cause benign and cancerous lesions [3, 4]. Cervical carcinogenesis is a long process evolving from cervical intraepithelial neoplasia (CIN) to invasive cervical cancer caused by epigenetic and genetic abnormalities, and studies have shown that hrHPV infection alone is insufficient to lead to the pathogenesis of cervical cancer because only a minority of HPV-infected women develop cervical cancer during their lifetime [5, 6]. Other causative agents such as viral and host factors should be taken into account for better understanding the progression of cervical cancer.

Epstein-Barr virus (EBV), as an ubiquitous oncogenic herpes virus, has been suggested as a possible viral cofactor in cervical cancer but its role remains controversial. Sixbey *et al.* first reported EBV infection in cultured ectocervical epithelial cells [7], following the findings of several studies reporting the detections of EBV DNA in endocervical scrapes or both in malignant and normal cervical biopsy tissues [8-10]. Despite all these, several other authors did not reach the same conclusion [11-13]. In this regard, the present authors performed this meta-analysis to explore the latent role of EBV in cervical cancer.

Materials and Methods

To identify all articles that examined the association of EBV and cervical cancer, the authors conducted a literature search of PubMed database, Embase, Web of Science, and China National Knowledge Infrastructure (CNKI). All the relevant articles retrieved were using the following terms: ‘‘Epstein-Barr virus’’, “cervical cancer” or “cervical carcinoma”. References of the retrieved publications were also screened for other relevant studies. When there were multiple publications from the same population, only the largest-scale study was included. Study selection was achieved by two investigators independently, according to the inclusion and exclusion criteria by screening the title, abstract, and full-text. Any dispute was solved by discussion. The language of publication was restricted to English and Chinese.

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Table 1. — *Prevalence of EBV in cervical cancer.*

Category	Subcategory	No of studies	No of cases	%	Prevalence (%)(95% CI)	Adjusted OR
Overall	Total	21	1260	100	17.00 (16.00-19.00)	
Region	Asia	14	1076	85.40	15.00 (13.00-17.00)	Ref
	Europe	3	50	4.00	38.00 (25.00-52.00)	2.44 (1.25-4.76)
	North Africa	2	102	8.10	52.0 (43.00-61.00)	1.41 (0.83-2.40)
	South America	2	32	2.50	62.00 (46.00-79.00)	6.07 (2.65-13.90)
Year	1993-2000	6	219	17.38	45.00 (39.00-51.00)	Ref
	2001-2010	8	361	28.65	7.00 (5.00-10.00)	0.33 (0.23-0.48)
	2011-2015	7	680	53.97	29.00 (26.00-32.00)	0.95 (0.60-1.50)
Tissue	Tissue	17	750	59.52	16.00 (14.00-18.00)	Ref
	Cell	4	510	40.48	22.00 (18.00-25.00)	0.36 (0.24-0.54)

95% CI: confidence interval, OR: odds ratio, Ref: reference.

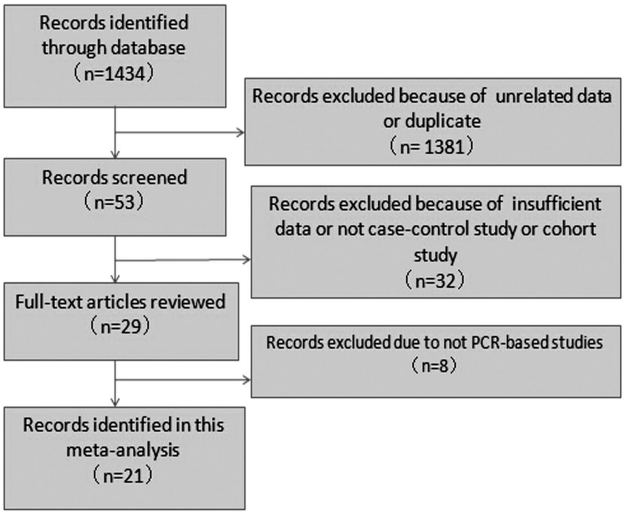

Figure 1. — Flow chart of literature selection.

Only research articles were included. If an article reported results including different studies, each study was treated as a separate comparison in this meta-analysis.

The flow chart of literature selection is shown in Figure 1. The following inclusion criteria were analyzed in selecting studies for the current meta-analysis, namely: (1) diagnosis of cervical cancer was proven by cytological or histopathological methods, (2) PCR-based technique was the only method used for detecting EBV infection, (3) for inclusion into the analysis, there was no limitation on the minimum number of patients of every single study. In instances where the research data were published in more than one article, only the publication with the most explicit description was included.

The exclusion criteria were defined as,overlapping of the data, case-only studies, and review articles. Studies using other methods to examine specimens were excluded.

Three investigators (Cao Hanyu,Wang Si, and Zhang Zhenyu) extracted all data independently and complied with the inclusion criteria listed above. Any discrepancy was resolved by discussion until an agreement was reached between the investigators. The following information was collected from each publication: the first author's name, publication year, patient's country, the sample types, techniques, number of patients, and the EBV prevalence.

First, the authors conducted an epidemiological evaluation of the EBV prevalence in cervical carcinoma cells or tissues and explored latent parameters for EBV infection or defection. An unconditional logistic regression was adopted to adjust odds ratios (ORs) according to several parameters of putative influence factors: region (Asia, Europe, North Africa, and South America), publication years (1993-2000, 2001-2010, and 2011-2015), and sample types (cell and tissue). Second, OR and 95% confidence intervals (CIs) were pooled to evaluate the association between EBV infection and risk of cervical cancer. Heterogeneity was investigated by the Cochran's chi-square Q test with a significance level of $p < 0.10$ and $I^2>50\%$. In this case, the random effects model would be used to estimate the pooled ORs [14]. Otherwise, the pooled ORs were estimated by the fixed effects model. Begg's funnel plots were performed to investigate publication bias [15]. All statistical tests were performed with Stata 12.0 software.

Results

As shown in Table 1, a total of 21 eligible studies published between 1993 and 2015 from different countries were included in this meta-analysis, with the number of patients ranging from nine to 464 per study, for a total population of 1,260 patients across studies [16-36]. Among them, 14 studies were conducted in Asia, three in Europe, two in North Africa, and two in South America. Six studies were published between 1993 to 2000, eight between 2001 to 2010, and seven between 2011 to 2015. In addition, four studies dealt with cell samples while the remaining undertook samples from fresh tissues or paraffin-embedded tissues.

All research detected prevalence of EBV using PCR-based technique and were divided into three subgroups based on their region, publication year and sample type. The overall prevalence was 17.00% with 95% CI of 16.00-19.00%. Among the first subgroup, the Asian subjects own the largest sample size of 1,076 cases with the lowest prevalence (15.00%, 95%CI = 13.00-17.00%). The European subjects and the South American subjects both have significantly higher prevalence of EBV infection compared to

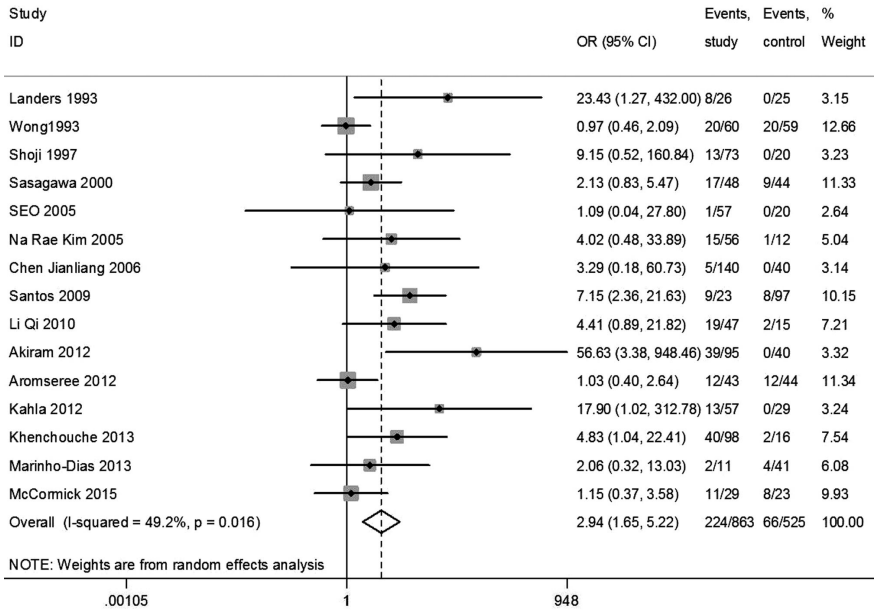

Figure 2. — Association between EBV infection and cervical cancer risk (ORs). Fifteen studies including control groups with normal tissues are adopted for calculating the pooled OR and 95% CI using a random-effects model due to the high heterogeneity (I2=49.2%, p = 0.016). An increased 2.94-fold (95% CI = 1.65-5.22) risk of cervical cancer compared to control groups is revealed.

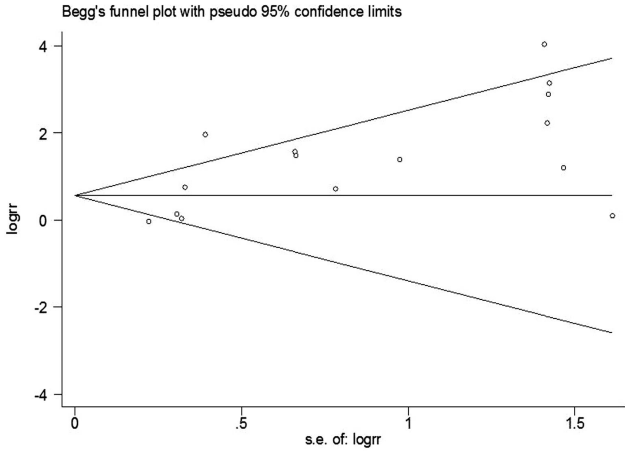

Figure 3. — Publication bias of estimated by Begg funnel plot. Begg's test was conducted for measuring publication bias of included studies in the meta-analysis of EBV infection in cervical cancer and shows a non-significant publication bias (p = 0.235).

the Asian studies (OR = 2.44, 95% CI = 1.25-4.76 and OR = 6.07, 95% CI = 2.65-13.90), while the North American showed no significant increase. Among the subgroup of publication year, only subjects in the period of 2001-2010 demonstrated significantly lower prevalence than that in the first period. With regards to the subgroup of sample type, subjects using cell sample (16.00%, 95% CI = 14.00-18.00%) presented significant lower (OR = 0.36, 95% CI = 0.24-0.54) prevalence than studies based on the tissue samples (16.00%, 95% CI = 14.00-18.00%).

Data from 15 studies included control groups with normal tissues were extracted for calculating the pooled OR and 95% CI. Considering evidence of high heterogeneity (I^2 = 49.2%, p = 0.016), a random-effects model was used, revealing an increased 2.94-fold (95% CI = 1.65-5.22) risk of cervical cancer compared to control groups (Figure 2). Visual inspection of the Begg funnel plot (Figure 3) revealed no obvious asymmetry (p = 0.235).

Discussion

It is now estimated that nearly 10% of worldwide cancers are attributable to viral infections [37]. Among the most common oncoviruses, EBV was the first virus that was isolated and found to be linked to human tumor. Recently EBV has been found to play an etiologic role in several human malignant tumors and was detected in nasopharyngeal carcinomas (NPC), gastric carcinomas, testicular carcinomas, and breast carcinomas [38-41]. The EBV latent membrane proteins have been reported to cause genetic changes and establish a latent transforming infection in epithelial cancer cells via contact with infiltrating EBV-positive B-cells with a possible effect of virus activation, due to the inflammatory milieu of the tumor, which could lead to increased expression of factors involved in angiogenesis and cell invasion and thus favor tumor progression [42-44]. However, correlation between EBV infection and risk of cervical cancer remained a controversial topic over the past decades, although there were many studies showing potential relationship between EBV viral replication and epithelial differential in uterine cervix. Explanation for this putative association is crucial to better understanding the cofactor role of EBV infection in the etiology of cervical cancer and contributes to its early detec-

tion and better prognosis.

To the best of the present authors' knowledge, this is the first meta-analysis that described the prevalence of EBV infections in cervical cancer. Possible association was explored by pooling data extracted from the included studies detecting EBV with PCR techniques. The authors detected the prevalence of EBV infection in this study adjusted by parameters such as region, publication year, and sample types. Statistics revealed that there was a significantly tighter link between EBV prevalence and cervical cancer in Europe and South America than in Asia (OR = 2.44, 95% CI = 1.25-4.76; OR = 6.07, 95% CI = 2.65-13.90, respectively). This heterogeneity is in accordance with the epidemiological evidence that incidence rates of EBV-positive nasopharyngeal carcinoma is much higher in Southern Asia than in Western countries [45]. In addition, EBV infection tended to influence population in less developed countries at an earlier stage than in more developed countries. The prevalence of EBV infection was at the highest level during publication year of 1993 to 2000, then declined sharply during period of 2001 to 2010 (OR = 0.33, 95% CI = 0.23-0.48) and was slightly elevated during the past few years. The previous decrease in the 2000-2010 may be due to more concentration on this oncogenic virus in some endemic areas and better treatment to control its spread. However, the latter slight increase indicated improved methods for detecting and quantifying EBV DNA and the evolving biological characteristics of the virus itself such as more frequent transmission through oropharyngeal secretion. Results concerning the sample types showed a significantly higher prevalence in tissue sample compared with cell sample (OR = 0.36, 95% CI = 0.24-0.54), which suggested variations derived from different sample preparation that makes comparisons of EBV infection prevalence from different studies difficult and should be taken into special consideration. Furthermore, the present authors found that EBV infection correlated more closely with an increased risk of cervical cancer (OR = 2.94, 95% CI = 1.65-5.22).

Despite the positive finding between EBV and cervical cancer, there are some limitations that should be taken into consideration. The first one is small sample size. A total of 1,260 patients may not be able to provide statistical evidence convincing enough to prove the role of EBV infection in cervical cancer. In addition, populations of studies from different regions varied greatly and were relatively small in Europe and South America, which made it difficult to evaluate geographic variation. Furthermore, the inclusion criteria placed restrictions on language of studies and only publications in English and Chinese were included, which may have led to incomprehensive collection of information. Moreover, the limited comparability of priority and merits of sample type resulted from the fact that no patients were detected using the two sample types at the same time .In this sense, further studies concerning this contentious topic should consider and try to avoid these potential confounding factors.

In conclusion, despite the limitations, results of the present meta-analysis suggested that EBV infection was associated with increased risk of cervical cancer, which raised the possibility that EBV contributes to the development of cervical cancer. Successful efforts to identify prevalence of EBV infection in cervical cancer may lead to further insight into etiology and pathogenesis as well as to new methods for therapeutic and prophylactic intervention.

References

[1] Fitzmaurice C., Dicker D., Pain A., Hamavid H., Moradi-Lakeh M., MacIntyre M.F., *et al.*: "The Global Burden of Cancer 2013". *JAMA Oncol.*, 2015, *1*, 505.

[2] Torre L.A., Bray F., Siegel R.L., Ferlay J., Lortet-Tieulent J., Jemal A.: "Global cancer statistics, 2012". *CA. Cancer J. Clin.*, 2015, *65*, 87.

[3] Kjaer S.K., Frederiksen K., Munk C., Iftner T.: "Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence". *J. N. C. I.*, 2010, *102*, 1478.

[4] Kadaja M., Sumerina A., Verst T., Ojarand M., Ustav E., Ustav M.: "Genomic instability of the host cell induced by the human papillomavirus replication machinery". *EMBO J.*, 2007, *26*, 2180.

[5] zur Hausen H.: "Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis". *J. N. C. I.*, 2000, *92*, 690.

[6] Missaoui N., Hmissa S., Trabelsi A., Frappart L., Mokni M., Korbi S.: "Cervix cancer in Tunisia: clinical and pathological study". *Asian Pac. J. Cancer Prev.*, 2010, *11*, 235.

[7] Sixbey J.W., Vesterinen E.H., Nedrud J.G., Raab-Traub N., Walton L.A., Pagano J.S.: "Replication of Epstein-Barr virus in human epithelial cells infected in vitro". *Nature*, 1983, *306*, 480.

[8] Szkaradkiewicz A., Wal M., Kuch A., Pieta P.: "Human papillomavirus (HPV) and Epstein-Barr virus (EBV) cervical infections in women with normal and abnormal cytology". *Pol. J. Microbiol.*, 2004, *53*, 95.

[9] Li L.,Zhang H.N., Zhang Y.K.: "Significance of HPV and EBV infections in cervical carcinogenesis". *Journal of Dalian Medical University*, 2011, 554.

[10] Se Thoe S.Y., Wong K.K., Pathmanathan R., Sam C.K., Cheng H.M., Prasad U.: "Elevated secretory IgA antibodies to Epstein-Barr virus (EBV) and presence of EBV DNA and EBV receptors in patients with cervical carcinoma". *Gynecol. Oncol.*, 1993, *50*, 168.

[11] Weinberg E., Hoisington S., Eastman A.Y., Rice D.K., Malfetano J., Ross J.S.: "Uterine cervical lymphoepithelial-like carcinoma. Absence of Epstein-Barr virus genomes". *Am. J. Clin. Pathol.*, 1993, *99*, 195.

[12] Elgui de Oliveira D., Furtado Monteiro T.A., Alencar de Melo W., Amaral Reboucas Moreira M., Alvarenga M., Bacchi C.E.: "Lack of Epstein-Barr virus infection in cervical carcinomas". *Arch. Pathol. Lab. Med.*, 1999, *123*, 1098.

[13] Payne S., Kernohan N.M., Walker F.: "Absence of in situ hybridization evidence for latent- or lytic-phase Epstein-Barr virus infection of preinvasive squamous lesions of the cervix". *J. Pathol.*, 1995, *176*, 221.

[14] DerSimonian R., Kacker R.: "Random-effects model for meta-analysis of clinical trials: an update". *Contemp. Clin. Trials*, 2007, *28*, 105.

[15] Begg C.B.: "A measure to aid in the interpretation of published clinical trials". *Stat. Med.*, 1985, *4*, 1.

[16] Landers R.J., O'Leary J.J., Crowley M., Healy I., Annis P., Burke L., *et al.*: "Epstein-Barr virus in normal, pre-malignant, and malignant lesions of the uterine cervix". *J. Clin. Pathol.*, 1993, *46*, 931.

[17] Tseng C.J., Pao C.C., Tseng L.H., Chang C.T., Lai C.H., Soong Y.K.,

et al.: "Lymphoepithelioma-like carcinoma of the uterine cervix: association with Epstein-Barr virus and human papillomavirus". *Cancer*, 1997, *80*, 91.

[18] Shoji Y., Saegusa M., Takano Y., Hashimura M., Okayasu I.: "Detection of the Epstein-Barr virus genome in cervical neoplasia is closely related to the degree of infiltrating lymphoid cells: a polymerase chain reaction and in situ hybridization approach". *Pathol. Int.*, 1997, *47*, 507.

[19] Guo F., Shi J.Y., Hu Y.F.: "Detection of Epstein-Barr virus in carcinoma of the cervix". Cancer Res. Prev. Treat, 1998, 81.

[20] Wong K.Y., Collins R.J., Srivastava G., Pittaluga S., Cheung A.N., Wong L.C.: "Epstein Barr virus in carcinoma of the cervix". *Int. J. Gynecol. Pathol.*, 1993, *12*, 224.

[21] Sasagawa T., Shimakage M., Nakamura M., Sakaike J., Ishikawa H., Inoue M.: "Epstein-Barr virus (EBV) genes expression in cervical intraepithelial neoplasia and invasive cervical cancer: a comparative study with human papillomavirus (HPV) infection". *Hum. Pathol.*, 2000, *31*, 318.

[22] Kim N.R., Lin Z., Kim K.R., Cho H.Y., Kim I.: "Epstein-Barr virus and p16INK4A methylation in squamous cell carcinoma and precancerous lesions of the cervix uteri". *J. Korean Med. Sci.*, 2005, *20*, 636.

[23] Seo S.S., Kim W.H., Song Y.S., Kim S.H., Kim J.W., Park N.H., *et al.*: "Epstein-Barr virus plays little role in cervical carcinogenesis in Korean women". *Int. J. Gynecol. Cancer*, 2005, *15*, 312.

[24] Chen J.L., Xiao Q.: "Expression and significance of high risk HPV and EBV gene in cervical cancer". *Journal of Nanhua University*, 2006, 244.

[25] Lau H.Y., Twu N.F., Chen P.C., Lai C.R., Juang C.M., Yen M.S., *et al.*: "The relationship between human papillomavirus and Epstein-Barr virus infections in relation to age of patients with cervical adenocarcinoma". *Taiwan J. Obstet. Gynecol.*, 2009, *48*, 370.

[26] Chao A., Tsai C.N., Hsueh S., Lee L.Y., Chen T.C., Huang S.L., *et al.*: "Does Epstein-Barr virus play a role in lymphoepithelioma-like carcinoma of the uterine cervix?". *Int. J. Gynecol. Pathol.*, 2009, *28*, 279.

[27] Szostek S., Zawilinska B., Kopec J., Kosz-Vnenchak M.: "Herpesviruses as possible cofactors in HPV-16-related oncogenesis". *Acta Biochim. Pol.*, 2009, *56*, 337.

[28] Santos N.B., Villanova F.E., Andrade P.M., Ribalta J., Focchi J., Otsuka A.Y., *et al.*: "Epstein-Barr virus detection in invasive and preinvasive lesions of the uterine cervix". Oncol. Rep., 2009, 21, 403.

[29] Li Q., Qiang X.: "Expression of EBV in cevical cancer". *Journal of Mudanjiang Medical University*, 2010, 47.

[30] Silver M.I., Paul P., Sowjanya P., Ramakrishna G., Vedantham H., Kalpana B., *et al.*: "Shedding of Epstein-Barr virus and cytomegalovirus from the genital tract of women in a periurban community in Andhra Pradesh, India". *J. Clin. Microbiol.*, 2011, *49*, 2435.

[31] Aromseree S., Pientong C., Swangphon P., Chaiwongkot A., Patarapadungkit N., Kleebkaow P., *et al*.: "Possible contributing role of Epstein-Barr virus (EBV) as a cofactor in human papillomavirus (HPV)-associated cervical carcinogenesis". *J. Clin. Virol.,* 2015, *73*, 70.

[32] Akiram H., Yushanjiang M., Niyazi M., Abudula A.: "The role of EBV AND HPV infection in cervical cancer development of Uyghur women". *Carcino Genesis, Terato Genesis & Muta Genesis*, 2012, 227.

[33] Kahla S., Oueslati S., Achour M., Kochbati L., Chanoufi M.B., Maalej M., et al.: "Correlation between ebv co-infection and HPV16 genome integrity in Tunisian cervical cancer patients". *Braz. J. Microbiol.*, 2012, *43*, 744.

[34] Marinho-Dias J., Ribeiro J., Monteiro P., Loureiro J., Baldaque I., Medeiros R., et al.: "Characterization of cytomegalovirus and epstein-barr virus infection in cervical lesions in Portugal". *J. Med. Virol.*, 2013, *85*, 1409.

[35] Khenchouche A., Sadouki N., Boudriche A., Houali K., Graba A., Ooka T., et al.: "Human papillomavirus and Epstein-Barr virus coinfection in cervical carcinoma in Algerian women". *Virol. J.*, 2013, *10*, 340.

[36] McCormick T.M., Canedo N.H., Furtado Y.L., Silveira F.A., de Lima R.J., Rosman A.D., *et al.*: "Association between human papillomavirus and Epstein - Barr virus DNA and gene promoter methylation of RB1 and CDH1 in the cervical lesions: a transversal study". *Diagn. Pathol.*, 2015, *10*, 59.

[37] Schiller J.T., Lowy D.R.: "Virus infection and human cancer: an overview". *Recent Results Cancer Res.*, 2014, *193*, 1.

[38] Pratesi C., Bortolin M.T., D'Andrea M., Vaccher E., Barzan L., Bidoli E., *et al.*: "Quantitative plasma/serum EBV DNA load by LMP2A determination in an Italian cohort of NPC patients". *J. Clin. Virol.*, 2003, *28*, 155.

[39] Chapel F., Fabiani B., Davi F., Raphael M., Tepper M., Champault G., et al.: "Epstein-Barr virus and gastric carcinoma in Western patients: comparison of pathological parameters and p53 expression in EBV-positive and negative tumours". *Histopathology*, 2000, *36*, 252.

[40] Shimakage M., Oka T., Shinka T., Kurata A., Sasagawa T., Yutsudo M.: "Involvement of Epstein-Barr virus expression in testicular tumors". *J. Urol.*, 1996, *156*, 253.

[41] Hippocrate A., Oussaief L., Joab I.: "Possible role of EBV in breast cancer and other unusually EBV-associated cancers". *Cancer Lett.*, 2011, 305, 144.

[42] Raab-Traub N.: "Epstein-Barr virus in the pathogenesis of NPC". *Semin. Cancer Biol.*, 2002, *12*, 431.

[43] Fawzy S., Sallam M., Awad N.M.: "Detection of Epstein-Barr virus in breast carcinoma in Egyptian women". *Clin. Biochem.*, 2008, *41*, 486.

[44] Huang J., Chen H., Hutt-Fletcher L., Ambinder R.F., Hayward S.D.: "Lytic viral replication as a contributor to the detection of Epstein-Barr virus in breast cancer". *J. Virol.*, 2003, *77*, 13267.

[45] Oh J.K., Weiderpass E.: "Infection and cancer: global distribution and burden of diseases". *Ann. Glob. Health*, 2014, *80*, 384.

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