Wien Med Wochenschr (2023) 173:57–61
https://doi.org/10.1007/s10354-021-00907-w

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Wiener Medizinische Wochenschrift

Interleukin 10 *rs1800896* and interleukin 1B *rs16944* polymorphisms and the risk of cervical cancer

Jasenka Wagner · Sanela Štibi · Nikica Selak · Ilija Alvir · Ivica Mamić · Luka Marcelić · Lucija Šušnjar · Mario Puljiz · Marija Heffer · Damir Danolić

Received: 29 January 2021 / Accepted: 9 December 2021 / Published online: 18 January 2022

Summary
Background The purpose of this study was to evaluate the relationships between interleukin 10 (*IL10*) (*rs1800896*) and interleukin 1B (*IL1B*) (*rs16944*) genetic polymorphisms and the risk for cervical cancer in a cohort of women from Croatia.
Methods A case–control study of 81 patients with cervical cancer and 80 age-matched healthy controls was performed. We collected peripheral blood samples, extracted deoxiribonucleic acid (DNA), and analyzed two single-nucleotide polymorphisms (SNPs) *rs1800896* and *rs16944* using TaqMan assays (Fa. Thermo Fisher Scientific, Waltham, MA, USA) and real-time polymerase chain reaction (PCR). We investigated a possible association between two cytokine genetic polymorphisms and the occurrence of cervical cancer.
Results Our results showed no significant difference in the frequency of *IL10* (*rs1800896*) and *IL1B* (*rs16944*) genotypes between the patients and the controls ($\chi 2$ test, $P < 0.05$).
Conclusion In this study, no association was found between *IL10 rs1800896* and *IL1B rs16944* polymorphisms and cervical cancer development.

Keywords Cytokines · Single-nucleotide polymorphism · Genes · Immune response · Chronic inflammation

J. Wagner · S. Štibi · N. Selak · M. Heffer
Department of Medical Biology and Genetics, Faculty of Medicine, University of Osijek, Osijek, Croatia

I. Alvir · I. Mamić · L. Marcelić · L. Šušnjar (✉) · M. Puljiz · D. Danolić
Department of Gynecologic Oncology, University Hospital for Tumors, Sestre milosrdnice University Hospital Center, Ilica 197, 10000 Zagreb, Croatia
lucija105@gmail.com

Interleukin-10-*rs1800896*- und Interleukin-1B-*rs16944*-Polymorphismen und das Risiko eines Zervixkarzinoms

Zusammenfassung
Grundlagen Das Ziel der vorliegenden Studie war, die Beziehung zwischen den genetischen Polymorphismen *IL10 (rs1800896)* und *IL1B (rs16944)* und dem Risiko für Gebärmutterhalskrebs in einer Kohorte von Frauen aus Kroatien zu untersuchen.
Methodik Es wurde eine Fall-Kontroll-Studie mit 81 Frauen mit invasivem Gebärmutterhalskrebs und 80 altersentsprechenden gesunden Kontrollen durchgeführt. Dazu wurden periphere Blutproben gesammelt, DNA extrahiert und 2 Einzelnukleotidpolymorphismen (SNP; *rs1800896* und *rs16944*) unter Verwendung von TaqMan-Assays (Fa. Thermo Fisher Scientific, Waltham, MA, USA) und der Echtzeit-Polymerasekettenreaktion (PCR) analysiert. Die Autor*innen untersuchten einen möglichen Zusammenhang zwischen dem genetischen Polymorphismus zweier Zytokine und dem Auftreten des Zervixkarzinoms.
Ergebnisse Dabei zeigte sich kein signifikanter Unterschied in der Häufigkeit der Genotypen *IL10 (rs1800896)* und *IL1B (rs16944)* zwischen den Patienten und gesunden Kontrollen (χ^2-Test, $p < 0{,}05$).
Schlussfolgerung In dieser Studie wurde kein Zusammenhang zwischen den Polymorphismen *IL10 rs1800896* und *IL1B rs16944* und der Entwicklung von Gebärmutterhalskrebs festgestellt.

Schlüsselwörter Zytokine · Einzel-Nukleotid-Polymorphismus · Gene · Immunantwort · Chronische Entzündung

Introduction

Cervical cancer (CC) is considered to be the fourth most common type of cancer in women worldwide. Human papillomavirus (HPV) is central to the development of CC and can be detected in 99.7% of cervical cancers [1]. Most genital HPV infections are transient and the majority of HPV-infected women do not develop CC, because HPV alone is not sufficient to cause invasive disease. The key factor in controlling HPV infection is the immune response [2]. Chronic inflammation and the local immune microenvironment also participate in the neoplastic process [3, 4]. Mechanisms that link virus infection, immune response, chronic inflammation, and cancer development include cytokines produced by activated innate immune cells [3, 5]. The cell-mediated immune response can be reflected as changes in cytokine levels in the cervix [6]. During chronic inflammation, cytokines modulate anti-tumor responses and promote cell transformation [7]. Numerous studies have demonstrated that genetic variants play a critical role in cervical cancer development [8–10]. It has been reported that several interleukin genes are associated with development of cervical cancer [11].

Interleukin 10 (IL-10) is the central immune regulator and mediator of the anti-inflammatory response that inhibits production of TNF-α, IL-6, and IL-12, which are major proinflammatory cytokines [5, 12]. IL-10 is an important negative regulator of the tumor immune microenvironment [13, 14]. The interaction between HPV and IL-10 can lead to an immunosuppressive environment in the cervix and, consequently, to the persistence of HPV infection, progression of cervical intraepithelial lesions, and finally to cervical cancer development [13–15]. Also, accumulating data have shown that during HPV infection, IL-10 levels are enhanced, and that IL-10 stimulates HPV E6 and E7 expression [13]. In the past few decades, the association between *IL10* polymorphisms and cervical cancer risk has been the subject of a large number of studies, but with contradictory results [14, 16].

Interleukin 1B (IL-1B) is a proinflammatory cytokine mainly produced during inflammation. Recent studies suggest the important role of chronic inflammation in HPV pathogenesis and promotion of carcinogenesis [17]. Higher levels of proinflammatory cytokines, including IL-1B, were found in women with persistent HPV infection [18]. Several studies have shown an association between *IL1B* polymorphisms and the risk of gastric and prostate cancers [19–21]. Studies regarding CC have revealed that serum IL-1B levels and *IL1B* polymorphisms may be considered as biomarkers for CC [11, 22].

Considering that many studies have previously shown the association between polymorphisms in cytokine genes and cancer development, we decided to investigate selected single-nucleotide polymorphisms (SNPs) as potential risk factors for cervical cancer development. Our objective was to evaluate the possible correlation of *IL10* polymorphism (*rs1800896*) and *IL1B* polymorphism (*rs16944*) with the risk of cervical cancer.

Materials and methods

Among 161 participants, 81 patients with cervical cancer (mean age 53 ± 12 years) were compared with 80 healthy women (mean age 53 ± 11 years). The cervical cancer diagnosis was confirmed by pathohistological examinations of tumor tissues. The control group was represented by age-matched healthy women without a history of malignant disease, chronic systemic inflammatory disorders, conization, or a pathohistologically proven cervical intraepithelial lesion. Only women with three or more consecutive normal Pap smears were included in the control group.

The blood samples were collected at the University Hospital for Tumors, Sestre Milosrdnice University Hospital Center, (Zagreb, Croatia) after obtaining written informed consent from each patient.

According to the manufacturer's instructions, DNA was extracted from 200 µl of EDTA-anticoagulated peripheral whole blood using spin colons for DNA extraction QIAamp DNA Blood Mini Kit (Qiagen GmBH, Germany) and samples were stored at –20 °C. SNP genotype analysis (*rs1800896* and *rs16944*) was performed using TaqMan-based fluorescent probes (TaqMan SNP Genotyping Assays, Fa. Thermo Fisher Scientific, Waltham, MA, USA) on the ABI PRISM 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The thermocycling procedure consisted of 10-minute holds at 95 °C, 15-second denaturation at 92 °C in 40 cycles, and 60-second primer annealing and extensions at 60 °C. Control samples were run simultaneously within each analyzed real-time PCR plate. The total reaction volume was 25 µL with 5 µL of DNA used as a template.

Statistical analyses were performed using the SHEsis software [23]. Analyses of genotypes were conducted using SNPstats software [24]. Pearson chi-square test was used for the analysis of allele frequency differences.

Results

A total of 161 subjects participated in the study, including 81 patients with a diagnosis of cervical cancer and 80 patients in the control group.

There was no statistically significant difference in *IL10* gene polymorphism *rs1800896* (–1082A > G) and *IL1B* gene polymorphism *rs16944* (–511C/T) between patients with cervical cancer and the control group (Table 1).

The genotype distributions and frequencies of polymorphism *rs1800896* (–1082A > G) in the *IL10* gene are shown in Table 2, while genotype distributions and frequencies of the *IL1B* gene polymorphism *rs16944*

Table 1 Minor allele frequency in the *IL10* and *IL1B* gene polymorphism cases and controls

Gene	SNP	Minor allele	Frequency of minor allele in the control group (*N* = 80)	Frequency of minor allele in the case group (*N* = 81)	χ^2	*P*-value
IL10	*rs1800896*	C	0.43	0.39	0.633	0.426
IL1B	*rs16944*	A	0.39	0.3	3.104	0.078

SNP single-nucleotide polymorphism

Table 2 Genotype distributions and frequencies of the *IL10* gene polymorphism *rs1800896* (–1082A > G) cases and control groups

Genotype	Control (%)	Case (%)	χ^2	*P*-value
TT	22 (27.5)	29 (36.2)	1.524	0.467
TC	47 (58.8)	40 (50)		
CC	11 (13.7)	11 (13.8)		

CC cytosine-cytosine, *TC* thymine-cytosine, *TT* thymine-thymine

Table 3 Genotype distributions and frequencies of the *IL1B* gene polymorphism *rs16944* (–511C/T) cases and control groups

Genotype	Control (%)	Case (%)	χ^2	*P*-value
GG	29 (36.2)	41 (51.2)	3.659	0.161
GA	39 (48.7)	30 (37.5)		
AA	12 (15.1)	9 (11.3)		

AA adenine-adenine, *GA* guanine-adenine, *GG* guanine-guanine

Table 4 Relationship between genetic models of the *IL10* *rs1800896* (–1082A > G) polymorphism and the risk for cervical cancer

Model	Genotype	Control (%)	Case (%)	*P*-value
Codominant	TT	22 (27.5)	29 (36.2)	1
	TC	47 (58.8)	40 (50)	
	CC	11 (13.8)	11 (13.8)	
Dominant	TT	22 (27.5)	29 (36.2)	1
	TC + CC	58 (72.5)	51 (63.8)	
Recessive	TT + TC	69 (86.2)	69 (86.2)	1
	CC	11 (13.8)	11 (13.8)	

CC cytosine-cytosine, *TC* thymine-cytosine, *TT* thymine-thymine

Table 5 Relationship between genetic models of the *IL1B* *rs16944* (–511C/T) polymorphism and the risk for cervical cancer

Model	Genotype	Control (%)	Case (%)	*P*-value
Codominant	GG	29 (36.2)	41 (51.2)	1
	GA	39 (48.8)	30 (37.5)	
	AA	12 (15)	9 (11.2)	
Dominant	GG	29 (36.2)	41 (51.2)	1
	GA + AA	51 (63.8)	39 (48.8)	
Recessive	GG + GA	68 (85)	71 (88.8)	1
	AA	12 (15)	9 (11.2)	

AA adenine-adenine, *GA* guanine-adenine, *GG* guanine-guanine

(–511C/T) are presented in Table 3. The data showed no significant difference in distributions and frequencies of the genotypes between the case and the control groups.

Furthermore, the analysis of the three genetic models by SNPStats software showed that the *IL10* *rs1800896* (–1082A > G) polymorphism and the *IL1B* *rs16944* (–511C/T) polymorphism are not associated with the risk for cervical cancer development (Tables 4 and 5).

Discussion

Chronic inflammation has a significant influence on the pathogenesis of various disorders, especially carcinogenesis [17, 25]. Inflammation-related genetic polymorphism could be associated with the development of cervical cancer.

Several studies have demonstrated a correlation between genetic variants of *IL10* and the cervical cancer risk [14, 16, 26]. The *IL10* gene is located on chromosome 1 and contains five exons and four introns that encode 178 amino acids [16, 27]. The three most common *IL10* SNPs that have been reported to significantly influence cervical cancer risk are –819T > C (*rs1800871*), –1082A > G (*rs1800870*), and –592C > A (*rs1800872*) [14, 16]. In 2018, meta-analyses found a significant association of *rs1800870* and *rs1800871* polymorphisms with the risk of cervical cancer development, while an association between *rs1800872* polymorphism and the risk of cervical cancer was not found [16].

In our study, we decided to investigate a less-commonly researched *IL10* single-nucleotide polymorphism, *rs1800896*, and its association with cervical cancer susceptibility. Kingo et al. found that the *rs1800896* polymorphism may have an influence on *IL10* mRNA expression and consequently on the production of IL-10 [28]. Recently, an *IL10 rs1800896* polymorphism has been reported to increase the risk of acute pancreatitis, liver cirrhosis, esophageal cancer, oral cancer, and colorectal cancer [29–31], and to decrease the risk of prostate cancer [30]. Only a few studies have examined the association between the *rs1800896* polymorphism and the risk for cervical cancer. Singhal et al. found a fourfold higher risk of cervical cancer associated with the *rs1800896* polymorphism (–1082 variant genotype GG) [32]. Other studies did not find an association between the *IL10* *rs1800896* polymorphism and the cervical cancer risk [33–35]. At present, the genetic association between this particular SNP and cervical cancer susceptibility has not been finally determined. Hao et al. concluded in their meta-analysis that the *IL10* gene has an ethnicity-specific effect [30] and that studies with more diverse ethnic populations should be performed. Our

study failed to find an association between the *IL10* gene *rs1800896* polymorphism and the risk of cervical cancer in the Croatian population.

Due to the association between increased IL-1B levels and development of cervical cancer [22], several studies attached importance to evaluating whether polymorphisms of the *IL1B* gene, which is highly polymorphic, have an effect on the development of cervical cancer. The base transition between C and T at position –511 (C–T; dbSNP: *rs16944*) located in the promoter region has been widely reported. That transition may influence IL-1B protein expression. Previously, the association between the *IL1B rs16944* polymorphism and various human cancers, such as gastric, lung, and breast cancer, was investigated, but results are inconsistent and inconclusive [36]. Several studies showed that certain genotypes of the *IL1B* gene are associated with cervical cancer development [11]. Kang et al. first reported that the *IL1B* –511 polymorphism is related to cervical cancer risk [37]. Their results confirmed the hypothesis that the –511 T allele causes an increased production of IL-1B and consequently influences cervical carcinogenesis [37]. In their meta-analysis, Xu et al. showed that the *IL1B*+3954C/T polymorphism was associated with an overall increased risk for cancer and that the *IL1B* –511C/T *(rs16944)* polymorphism was associated with an increased risk of cervical cancer development [36]. Qian et al. found that genotypes *IL1B* T-31C TC/CC and C-511T CT/TT were associated with an increased risk of cervical cancer, especially among patients with higher IL-1B levels, and concluded that the functional *IL1B* genotypes may modify plasma IL-1B concentrations and consequently contribute to cervical cancer development [38]. Wang et al. also showed that the *IL1B rs16944* polymorphism significantly increased cervical cancer risk in the Chinese Uygur female [11]. The *IL1B rs16944* polymorphism is also associated with a higher risk of cervical cancer development in the Egyptian population [22]. Our study did not provide evidence for an association between the *IL1B rs16944* polymorphism and the risk for cervical cancer in the Croatian population.

To the best of our knowledge, this is the first study that has investigated the association between *IL10 rs1800896* and *IL1B rs16944* polymorphisms and cervical cancer susceptibility in the Croatian/European population. A major limitation of the present study is the small sample size, which can reduce reliability of the results. Furthermore, additional risk factors for cervical cancer epidemiology, such as smoking, use of oral contraceptives, and sexual behavior, were not included in the analysis. It would therefore be worthwhile to perform further studies on *IL10 rs1800896* and *IL1B rs16944* polymorphisms in European populations, which would include larger number of patients and take into account additional risk factors for cervical cancer.

Conclusion

In conclusion, within the limitations, our study did not provide evidence of an association between *IL10 rs1800896* and *IL1B rs16944* polymorphisms and the risk for cervical cancer. These results need to be taken with caution and further studies with a larger sample size are warranted to establish the role of *rs1800896* and *rs16944* in cervical carcinogenesis in the European population.

Funding This work was supported by the Faculty of Medicine in Osijek grant No. 2018-VIF-12 ("IL-1B and IL-10 genetic polymorphism in cervical cancer," project leader Jasenka Wagner).

Conflict of interest J. Wagner, S. Štibi, N. Selak, I. Alvir, I. Mamić, L. Marcelić, L. Šušnjar, M. Puljiz, M. Heffer, and D. Danolić declare that they have no competing interests.

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