Conjecturas

DOI: 10.53660/CONJ-582-211

IL-10 and *TNF-α* genes polymorphisms and the development of cervical lesions and cervical adenocarcinoma: a case-control study.

Polimorfismos nos genes *IL-10* and *TNF-α* e o desenvolvimento de lesões cervicais e adenocarcinoma: Um estudo caso controle.

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ABSTRACT

Cytokines have an important role in the progression of cervical lesions and/or adenocarcinoma. We investigated whether polymorphisms at the promoter regions of the *IL-10* -1082 (A> G, rs1800896) and *TNF*-α -308 (G>A, rs1800629) genes were associated with susceptibility to progression of cervical dysplasia and adenocarcinoma. The study consisted of 240 women infected with HPV (72 with adenocarcinoma and 168 with cervical intraepithelial lesions), and 169 healthy control women. There was a significant increase in the frequency of the *IL10* -1082G allele in both cervical dysplasia (OR = 1.39; P = 0.0372) and adenocarcinoma patients (OR = 2.19; P = 0.0002). For the *TNF-α* -308 polymorphism, there was higher susceptibility to cervical lesions, in relation to risk factors such as: age > 35 years old (OR = 2.57; p = 0.0057), age of first sexual intercourse 1st < 18 years old (OR = 6.6224, p < 0.0001), smoking (OR = 3.80; P = 0.0003), African ancestry (OR=5.18, p < 0.0001) and co-infection with *Chlamydia trachomatis* (OR=2.41, p=0.0315). Our findings suggest that polymorphisms in the *IL-10* and *TNF-α* genes may play a role in the susceptibility or severity of cervical disease in the study population.

Keywords: Tumor Necrosis Factor alpha; Interleukin 10; Cervical lesions; Adenocarcinoma; HPV.

RESUMO

As citocinas tem uma importante função na progressão de lesões cervicais e/ou adenocarcinoma. Nó destes investigamos se polimorfismos na região promotora dos genes *IL-10* -1082 (A> G, rs1800896) e *TNF*-α -308 (G>A, rs1800629) estavam associados com o a susceptibilidade ou progressão de lesões cervicais e adenocarcinoma. O estudo consistiu de 240 muheres infectadas pelo HPV (72 com adenocarcinoma e 168 com lesões intraepiteliais cervicais), e 169 mulheres controle saudáveis. Houve um aumento significativo na frequência do alelo *IL10* -1082G, tanto em pacientes com lesões cervicais (OR = 1,39; P = 0,0372), como aqueles com adenocarcinoma (OR= 2,19; P = 0.0002). Para o polimorfismo *TNF-α* -308, houve uma maior susceptibilidade às lesões cervicais, em relação aos seguintes fatores de risco: idade > 35 anos (OR = 2,57; p = 0,0057), idade na primeira relação sexual < 18 anos (OR = 6,6224; p < 0,0001), ser fumante (OR = 3,80; P = 0,0003), ancestralidade africana (OR=5,18; p < 0,0001) e co-infecção com *Chlamydia trachomatis* (OR=2,41; p=0,0315). *N*ossos achados sugerem que polimorfismos nos genes *IL-10* and *TNF-α* podem ter uma função na susceptibilidade ou severidade da doença cervical na população em estudo.

Keywords: Fator de Necrose tumoral alfa; Interleucina 10; Lesões cervicais; adenocarcinoma; HPV.

INTRODUTION

Despite infection by one of the types of human papillomavirus of high oncogenic risk (HR-HPV) being recognized as the main cause of cervical cancer and its precursor lesions (Cervical Intraepithelial Neoplasms CIN), Ferlay *et al.*(2008) indicated that the presence of other cofactors plays an important role in viral etiology, such as: alcoholism, multiple partners, multiparity, use of oral contraceptives, age, and co-infections with other types of pathogens [Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV) and *Chlamydia trachomatis* (CT)]. Furthermore, Moscicki et al. (2012) pointed out the need to also take into consideration genetic factors of the host. The type and quality of the immune response are factors that favor an enabling environment for viral replication, and individuals with impaired cellular immune response have a high prevalence of cervical lesions induced by HPV (Stanley, 2006).

Interleukin 10 (IL-10) and tumor necrosis factor alpha (TNF-α) are two multifunctional cytokines involved in the immune response of the host (Tavares et al. 2016; Jin, 2015; Li et al. 2018; Zidi et al. 2015; Lesiak et al. 2014). IL-10 is an anti-inflammatory cytokine excreted by a variety of cells and has dual activity in the immune response by suppressing the cellular immune response (Th-1) and stimulating the humoral immune response (Th-2) (Mege et al. 2006). Patients with severe squamous intraepithelial lesions (SIL) have been found with increased levels of IL-10 expression (El-sherif et al. 2001; Zoodsma et al. 2005). TNF-α is a pro-inflammatory cytokine secreted mainly by macrophages, and has a key role in inflammation, immune homeostasis and host defense. Furthermore, increased expression of adhesion molecules and activation of neutrophils stimulate the production of cytokines and act in a T-cell co-stimulatory activation and antibody production (Wajant, 2009). Additionally, TNF-α is involved in the defense against HPV infection by modulating viral replication (Zur Hausen, 2000).

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Polymorphisms in genes related with the immune response, such as *IL-10* and *TNF*-α genes may be involved in the etiology of cervical disease (Tavares et al. 2016; Jin, 2015; Li et al. 2018; Zidi et al. 2015; Berrington et al, 2004). Several polymorphisms have been described in the *IL-10* gene, in particular three in the promoter region (-1082 A/G, -819 T/C, and -592 A/C) may influence the transcriptional level of mRNA and protein expression *in vitro* and consequently they contribute to the development of cancer (Turner et al. 1997).

TNF-α also has several genic polymorphisms located in its promoter region: -1031 (T/C), -863 (C/A), -857 (C/T), -308 (G/A), -238 (G/A), -1196 (C/T), -1125 (G/C), -572 (A/C), -316 (G/A), -163 (G/A) and -70 (G/A). However, single nucleotide polymorphisms (SNP) located at position -308 (G> A) of the *TNF*-α gene causes variation in the serum concentration of the protein encoded by this gene. The *TNF*-α -308 GG genotype was associated with low protein production, while -308 AA and -308 AG genotypes were associated with middle and high protein production, respectively (Fernandes et al. 2008).

In relation to cervical lesions, the presence of the *TNF*-α -308 GG genotype has been associated with the induction of squamous cervical intraepithelial lesions (SCIL), whereas the presence of *TNF*-α -308AG and *TNF*-α -308AA genotypes were associated with the progression to cervical lesions and even in the formation of invasive cervical cancer (ICC), however there have been conflicting results (Tavares et al. 2016; Jin, 2015; Crilly et al. 2003; Li et al. 2013).

Despite *IL-10* -1082 (A/G) and *TNF-α* -308 G/A polymorphisms having been studied with respect to their susceptibility to many infectious diseases, the functional importance of these polymorphisms needs to be clarified. Therefore, this research aimed to investigate the possible association among *IL-10* -1082 (rs1800896) and *TNF*-α -308 (rs1800629) gene polymorphisms and susceptibility to cervical intraepithelial lesions as well as to adenocarcinoma in a population from the Northeast of Brazil.

MATERIALS AND METHODS

Design and study site

A cross-sectional study was performed aimed at analyzing *IL-10* and *TNF-α* gene polymorphisms in women with squamous cervical intraepithelial lesions (SCIL) and

cervical adenocarcinoma. Women were recruited at the Lower Genital Tract Pathology Clinic at Women´s Healthcare Center of the Prof. Fernando Figueira Institute of Integrated Medicine between January 2008 and April 2010.

The laboratorial analyses were conducted at the Professor Tânia Falcão Laboratory of Genetics, Biochemistry and DNA Sequencing at the Rural Federal University of Pernambuco. The local Ethics Committee for Research (nº 3326/13) approved the study and all patients and controls agreed to participate, signing the Terms of Free and Informed Consent.

Patients and Controls

The study population consisted of 3 groups: group 1: 96 women with cervical lesions and HPV-positive; Group 2: 72 women with cervical adenocarcinoma and HPV-positive; Group 3: 169 healthy control women, HPV-negative. The samples of this study were selected from a DNA bank originated from cervical smears of women aged 16 to 75 years assisted by spontaneous demand in the Central Public Health Laboratory of Pernambuco (LACEN) and Professor Fernando Figueira Integrative Medicine Institute (IMIP), from January 2008 to April 2010. As regards the samples of Group 2, women between 31 and 76 years of age with histopathological diagnosis of adenocarcinoma *in situ* and cervical cancer were included in this group, as identified in the file Cervical Pathology Service of the CAM-IMIP, in the period between 2001 and 2014. The study excluded women who had undergone treatment of the cervix or diagnosed with HIV and patients with adenocarcinoma included in paraffin for whom there was no nail biopsy or surgical piece blocks. For the healthy control group women were excluded who had the cytology diagnosed with atypical cells and colposcopy with abnormal colposcopic findings or suggestive of cervical lesions and positive for HPV infection. For all groups, information was collected from medical records of patients in relation to biological, sociodemographic, reproductive, and lifestyle habit factors.

IL-10 and *TNF-α* gene polymorphism analysis

The presence of polymorphisms in the promoter regions of *IL-10* -1082 (G> A) and *TNF*-α -308 (G> A) genes were analyzed by the sequence-specific PCR technique (PCR-SSP). The analyses of the *IL-10* -1082 (rs1800896) polymorphism gene were

performed as described by Crilly *et al*. (2003), while analyses of the *TNF*-α -308 (rs1800629) polymorphism gene were performed as described by Perrey *et al*. (1999).

The amplification reactions for analysis of both polymorphic sites were made to a final volume of 15 µl. The reaction mixture contained approximately 50 ng of DNA vaginal secretions, 1X Platinum Taq Buffer (Invitrogen Life Technologies), 200 µM dNTPs, 2.5 mM $MgCl_2$, 1U Taq DNA Platinum DNA polymerase (Invitrogen Life Technologies) and 1 µM of each primer (common and specific allele). The cycling conditions used were the same as previously described in the literature cited above.

Statistical analysis

The genotype distribution and allele frequencies of the polymorphisms were obtained by direct counting. The Hardy-Weinberg equilibrium test was applied to datasets using the Biostat 5.0 (Manirauá, Amazônia, Brazil) program. Univariate statistical analyses and logistic regression were performed using the R software, version 3.0.2 (http://www.R-project.org/). The Chi-square ($\chi 2$) test was used for analyzing categorical variables. The Student's t-test was used for continuous data with normal distribution and the Wilcoxon–Mann–Whitney test was performed for data without normal distribution. The Kolmogorov–Smirnov test was used to evaluate normality. The genotypes and their combinations were analyzed by the $\chi 2$ test and the odds ratio (OR) with 95 % confidence interval (CI). P-values under or equal to 0.05 were considered statistically significant.

Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The local Ethics Committee for Research (nº 3326/13) approved this study.

RESULTS

In Table II are shown the distribution of allelic and genotypic frequencies of *IL-10* -1082 A> G and *TNF*-α -308 G> A gene polymorphisms, between cases and healthy control groups. Individuals with the *IL-10* -1082GG genotype and *IL-10* -1082 G allele were significantly associated with increased risk for cervical lesions [OR = 2.17, p =

0.047; OR = 1.39, p = 0.037, respectively], as well as for the development of adenocarcinoma [OR = 3.8; p = 0.0029; OR = 2.19, p = 0.0002]. However, no significant difference in the distribution of allelic and genotypic frequencies were observed in relation to the *TNF*-α -308 G>A gene polymorphism, when compared with cervical lesions (p> 0.05) and adenocarcinoma (p> 0.05).

In Table III, we relate clinical, sociodemographic, reproductive and lifestyle habits feature of selected patients with the two polymorphisms analyzed. Seven risk factors (age> 35 years, age at first sexual intercourse 1st <18 years, pregnancies> 3, use of OCP, smoking, African ancestry, and coinfection with CT) were evaluated in relation to development of both cervical lesions and adenocarcinoma and they were correlated with *IL-10* -1082 A>G and *TNF*-α -308 G>A gene polymorphisms. Of all the cofactors analyzed, only patients aged over 35 years were associated with *IL-10* -1082 A> G gene polymorphism and the susceptibility to cervical lesions. Regarding the *TNF*-α -308 G> A gene polymorphism, significant differences were observed with respect to age > 35 years (OR = 2.57, p = 0.0057), first sexual intercourse < 18 (OR = 6.62; p <0.0001), smoking (OR = 3.80; p = 0.0003), use of OCP (OR = 14.17; p <0.0001), and coinfection by *Chlamydia trachomatis* (OR = 2.41; p = 0.0315), also related to cervical cancer. On the other hand, no significant difference was observed among the cofactors and the presence of adenocarcinoma for both the polymorphisms analyzed in this study (p> 0.05).

Table I. Univariate analysis of the relation of *the IL10 -1082* A>G (rs1800896) and *TNF-α* -308 G/A (rs1800629) polymorphisms on the occurrence of the cervical lesions and the cervical adenocarcinoma in women from Brazil.

SNPs		Control n=169 (%)	Cervical lesions n=168 (%)	Cervical Adenocarcinoma n= 72 (%)	χ^2	OR (95% CI)	*P**
IL10 -1082 (rs1800896)							
Genotypes	AA	28 (16.57)	36 (21.5)	14 (19)			
	GA	121 (71.6)	76 (45.2)	20 (28)	6.158[a] 7.844[b]	0.4885 (0.2760 – 0.0195) [a] 0.3306 (0.1490 – 0.7336) [b]	**0,0195[a]** **0,01[b]**
	GG	20 (11.83)	56 (33.3)	38 (53)	4.687 [a] 10.109[b]	2.1778 (1.0703 – 4.4311) [a] 3.8 (1.6413 – 8.7979) [b]	**0,0470[a]** **0,0029[b]**
Alleles	A	177 (52.37)	148 (44.0)	48 (33.3)			
	G	161 (47.63)	188 (56.0)	96 (66.7)	4.671[a] 14.698[b]	1.3965 (1.0312 – 1.8912) [a] 2.1988 (1.4637 – 3.3031) [b]	**0,0372[a]** **0,0002[b]**
TNF-α -308 (rs1800629)							
Genotypes	GG	95 (56.21)	103 (61.3)	44 (61.1)		Ref.	
	GA	74 (43.79)	58 (34.5)	26 (36.1)	2.07[a] 0.898[b]	0,7229 (0.4644 – 1.1254) [a] 0,7586 (0.4281 – 1.3444) [b]	0,1847[a] 0,4217[b]
	AA	0 (0)	7 (4.2)	2 (2.8)		----	NA
	GA+AA	74 (43.79)	65 (38.7)	28 (38.9)	0.903[a] 0.496[b]	0,8102 (0.5247 – 1.2510) [a] 0.8170 (0.4653 - 1.4344) [b]	0.401[a] 0,5741[b]
Alleles	G	264 (78.1)	264 (78.6)	114 (79.2)		Ref	
	A	74 (21.9)	72 (21.4)	30 (20.8)	0.021[a] 0.067 [b]	0.9730 (0.6744 – 1.4038) [a] 0.9388 (0.5823 - 1.5138) [b]	0.9577[a] 0.8902[b]

[a]Comparative results between cervical lesion and healthy control group; [b]Comparative results between adenocarcinoma and healthy control group; χ^2 – chi-square test; OR – Odds ratio; CI – Coincidence Intervals; P* – P value of odds ratio bold numbers represents significant values. NA= Not analyse

Table II. Compararison between genotypic distribution for *IL-10 -1082 A>G* and *TNF-α -308 G>A* gene polymorphisms and clinical features of the women with cervical lesions from Pernambuco- Brazil.

Socio-demographic characteristics	*IL-10* genotype AA (n)	AG+GG (n)	χ^2	OR (95%IC)	P*	*TNF-α* genotype GG (n)	GA+AA (n)	χ^2	OR (95%IC)	P*
Age > 35 years										
Yes	25	24				49	44			
No	11	108	35.98	0.098 (0.0424-0.2255)	**<0.0001**	54	21	6.53	2.571 (1.3545-4.8815)	**0.0057**
1^0 sexual intercourse <18 years										
Yes	26	90				44	55			
No	10	42	0.54	0.824 (0.3644-1.8681)	0.3937	59	10	28.90	6.622 (3.0571-14.3458)	**<0.0001**
Pregnancies										
≤ 3	19	66				45	35			
>3	17	66	0.09	0.895 (0.4278-1.8715)	0.9144	58	30	1.65	1.504 (0.8057-2.8066)	0.2605
OCP[1] use										
Yes	19	64				48	33			
No	17	68	0.21	0.842 (0.4026-1.765)	0.7882	55	32	0.28	14.179 (4.6013-43.6973)	**<0.0001**
Smoking										
Yes	14	36				18	29			
No	22	96	1.83	0.589 (0.2723-1.2751)	0.252	85	36	14.58	3.804 (1.8786-7.7030)	**0.0003**
African derivation										
Yes	8	50				22	38			

No	28	82	3.07	2.134 (0.9023-5.0477)	0.1203	81	27	18.58	5.182 (2.6194-10.2510)	**<0.0001**
Chlamydia trachomatis infection										
Yes	4	20	0.38	1.429 (0.4554-4.4810)	0.7298	16	20			
No	32	112				87	45	5.49	2.417 (1.1422-5.1134)	**0.0315**

[1]Anticoncepcional Oral; χ^2 – chi-square test; χ^2; OR=Odds ratio; CI – Coincidence Intervals; P* – P value of odds ratio; bold characteristics represents significant values.

DISCUSSION

In this study, we evaluated the correlation between the distribution of genotypic and allelic frequencies for both *IL-10* (rs1800896) and *TNF-α* (rs1800629) polymorphisms and susceptibility to cervical lesions and development of adenocarcinoma. Our findings suggest that the presence of the GG and GA genotypic variants as well as the G allele in the promoter region of the *IL-10* (rs1800896) gene influence the development of cervical lesions and adenocarcinoma. Some studies were conducted in order to verify the associations between *IL-10 -1082 G/A* polymorphism and cervical cancer and/or CIN, but the results have been inconsistent. Matsumoto *et al.* (2010) and Stanczuk et al. (2001) reported that, the *IL-10* G allele was associated with a higher cervical cancer risk in Japanese and Zimbabwean populations, compared to *IL-10* A allele. These findings can be explained by the increase in the transcriptional level of IL-10 and, as IL-10 is a cytokine produced by Th-2 cells and possesses immunosuppressive and antiangiogenic activities, it may lead to an increased susceptibility to both cervical lesions and adenocarcinoma. However, other studies did not find association of this allele with cervical cancer or CIN in Chinese (Wang et al. 2011; Yu et al. 2011), Korean (Roh et al. 2002), British (Farzaneh et al. 2006), Dutsh (Zoodsma et al. 2005) and Argentinian populations (Barbisan et al 2012). A possible explanation for different finds among the studies is heterogeneity of them (case definition and sampling, methods of genotyping, and differences in ethnicity).

In relation to the *TNF*-α -308 G/A gene polymorphism, it is known that the transcriptional level of mRNA of the TNF-α protein increases from 6 to 9 times *in vitro* in the presence of the transition from G to A (Cabrera et al 1995), and may affect the susceptibility to various diseases, including cervical cancer (Tavares et al. 2016; Pasha et al. 2013; Radwan et al. 2012; Gen-Selma et al. 2011; Kroeger et al. 1997). However,

reports in the literature are still contradictory when related to susceptibility for cervical lesions and progression to cervical cancer. In this study, no association was found among the *TNF*-α -308 G/A gene polymorphism and the susceptibility to cervical lesions or development of adenocarcinoma ($p > 0.05$). Souza *et al.* (2014) studying a Portuguese population, also found no significant association with the development of pre-invasive cervical lesions. However, in this same study they found that individuals with the -308A allele and -308AA genotype had increased risk for developing cervical cancer.

Kirkpatrick *et al.* (2004) found a significant association between individuals who were carriers of the GG genotype (low secretory) with the development of low-grade cervical intraepithelial lesions, but they did not find an association with respect to the development of cervical cancer. This last result agreed with others that have also shown no association (Wang et al. 2012; Govan et al. 2006). Furthermore, a meta-analysis performed by Liu *et al*. (2012) showed that individuals with African ancestry carrying the -308 AA genotype had been protected against developing cervical cancer, but no association in relation to Caucasian ancestry was observed. These discordant results may be explained by the difference in genetic background between the different populations analyzed.

Regarding socio-behavioral factors, our results showed that the *IL-10* gene polymorphism was associated only with age above 35 years. On the other hand, when we analyzed the *TNF*-α -308 polymorphism, individuals carrying the GA and AA genotypes were significantly associated to several risk factors, such as age > 35 years, age at first intercourse <18 years, prolonged use of oral contraceptives (ACO), smoking, and presence of co-infection with CT ($p < 0.05$). Duarte *et al.* (2011) found similar results and suggested that these results could be explained by HPV latency in the body, causing histological changes in women of older age. Bezerra *et al.* (2005) showed that the early age of first sexual intercourse, considering the malformation of the female reproductive system contributes directly to an increase in the chance of developing cervical lesions. A study performed by Rosa *et al.* (2009) found that the use of ACO potentiates the onset of cervical lesions by about three times. In this same study, they found an increased risk for women with more parities.

Furthermore, Simonetti *et al*. (2009) suggested in their study that the co-infection by *Chlamydia trachomatis* may cause inflammatory responses that damage the cervical

mucosa, leading to lesions or even facilitate the HPV infection. In our study, we found an association of *IL-10* (rs1800896) polymorphisms with the predisposition to develop cervical lesions and adenocarcinoma. In addition, the clinical data of the patients presented significant differences for both *IL-10* (rs1800896) and *TNF-α* (rs1800629) polymorphisms. Thus, our data suggest that the *IL-10* and *TNF*-α genes can be used as molecular markers in patients predisposed to the screening of cervical disease.

Acknowledgements

We thank the patients and their families by collaboration and understanding to achievement of this work. This research was financed by FACEPE (Foundation for Science and Technology State of Pernambuco) and CNPq (National Council for Scientific and Technological Development).

Conflicts of interests

The authors declare no conflicts of interest.

Author´s contributions

APS, EUDS, TML conceived the idea, APS performed the literature search and wrote the manuscript. ASRS, PRES and MMDM provided inputs for strategy and final edition of the article. All authors read and approved the final manuscript.

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Recebido em: 03/01/2022

Aprovado em: 30/01/2022

Publicado em: 03/02/2022