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Association of Tumor Necrosis Factor *a-2* and *a-8* Microsatellite Alleles with Human Papillomavirus and Squamous Intraepithelial Lesions among Women in Brazil

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Infection with oncogenic human papillomavirus (HPV) is considered to be the major risk factor for cervical cancer. Tumor necrosis factor (TNF) is a pluripotent cytokine that plays an important role in inhibiting the action of microbial agents, and *TNF* microsatellite polymorphisms have been associated with several diseases, including cancer and viral infections. This study analyzed the associations between *TNFa* to *-e* microsatellite polymorphisms and the severity of squamous intraepithelial lesions (SIL), according to the presence of the oncogenic HPV16 and HPV18 types. Samples from 146 HPV-positive women with low-grade SIL (LSIL) and high-grade SIL (HSIL) and samples from 101 healthy women were studied. *TNF* microsatellite polymorphism typing and HPV detection and typing were performed using PCR-amplified DNA hybridized with sequence-specific primers. Data were analyzed by Fisher's exact test using the GENEPOP software. Significant associations were observed between LSIL and the *TNFa-8* allele (4/166; $P = 0.04$), as well as between *TNFa-2* with HPV18 only (16/44; $P = 0.002$) and *TNFa-2* with HPV18 coinfection with HPV16 (16/44; $P = 0.001$). Patients exhibiting the *TNFa-2* allele and harboring HPV18, in the presence or absence of coinfection with HPV16, had an increased risk of HSIL occurrence (13/38; $P = 0.04$; 5/10; $P = 0.04$) compared to patients with other HPV types. These results suggest that the *TNFa-8* allele is associated with increased susceptibility to the occurrence of LSIL and that despite the presence of a high TNF-α production allele, the ability of HPV18 to resist the inhibitory effects of TNF-α may contribute to the occurrence of infection and consequently to HSIL in women with cervical HPV18 infection.

Human papillomaviruses (HPV) are DNA viruses that induce epithelial cell transformation during the course of infection (31). Infections with specific HPV types appear to be an essential step in the development of invasive cervical cancer and precursor lesions, i.e., squamous intraepithelial lesions (SIL) (48). HPV DNA can be identified in more than 90% of invasive cervical cancers, and there is compelling evidence that development of cervical cancer without the involvement of HPV is exceptional (7). To date, more than 100 HPV types have been identified, of which at least 35 have been found in the female genital tract (7, 10). HPV types that infect the genital tract can be classified into two groups, high-risk (HPV16 and -18, among others) and low-risk (HPV6 and -11, among others) types, based on the relative risk of the occurrence of a high-grade lesion and invasive cervical cancer (29).

The immune system is important for the surveillance of HPV-related cervical neoplasia. In almost all cases, the viral infection is cleared and SIL regresses when an appropriate host immune response is activated (3, 31). However, occasionally, the lesions do not regress and malignant progression can follow under appropriate environmental conditions (9). Persistent viral infection is required for neoplastic progression, and failure of virus clearance has been attributed to a poor immunological response (32). Triggering of the adaptive immune response is an important feature of protection against HPV infection and, consequently, neoplastic progression. In an immunocompetent host, the immune surveillance mechanisms against HPV infection include the participation of different anti-inflammatory and antitumor cytokines that affect the growth of HPV-infected cells, facilitating lesion regression (22, 26). One of the most relevant cytokines is tumor necrosis factor alpha (TNF-α), which plays a striking role in viral clearance and inflammatory reactions (1).

TNF-α is a proinflammatory cytokine secreted by several cell types, including macrophages and T cells, playing a pivotal role in the pathogenesis of many infectious and inflammatory diseases (16). TNF-α has antitumor properties and is spontaneously released by HPV-harboring cells, inhibiting the growth of some HPV-transformed cell lines (24, 25, 45). TNF is the major mediator of inflammation in the skin and mucosa, the first barrier against epitheliotropic viruses (5). Studies evaluating cytokine levels in cervicovaginal washings reported increased levels of TNF-α and interleukin-1β in patients with

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cervical cancer compared to those observed in patients with SIL or in healthy controls (2, 41).

The TNF-α gene cluster is located within the class III region of the highly polymorphic major histocompatibility complex on human chromosome 6p21. The TNF-α gene lies between the lymphotoxin-α (TNF-β) and lymphotoxin-β genes, occupying a 7-kb region 250 kb centromeric to the HLA-B locus, 400 kb telomeric to the C2/BF locus, and approximately 1,000 kb from the HLA-DR genes (16). Several polymorphisms have been described within the *TNF* gene cluster. Originally, Udalova et al. (43) described five microsatellites in this gene cluster (*TNFa* to *-e*), and recently Tsukamoto et al. (42) described a sixth microsatellite (*TNFf*) upstream of the lymphotoxin-β gene, a highly polymorphic (CA) dinucleotide repeat presenting with 10 alleles. The associations of the original five polymorphic microsatellites, *TNFa* to *-e*, with disease have been extensively studied in different populations (11, 35). *TNFa*, *-b*, and *-d* are highly polymorphic, multiallelic genetic markers (14, 7, and 7 alleles, respectively), whereas *TNFc* is biallelic and *TNFe* is triallelic (19). These alleles have been associated with genetic predisposition to differential secretion of TNF-α, but there are conflicting results on the associations between TNF microsatellites and in vitro TNF-α production (13, 33).

Several reports have shown associations between *TNF* microsatellites and cancer, as well as infectious diseases (20, 23). However, only a few investigations have studied the association between the TNF-α microsatellite and HPV infection, two of which were conducted with Swedish women (17, 18). In a previous report studying Brazilian patients with HPV infections, we showed a significant association between the *TNFa-8* allele and HPV infection ($\chi^2 = 4.79$; relative risk = 10.73; 95% confidence interval [CI], 1.28 to 89.9; $P = 0.033$), irrespective of the HPV type and the severity of the lesion (38). In the present study, we further analyzed the possible association between *TNFa*, *-b*, *-c*, *-d*, and *-e* microsatellite polymorphisms and SIL, stratifying these patients according to the severity of the lesion and according to the presence of high-risk HPV16 and HPV18 types.

MATERIALS AND METHODS

Study outline and sample population. The study was conducted with 146 HPV-infected nonpregnant women aged 15 to 75 (median = 45) years with suggestive cytological results and colposcopic suspicion of HPV infection. The control group was composed of 101 healthy, sexually active women aged 15 to 75 (median = 45) years. These patients were followed up for routine gynecological examination at the Division of Infectious Diseases in Gynecology and Obstetrics of the University Hospital, School of Medicine, University of São Paulo (HC-FMRPUSP), Ribeirão Preto, São Paulo, Brazil, from February 1999 to October 2000.

Sample collection. All women submitted to blood sample, cervical scraping, and biopsy sample collection whenever indicated, at the time of the medical visit. To obtain DNA for HPV detection and typing, cervicovaginal scrapings were collected with appropriate brushes and placed into microtubes containing adequate buffer (0.01 M Tris/HCl [pH 7.6], 5 mM $MgCl_2$, 1% Triton X-100). DNA for microsatellite polymorphism analysis was obtained from peripheral blood. DNA extraction from blood and scraping samples was performed as previously described (21). Cervical biopsies were stratified into two groups: LSIL (83 patients with cervical intraepithelial neoplasia grade I and HPV DNA positive) and HSIL (63 patients with cervical intraepithelial neoplasia grade II or III and in situ cervical carcinoma). Tissues were sectioned and evaluated histologically after staining with hematoxylin and eosin, and two experienced histopathologists determined the lesion stage in a double-blind protocol.

The local and Brazilian Institutional Ethics Committees on human experimentation approved the study.

TABLE 1. Distribution of HPV types stratified by lesion grade

Lesion grade	No. (%) of patients				
	HPV16	HPV18	HPV16-HPV18	HPVX	Total
LSIL	28 (33.73)	17 (20.48)	19 (22.89)	19 (22.89)	83 (100)
HSIL	25 (39.68)	05 (7.94)	14 (22.22)	21 (30.16)	65 (100)

TNF microsatellites. The primer sequences used to amplify HPV and TNF microsatellites were identical to those reported by Manos et al. (27) and Udalova et al. (43), respectively. A thermocycler (MJ Research, San Francisco, CA) was used for all amplification procedures.

All *TNF* microsatellite amplifications were performed using similar reagents and cycling conditions. The final volume of the reaction mixture for each sample was 25 μl, including 20 ng of genomic DNA, 400 μM deoxynucleoside triphosphate (Pharmacia, Uppsala, Sweden), 0.2 mM TNF primer (Biosynthesis, Lewisville, TX), 0.5 U DNA polymerase (Biotools, Palo Alto, CA), and 1× buffer (0.2 M Tris-HCl [pH 8.5], 0.5 M KCl, 0.02 M $MgCl_2$). After an initial denaturation step of 94°C for 5 min, the samples were submitted to 28 cycles of 94°C for 1 min, 61°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min.

HPV detection and typing. Primers MY09 and MY11 (27), which amplify a 450-bp DNA fragment, were used for generic HPV amplification. Primers HPV16E7.667 and HPV16E7.774 (specific for HPV16) and primers HPV18E7.696 and HPV18E7.799 (specific for HPV18) were also used (46). Since HPV16 and HPV18 are the HPV types most frequently associated with SIL and invasive cervical neoplasia, attention was focused on them. The absence of HPV16 or HPV18 did not exclude the presence of other HPV types, which in this study were called HPVX.

The amplification procedure for HPV detection and typing was carried out using a final volume of 25 μl. The reaction mixture included 20 ng of genomic DNA, 400 μM deoxynucleoside triphosphate (Pharmacia, Uppsala, Sweden), 0.8 mM (Gibco, Lewisville, TX), 1.5 U *Taq* polymerase (Biotools, Palo Alto, CA), 3 mM $MgCl_2$, and 1× buffer (Biotools, Palo Alto, CA). The cycling conditions consisted of an initial denaturation step of 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 40 s, and 72°C for 45 s and a final extension cycle of 72°C for 10 min.

Amplicons were separated by 12% denaturing polyacrylamide gels for *TNF* (*a* to *e*) typing and 10% nondenaturing polyacrylamide gels for HPV typing. The silver staining procedure for DNA fragments on polyacrylamide gels was performed using the protocol described by Sanguinetti et al. (37).

Statistical analysis. GENEPOP (34) software was used to verify the allelic frequencies of *TNF* microsatellites at each locus. The associations between microsatellite polymorphism and HPV type infection, the association between the control group and patients group stratified by the severity of the lesions and HPV types, and the association between the severity of the lesions and HPV types in the patient group were evaluated using the two-sided Fisher exact test and the odds ratio (OR), considering P values significant if ≤0.05 after Bonferroni correction (Instat software; GraphPad, San Diego, CA).

RESULTS

HPV typing and SIL severity. HPV16 alone was identified in 53/146 (36%) and HPV18 alone was identified in 22/146 (15%) of the scrapings. Were detected both HPV16 and -18 in 33/146 (23%) and HPVX in 38/146 (26%) of the cases. With respect to the lesion grade, 83/146 (56.8%) patients had LSIL and 63/146 (43.2%) had HSIL. The distribution of HPV types according to the severity of the lesion is shown in Table 1.

***TNF* microsatellite alleles according to HPV types.** Overall, the *TNFa-2* (36.4%) and *-a-10* (12.3%) alleles were the most frequently observed in patients harboring exclusively an HPV18 infection, whereas the *TNFa-2* (16%), *TNFa-4* (15.5%), *TNFa-7* (14.2%), and *TNFa-10* (12.3%) alleles were those most frequently observed in patients harboring exclusively an HPV16 infection. *TNFa-2* (15.2%), *TNFa-6* (28.8%), and *TNFa-10* (19.7%) were the most frequent alleles in pa-

tients harboring a coinfection with the HPV16 and -18 types, whereas *TNFa-4* (11.8%), *TNFa-6* (27.6%), *TNFa-7* (15.8%), and *TNFa-10* (11.8%) were observed in women infected with HPVX.

The *TNFa-2* allele was overrepresented in patients harboring exclusively HPV18 infection compared to patients exhibiting other HPV types, including a coinfection with HPV16 and -18 (OR, 3.36; $P = 0.001$; 95% CI, 1.543 to 4.509). After excluding patients harboring a coinfection with HPV16 and -18, the *TNFa-2* allele remained overrepresented in HPV18-infected patients compared to other HPV types (OR, 3.42; $P = 0.002$; 95% CI = 1.496 to 4.189).

***TNF* microsatellite alleles according to SIL severity.** Although the *TNFa-6* (24.1%) and *TNFa-2* (22.2%) alleles were the most frequently observed in patients with LSIL or HSIL, respectively, a significant association was observed only for the *TNFa-8* allele when LSIL patients were compared with healthy individuals (OR, 11.22; $P = 0.04$; 95% CI, 2.003 to 2.520).

No significant association was observed between *TNF* microsatellite alleles and HSIL.

***TNF* microsatellite alleles and HSIL stratified according to HPV type.** The *TNFa-2* allele was primarily associated with the presence of HSIL and infection with HPV18, even after performing several comparisons with others HPV types. A significant association was observed when HSIL-HPV18 patients, including those coinfected with HPV16, were compared with other HPV types (OR = 2.53; $P = 0.04$; 95% CI, 1.079 to 3.69). When HSIL patients harboring exclusively HPV18 were compared with HSIL patients harboring other HPV types, including a coinfection with HPV16, the risk of HSIL occurrence doubled (OR, 4.04; $P = 0.04$; 95% CI, 1.090 to 11.238). Finally, when HSIL patients harboring exclusively HPV18 were compared with patients harboring other HPV types, excluding a coinfection with HPV16, the risk of HSIL occurrence increased slightly (OR, 4.867; $P = 0.03$; 95% CI, 1.249 to 12.175).

No significant association between *TNF* microsatellite alleles and HSIL with either HPV16 or HPVX was detected. Table 2 illustrates the frequencies of five *TNF* microsatellites observed in patients and control individuals.

DISCUSSION

TNF-α and related cytokines bind to specific members of the TNF receptor superfamily, initiating various signaling pathways that lead to growth arrest, proliferation, or cell death (30). The effects of TNF include activation of cytotoxic effector cells in the recognition and destruction of virus-infected cells (1). TNF-α can also contribute to the tumorigenesis process, due to the activation and induction of the expression of genes involved in proliferation, invasion, and tumor cell spreading (1, 12).

Many studies have reported associations between *TNF* microsatellite alleles and some types of cancer (17, 18, 35); however, the associations of these markers with cervical cancer and cervical HPV infection have rarely been studied. To address the question of whether the severity of the lesion or the oncogenic HPV type is associated with *TNF* microsatellites, we analyzed the association of the five *TNF* microsatellite alleles (*a* to *e*) with HPV16 and HPV18 infections.

Ghaderi et al. analyzed the *TNFa* microsatellite alleles in HPV16- and HPV18-seropositive women with SIL. Those authors reported that the *TNFa-11* allele was associated with an increased risk of SIL progression in patients harboring an HPV16 infection (17). In addition, the concomitance of *TNFa-11* and HLA-DQ6 increased 15-fold the relative risk of SIL development in Swedish patients with HPV16 infection. In a subsequent study, the same group of authors reported that the extended HLA DQ6-*TNFa-11* haplotype significantly increased the risk of development of cervical cancer (18). We have previously reported that the *TNFa-8* allele was associated with HPV infection in a series of Brazilian women (38), yielding a relative risk of 10.7 compared to healthy women.

In the present series, significant associations were detected between LSIL and the *TNFa-8* allele and the *TNFa-2* allele in HSIL patients harboring an HPV18 infection. In addition, the *TNFa-11* allele presented a low frequency in both HPV types and a low frequency in the LSIL, HSIL, and control groups. Indeed, the *TNFa-11* allele has been reported to be present in approximately 10% of our Brazilian population (M. W. P. Carvalho and A. L. Simões, unpublished data), and a low frequency of this allele has also been reported in European and Japanese populations (11, 40). Up to now, there have been no reports regarding the association of the *TNFa-8* allele with TNF-α production or its association with cancer. On the other hand, the *TNFa-2* allele has been associated with high in vitro TNF-α production (33) and several studies have reported high levels of TNF-α in cervical washings of patients with SIL or cervical cancer lesions (2, 41).

In the present study, irrespective of the lesion grade, the *TNFa-2* allele was associated with the presence of HPV18, with coinfection with HPV16 (HPV16 and -18) ($P = 0.001$) or without coinfection with HPV16 (only HPV18) ($P = 0.002$). This allele was the most frequently observed in patients with HPV18 and HSIL. In addition, when HSIL patients harboring exclusively HPV18 were compared with other HPV types, the risk of HSIL occurrence doubled ($P = 0.04$). HPV18 has been shown to be more aggressive than HPV16 because of its ability to integrate into the cell genome in preneoplastic lesions (4). Once integrated into the cell genome, HPV18 leads to disruption of normal epithelial differentiation, contributing to viral persistence and to the eventual development of SIL (28). The ability of HPV18 to integrate into the cell genome in preneoplastic lesions leads to replication of the damaged HPV DNA expressing only oncogenic proteins (E6 and E7). These oncogenic proteins bind and induce the degradation of tumor suppressor proteins, such as p53 and pRB, that regulate cellular growth and apoptosis (8, 14, 47). After integration of the HPV18 DNA into the host cell and expression of the oncoproteins in HSIL, the proapoptotic effects of TNF-α may be abrogated further, contributing to tumorigenesis.

Organotypic cultures of normal or HPV18-immortalized (E6 plus E7 and whole genome) keratinocytes are resistant to the growth-inhibiting effect of TNF-α in cell culture (6, 39, 44), and distinct TNF-α responses have been observed in HeLa cell line variants. The HPV18-positive HeLa cell has been reported to be sensitive to TNF-α effects, whereas the tumorigenic variant of HeLa cells has not (36, 39). High TNF-α levels were observed in cervical cancer specimens, but no association was found between TNF-α and HPV16 infection (2). However, decreased intralesional TNF-α levels have been described in

TABLE 2. Allelic distribution of *TNF* microsatellites among HPV types stratified by lesion grade in 146 HPV-positive patients ($n = 292$) and 101 healthy controls ($n = 202$)

Locus and allele	No. (%) of controls	No. (%) of patients							
		HPV16		HPV18		HPV16-HPV18		HPVX	
		LSIL	HSIL	LSIL	HSIL	LSIL	HSIL	LSIL	HSIL
TNFa									
1	6 (3)	1 (1.8)	3 (6.0)		1 (10.0)			1 (2.6)	
2	41 (20.3)	9 (16.1)	8 (16.0)	11 (32.4)	5 (50.0)	2 (5.3)	8 (28.6)	2 (5.3)	7 (18.4)
3	7 (3.5)	2 (3.6)	2 (4.0)	1 (2.9)		1 (2.6)			
4	13 (6.4)	9 (16.1)	7 (14.0)		2 (20.0)	3 (7.9)	1 (3.6)	3 (7.9)	6 (15.8)
5	11 (5.4)	2 (3.6)	3 (6.0)	1 (2.9)		3 (7.9)	3 (10.7)	4 (10.5)	3 (7.9)
6	39 (19.3)	10 (17.9)	8 (16.0)	6 (17.6)		13 (34.2)	6 (21.4)	11 (28.9)	10 (26.3)
7	31 (15.3)	7 (12.5)	8 (16.0)	2 (5.9)		2 (5.3)	4 (14.3)	9 (23.7)	3 (7.9)
8		1 (1.8)		1 (2.9)		1 (2.6)	1 (3.6)	1 (2.6)	1 (2.6)
9	5 (2.4)	4 (7.1)	1 (2.0)						1 (2.6)
10	25 (12.4)	8 (14.3)	5 (10.0)	6 (17.6)	1 (10.0)	11 (28.9)	2 (7.1)	3 (7.9)	6 (15.8)
11	16 (8)	2 (3.6)	4 (8.0)	4 (11.8)		2 (5.3)	2 (7.1)	4 (10.5)	1 (2.6)
12	3 (1.5)	1 (1.8)		1 (2.9)					
13	5 (2.5)		1 (2.0)				1 (3.6)		
14				1 (2.9)	1 (10.0)				
Total no. (%)	202 (100)	56 (100)	50 (100)	34 (100)	10 (100)	38 (100)	28 (100)	38 (100)	38 (100)
TNFb									
1	35 (17.3)	9 (16.1)	10 (20.0)	5 (14.7)	5 (50.0)	3 (7.9)	2 (7.1)	3 (7.9)	5 (13.2)
2							2 (7.1)		
3	18 (9)	3 (5.4)	1 (2.0)	9 (26.5)		4 (10.5)	3 (10.7)	2 (5.3)	6 (15.8)
4	68 (33.7)	18 (32.1)	16 (32.2)	10 (29.4)	2 (20.0)	13 (34.2)	10 (35.7)	14 (36.8)	6 (15.8)
5	73 (36.1)	23 (41.1)	20 (40.0)	10 (29.4)	3 (30.0)	17 (44.7)	7 (25.5)	17 (44.7)	18 (47.4)
6	1 (0.5)						1 (3.6)		
7	7 (3.4)	3 (5.4)	3 (6.0)			1 (2.6)	3 (10.0)	2 (5.3)	3 (7.9)
Total no. (%)	202 (100)	56 (100)	50 (100)	34 (100)	10 (100)	38 (100)	28 (100)	38 (100)	38 (100)
TNFc									
1	134 (66.4)	38 (67.9)	31 (32.1)	20 (58.8)	4 (40.0)	30 (78.9)	24 (85.7)	31 (81.6)	24 (63.2)
2	68 (33.6)	18 (62.0)	19 (38.0)	14 (41.2)	6 (60.0)	8 (21.1)	4 (14.3)	7 (18.4)	14 (36.8)
Total no. (%)	202 (100)	56 (100)	50 (100)	34 (100)	10 (100)	38 (100)	28 (100)	38 (100)	38 (100)
TNFd									
1	1 (0.5)		2 (4.0)			1 (2.6)			2 (5.3)
2	17 (8.4)	3 (5.4)	1 (2.0)	3 (9.4)		1 (2.6)	6 (21.0)	2 (5.3)	3 (7.9)
3	11 (5.4)	4 (7.1)	3 (6.0)	1 (3.1)				2 (5.3)	1 (2.6)
4	101 (50)	25 (44.6)	19 (38.0)	16 (50.0)	4 (40.0)	19 (50.0)	14 (50.0)	25 (5.3)	16 (42.1)
5	47 (23.2)	14 (25.0)	20 (40.0)	10 (31.2)	5 (50.0)	9 (23.7)	5 (17.9)	5 (65.8)	11 (28.9)
6	22 (11)	7 (12.5)	4 (8.0)	2 (6.2)	1 (10.0)	8 (21.1)	3 (10.0)	3 (13.2)	4 (10.5)
7	3 (1.5)	3 (5.4)	1 (2.0)					1 (2.6)	1 (2.6)
Total no. (%)	202 (100)	56 (100)	50 (100)	32 (100)	10 (100)	38 (100)	28 (100)	38 (100)	38 (100)
TNFe									
1	32 (15.8)	8 (14.3)	8 (16.0)	5 (15.6)	5 (50.0)	2 (5.3)	2 (7.1)	1 (2.6)	5 (13.2)
2	3 (1.5)	1 (1.8)						1 (2.6)	1 (2.6)
3	167 (82.7)	47 (83.9)	42 (84.0)	27 (84.4)	5 (50.0)	36 (94.7)	26 (92.9)	36 (94.7)	32 (84.2)
Total no. (%)	202 (100)	56 (100)	50 (100)	32 (100)	10 (100)	38 (100)	28 (100)	38 (100)	38 (100)

patients harboring HPV18 (15). In the present study the *TNFa-2* allele was overrepresented in Brazilian women with HSIL and HPV18. Although this allele was described as a high producer of TNF-α (33), it does not seem to protect HPV-infected women from the occurrence of HSIL. Indeed, HPV18 has been reported to be resistant to the inhibitory effects of TNF-α (44).

Several issues need to be addressed before generalizing the results presented here. The present study was based on a cross-sectional design, and individual allelic associations were based on a small number of observations, not allowing inference of the clinical relevance of these results or further comparisons with other studies. In addition, the scarce information about the effects of *TNFa-8* on TNF production does not permit us to establish further comparisons of the probable effects of this allele on cervical lesions and on HPV infection.

Based on our previous study and on the literature (6, 15), we conclude that despite the presence of a high TNF-α production allele, the ability of HPV18 to resist the inhibitory effects of TNF-α may contribute to the occurrence of the infection and consequently to HSIL in women with cervical HPV18 infection. In conclusion, these observations may have important implications for the understanding of the pathogenesis of HPV-mediated lesions and for the design of strategies to prevent SIL development, especially in susceptible individuals. However, considering that this study was originally designed as a transversal study, further studies including larger numbers of patients of distinct ethnic backgrounds should be performed to address the specific issue of how much the specific HPV types might influence cytokine levels.

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