Detection of Zika Virus and Human Papilloma Virus in Cervical Cytology Samples using Two Real Time PCR Based Techniques in Ecuadorian Women diagnosed with ASCUS

Pool Tobar, MD; Marcela Vega, MSc; Cathy Ordoñez, BA; Lisette Rivera, MSc; José Landivar, QF; Hector Zambrano, MD

Objective: Atypical Squamous Cells of Undetermined Significance(ASCUS) is the most reported result on pap smears. It is most commonly related to Human Papillomavirus (HPV) infections. However, other pathogens (EBV, Chlamydia) have been reported on cervical samples with abnormal results. Zika virus(ZIKV) has been found to induce cellular abnormalities on different tissues and its presence has been identified on genital secretions.

Methods: Two real-time PCR has been made on cervical samples from the gynecological service of a third level hospital on Guayaquil-Ecuador. This work was part of a bigger study of ZIKV presence on different body fluids.

Results: From 89 samples obtained, 19 received an ASCUS result. From these 5 were positive for ZIKV and 5 were positive for HPV, there were no co-infections.

Conclusion: Given the presence of ZIKV RNA and the absence of HPV DNA on cervical samples diagnosed as ASCUS, it might be plausible that ZIKV could be a triggering factor for the induction of cellular changes observed on these cells. [*P R Health Sci J 2018;37(Special Issue):S96-S98*]

Key words: Zika, HPV, ASCUS

Atypical Squamous Cell of Undetermined Significance (ASC-US) is the most reported abnormal result on gynecological cytology tests (Pap-smear) (1). ASC-US diagnosis determines cellular abnormalities more prominent than those attributable to reactive changes, but that do not meet the requirements for a squamous intraepithelial lesion (SIL) (2). Nevertheless, this result is not always consistent even among experienced pathologists (3). Because the diagnosis of ASCUS might be equivocal, the molecular detection of HPV is used as triage for further management, in accordance to the American College of Obstetricians and Gynecologist (ACOG) guidelines (4). Cellular changes that grant a diagnosis of ASC-US may be associated to the presence of pathogens such as the aforementioned HPV, Chlamydia Trachomatis (5) or Epstein Barr virus(EBV) (6).

Zika virus (ZIKV) is a flavivirus that recently received attention due to the outbreaks of 2007 in French Polynesia and the outbreak of 2015, when it spread to the whole Western Hemisphere. ZIKV has the capacity to induce cellular abnormalities on progenitor cells and in the cerebral cells of animal models (7). ZIKV RNA has been found on cervico-vaginal fluids (8), where the RNA of ZIKV has been detected up to 11 weeks after the initial diagnosis (9). ZIKV sexually transmitted infections have been reported since 2011, in both male-to-female (10) and female-to-male (11).

Although there is a lot of evidence of the presence of HPV in ASC-US diagnosed cervical samples, there is little to no evidence of the association of ZIKV with ASC-US.

The aim of this work was to determine the presence of HPV and ZIKV in a group of Ecuadorian patients with the cytology diagnostics of ASC-US. These samples were obtained during the first ZIKV outbreak in Guayaquil, Ecuador.

Methods

This study was done in a reference center from Guayaquil, the most populated city in Ecuador, located on a region with humid tropical climate with previous outbreak of Dengue and Chikungunya and the presence of vectors of these viruses. The present work has been approved by the research committee of the hospital. The Hospital Review Board approved and reviewed this manuscript. The study was part of a larger study aimed for the detection of ZIKV in different body fluids.

Luis Vernaza Hospital, Molecular Biology Department Medical Director, International Centre of Reproductive Health U Gent, Belgium

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Address correspondence to: Hector Zambrano, MD. Email: hzambrano@jbgye.org.ec

Patients

Women included in this study were attending gynecology consultation for routine control. As such, these women underwent both Pap smear and HPV testing. For Pap smear a sample was obtained using an Ayre spatula and cervical brush for each woman. For HPV testing, a liquid cytology sample was obtained using a Rovers Cervex brush in the same consultation. Liquid cytology samples were kept in Presercyt Transportation Medium and stored at room temperature until tested.

Molecular detection of HPV and ZIKV

Samples were tested for high risk HPV genotypes (HPV-HR) using Cobas HPV 480 (Roche Diagnostics) following manufacturer guidelines. Cobas HPV has specific call-outs for HPV 16 and HPV 18 and combines detection of the remaining 12 genotypes.

Detection of ZIKV was done using a validated, laboratory-developed real-time rt-PCR (the ZCD assay) as described previously (12). All samples were taken at the outpatient gynecologic clinic by two gynecologists.

Results

We reviewed the records of women who underwent gynecology routine examination in the Gynecologic service of Luis Vernaza Hospital between the months of June and July 2016.

We retrieved records from 89 women tested for HPV through Pap Smear. Convenience cervical cytology samples from those women were tested for ZIKV virus.

Out of these 89 patients, nineteen (n=19) had pap smears results of ASCUS. Data from these 19 patients were included in this study (Table 1). The mean patient age was 36.3 years (SD 17.4). Results of testing using the Cobas HPV are shown in Table 1. Five Samples tested positive for HR-HPV. Results of testing using the ZCD assay are also shown in Table 1. Five patients tested positive for ZIKV. Fourteen women tested negative in the ZCD assay but had detectable RNase P, indicating sufficient nucleic acid extraction and the absence of PCR inhibitors. We did not find any HPV-ZIKV co-infection. There were nine cases negative for both HPV and ZIKV.

Table 1. Demographic information

Patient	Age	Number of Pregnancies	HR-HPV	ZIKV	ASC-US
1	41	0	NR	NR	R
2	32	0	R	NR	R
3	34	1	NR	R	R
4	35	0	NR	R	R
5	34	0	NR	NR	R
6	26	0	NR	R	R
7	58	1	NR	R	R
8	30	0	R	NR	R
9	33	0	NR	NR	R
10	31	0	NR	NR	R
11	39	0	NR	NR	R
12	44	0	R	NR	R
13	28	1	NR	R	R
14	52	0	R	NR	R
15	37	1	R	NR	R
16	40	0	NR	NR	R
17	30	0	NR	NR	R
18	40	0	NR	NR	R
19	29	1	NR	NR	R

R: Reactive, NR: Not-Reactive

Discussion

In the current paper, we present 19 patients who attended to a single center in Guayaquil, Ecuador, and received a diagnosis of ASCUS in Pap smear. We found five women positive for HPV-HR. We also identified five individuals with detectable RNA from ZIKV, in cervical cytology.

ASC-US diagnosis reflects a subjective-prone result, in which it is difficult to differentiate between reactive changes and Low Grade Squamous Intraepithelial Lesion (LSIL) (2). Inter-observer inconsistency among different pathologist for the same sample has been described (3). In a related matter, Macêdo et al. analyzed 103 cervical specimens initially classified as ASC-US, confirming the ASC-US diagnosis in only 70/103 (68%) of the cases (4). Thus, AS-CUS can be considered in a large proportion of cases, a subjective diagnostic.

An AS-CUS diagnosis, in despite of its inherent inter observer inconsistency and subjectivity, may be also the result of real cervical cytology changes due to the action of pathogens. Therefore, following ACOG recommendations an ASC-US result grants a triage for HPV, because of their well-established relationship (4). Nevertheless, Pirog et al. found only a 49% prevalence of HPV DNA on their ASC-US set of samples (13). We found a 26% of HPV DNA on this study samples.

Macedo et al. found on their 70 confirmed ASC-US samples, that 40% of their cervical samples had a concomitant non-HPV infection (5). Cells from the Cervical Transformation Zone (CTZ), seem to have infectious susceptibility towards viral agents (14). Thus, cervical cytological sampling focus on this area. EBV has been link to cytopathological changes in cervical specimens (6). It seems reasonable to think that viruses other than HPV and EBV may induce cellular changes in the cervix. Those cellular changes may eventually be detected during a routine pap smear and receive the diagnostics of ASCUS.

ZIKV has shown to induce apoptotic, aplastic and dysfunctional changes on different cellular and animal models (7,15). Cellular models of ZIKV infection in cerebral human cells cultures displayed evident morphological abnormalities including signs of cell death (16). There is, however, little evidence of cellular changes induced in cervical tissue of women. The presence of ZIKV ARN has been detected on cervical secretions long after the resolution of symptoms (9). Although, all our patients were asymptomatic for arboviral related symptoms at the time of the sample recollection. 5/19

had a positive ZIKV result when analyzing their samples through rt-PCR. Their ASCUS diagnosis may be explained by the action of ZIKV. This provocative statement, however, needs to be confirmed by more evidence.

Sexual transmission of ZIKV has been described almost since the epidemic started, given the possible vertical exposure (7,10,11). In vitro studies have shown that uterine fibroblast is not only susceptible to ZIKV infection, but they remain viable after the infection, theoretically increasing vertical infectious risk (17).

To conclude, we are reporting a group of 19 women diagnosed with ASC-CUS. Five of these 19 women were positive for HPV and other five were positive for ZIKV. The presence of both of these viruses may explain the cytology chances associated to ASC-US diagnosis. Furthermore, the 5 patients for ZIKV are in their reproductive ages, thus, risen concern of the potential sexual and vertical transmission of ZIKV in this population. However, more prospective observational studies are required to stablish if the ZIKV is capable by itself to cause cervical cytology changes compatible with ASC-US.

Resumen

Objetivo: Células escamosas atípicas de significancia indeterminada(ASCUS) es el diagnóstico más reportado en pap smears. Comúnmente se relaciona a infecciones por Virus del papiloma Humano(HPV). Sin embargo, otros patógenos (EBV, Chlamydia) se han reportado en muestras cervicales anómalas. Se ha observado que el virus del Zika(ZIKV) puede inducir anormalidades celulares en diferentes tejidos y su presencia se ha identificado en muestras genitales. Métodos: Se han realizado dos pruebas de PCR en tiempo real en muestras cervicales obtenidas del servicio de ginecología de un hospital de tercer nivel en Guayaquil-Ecuador. Este estudio es parte de una investigación mayor que identifico la presencia de ZIKV en muestras de diferentes fluidos corporales. Resultados: De 89 muestras obtenidas, 19 recibieron el diagnostico de ASCUS. De estas 5 fueron positivas para ZIKV y 5 fueron positivas para HPV. No hubo ningún caso de co-infección. Conclusión: Dada la presencia de RNA de ZIKV y la ausencia de DNA de HPV en muestras diagnosticadas como ASCUS, es posible que el ZIKV pueda ser un factor que induzca cambios celulares anómalos en este grupo celular.

References

1. Davey D, Neal M, Wilbur D, et al. Bethesda 2001 implementation and reporting rates: 2003 practices of participants in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. Arch Pathol Lab Med 2004;128:1224-1229.
2. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002;287: 2114-2119.
3. Stoler M, Schiffman M. Atypical Squamous Cells of Undetermined Significance-Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS) Group. Interobserver reproducibility of cervical cytology and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study. JAMA 2001;285:1500-1505.
4. The American College of Obstetricians and Gynecologist. Cervical Cancer screening and prevention. Obstet Gynecol 2016;128:111-130.
5. Macêdo A, Márcia M, Sheila A, et al. Atypical Squamous Cells of Undetermined Significance: Bethesda Classification and Association with Human Papillomavirus. Infect Dis Obstet Gynecol 2011;2011:904674
6. Khenchouche A, Sadouki N, Boudriche A, et al. Human papillomavirus and Epstein-Barr virus co-infection in cervical carcinoma in Algerian women. Virol J 2013;10:340.
7. Miner J, Diamond M. Zika Virus Pathogenesis and Tissue Tropism. Cell Host Microbe 2017;21:134-142.
8. Zambrano H, Waggoner J, León K, et al. High incidence of Zika virus infection detected in plasma and cervical cytology specimens from pregnant women in Guayaquil, Ecuador. Am J Reprod Immunol 2017;77:e12630.
9. Murray K, Gorchakov R, Carlson R, et al. Prolonged Detection of Zika Virus in Vaginal Secretions and Whole Blood. Emerg Infect Dis 2017;23: 99-101.
10. Foy DB, Kobylinski KC, Joy LC , et al. Probable Non–Vector-borne Transmission of Zika Virus, Colorado, USA. Emerg Infect Dis 2011;17:880-882.
11. Davidson A, Slavinski S, Komoto K, et al. Suspected Female-to-Male Sexual Transmission of Zika Virus — New York City, 2016. MMWR Morb Mortal Wkly Rep 2016;65:716-717.
12. Waggoner J, Gresh L, Mohamed-Hadley A, et al. Single-reaction multiplex reverse transcription PCR for detection of Zika, Chikungunya, and Dengue Viruses. Emerg Infect Dis 2016;22:1295-1297.
13. Pirog EC, Erroll M, Harigopal M, et al. Comparison of human papillomavirus DNA prevalence in atypical squamous cells of undetermined significance subcategories as defined by the original Bethesda 1991 and the new Bethesda 2001 Systems. Arch Pathol Lab Med 2004;128:527-532.
14. Herfs M, Yamamoto Y, Laury A, et al. A discrete population of squamocolumnarjunction cells implicated in the pathogenesis of cervical cancer. Proc Natl Acad Sci U S A 2012;109:10516-10521.
15. Ezinne CC, Veronika T, Katharina SL, et al. Zika virus infection in pregnancy: a systematic review of disease course and complications. Reprod Health 2017;14:28.
16. Cugola FR, Fernandes IR, Russo FB, et al. The Brazilian Zika virus strain causes birth defects in experimental models. Nature 2016;534:267-271.
17. Chen JC, Wang Z, Huang H, Weitz SH, et al. Infection of human uterine fibroblasts by Zika virus in vitro: implications for viral transmission in women. Int J Infect Dis 2016;51:139-140.