Coinfection of torque teno virus (TTV) and human papillomavirus (HPV) in cervical samples of women living in Tehran, Iran

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ABSTRACT

Background and Objectives: Torque Teno virus or transfusion-transmitted virus (TTV) is a non-enveloped virus with a single strand circular DNA genome that currently is classified in the Alphatorquevirus genus and the family of *Anelloviridae*. Unlike other DNA viruses, TTV has an extremely wide genomic diversity. This virus, based on previous studies, infects both healthy people, as well as those who have HCV and human papillomavirus (HPV). This study aimed to evaluate the coinfection of torque teno virus (TTV) and HPV in cervical samples from Iranian women.

Materials and Methods: In this case-control study, the fresh cervical cytobrush specimens were collected from 150 women referred to Dena laboratory in Tehran. Viral DNA was extracted from samples. The HPV-DNA was detected and genotyped. Then, nested polymerase chain reaction (Nested PCR) was performed for TTV using specific primers.
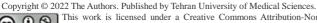
Results: Among 50 cervical specimens without HPV, 14 were TTV positive (28%); among 50 low-risk HPV cervical specimen, 23 were TTV positive (46%), and from 50 high-risk HPV cervical specimen, 48 were TTV positive (96%). There is a significantly higher prevalence of TTV virus in low-risk and high-risk papillomavirus-infected specimens than in healthy specimens (p 0.0001). Additionally, TTV is more prevalent in samples containing high-risk papillomaviruses than in samples with low-risk papillomaviruses (P = 0.048).

Conclusion: The higher prevalence of TTV among people infected with papillomavirus than in non-infected people indicates that both viruses are transmitted by the same mechanism (sexual route). In addition, the prevalence of TTV in samples containing high-risk papillomavirus is significantly higher than that in samples containing low-risk papillomavirus. The presence of papillomaviruses, particularly high-risk types, may be associated with TTV proliferation, which requires further research in the future.

Keywords: Coinfection; Papillomaviridae; Torque teno virus

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INTRODUCTION

Torque teno virus (TTV) or transfusion-transmitted virus is a ubiquitous, naked single-stranded DNA virus that belongs to the *Anelloviridae* family. Human TTV (huTTV) and other anellovirus genera such as the beta-torque virus, torque teno mini virus (TTMV), and the gamma-torque virus have high infection rates but no recognizable pathogenicity in humans (1, 2). There are ten genera in the *Anelloviridae* family. Alphatorquevirusis significantly more prevalent among human populations worldwide. It is classified into seven phylogenetic or genogroups (1). Since TTV was first detected in 1997 in a Japanese patient who died from post-transfusion hepatitis, the disease has been detected in humans worldwide. It is the most common component of the human blood virome (1-3). The first time, TTVs were found in the serum of a patient with non-A-E hepatitis, but later, TTVs were found in various human tissues (4). Studies have shown a link between TTVs and different diseases. For example, Genogroup 3 TTV was reported in gastritis associated with *Helicobacter pylori*, and Genotype 1 is linked to Epstein-Barr virus (EBV)-associated Hodgkin lymphoma, as well as its relationship with CD4-positive cells in HIV-infected patients (4).

A comparable study suggests that the cervical mucosa may be a more approving site for TTVs than the respiratory tract mucosa (4). In females, the lower genital tract mucosal barrier is the first line of defense against pathogens. In order to prevent pathogens from entering the body, epithelial cells create a physical barrier; furthermore, dendritic cells, macrophages, NK cells, and chemical elements are the main parts of the innate immune system. However, many common infectious pathogens such as HPV enters to the body through the mucous membrane. HPV is one of the most common viral pathogens of cervical epithelial cells (5). *TTV ORF1* has a role in immune evasion and persistence of the virus (6). New data indicate that the TTV ORF2 protein may be involved in regulating the host's innate and adaptive immunity (7). The ORF3 genomic region encodes some proteins such as ORF3 protein and TTV-derived apoptosis-inducing protein (TAIP). The researchers have indicated that inhibiting interferon signaling by TTV microRNAs (miRNAs) improves TTV evasion and aids TTV persistence in the host (6). Inflammation pathways or the presence of ma-

lignant tissue can provoke genogroup 1 TTV-linked immunological changes. Studies have shown the potential role of TTV in the downregulation of inflammatory response and suppressor tools for interferon production and interferon-related gene expression. These modifications may convert HPVs infection cycle towards non-productive persistent and provide appropriate circumstances for viral carcinogenesis activities (4).

Human papillomavirus (HPV), ubiquitous dsD-NA virus, is a member of the *papillomaviridae* family with more than 200 identified genotypes. High-risk HPV genotypes include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 that are associated with cervical malignancies and anogenital, neck, and head cancers. Low-risk HPV genotypes 6 and 11 are usually detected in benign or low-grade cancerous tissues (8). Approximately 70% of cervical cancers and 50% of cervical intraepithelial neoplasia grade 3 (CIN3) are linked to HPV16 and HPV18 (9).

The main transmission routes of TTV infection are parenteral, fecal-oral, and possibly saliva. Following TTV's DNA was detected in semen and cervical smears, researchers noted sexual transmission as another possible route of infection, mother-to-infant transmission is controversial. Moreover, a study has demonstrated a direct link between TTV prevalence increasing, and the rising number of sexual partners among drug users with a history of liver disorder Mother-to-infant transmission is controversial (5, 10). Therefore, the role of sexual transmission needs further study to confirm. In the present study, co-infection TTV and HPV is investigated to evaluate the hypothesis of the sexual route of TTV transmission. The nature of cooperation between HPV and TTV needs to be investigated further. To clarify the relationship between these two viruses, another aim of the present study was to evaluate the difference in the prevalence of TTV between low-risk and high-risk HPV samples.

MATERIALS AND METHODS

In this case-control study, the fresh cervical cytobrush specimens were collected from 150 women referred to Dena laboratory in Tehran from January 2020 to January 2021. The inclusion criteria were as follows: (1) agreement to participate; (2) no preg-

nancy. Women were excluded from the study if they had undergone a procedure on the cervix. DNA was extracted from 150 samples using commercial FavorPrep™ Blood / Cultured Cell Genomic DNA Extraction Mini Kit following the procedure provided by the manufacturer.

The HPV-DNA was detected and genotyped by the MolecuTech REBA HPV-ID kit (Molecules and Diagnostics, Wonju, Korea), which is a PCR-based reverse blot hybridization assay. This kit detects 18 high-risk HPV genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, and 73), one probable high-risk HPV (34), and 13 low-risk HPV genotypes (6, 11, 32, 40, 42, 43, 44, 54,70, 72, 81, 84, and 87). The nested PCR was performed with MY11 and MY9 primers in step 1, followed by the GP5/GP6 primer pair in step 2, using the Labcycler PCR system (SensoQuest, Germany). Then, PCR products were denatured with a denaturation solution and added into the membrane strip labeled with HPV genotype-specific probes. After washing unhybridized PCR products, the alkaline phosphatase enzyme reaction was performed, and chromogen was added to develop detection signals. Finally, the strips were scanned using REBASCAN (YD Diagnostics Corp).
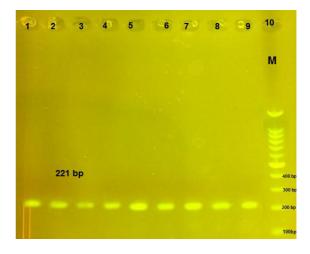
A nested-PCR test was performed for the TTV detection using primers (11) summerizad in Table 1. The reaction mix contained 5 µM of template DNA or controls, 8 µM of master mix (2×), 1 µM of primers mix (F1+R1), 1 µM dNTP, and 10 µM of sterilized DW. PCR protocol was included 5 min at 94℃ (for both first and second round), 35 cycles of the 45s at 94℃ (for both first and second round), 45s at 60℃ (for the first round) and 30s at 60℃ (for the second round), 45s at 72℃ and one final extension step at 72℃ for 10 min (for the first round) and 5 min (for the second round). PCR products were run on a 2% agarose gel and visualized using a UV transilluminator after staining with SYBR Safe DNA gel stain. A chi-square test used for statistical analysis through SPSS software version 20.

This study was approved by Research Ethics Committees of Islamic Azad Tehran Medical Sciences University (IR.IAU.PS.REC.1398.084).

RESULTS

We studied cervical samples from 150 women aged between 20 and 58 years old. Fifty women were HPV negative and 100 women were HPV positive. We detected the TTV genome in 87 cervical samples (Fig. 1) and according to statistical analysis, there was no association between age and TTV virus infection (P> 0.05). TTV DNA was found in 28% of HPV negative cases, in 96% of High-risk HPV positive cases, and in 46% of Low-risk HPV positive patients (Table 2). Based on data comparison in the three groups, it is shown that TTV DNA is significantly more prevalent in HPV-infected individuals (both high-risk and low-risk) than in HPV-negative individuals. (P <0.0001). The prevalence of TTV in people with low-risk or high-risk HPV is higher than in healthy HPV-negative people. Moreover, TTV is significantly higher in high-risk HPV-infected individuals than in low-risk HPV-infected individuals (P =0.048).

Fig. 1. Agarose gel electrophoresis Image of TTV DNA amplification with NG054 and NG1321 primers. Lan 1-8: positive samples, Lan 9: positive control, Lan10: ladder 100 bp.

Table 1. Oligonucleotide primer sequences

Primer	Polarity	Nucleotide position	Nucleotide sequence (5′-3′)	Reference
NG054	Sense	3-22	TTTGCTACGTCACTAACCAC	(11)
NG147	Antisense	211-233	GCGAGTCCCCGAGCCCGAATTGCC	(11)
NG132	Antisense	204-223	AGCCCGAATTGCCCCTTGAC	(11)

Table 2. Number and percentage of TTV DNA positive cases in both HPV positive and HPV negative group

		TTV Positive N (%)	TTV Negative N (%)	Total N (%)
HPV Negative		14 (28%)	36 (72%)	50 (100%)
HPV Positive	High risk HPV	48 (96%)	2 (4%)	50 (100%)
	Low risk HPV	23 (46%)	27 (54%)	50 (100%)

DISCUSSION

In the present study, 87 Iranian women were found to have TTV DNA in their samples, 28% of whom were HPV negative, while 96% were high-risk HPV positive and 46% were low-risk HPV positive. The prevalence of TTV in cervical smears was high in our study, as observed previously by Chan et al. Calcaterra et al., Salakova et al. and Fornai et al., (5, 12, 13). The prevalence of TTV-DNA in cervical smears in Calcaterra's study was 16.4%, without a meaningful difference between HPV-positive (18.6%) and negative (14.9%) samples. The number of infected cases with high/middle and low-risk HPV types was similar in TTV-positive and negative samples. Women with multiple HPV types had a higher TTV-DNA prevalence (60.0%) than HPV-negative women (12). In the study of Fornai et al. 75% of high-risk HPV infected individuals and 75% of low-risk HPV infected individuals were TTV positive (13). The HPV-positive cases in Salakova's study had significantly higher TTV DNA prevalence (71.6%) than HPV negatives (48.8%). TTV DNA has a significantly higher prevalence in cervical smears of symptomatic women (74.7%), and the prevalence rate was slightly higher in women with high-grade lesions (76.5%) than in those with low-grade lesions (68.2%). However, the difference was not statistically notable in this study. Based on the high prevalence of TTV in cervical smears, they suggested that sexual transmission might serve as another mode of TTV transmission (5). Zheng et al. demonstrated that In cervical smears of patients with cervical lesions and healthy women, the prevalence of TTV DNA was 52.7% (29/55) and analogous with that of matched serum samples (50%); however, women with cervical abnormality had a significantly higher prevalence of TTV DNA in their cervical smears (74.7%) than healthy women. HPV16, 18, 33 genotypes were frequent in high grade squamous intraepithelial lesions (HSIL), and HPV6 was more prevalent in low-grade squamous intraepithelial lesions (LSIL). This study demonstrated that HPV-positive cases had significantly higher TTV DNA prevalence than HPV negatives. In cervical smears, the TTV viral titer was 10 to 1000 times higher than in serum, suggesting active TTV replication in the female genital tract (14). Eniko″ Feher's study suggested that genogroup 1 TTV may be explicitly connected to some head and neck mucosal diseases, but refutes a (co) carcinogenic role of TTV in oral or cervical cancer and its association with HPV or HPV-associated malignancies (4). In the study from Isfahan/Iran, 62% of the tested samples were positive for TTMV, and virus genome was detected in 52.4% of adenocarcinoma, 68.4% of cervical intraepithelial neoplasia (CIN), and 100% squamous cell carcinoma (SCC) cases (15). Szladek et al. informed that co-infection of TTV and HPV promoted larynx squamous cell carcinoma (16). In this way, Suzuki et al. investigated the TTV genome in 18.6% of healthy donors and 24.32% of HPV-infected Brazilian women. TTV prevalence was notably higher among the HPV-positive patients with cervical cancer (57.14%) than in HPV noncancerous patients (16.67%). Consequently, they hypothesized that TTV infection could contribute to cancer progression in patients with HPV-TTV co-infection (16).

A significant limitation of our study was the lack of genotyping information of HPV/TTV-positive cases to assess the possible correlation between them. Moreover, we did not have access to patients' marital status, jobs, and clinical history data.

CONCLUSION

There is a direct correlation between the TTV prevalence and HPV infection in women. In the present study, a significant higher prevalence of TTV virus was found in HPV-infected individuals (high-risk and low-risk) than in HPV-negative cases, indicating a similar route of transmission for both viruses (sexual). The TTV is significantly lower in low-risk

HPV-infected cases than in high-risk HPV-infected cases. There are many aspects of TTV pathological roles that remain unclear and can be investigated in the future.

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