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ORIGINAL ARTICLE

Association of *IL-10* (rs1800872) and *IL-4R* (rs1805010) polymorphisms with cervical intraepithelial lesions and cervical carcinomas

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Summary

Purpose: *Genetic characteristic of cytokines may influence cervical intraepithelial neoplasia (CIN) and cervical cancer (CCa) susceptibility. We analysed an association of IL-10-592 A/C, IL-4R I75VA/G polymorphisms with susceptibility to human papillomavirus (HPV) positive CIN and CCa.*

Methods: *Using multiplex PCR- SNaPShot analysis, 134 cases (HPV positive CINs and CCa) and 113 controls (HPV negative NILM) were genotyped for these two cytokine variants.*

Results: *Data analyzed using odds ratio (OR) and chi-square (x^2) test showed that the frequency of CC of IL-10-592 genotype was significantly higher in cases (67.2%) than in controls (49.6%) [CC vs CA+AA; p=0.005, OR=2.08 (95%CI: 1.24-3.49)] as well as the allelic frequency of major C allele (82.1%) in cases than in controls (72.6%) [p=0.01, OR=1.73 (95%CI: 1.13-2.66)]. Furthermore, AA genotype of IL-4RI75V had significantly lower frequency in CIN1 (25.0%) compared with CIN2+ group (30.8%) (p=0.03, OR=0.39, 95%CI: 0.14-1.11) after the stratifications of the cases in low grade and high grade with CCa as separate groups.*

Conclusion: *We concluded that IL-10-592 A/AA variant indicates a protective role in cervical cancer development and the GG genotype of IL-4RI75V conferred protection against progression of CIN1 to CIN2+ or CCa among women from Republic of North Macedonia.*

Key words: *cervical cancer, cervical intraepithelial lesions, IL-4R, IL-10, polymorphisms*

Introduction

Cervical cancer is the second most frequent malignant disease in women worldwide and the fifth most deadly malignancy in mortality from cancer among females in developing countries [1]. HPV is the most important etiological factor of initiation of cervical intraepithelial abnormalities but still the majority of HPV infections are spontaneously resolved and do not contribute to progression to disease.

The host variations in immune regulating genes, such as cytokine genes, can influence the outcome of HPV infection as well as CIN progression. Cytokines play a crucial role in the regulation of key pathways of immunity and the balance between cell-mediated (Th1) and humoral (Th2) responsiveness. Th1 cells drive cellular immunity to fight intracellular pathogens including viruses and to remove cancer cells, whereas Th-2 cells control

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humoral immunity by up-regulating antibody production to protect against extracellular pathogens [2,3].

Interleukin 10 (*IL-10*) is a key cytokine that determines viral clearance or viral persistence [4,5]. It is a multifunctional Th2-cytokine that acts as an immune response modulator and anti-inflammatory agent in HPV infection associated with of E6 and E7 viral oncoproteins production [6,7] and it also interferes in the process of carcinogenesis [8,9]. Some studies showed that its higher production related to different *IL-10* genetic characteristics has immunosuppressive effect and support viral persistence [5,10,11]. Single nucleotide polymorphisms (SNPs) within the promoter of *IL-10* gene were reported to be associated with HPV persistence and CIN progression by changing the immune response to HPV and modifying the risk for CCa development. The variants in this region have been shown to be associated with differential gene transcription that can produce low, medium or high amount of gene product [11,12] and different cell clearance from HPV infection [13] followed by susceptibility to infections [14] in some malignancies.

Additionally, Interleukin 4 (IL-4) is another immune factor that may influence the course of the disease exerting its biological effects via signalling through its receptor, *IL-4R*. Polymorphism in *IL-4R* I75V could have influence in HPV viral persistence as well, based on assumption that it could alter Th1/Th2 balance, rising Th2 response. A recent report showed that autocrine secretion of IL-4 alters the apoptosis [15], so IL-4 interaction with its receptor variant could be additional mechanism that influences the cervical carcinogenesis, changing the rate of apoptosis.

Therefore, we conducted a case-control study carried out to find whether *IL-10*-592 and *IL-4R* I75V polymorphisms associate with HPV positive CIN1+ and CCa in a group of women from Republic of North Macedonia.

For this purpose we used SNapShot method and we designed primers for multiplex reaction guided by previous scientific experience [16-18].

Methods

Patient selection

A total of 247 women who underwent cervical cancer screening, both with cytological and HPV testing of cervical specimens and/or underwent surgical treatment from private gynaecological clinics in Skopje and the University Clinic for Obstetrics and Gynecology of the Medical Faculty in Skopje, were approached to participate in this study. The inclusion criteria for the cases were to be twice successively HPV positive in a period of 12 months and have histologically confirmed CIN1+ and for control group to be HPV negative NILM. The cytological and/or histological status was confirmed by pathological diagnosis on cervical swabs and surgical specimens, respectively. The cytological diagnosis was done by cytopathologists using the Bethesda classification system. Histology was performed on specimens

Table 1. Genetic characteristics of the analyzed polymorphisms

dbSNP	*Reference sequence*	*Genome position*	*Transcript*	*cDNA*	*Protein sequence*	*Aa**	*Trivial name*
rs1800872	NC_000001.10	g.206946407T>G	NM_000572.2	c.-627A>C			IL-10-592 C/A

*Aa: amino acid change

Table 2. Primers used in multiplex reaction in detection of the four SNPs (rs1800872, rs1805010)

Gene variant	*	*5'-3' base pair*	*PCR-fragment*
IL-10-592 C/A	F	GGGGTCATGGTGAGCACTAC	230
	R	CAAGCAGCCCTTCCATTTTA	
IL-4R-I75V G/A	F	CCCCAGATCTGTCCTCACAT	242
	R	AGCCCACAGGTCCAGTGTAT	

*primer orientation

Table 3. Primers for single-nucleotide primer extension reaction

Gene polymorphism	*5'-3'*	*Bp#*	*P**	*SNP*	*E***	*C(μM)*
IL-10-592 C/A	CAGAGACTGGCTTCCTACAG	20+16 (C)	reverse	G/T	36.8/37.0	2
IL-4R I75V G/A	GCCTCCGTTGTTCTCAGGGA	20 (C)	reverse	C/T	28.5/30.7	2

#Bp: base pairs, *p: primer orientation, **E: electrophoregram position (referent/variant), C(μM): concentration in μM

collected by a colposcopy-directed biopsy and/or cone specimens collected by loop excision procedure in patients where it was indicated or on surgical specimens. HPV DNA and SNP typing were done in the Laboratory for Virology and Molecular Diagnostics of the Institute of Public Health of Republic of North Macedonia (IPHRNM) and in the Research Center for Genetic Engineering and Biotechnology (RCGEB), Academy of Sciences and Arts of Republic of North Macedonia (MASA). The pathologists involved in the cytological and histological assessments were not involved in testing for HPV. Signed informed consent was obtained from all individual participants included in the study. The control group consisted of 113 HPV and cytologically NILM findings, while the case group consisted of 134 HPV positive women with histologically confirmed CIN or CCa. The median age of study participants was 42.6 years (range 21-68). The study was approved by the IPHRNM Ethics Committee.

DNA isolation

QIAamp® DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) was used for DNA extraction from peripheral blood (case group) and cervical swabs (control group) according to the manufacture's instructions.

HPV detection

Cervical specimens for nucleic acid analyses were collected with a cervical brush via standard procedures. HPV typing was performed using HPV4ACE 4 Screening (Seeplex, Seegene, Korea) on DNA isolated from cervical swabs.

Primer design, SNP genotyping

For SNPs analysis we used exfoliated cervical cells obtained from cervical swabs specimens from the control group and CIN1 patients. Blood specimens were obtained from CIN2+ and CCa patients. The primers were designed using primer design software - Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Secondary structures and potential primer dimers were predicted using the PriDimerCheck (http://biocompute.bmi.ac.cn/MPprimer/primer_dimer.html). The characteristics of selected polymorphisms are presented in Table 1.

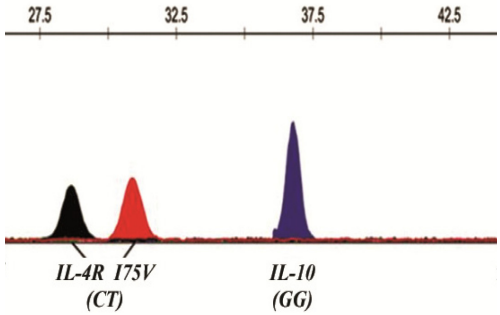

Figure 1. Electrophoregram after multiplex PCR followed by SNaPShot analysis on capillary electrophoresis. The Figure shows heterozygous AG genotype for IL-4R175V and homozygous CC genotype for IL-10-592.

Multiplex polymerase chain reaction (PCR) followed by single-nucleotide primer extension assay were done for SNP genotyping. PCR primers were designed to give amplicons with different fragment sizes (Table 2).

The mutation-specific primers were 5' tailed with a poly-C sequence to produce extension products of 21 and 44 nucleotides long allowing separation by capillary electrophoresis (Table 3). After SNaPShot reaction, SAP-inactivated single-nucleotide extension reaction, diluted with HiDi Formamide (Life Technologies, Carlsbad, CA, USA) and supplied with 0.5 µl GeneScan 120 LIZ Size Standard (Life Technologies) was denatured at 95°C for 5 min and loaded onto an ABI PRISM 3130 Genetic Analyzer (Life Technologies). Extension products were visualized and analyzed using GeneScan 4.0 (Life Technologies) (Figure1). Initial validation of the method was performed against DNA sequencing of the amplified regions.

Statistics

All statistical analyses were done using SPSS software 1.0 (IBM SPSS Chicago, IL, USA). The possible association between polymorphisms and stage of cervical lesions was done using Pearson x^2 test and the Fisher exact test, where indicated. The significance of association was assessed by odds ratio (OR) and 95% confidence interval (95%CI). Multivariate binary logistic regression analysis was used to examine the effect of the studied polymorphisms after adjusting for age and tobacco smoking. Deviation from Hardy-Weinberg equilibrium (HWE) in the studied groups was examined by x^2 test. P value <0.05 was considered as statistically significant for both analyses.

Results

An association was observed between SNP *IL-10*-592C/A (rs1800872) and *IL-4R*I75VA/G (rs1805010) with HPV positive cervical lesions and CCa. The alleles in the control group were within HWE. The characteristics of the women tested after gynaecological examinations are presented in Table 4.

For further association analysis the study group was stratified in groups based on the grade of cervical lesions: all cases CINs and CCa in group C1 (n=134); CIN2+ and CCa in C2 group (n=94: 20 CIN2, 37 CIN3, 17 Ca *in situ*, 20 microinvasive and invasive cervix uteri) and C3 group (n=40) consisted only of CIN1.

The results from the SNP analyses indicated an *IL-10*-592C/A association with HPV positive CIN and CCa. The allelic frequency of major C allele was higher within C1 and C2 group (82.1 and 83.0%, respectively) compared to N group (NILM and no HPV infection) (72.6%): [p=0.01, OR=1.73 (95%CI: 1.13-2.66)] and [p=0.01, OR=1.8 (95%CI: 1.1-3.0)], respectively (Table 5).

Moreover, the frequency of CC genotype was significantly higher in all cases - C1 group (67.2%) and in C2 group (CIN2+ and CCa) (70.2%), compared to the N group (49.6%): [CC vs CA+AA; p=0.005, OR=2.08 (95%CI: 1.24-3.49)] and [CC vs CA+AA; p=0.002, OR 2.39 (95%CI; 1.35-4.27], respectively (Table 6). Similar results for all cases compared to the controls were observed after adjustment for age and tobacco smoking. Summary of allele and genotype distribution between cases and controls is shown in Figure 2.

The results from *IL-4R*I75V polymorphism showed that the allele frequencies of 134 case subjects [A (17.9%) and G (82.1%)] were not signifi-

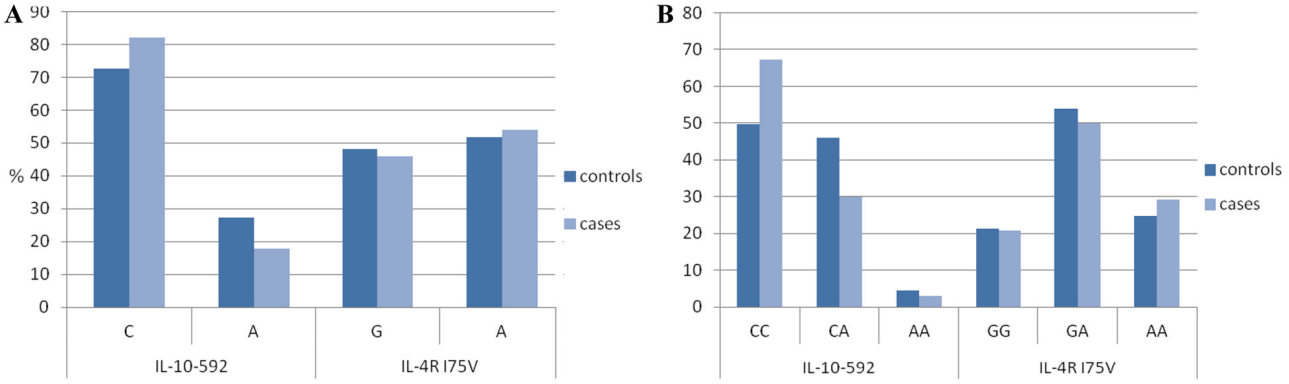

Figure 2. Summary from comparison of allele and genotype frequencies in cases and controls for both polymorphisms. **A:** IL-10-592 C allele frequency compared to A allele in cases and controls, p=0.01; **B:** IL-10-592 CC genotype frequency compared to CA and/or CA+AA genotypes in cases vs controls, p=0.005. General distribution of allele and genotype frequencies in IL-4R 175V doesn't show statistically significant difference between cases and controls.

Table 4. Characteristics of study group (age, histological results and HPV type presence)

Patient characteristics	*n*		*%*
Age, years			
<30	40		16.2
≥30	207		83.8
Tobacco smoking	40		16.2
No smoking	207		83.8
NILM, no HPV	113		45.7
Histology			
CIN1	40		16.2
CIN2, CIN3	57		23.1
Ca *in situ*	17		6.9
Ca micro invasive and invasive	20		8.1
HPV type	*In CIN1 (%)*	*In CN2+, CCa (%)*	*Total*
HPV 16 and/or 18	6 (15.0)	58 (61.7)	64 (47.8)
Other High risk type	24 (60.0)	20 (21.3)	44 (32.8)
Multiple infections	10 (25.0)	16 (17.0)	26 (19.4)
Total	40 (100.0)	94 (100.0)	134 (100.0)

Table 5. Characteristics of study group (age, histological results and HPV type presence)

SNP alleles		*C1* *n (%)*	*C2* *n (%)*	*C3* *n (%)*	*N* *n (%)*	*p1(x^2)*	*p2(x^2)*	*p3 (x^2)*	*p4 (x^2)*
IL10-592	A	48 (17.9)	32 (17.0)	16 (20.0)	62 (27.4)	0.01	0.01	0.18	0.56
C		220 (82.1)	156 (83.0)	64 (80)	164 (72.6)				
IL-4R	A	145 (54.1)	108 (57.4)	37 (46.3)	117 (51.8)	0.60	0.30	0.39	0.1
G		123 (45.9)	80 (42.6)	43 (53.7)	109 (48.2)				

p1: association C1 vs N; p2 (C2 vs N); p3 (C3 vs N); p4 (C3 vs C2)

cantly different from those of 113 control subject [A (27.4%) and G (72.6%;Table 5). However, after analysis of stratified cases in 3 groups we found that GG genotype has significantly higher frequency in C3 (32.5%) than in C2 (16.0%) group [recessive model: GG vs AG+AA; p=0.03, OR=0.39 (95%CI: 0.14-1.1)]. The frequency distribution of different genotypes for the *IL-4R*I75VA/G polymorphism is presented in Tables 6 and 7.

The OR and 95% CI values only from the significant association analysis done with Pearson x^2 test, are presented in Table 8. For the other results we show only the p value as indicator of significance in the previous Table.

Table 6. Genotype frequencies of different SNPs and results after statistical analyses using x^2 test and Odds ratios (OR) adjusted by age and tobacco smoking

SNPs	*Genotype*	*C1 (%)*	*n (%)*	*p (x^2)*	*OR*	*95% CI*	*p*(x^2)*	*OR**	*95% CI*
IL10-592 C/A	CC	90(67.2)	56 (49.6)	ref	-	-	ref	-	-
	CA	40(29.9)	52 (46.0)	**0.005**	0.47	0.28-0.81	**0.006**	0.47	0.27-0.80
	AA	4 (2.9)	5 (4.4)	0.3	0.49	0.12-1.9	0.29	0.48	0.12-1.90
	AC+AA/CC	44(32.8)/90 (67.2)	57 (50.4)/56 (49.6)	**0.005**	0.48	0.28-0.8	**0.006**	0.48	0.27-0.81
	AA/AC+CC	4 (2.9)/130 (97.1)	5 (4.4)/108 (95.6)	0.39	0.66	0.17-2.53	0.37	0.64	0.15-2.5
IL-4R I75V G/A	GG	28 (20.9)	24 (21.2)	0.63	0.83	0.4-1.7	0.63	1.19	0.57-2.47
	GA	67 (50.0)	61 (54.0)	0.43	0.78	0.4-1.4	0.85	0.93	0.49-1.8
	AA	39 (29.1)	28 (24.8)	ref	-	-	ref	-	-
	AG+AA/GG	106 (79.1)/28 (20.90)	89 (78.8)/24 (21.2)	0.90	1.02	0.55-1.88			
	AA/AG+GG	39 (29.1)/95 (70.9)	28 (24.8)/85(75.2)	0.44	1.2	0.7-2.19			

*p: chi square value and odds ratio (OR) value adjusted by age and tobacco smoking. Bold numbers denote statistical significance.

Table 7. Results after stratification in groups depending of grade of the lesions.

SNPs	*Genotype*	*C2* *n (%)*	*C3* *n (%)*	*N* *n (%)*	*p 2(x^2)*	*p 3(x^2)*	*p 4(x^2)*
IL10-592	CC	66 (70.2)	24 (60.0)	56 (49.6)	Ref	Ref	Ref
	CA	24 (25.5)	16 (40.0)	52 (46.0)	**0.01**	0.37	0.13
	AA	4 (4.3)	0	5 (4.4)	0.6	0.6	0.11
	AC+AC /CC	28 (29.8)/66 (70.2)	1640.0)/24 (60.0)	57 (50.4)/56 (49.6)	**0.002**	0.25	0.24
	AA/CC+CA	4/90	0/40	5/108	0.6	0.2	0.08
IL-4R I75V	GG	15 (16.0)	13 (32.5)	24 (21.2)	0.23	0.4	0.08
	GA	50 (53.2)	17 (42.5)	61 (54.0)	0.47	0.59	1
	AA ref	29 (30.8)	10 (25.0)	28 (24.8)	Ref	Ref	Ref
	AG+AA /GG	79 (84.0)/15 (16.0)	**27(67.5)/13 (32.5)**	**89 (78.8)/24 (21.1)**	0.40	0.15	**0.03**
	AA/GG+GA	29 (30.8)/65 (69.2)	**10 (25.0)/30 (75.0)**	**28 (24.8)/85 (75.2)**	0.40	0.61	0.5
	n-total	94 (100)	40 (100)	113 (100)			

p: Values obtained by chi-square test, C: case group, N: control group, n: number. p2 (association C2 vs N); p3 (C3 vs N); p4 (C3 vs C2). Bold numbers denote statistical significance.

Table 8. Significant statistical associations between the SNPs and the grade of CIN lesions or CCa presented by OR and 95% confidence interval

SNPs	*Comparison*	*Model*		*p(x^2)*	*OR*	*95% CI*
IL-10-592	Cases/controls	allelic	A vs C	0.01	1.73	1.1-2.6
	Cases/controls	heterozygous	CA vs CC	0.005	0.47	0.3-0.8
	Cases/controls	dominant	CC vs AC+AA	0.005	2.08	1.2-3.4
	CIN2+, CCa /controls	allelic	A vs C	0.01	1.8	1.1-3.0
	CIN2+,CCa /controls	heterozygous	CA vs CC	0.01	3.5	1.4-4.6
	CIN2+CCa/controls	dominant	CC vs CA+AA	0.02	2.4	1.3-3.2
IL-4R I75V	CIN2+, CC/CIN1	recessive	GA+AA vs GG	0.03	0.4	0.1-1.1

Discussion

The IL-4 and *IL-10* are cytokines known to be involved in anti-inflammatory processes which are potentially cancer-promoting. They have antiangiogenic function (potentially cancer-inhibiting factor) as well, so their role in cervical cancer may be dual [7]. In fact, there are many contradictory studies about the association of the variants of these 2 genes with HPV and cervical cancer, so we did an investigation whether Northern-Macedonian women with particular genotypes are at risk for HPV positive CIN or CCa.

Two E6 and E7 viral oncoproteins of high risk HPV types, play important role in the malignant transformation of cervical cancer cells and during their high activity, *IL-10* influences the promotion of tumor growth [7]. Based on this knowledge, for further analyses, we selected only HPV persistently infected CIN as cases that are theoretically at higher risk for progression to CIN2+ or CCa. The *IL-10* gene promoter is highly polymorphic and different variants of this promoter gene have implication on its activity. Some important SNPs in the promoter's region are supposed to influence the transcription of *IL-10* mRNA and its expression *in vitro* (rs1800896, rs1800871 and rs1800872) [10,19-21] and have been identified in many diseases. Positive correlation of rs1800872 polymorphisms with HPV and cervical cancer susceptibility was observed, especially in Asian populations [22]. These polymorphisms could have influence on the immune response to HPV and could modify the risk for CCa. We analyzed this polymorphism based on the model that *IL-10*-592 A variant is associated with lower *IL-10* expression [23,24]. The higher expression leading to higher IL10 (related to CC genotype) is an event that may support the immunosuppressive effect of *IL-10* and the onset of persistent infection.

Another suggested mechanism is that E6 and E7 oncoproteins binding to the SP1 transcription factor of TGFb promoter, enhance the genetic regulation through GGGGCGG consensus sequence located on 180-172 nucleotides from TGF-β gene [25]. According to this finding and according the study of Torres-Proveda et al [26], E6 and E7 increase the regulation of *IL-10* expression resulting in lower *IL-10* load and support the thesis that A allele carriers have two-fold higher risk for cervical cancer (lower blood concentration of *IL-10*). The exact mechanism of *IL-10* CC variant influence on CIN and CCa development and HPV persistence is still unknown and left to be resolved.

Our results are similar with the results obtained in Indian and Asian populations [27-29] and from studies which indicated that the CC genotype is the risk factor for CIN and CCa, and the variant A allele and variant homozygote AA genotype have protective effects. We did not measure the IL10 blood concentration, we only assumed that CC genotype, as higher inducer of IL10, could have influence on cervical lesions.

In our study the frequency of CC genotype was confirmed to be significantly higher in all cases and, after stratification of the cases in two additional groups, significant association was found within CIN2+ and CCa group too. The different results regarding C variant as a protective variant in some populations [22] and a risk for North Macedonian women might be explained with possible influence of different genetic or epidemiological factors that were not analysed in this study. Different and inconclusive results are reported in other studies too [9,20,26,30,31]. This inconsistency could be also explained by the differences in study design and/or different genetic background of the population examined. Different allelic frequency of these alleles in different populations could be a factor for reaching different conclusion as well: in Asian population, the *IL-10*-592 A allele frequency is 0.87% which is significantly higher than in European population (0.31%; p<0.001) [28].

Finding that CC genotype is associated with significantly higher risk for CCa in North Macedonian women shows that different biological mechanisms could have influence in this association as well as the impact of the influence of epidemiological factors.

Regarding *IL-4R* variant, it is well known that its interaction with IL-4 (Th2 cytokine) has important role in the immune response and this IL-4/*IL-4R* signalling pathway is a strong promoter of prometastatic phenotypes in epithelial cancer cells, including enhanced migration, invasion, proliferation and lower survival [32].

The *IL-4R* I75V polymorphism (rs1805010) A/G located in gene coding for α chain of the receptor, described as 'gain-of-function' mutation, results from substitution of isoleucine for valine (I75V) in the extracellular domain of *IL-4R*α subunit. A recent study showed that this variant was associated with enhanced receptor sensitivity to IL-4 and this enhanced IL-4 signalling influences the promotion of a sustained phosphorylation, leading to abnormally active STAT6 transcription factor, even upon withdrawal (stopping) IL-4 stimulation [33].

On the other hand, 75V variant could contribute to CCa development through its effect on Th1/Th2 balance that can result in higher HPV and CCa susceptibility. This is based on the evidence that

cell mediated immunity, especially Th1, is the most important for HPV clearance [34].

Thus, we hypothesised that the 75V variant could be linked to a T_H2-based response and assumed that it could influence viral persistence or progression due to the effect on Th1/Th2 balance that lead to increased susceptibility to cervical cancer. As it is already known, IL-4 can inhibit the differentiation of Th1 lymphocytes and downregulate the immune response of immune cells against malignant cells enabling tumor cells surveillance [35]. Still no scientific evidence confirmed that Th1/Th2 cytokines balance is the result of genetic characteristics.

Up to now only few studies have analysed an association of this variant with CCa susceptibility. Some studies of *IL-4R*I75V variant showed its association with elevated risk for IgE production in cell lines [36] and in human immunodefinciency virus (HIV) positive carrier that leads to slow progression from HIV positivity to AIDS [37]. Carriers of this SNP also have higher risk for an erosive illness in rheumatic arthritis patients [38]. A Swedish study [39] concluded that *IL-4R* 75V variant is more frequent in CCa cases than in controls and they examined two additional SNPs in *IL-4R*: Q576R and V64I polymorphisms, that showed not be important for CCa development either individually or after analyzing the haplotypes. Another Asian study [40] provided opposite results, showing that the carriers of this polymorphism are less susceptible to CCa in this population.

Our study showed an association between AA genotype (75I-variant) and progression of HPV-positive CIN1 to higher grade CIN2+ or CCa, and GG genotype (75V variant) may have a protective role in the progression of CIN1 to CIN2+ and CCa, but there was no evidence of any association between this polymorphism and initiation of HPV-positive CINs and cervical cancer. Preliminary results of our study suggest that *IL-4R* I75V G/A might be a good marker for assessing this progression.

Our results for *IL-10*-592 and *IL-4R* 175V variants and their association with HPV positive CIN and CCa were obtained only from clinically defined factors and the power for explanation the biological mechanism is limited and its functional relation remains unsolved. Furthermore, for proper evaluation of the importance of this association, explanation of the exact functional mechanism is necessary.

Confirmation of the prognostic value for these cytokine variants needs inclusion of additional epidemiological and biological risk factors in more comprehensive studies with larger study groups.

Conclusion

To our knowledge, this is the first study to report an association between *IL-10*- SNP592 C/A and *IL-4R*I75V A/G polymorphisms and the risk of CCa and CIN in women from Republic of North Macedonia, which indicates these genotypes could be used as predictive markers. Still, further epidemiologic investigations involving larger studies and control groups with wider ethnic background should be conducted to explain the conflicting results and determine whether these variants are risk factors alone or if additional factors, such as exposure to dietary carcinogens, smoking, using contraceptives or factors influencing IL and cytokine production could interfere with this association.

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Ethical approval

The presented study was approved by the ethics committee of the Institute of Public Health of Republic of North Macedonia. 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. Informed consent was obtained from all individual participants included in the study.

Conflict of interests

The authors declare no conflict of interests.

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