Cytomegalovirus Infection and Cervical Cancer: from Past Doubts to Present Questions

Infeção por Citomegalovirus e Cancro do Cólo do Útero: de Dúvidas Passadas a Questões Presentes

Joana MARINHO-DIAS[1,3], Hugo SOUSA[1,2]
Acta Med Port 2013 Mar-Apr;26(2):154-160

ABSTRACT

Background: Since early 60's that Cytomegalovirus was studied for its possible role in cervical cancer development. Despite several decades of studies and the description of CMV DNA in cervical samples, it is still doubtful what is the prevalence of Cytomegalovirus in cervix and if CMV can act as a co-factor in cervical carcinogenesis.

Methods: In this Systematic Review we intend to summarize the frequency of Cytomegalovirus in cervical samples by revising all published studies between 1980 and 2011 regarding the detection of Cytomegalovirus in cervical samples and the development of lesions/invasive cervical cancer. Crude and adjusted frequencies of Cytomegalovirus infection were calculated according to country and world region.

Results: This study revealed that the worldwide crude frequency of Cytomegalovirus infection in the cervix was 18.9% in all cervical samples and 36.5% in HPV positive women. Cytomegalovirus infection was present in all different types of lesions: 17.4% in normal/cervicitis, 28.0% in LSIL, 19.7% in HSIL and 44.4% in CIS/ICC. The overall rate of Cytomegalovirus infection varied from 1.58% to 61.0% with an increased incidence in less developed countries.

Conclusion: In this study we described a high frequency of positive Cytomegalovirus cases in all types of cervical samples, with increased incidence in both HPV-infected women and CIS/ICC cases. Hence, despite results showed that Cytomegalovirus shedding in cervical samples is frequent more studies should be performed to clarify if Cytomegalovirus infection is an opportunistic infection in HPV-infected cases, or if it contributes for cervical immunosuppression that will favor HPV-associated carcinogenesis.

Keywords: Cytomegalovirus; Cytomegalovirus Infections; Uterine Cervical Neoplasms.

RESUMO

Introdução: Desde os anos 60 que o Citomegalovírus tem sido estudado pelo potencial papel no desenvolvimento de cancro do colo do útero. Após várias décadas de estudos e relatos da presença de DNA viral em amostras cervico-vaginais, ainda permanecem dúvidas sobre qual a prevalência do Citomegalovírus no cólo do útero e se este pode ser um cofator da carcinogénese.

Métodos: Nesta Revisão Sistemática pretendemos descrever a prevalência de Citomegalovírus em amostras cervicais, revendo todos os estudos publicados entre 1980 e 2011 que descrevem a infecção por Citomegalovírus em amostras cervicais e o desenvolvimento de lesões/carcinoma invasor. As frequências da infecção por Citomegalovírus foram calculadas de acordo com a região no mundo.

Resultados: Este estudo revelou que a frequência bruta da infecção por Citomegalovírus no cólo do útero foi de 18,9% em todas as amostras e 36,5% em mulheres HPV positivas. A infecção por Citomegalovírus está presente em todas as diferentes lesões: 17,4% em normais/cervicite, 28,0% em LSIL, 19,7% em HSIL e 44,4% em CIS/ICC. A frequência global variou de 1,58% a 61,0%, com uma maior incidência em países menos desenvolvidos.

Conclusão: Neste estudo, verificou-se uma elevada frequência de casos positivos para Citomegalovírus em todos os tipos de amostras cervico-vaginais, com maior incidência em mulheres infectadas por HPV e em casos de cancro. Assim, são necessários mais estudos para esclarecer se a infecção por Citomegalovírus é uma infecção oportunista ou se contribui para a imunossupressão favorecendo a carcinogénese associada ao HPV.

Palavras-chave: Citomegalovírus; Infecções por Citomegalovírus Neoplasias do Colo do Útero.

INTRODUCTION

Cervical cancer is the third most common female cancer worldwide. Although there have been advances in detection and treatment, cervical cancer is still a major health issue, especially in developing countries.[1] In developed countries several enhancements have been made in prevention and treatment leading to a decline of both incidence and mortality.[2] Persistent infection by oncogenic types of HPV, such as HPV16, 18, 31, 45 and others, are considered the etiological factor for cervical carcinoma development. Although HPV is not a sufficient factor for developing cervical cancer, several other co-factors were identified, such as: smoking, infection by other sexually transmitted diseases (STI) (Human Immunodeficiency Virus, Chlamydia trachomatis, Cytomegalovirus, etc.), long term use of oral contraceptives, intrauterine device use, multiple full-term pregnancies, young age at full-term pregnancy and poverty.[2-4]

Human Herpesviruses (HHVs) can induce cell transformation by production of oncogenic proteins or by interacting with cell replication, which are important steps for cancer development[5] For many years, CMV was one of the HHVs that was thought to have a role in the development of cervical lesions.[6] CMV infects between 50 to 85% of the worldwide population by early adulthood. It can be transmitted orally, sexually and parenterally.[7] Although CMV is not cor-

1. Virology Service. Institute of Oncology of Porto. Porto. Portugal.
2. Molecular Oncology Group of Portuguese. Institute of Oncology of Porto. Porto. Portugal.
3. Superior School of Biotechnology (Universidade Católica Portuguesa). Porto. Portugal.
Recebido: 11 de Novembro de 2012 - Aceite: 16 de Abril de 2013

related to human cancer some studies suggest that it may be specifically associated with some human malignancies.[8] CMV DNA has been found in cervico-vaginal secretions; however no correlation has been made between this virus and the development of cervical carcinoma.[6,9-12]

The aim of this study was to perform a systematic review of all published data regarding CMV infection in the cervix, to determine what is the relationship between CMV infection and the development of lesions/invasive cervical cancer.

METHODS

Articles search and Selection criteria.

This systematic review was performed by searching Pubmed/Medline databases using the following query: ("Cytomegalovirus"[Mesh] OR "cytomegalovirus"[All Fields] OR "CMV"[All Fields] OR "Herpesvirus 5"[All Fields] OR "Human Herpesvirus 5"[All Fields] OR "HHV-5"[All Fields] OR "human cytomegalovirus"[All Fields] OR "HCMV"[All Fields] OR "citomegalovirus"[All Fields] OR "Cytomegalovirus Infections"[Mesh] OR "Cytomegalic"[All Fields]) AND ("uterine cervical neoplasms"[Mesh] OR "cervical intraepithelial neoplasia"[Mesh] OR "uterine cervical dysplasia"[Mesh] OR "uterine cervical diseases"[Mesh] OR "cervix infection"[All Fields] OR "cervix uteri"[MeSH] OR "cervix uteri"[All Fields] OR ("cervix"[All Fields] AND "uteri"[All Fields]) OR "cervix"[All Fields] OR "Uterus"[Mesh] OR "uterine"[All Fields]).

To be included in the study articles should clearly include data from CMV detection in cervical samples. Article search was restricted to data published after 1980 and included all articles available in English, French, Spanish, and Portuguese. We have also considered the references list of selected articles to identify additional studies.

Data extraction

Included studies were revised by the authors, in order to extract the necessary information to perform the analysis: design of study (case-control and case study), source of sample (cervical swabs or smears and biopsies), population, geographical location (region and continent), type of cervical lesions, presence or absence of Human Papillomavirus infection, CMV diagnostic methodology (Polymerase Chain Reaction, Nested, Real-time PCR, *in situ* Hybridization, Culture followed by indirect immunofluorescence) and CMV status.

RESULTS

Study Characteristics

The literature searches yielded a total of 376 articles from which we have obtained 26 published articles[3,11-35] to include in the systematic review (Fig. 1). Studies were excluded considering the following criteria: 1) articles older than 1980 (*n* = 30); 2) language, such as Russian, Polish, Chinese, Japanese or Italian (*n* = 21); 3) study performed in male population (*n* = 3); 4) other types of articles such as case reports (*n* = 25), reviews (*n* = 57) and letters (*n* = 7); 5) other types of samples than cervico-vaginal (*n* = 60); 6) other types of studies, such as *in vitro* studies (*n* = 24) and *in vivo* studies (*n* = 20); and 7) studies not related to the aim of this study (*n* = 66). Some of the 63 articles included were not available after request to authors (*n* = 37).

The 26 studies were performed in 17 different countries: 8 from Asia (China, India, Japan, Thailand and Turkey), 12 from Europe (Croatia, Denmark, Finland, Germany, Greece, Italy, Netherland and Poland), 2 from Africa (Kenya), 1 from Oceania (Australia) and 6 from North-America (Canada, USA). Studies were very different in the number of cases included, ranging from 17 to 1685 cases (mean 239 ± 337; median 143). The total number of cases was 6936 with the great majority of studies to be was conducted in Europe with a total of 1825 individuals, followed by Asia with 1490 individuals, North-America with 3118 individuals, Africa with 328 individuals and Oceania with 175 individuals (Table 1).

From the final 26 studies it was possible to identify a total of 29 individual populations on which CMV infection was characterized in cervical samples with different types of lesions. These individual populations include one group of women with with endometriosis, three from Human Immunodeficiency Virus (HIV) positive women, four from pregnant women, two from non-pregnant, two from women attending a STD clinic and seventeen regarding general population. The type of sample used to test CMV was also

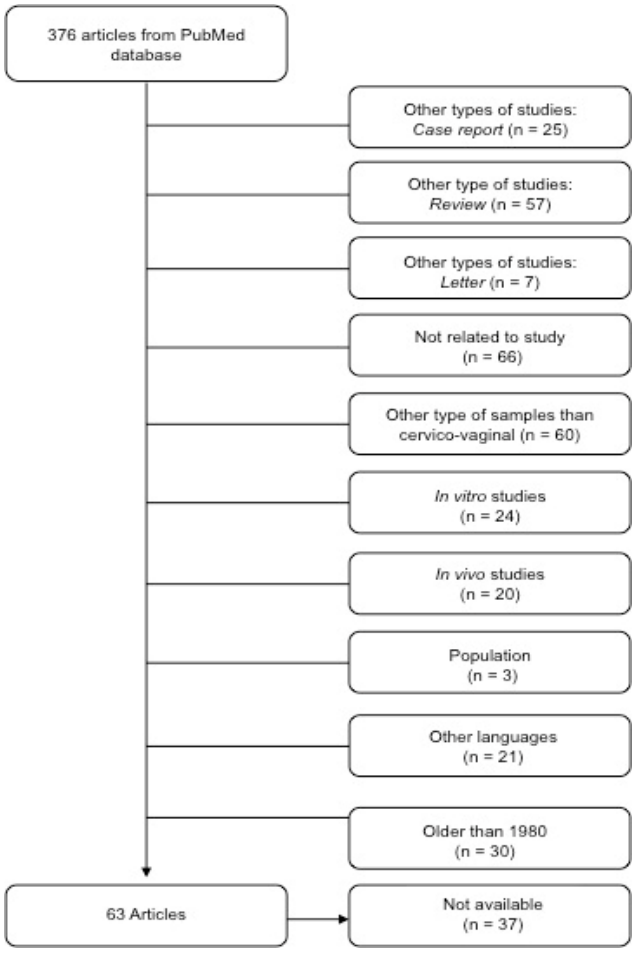

Figure 1 – Flowchart of application of inclusion/exclusion criteria.

different among the different populations: 16 with cervical swabs, 10 with cervical smears and 3 with biopsies.

Regarding the type of cervical lesions we found: seven studies with normal/cervicitis cases from Europe (*n* = 4), Asia (*n* = 2) and America (*n* = 1); six studies with low-grade squamous intraepithelial lesions (LSIL) from Europe (*n* = 5) and Asia (*n* = 1); nine studies with high-grade squamous intraepithelial lesions (HSIL) from Europe (*n* = 7) and Asia (*n* = 2); six studies with CIS/ICC from Europe (*n* = 3) and Asia (*n* = 3); and three studies with atypical squamous cells (ASC) from Europe. Moreover, considering the HPV infection status, we observed that only a minority of studies provided these data (n = 7), including a total of 535 cases, of which 302 individuals were from Asiatic countries and 233 individuals from Europe (Table 1).

Finally, we observed that the most common methodology for CMV diagnosis was Polymerase Chain Reaction (PCR) (*n* = 16), followed by Culture with indirect immunofluorescence (*n* = 7), Real-time PCR (*n* = 3), *In situ* Hybridization (*n* = 2) and one Nested-PCR (*n* = 1).

Data Analysis

Table 1 and 2 show the crude and adjusted frequency of CMV in different cervical lesions with pooled frequencies to be estimated by country and continent: crude rates were calculated by $(\sum_{CMV+cases}/\sum_{all\ cases})$ x 100; and adjusted rate was calculated as the average frequency of CMV positive cases.

The analysis of CMV infection status among all studies, revealed an overall crude frequency of 18.9% and adjusted frequency of 24.5% in cervical samples. Studies from Africa revealed the highest frequency, followed by Asia, Europe, North-America and Oceania (61.0%, 26.9%, 16.6%, 12.8% and 6.29%, respectively). Considering HPV infection, we observed that only studies from Europe and Asia showed data concerning HPV status, revealing an overall frequency of CMV infection of 36.4% in HPV positive women and adjusted frequency of 40.8%. Curiously, the crude frequency was significantly different from Europe to Asia (52.8% vs. 23.8%, respectively), while adjusted frequency revealed similar data. Considering the different cervical lesions, our study revealed an overall crude CMV frequency of 27.0% in Europe, 10.2% in China and 11.3% in USA in normal/cervicitis cases, with an overall global rate of 17.4%; in LSIL cases, CMV infection was present in 32.6% for Europe and 16.1% for China, with a global rate of 28.0%; in HSIL, data sowed an overall rate of 23.9% for Europe and 10.0% for China with a global rate of 19.7%; finally, in CIS/ICC cases, the analysis revealed a CMV frequency of 48.6% in Europe and 41.2% for Asia, with a global rate of 44.4%.

DISCUSSION

Cytomegalovirus is recognized as one of the most frequent viruses to infect the genital tract. Nevertheless, there is no study that clearly characterizes CMV infection in cervical epithelium.[3,32] After primary infection CMV is thought to develop a persistent infection in the genital tract with chronicle viral shedding in the cervix.[36] Moreover, several studies revealed that CMV can induce transformation of cells *in vitro* which could lead to cancer development. Despite the evidences and even considering that CMV could be found in biopsies of cervical carcinomas, CMV has not proved to be the causative agents of ICC. Nevertheless, CMV infection has been alleged to increase the risk of developing cervical cancer and although there are studies that report the presence of CMV in the cervix, only few studies refer the use of cervico-vaginal swabs or scrapes.[9,32,36]

An association between CMV infection and the development of cervical lesions/cancer is still the main question nowadays. In hospitalized patients the diagnosis of CMV infection is mainly performed by antigenemia or polymerase chain reaction (PCR). Antigenemia is frequently applied to follow-up patients and is the gold-standard technique in CMV detection, although, PCR is being described as more reliable and with higher sensitivity associated. Despite CMV detection is more often performed by serological characterization with about 50-85% of positive cases.[7] Several studies indicate that CMV DNA may be found in cervico-vaginal samples even when undetectable in the blood.[6,9,11,12] In fact, data points to a variation between only 2 to 66% of CMV positivity in cervico-vaginal samples and PCR or Real-time PCR are being suggested as the best approach for CMV detection.[3,32,37-39]

In this study, we have performed a systematic review of literature considering CMV and cervical lesions/cancer. Throughout acquisition of articles there were some major concerns: of the 376 articles obtained by the query, only 63 met the selection criteria. Of these 63 articles, 37 were not available mainly because articles were not accessible for free or institution subscription or even because authors did not reply to emails; another concern was the types of samples leading to exclusion of 60 articles because prevalence characterization of CMV infection was performed by serology/antigenemia in blood or serum samples.

Of all articles published we identified a total of 26 articles corresponding to 29 individual populations where CMV was analysed in cervico-vaginal samples. Considering these studies distribution of CMV infection by region varies significantly. The overall CMV infection frequency was of 18.9%: Africa showed the higher crude frequency (61.0%), followed by Asia with 26.9%, Europe with 16.6%, North-America with 12.8% and finally Oceania with 6.29%. Considering that there is a significant variation of number of individuals in the different we have calculated an adjusted frequency for CMV infection (24.5% worldwide): Africa remains the leader with the higher adjusted frequency (79.4%), followed by Asia (31.1%), Europe (16.7%) and North-America (16.1%). This variation in CMV infection frequency may be either correlated with specific population distribution or it may be explicated by the significant variations in the numbers of individuals analysed in each study. Nevertheless, these data show that CMV infection is significantly more prevalent amongst developing countries than developed.

CMV infection might not be the cause of cervical cancer

Table 1 – Prevalence of Cytomegalovirus in cervical samples by Country and World Region

	All cases					HPV+ cases					Normal/Cervicitis				
	n	Total cases	CMV + cases	Crude Frequency	Adjusted Frequency	n	Total cases	CMV + cases	Crude Frequency	Adjusted Frequency	n	Total cases	CMV + cases	Crude Frequency	Adjusted Frequency
EUROPE	12	1825	303	**16.6%**	16.7%	4	233	123	**52.8%**	43.5%	4	282	76	**27.0%**	27.3%
East Europe															
Poland	1	60	31	51.7%	---	1	60	31	51.7%	---	---	---	---	---	---
Central Europe	3	516	55	10.7%	15.8%										
Germany	2	339	36	10.6%	10.4%	---	---	---	---	---	1	35	6	17.1%	---
Netherlands	1	177	19	10.7%	---	---	---	---	---	---	---	---	---	---	---
North Europe	3	582	28	4.81%	2.7%										
Denmark	2	52	1	1.92%	---	---	---	---	---	---	---	---	---	---	---
Finland	1	530	27	5.09%	---	---	---	---	---	---	---	---	---	---	---
South Europe	5	667	189	28.3%	21.8%	3	173	92	53.2%	40.8%	3	247	70	28.3%	30.7%
Croatia	2	218	9	4.12%	2.7%	---	---	---	---	---	---	---	---	---	---
Greece	1	98	23	23.5%	---	1	50	15	30.0%	---	1	38	2	5.26%	---
Italy	2	351	157	44.7%	39.9%	2	123	77	62.6%	42.2%	2	209	68	32.5%	43.4%
ASIA	8	1492	401	**26.9%**	31.1%	3	302	72	**23.8%**	37.1%					
South-East Asia	6	893	278	31.1%	36.9%	2	209	45	21.5%	41.1%					
Thailand	1	42	28	66.7%	---	1	33	23	69.7%	---	---	---	---	---	---
Japan	3	379	192	50.7%	40.0%	---	---	---	---	---	---	---	---	---	---
China	2	472	58	12.3%	17.3%	1	176	22	12.5%	---	2	255	26	10.2%	12.6%
South Asia															
India	1	464	121	26.1%	---	1	93	27	29.0%	---	---	---	---	---	---
Central-western Asia															
Turkey	1	135	2	1.48%	---	---	---	---	---	---	---	---	---	---	---
NORTH AMERICA	6	3118	399	**12.8%**	16.1%										
Canada	1	247	21	8.05%	---	---	---	---	---	---	---	---	---	---	---
USA	5	2871	378	13.0%	19.6%	---	---	---	---	---	1	142	16	11.3%	---
AFRICA															
Kenya	2	328	200	61.0%	79.4%	---	---	---	---	---	---	---	---	---	---
OCEANIA															
Australia	1	175	11	6.29%	---	---	---	---	---	---	---	---	---	---	---
Global Analysis	29	6936	1310	**18.9%**	24.5%	7	535	195	**36.5%**	40.8%	7	679	118	**17.4%**	20.8%

Table 2 – Prevalence of Cytomegalovirus in cervical samples by Country and World Region (continuation)

	LSIL					HSIL					CIS/ICC				
	n	Total cases	CMV + cases	Crude Frequency	Adjusted Frequency	n	Total cases	CMV + cases	Crude Frequency	Adjusted Frequency	n	Total cases	CMV + cases	Crude Frequency	Adjusted Frequency
EUROPE	5	144	47	**32.6%**	29.4%	7	243	58	**23.9%**	19.0%	3	72	35	**48.6%**	49.4%
East Europe															
Poland	1	27	10	37.0%	---	1	10	3	30.0%	---	1	23	18	78.3%	---
Central Europe															
Germany	---	---	---	---	---	1	18	2	11.1%	---	---	---	---	---	---
South Europe	4	117	37	31.6%	27.4%	3	215	53	24.7%	29.0%					
Croatia	2	60	3	5.00%	4.06%	2	158	6	3.80%	2.34%	---	---	---	---	---
Greece	1	11	4	36.4%	---	---	---	---	---	---	2	49	17	34.7%	35.0%
Italy	1	46	30	65.2%	---	1	57	47	82.5%	---	---	---	---	---	---
ASIA															
South-East Asia											3	97	40	41.2%	35.1%
Thailand	---	---	---	---	---	---	---	---	---	---	1	42	28	66.7%	---
China	1	56	9	16.1%	---	2	107	11	10.0%	10.0%	2	55	12	21.8%	19.4%
Global Analysis	6	200	56	**28.0%**	27.1%	9	350	69	**19.7%**	17.0%	6	169	75	**44.4%**	42.3%

development, but several authors state that it might act as an inductor in HPV carcinogenesis. In fact, there are studies that refer that persistent infection by CMV leads to a higher predisposition to acquire HPV infection.[23] In our study, the overall crude CMV frequency amongst HPV positive women was of 36.5% and the adjusted frequency of 40.8%. This analysis was only possible for 7 populations, 3 from Europe and 4 from Asia, yet, this analysis revealed that co-infection of CMV with HPV was more prevalent in Europe than Asia (52.8% *vs.* 23.8%, respectively). This data might constitute a bias in the overall analysis of CMV association with HPV infection and the possible explanations for this fact are: number of individuals, age distribution or type of lesions.

CONCLUSION

Since our study discriminated the data of CMV frequency according to the different types of cervical lesions, we have observed that CMV is frequently found in the different lesions. Moreover, we observed that the frequency was lower in Normal/Cervicitis cases (17.4%) and that it is more prevalent in CIS/ICC cases (44.4%). The frequency of CMV infection did not show an increasing tendency as cervical lesions progress, although, the higher frequency amongst CIS/ICC cases may be correlated with a reactivation of CMV infection.

Overall, this systematic review revealed that CMV shedding in cervical samples is frequent and that it may be correlated with cervical cancer development, mainly after HPV-infection. Despite it was not possible to show a real role of CMV in the development of cervical cancer, the data of viral shedding in normal samples suggest that more studies should be performed to elucidate if CMV infection is an opportunistic infection in HPV-infected cases, or if it contributes for cervical immunosuppression that will favor HPV-associated carcinogenesis.

CONFLICT OF INTERESTS

There was none to be stated.

FUNDING SOURCES

There was none to be stated

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127:2893-917.
2. Chattopadhyay K. A comprehensive review on host genetic susceptibility to human papillomavirus infection and progression to cervical cancer. Indian J Hum Genet. 2011;17:132-44.
3. Broccolo F, Cassina G, Chiari S, Garcia-Parra R, Villa A, Leone BE, et al. Frequency and clinical significance of human beta-herpesviruses in cervical samples from Italian women. J Med Virol. 2008;80:147-53.
4. 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;11:235-8.
5. Salcedo Mde M, Silveira GP, Zettler CG. A expressão da proteína p16 e herpes simples vírus tipo 2 em lesões pré–neoplásicas e neoplásicas do colo do útero. Rev Bras Ginecol Obstet. 2008;30:61-6.
6. Clarke LM, Duerr A, Feldman J, Sierra MF, Daidone BJ, Landesman SH. Factors associated with cytomegalovirus infection among human immunodeficiency virus type 1-seronegative and -seropositive women from an urban minority community. J Infect Dis. 1996;173:77-82.
7. Tomtishen JP 3rd. Human cytomegalovirus tegument proteins (pp65, pp71, pp150, pp28). Virol J. 2012;9:22.
8. Johnsen JI, Baryawno N, Soderberg-Naucler C. Is human cytomegalovirus a target in cancer therapy? Oncotarget. 2011;2:1329-38.
9. Fox-Canale AM, Hope TJ, Martinson J, Lurain JR, Rademaker AW, Bremer JW, et al. Human cytomegalovirus and human immunodeficiency virus type-1 co-infection in human cervical tissue. Virology. 2007;369:55-68.
10. Landers RJ, O'Leary JJ, 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;46:931-5.
11. Lurain NS, Robert ES, Xu J, Camarca M, Landay A, Kovacs AA, et al. HIV type 1 and cytomegalovirus coinfection in the female genital tract. J Infect Dis. 2004;190:619-23.
12. Mostad SB, Kreiss JK, Ryncarz AJ, Overbaugh J, Mandaliya K, Chohan B, et al. Cervical shedding of cytomegalovirus in human immunodeficiency virus type 1-infected women. J Med Virol. 1999;59:469-73.
13. Biri A, Bozdayi G, Cicfti B, Dinc B, Yucel A, Rota S. The detection of CMV in amniotic fluid and cervicovaginal smear samples by real-time PCR assay in prenatal diagnosis. Arch Gynecol Obstet. 2006;273:261-6.
14. Chan PK, Chan MY, Li WW, Chan DP, Cheung JL, Cheng AF. Association of human beta-herpesviruses with the development of cervical cancer: bystanders or cofactors. J Clin Pathol. 2001;54:48-53.
15. Clarke LM, Duerr A, Yeung KH, Brockman S, Barbosa C, Macasaet M. Recovery of cytomegalovirus and herpes simplex virus from upper and lower genital tract specimens obtained from women with pelvic inflammatory disease. J Infect Dis. 1997;176:286-8.
16. Collier AC, Handsfield HH, Ashley R, Roberts PL, DeRouen T, Meyers JD, et al. Cervical but not urinary excretion of cytomegalovirus is related to sexual activity and contraceptive practices in sexually active women. J Infect Dis. 1995;171:33-8.
17. Coonrod D, Collier AC, Ashley R, DeRouen T, Corey L. Association between cytomegalovirus seroconversion and upper genital tract infection among women attending a sexually transmitted disease clinic: a prospective study. J Infect Dis. 1998;177:1188-93.
18. Eggert-Kruse W, Reuland M, Johannsen W, Strowitzki T, Schlehofer JR. Cytomegalovirus (CMV) infection--related to male and/or female infertility factors? Fertil Steril. 2009;91:67-82.
19. Faix RG, Zweig SE, Kummer JF, Moore D, Lang DJ. Cytomegalovirus-specific cell-mediated immunity during pregnancy in lower socioeconomic class adolescents. J Infect Dis. 1983;148:621-9.
20. Fujikawa T, Numazaki K, Asanuma H, Kudo R, Tsutsumi H. Frequency of human cytomegalovirus-specific T cells during pregnancy determined by intracellular cytokine staining. J Med Virol. 2003;71:527-31.
21. Furukawa T, Jisaki F, Sakamuro D, Takegami T, Murayama T. Detection of human cytomegalovirus genome in uterus tissue. Arch Virol. 1994;135:265-77.
22. Gradilone A, Vercillo R, Napolitano M, Cardinali G, Gazzaniga P, Silvestri I, et al. Prevalence of human papillomavirus, cytomegalovirus, and Epstein-Barr virus in the cervix of healthy women. J Med Virol. 1996;50:1-4.
23. Grce M, Husnjak K, Matovina M, Milutin N, Magdic L, Husnjak O, et al. Human papillomavirus, cytomegalovirus, and adeno-associated virus infections in pregnant and nonpregnant women with cervical intraepithelial neoplasia. J Clin Microbiol. 2004;42:1341-4.
24. Han CP, Tsao YP, Sun CA, Ng HT, Chen SL. Human papillomavirus, cytomegalovirus and herpes simplex virus infections for cervical cancer in Taiwan. Cancer Lett. 1997;120:217-21.
25. Koffa M, Koumantakis E, Ergazaki M, Tsatsanis C, Spandidos DA. Association of herpesvirus infection with the development of genital cancer. Int J Cancer. 1995;63:58-62.
26. McIver CJ, Rismanto N, Smith C, Naing ZW, Rayner B, Lusk MJ, et al. Multiplex PCR testing detection of higher-than-expected rates of cervical mycoplasma, ureaplasma, and trichomonas and viral agent infections in sexually active australian women. J Clin Microbiol. 2009;47:1358-63.
27. Mostad SB, Kreiss JK, Ryncarz AJ, Mandaliya K, Chohan B, Ndinya-Achola J, et al. Cervical shedding of herpes simplex virus in human immunodeficiency virus-infected women: effects of hormonal contraception, pregnancy, and vitamin A deficiency. J Infect Dis. 2000;181:58-63.
28. Pereira LH, Embil JA, Haase DA, Manley KM. Cytomegalovirus infection among women attending a sexually transmitted disease clinic: associa-

tion with clinical symptoms and other sexually transmitted diseases. Am J Epidemiol. 1990;131:683-92.
29. Schon HJ, Schurz B, Marz R, Knogler W, Kubista E. Screening for Epstein-Barr and human cytomegalovirus in normal and abnormal cervical smears by fluorescent in situ cytohybridization. Arch Virol. 1992;125:205-14.
30. Silver MI, 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;49:2435-9.
31. Huang SL, Liu ZQ, Zhang XN, Li MJ. Human papillomavirus, human cytomegalovirus and oncogene C-myc in cervical carcinoma and cervicitis. Chin Med J. 1993;106:208-10.
32. Szostek S, Zawilinska B, Kopec J, Kosz-Vnenchak M. Herpesviruses as possible cofactors in HPV-16-related oncogenesis. Acta Biochim Pol. 2009;56:337-42.
33. Vestergaard AL, Knudsen UB, Munk T, Rosbach H, Bialasiewicz S, Sloots TP, et al. Low prevalence of DNA viruses in the human endometrium and endometriosis. Arch Virol. 2010;155:695-703.
34. Wertheim P, Galama J, Geelen J, Buurman C, van der Noordaa J. Epidemiology of infections with cytomegalovirus (CMV) and herpes simplex virus in promiscuous women: absence of exogenous reinfection with CMV. Genitourinary Med. 1985;61:383-6.
35. Yliskoski M, Tervahauta A, Saarikoski S, Mantyjarvi R, Syrjanen K. Clinical course of cervical human papillomavirus lesions in relation to coexistent cervical infections. Sex Transm Dis. 1992;19:137-9.
36. Heggie AD, Wentz WB, Reagan JW, Anthony DD. Roles of cytomegalovirus and Chlamydia trachomatis in the induction of cervical neoplasia in the mouse. Cancer Res. 1986;46:5211-4.
37. Dinc B, Bozdayi G, Biri A, Kalkanci A, Dogan B, Bozkurt N, et al. Molecular detection of cytomegalovirus, herpes simplex virus 2, human papillomavirus 16-18 in Turkish pregnants. Braz J Infect Dis. 2010;14:569-74.
38. Farfan UM, Torres TJ, Vergara AA, Donoso WG, Alba GA, Paris DC, et al. Comparacion de las tecnicas de reaccion de polimerasa en cadena en tiempo real y antigenemia para la deteccion de citomegalovirus en sangre de ninos sometidos a trasplantes. Rev Chilena Infectol. 2011;28:113-7.
39. Leung AK, Loong EP, Chan RC, Murray HG, Chang AM. Prevalence of cytomegalovirus cervical excretion in pregnant women in Hong Kong. Asia Oceania J Obstet Gynaecol. 1989;15:77-8.