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RESEARCH

Interleukin-1 Gene Cluster Polymorphisms and its Haplotypes may Predict the Risk to Develop Cervical Cancer in Tunisia

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Abstract Our study aimed to evaluate the association between IL-1α (4845 G/T), IL-1β (-511C/T) and IL-1RN (VNTR) polymorphisms and risk of cervical cancer. This case-control study investigates three polymorphisms in 130 patients and 260 controls by PCR-restriction fragment length polymorphism (RFLP). The IL-1RN (VNTR) A1/A3 genotype appear as a cervical cancer risk factor (p=0.048; OR= 2.92; 95 % CI=1.00–8.74), moreover, the L/2* decreased the risk (p=0.011; OR=0.47; 95 % CI=0.25–0.88) and may be a protective factor against this pathology. Stratified analysis according to the FIGO stage subgroup revealed that the IL-1β-511 T/T genotype and T allele may be a protective factors against cervical cancer development for patients with early stage (p=0.030; OR=0.46; 95 % CI=0.22–0.96) (p=0.020; OR=0.68; 95 % CI=0.48–0.97). However, for the patients with advanced FIGO stage, IL-1RN-VNTR L/2* genotype appear as a protective factor for this pathology (p=0.023; OR=0.29; 95 % CI=0.08–0.99). The (G-T-L) haplotype showed a significant decreased frequency in cervical cancer patients as compared to controls (p=0.032; OR=0.53; 95 % CI=0.29–0.95). In contrast, the (T-T-2*) combination appear a risk factor for the development of cervical cancer (p=0.018; OR=1.57; 95 % CI=1.07–2.30). Our study suggested that IL1 cluster polymorphisms and haplotypes may be a genetic risk factor for cervical cancer.

Keywords Polymorphism · Haplotype · Interleukin-1beta · Interleukin-1alpha · Interleukin-1receptor antagonist · Cervical cancer · Tunisians

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Introduction

Chronic inflammation has been shown to be an important risk factor for a variety of epithelial cancers [1]. Cervical Cancer (CC) is one of the second most common cancers in women worldwide. Fairly 530,232 new cases are diagnosed annually and more than 275,000 women die every year and 85 % of them in developing countries [2].

Parkin et al., present estimates of the incidence and mortality of cancer in Africa in 2012, shows cumulative incidence by country and illustrates the very high risk in East Africa, with cumulative risk in Malawi, Zimbabwe, and Mozambique in excess of 6 %, whereas in some countries of North Africa (Egypt, Sudan and Tunisia) the cumulative risk is below 1 % [3].

Several lines of evidence indicate that the pathogenesis of cancer is largely dependent on the immune response. A number of previous reports suggested that chronic inflammation is associated with the precancerous intraepithelial lesion and cancer of uterine cervix [4, 5]. The inflammatory reaction in infectious and autoimmune diseases is regulated by a delicate balance between the pro-inflammatory and anti-inflammatory cytokines [6–8].

Interleukin-1 (IL-1) is a prototypic multifunctional cytokine and considered as a key mediator of inflammation [9]. The IL1 gene cluster is located on the long arm of chromosome 2q13-21 within a region of 430 Kb [10, 11].

The regulation of its activity involves three isoforms, two with overlapping proinflammatory effects (IL-1α and IL-1β) and a third form with no agonist activity, the IL-1 receptor antagonist (IL-1RA) [12]. These cytokines produced by several cell types, have multiple biological functions and mediate both acute and chronic inflammation [13]. IL-1 genes are polymorphic and there are at least one single nucleotide polymorphism (SNP) at each gene; The C-to-T transition at positions −511 and +3953 of IL-1β, −889 and G-to-T at position +4845 of IL-1α, IL-1RN +2018 and IL-1RN (VNTR) are the most studied [14–17]. However, a common polymorphic allele of the regulatory region of the IL-1B gene was found to be associated with increased IL-1 production [11]. SNPs at the following IL-1 gene loci; IL-1β-511 and IL-1RN +2018 have been implicated in increased severity and susceptibility to inflammatory diseases [18–20]. The penta-allelic polymorphism in intron 2 of the IL-1RN gene, containing a variable number of 86-bp tandem repeat (VNTR) sequence, has been described [14]. The more common allele 1 contains four repeats, whereas allele 2 contains two repeats, allele 3 contains five repeat, allele 4 contains three repeat and allele 5 contains six repeat [18].

In gynecological cancers, IL-1RN may modulate host immune response [16] and this modulation is different according alleles and genotypes of IL-1RA. IL-1RA A1/A2 genotype has been found to be associated with increased risk of CC [16, 21, 22]. IL-1β-511 C/T and T/T genotypes have also been associated with higher risk of CC in Korea [23]. In a previous study, we have analyzed IL-1α +4845 and IL-1β -511 polymorphisms and we showed that C/C genotype of IL-1β may be a risk factor for CC while no significant association was revealed for IL-1α among Tunisians [24].

These three IL-1A, IL-1B and IL-1RN genes are located on the same chromosome and considerable interaction has been reported between them [12, 21, 22]. Thus, studying those genes together in some cases would be more informative than studying them individually, which has been carried out in previous studies.

In this contest view of these findings, we investigated the potential involvement of the IL-1α (4845G/T) [rs17561], IL-1β (−511C/T) [rs16944] and IL-1RN-VNTR [rs2234663] polymorphisms and haplotypes among Tunisians, and compared our results with those of other published studies, as those polymorphisms have an essential role in CC susceptibility and/or severity of the outcome of the host immune response.

Materials and Methods

Subjects: Cases and Controls

This case-control study consisted of 130 incidents of CC patients approved by Salah Azeiz Oncology Institute (SAI, Tunisia) and 260 cancer-free controls.

Cancer diagnosis was established by clinical examination and biopsy, confirmed by two senior pathologists of the SAI. Clinical data were obtained by questionnaire, personal interviews and review of case records. Tumors were staged according to the FIGO classification (International Federation of Gynecology and Obstetrics, www.figo.org) [23].

Informed consent was obtained. Each subject was personally interviewed to obtain information on demographic data, menstrual and reproductive history and family history of cancer (no cancer in first-degree relatives was reported). The study protocol was approved by the Ethics Committee at Salah Azeiz Oncology Institute in Tunis, Tunisia.

Blood Collection

Five milliliters of venous blood with EDTA, as anticoagulant, were collected from each subject. For patients, the blood was obtained prior to radiation therapy or chemotherapy. Genomic DNA was extracted using QIAamp® DNA blood Mini Kit (Qiagen GmbH, Hilden).

Genotyping

Polymorphisms in the IL-1B and IL-1A genes at positions respectively −511 and +4845 of the promoter region were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as previously described [24].

As the IL-1RN has a variable numbers of an identical tandem repeat of 86 bp, we used the direct PCR with specific primers: forward, 5′CTCCAGCAACACTCCTAT 3′; reverse, 5′ TCCTGGTCTGCAGGTAA 3′. For each sample, the PCR was performed in a total reaction volume of 10 ml containing 1 ml (100 ng) of genomic DNA, 25 mM of dNTP, 1 μl of buffer 10X and 0,25U of Taq polymerase (Fermentas). The PCR cycling program comprised an initial denaturation at 95 °C for 20 s, annealing of primers at 51 °C for 30 s and extension at 72 °C for 50 s and a final extension at 72 °C for 10 min. Amplified DNA fragments were separated on 1.5 % ethidium bromide agarose gel, and visualized under ultraviolet light [24].

The IL-1RN alleles were coded as previously described: The 240 bp product contained two 86 bp repeats (allele 2), the 326 bp product contained three 86 bp repeats (allele 4), the 412 bp products contained four 86 bp repeats (allele 1), the 498 bp product contained five 86 bp repeats (allele 3) and the 595 bp product contained six 86 bp repeats (allele 5) [18]. The IL-1RN alleles were further divided into two categories: long genotype (L: including alleles 1, 3, 4, and 5) and short genotype (2: allele 2 only). The genotypes were classified as LL, 2L, and 22 [25].

Statistical Analysis

Allele and genotype frequencies were calculated by the gene counting method. The genotype distribution of the tested polymorphisms was consistent with Hardy-Weinberg equilibrium. Statistical analyses were performed by Epi info 7 (https://wwwn.cdc.gov/epiinfo/html/downloads.htm) and SPSS 17.0 statistic software system (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.).

Categorical data were analyzed by Pearson chi-square analysis and Fisher's exact test where appropriate. Binary logistic regression analysis and multinomial regression analysis were used for odds ratios (OR) and 95 % confidence intervals (CI) calculation. A P value of <0.05 was considered statistically significant.

Linkage disequilibrium (LD) analysis and haplotype reconstruction was performed using Haploview 4.1 (http://www.broad.mit.edu/mpg/haploview). Logistic regression analysis was performed in order to determine the odds ratios (OR) and 95 % confidence intervals (95%CI).

Results

Study Design

The selected characteristics of the cases and controls enrolled in this study were summarized as follow; the median age, was 52 years for patients with CC and 53 years for healthy controls with a range of 30–81. Among the 130 patients, 124 (95.40 %) are married, 115 (88.50 %) have used hormonal contraceptives and 21(16.15 %) have a cancer in their family. According to the menopausal status of women with CC, our sample was divided into two groups; 38 (29.25 %) were pre-menopausal and 92 (70.75 %) patients were post-menopausal.

Diagnoses of squamous cell carcinoma were confirmed by histopathological examination as International Federation of Gynecology and Obstetrics, the distribution of the sample according to the FIGO stage is as follow; stage I (30.00 %), II (37.70 %), III (24.60 %) and IV (7.70 %).Three histological types were identified: squamous cell carcinoma (83.08 %), adenocarcinoma (14.61 %) and sarcoma (2.31 %).

Polymorphisms Alleles and Genotypes Analysis

The observed genotype and allele frequency distribution of IL1-RN (VNTR) between cases and controls are depicted in Table 1 according to two models of study; For the first model, a significant difference of genotype A1/A3 was reveled (p= 0.048; OR=2.92; 95 % CI=1.00–8.74). The A1/A3 genotype is more common in patients compared to controls thereafter appear as a CC risk factor. No significant differences of allelic and the rest of genotypic distributions at IL1-RN VNTR were observed between the two groups. Among the second model, the L/2* is more frequent in control group. Moreover, this genotype decreased the risk of the tumor evolution (p= 0.011; OR=0.47; 95 % CI=0.25–0.88) and may be a protective factor against CC. Same, no significant association between alleles.

The genotyping of IL1-α+4845 and IL1-β-511 were reported in our previous study [24]. In the present study we increased the controls to 260 and we repeated the statistical analysis but no supplement significant association was found (data not shown).

Association of IL1-α+4845, IL1-β-511 and IL1-RN (VNTR) Gene Polymorphisms According the FIGO Stage

A stratification of cases according the FIGO stage was carried out to investigate whether any possible association exists between IL1-α+4845, IL1-β-511 and IL1-RN (VNTR) gene polymorphisms and stages of CC; early stage (stages I+II; n=88 cases) and advanced stage (stages III+IV; n=42 cases) (Table 2). The results revealed a significant association between the IL1-β-511 T/T genotype and T allele distribution and CC early FIGO stage (p=0.030; OR=0.46; 95 % CI= 0.22–0.96) (p=0.020; OR=0.68; 95 % CI=0.48–0.97), respectively. Early FIGO stage CC patients had a significantly higher frequency of the C allele in respect to T allele. Carriage of the T allele of IL-1β-511 was associated with a decreased risk and may be a protective factor from CC.

However, the distribution of genotypes for the advanced FIGO stage subgroup revealed that the IL-1RN (VNTR) L/2* genotype was more common in control group compared with cases. Our results showed that this genotype may be a protective factor against the development of CC in Tunisian population (p=0.023; OR=0.29; 95 % CI=0.08–0.99).

Nevertheless, no significant associations were revealed for the rest of data.

Haploview Analysis

Since IL-1A, IL-1B and IL1RA genes studied are located on the same chromosome, we aimed to test the possibility of an interaction between them by studying the haplotype combination of the associated SNPs. The estimated frequencies combinations are depicted in Table 3.

The (G-T-L) combination showed a decreased frequency in CC patients as compared to controls (p=0.032; OR=0.53; 95 % CI=0.29–0.95). In contrast, the (T-T-2*) combination appears as a risk factor for the development of CC (p=0.018; OR=1.57; 95 % CI=1.07–2.30).

The position of the tested SNPs are indicated in above the haploview output. The LD between specificpair of those SNPs is indicated by the color scheme, wich represented LD relationships based on D' values multiplied by 100; D' is

Table 1 Frequency distribution of IL1-RN (VNTR) genotypes/alleles and association with cervical cancer among two models of study

	Controls (*N*=260) (%)	Cases (*N*=130) (%)	p_{value}	OR (95 % CI)
Genotypes				
A1/A1	149 (57.30 %)	68 (52.30 %)	–	Reference
A2/A2	15 (5.80 %)	14 (10.77 %)	0.056	2.04 (0.93–4.73)
A3/A3	6 (2.30 %)	4 (3.08 %)	0.395	1.46 (0.39–5.34)
A4/A4	9 (3.46 %)	7 (5.40 %)	0.223	1.70 (0.60–4.76)
A1/A2	40 (15.38 %)	15 (11.53 %)	0.341	0.82 (0.42–1.58)
A1/A3	6 (2.30 %)	8 (6.15 %)	**0.048**	**2.92 (1.00–8.74)**
A1/A4	10 (3.85 %)	10 (7.70 %)	0.075	2.19 (0.87–5.51)
A1/A5	2 (0.77 %)	–	NS	–
A2/A3	8 (3.00 %)	–	NS	–
A2/A4	10 (3.84 %)	–	NS	–
A3/A4	5 (1.90 %)	3 (2.30 %)	0.488	1.31 (0.30–5.66)
A4/A5	–	1 (0.77 %)	NS	–
Alleles				
A1	356 (68.50 %)	169 (65.00 %)	–	Reference
A2	88 (16.92 %)	43 (16.54 %)	0.483	1.02 (0.68–1.54)
A3	31 (5.96 %)	19 (7.30 %)	0.246	1.29 (0.70–2.35)
A4	43 (8.26 %)	28 (10.78 %)	0.139	1.37 (0.82–2.28)
A5	2 (0.36 %)	1 (0.38 %)	0.689	1.05 (0.09–11.69)
General genotypes				
L/L	187 (72.00 %)	101 (77.70 %)	–	Reference
L/2*	58 (22.30 %)	15 (11.54 %)	**0.011**	**0.47 (0.25–0.88)**
2*/2*	15 (5.77 %)	14 (10.76 %)	0.114	1.72 (0.80–3.72)
Dominant genotypes				
L/L	187 (72.00 %)	101 (77.70 %)	–	Reference
L/2*+2*/2*	73 (28.00 %)	29 (22.30 %)	0.135	0.73 (0.44–1.20)
Alleles				
L	432 (83.07 %)	217 (83.45 %)	–	Reference
2*	88 (16.93 %)	43 (16.55 %)	0.489	0.97 (0.65–1.45)

OR odds ratio

N total number of cases (130) and controls (260); $p>0.05$ no significant association; degree of freedom=1; Values in bold are statistically significant at the 5 % level

calculated as D divided by the theoritical maximum for the observed alleles frequencies. Values approaching zero indicate absence of LD and those approaching 100 indicate compleate LD. The three studied polymorphisms are in low linkage disequilibrium (Fig. 1) (D′=0.13).

Discussion

Like many other malignancies, CC develops as a result of complex interactions between environmental risk factors, HPV infection and genetic alterations. This interaction can alter gene expression and promote cell growth and carcinogenesis such a molecular mechanism involves the human IL-1 gene and its associated polymorphisms linked to CC development.

IL-1cluster is involved in immune defense against infection and it has been suggested that polymorphisms of the IL-1A, -1B and -1RN genes may be associated with autoimmune diseases, such as rheumatoid arthritis, and human cancers, [1, 26–28]. Many studies investigated the association between IL-1α (4845 G/T) [rs17561], IL-1β (-511C/T) [rs16944], IL-1RN (VNTR) [rs2234663] polymorphisms and cancers but, to the best of our knowledge, our study is the first exploring the implication of those polymorphisms and haplotypes in the development of CC.

Previous research have reported that IL-1RN levels are useful predictors of host immune response [27] and others have shown that this molecule is also altered in cancer development [29, 30]. Up to date, there are controversial data regarding the role of this polymorphism in the development of CC [22, 31–34]. This may partly be owing to a small sample size of individual studies and distinct genetic background.

Table 2 Genotype and allele frequency distribution of IL1-α+4845, IL1-β-511 and IL1-RN-VNTR polymorphisms in the study subjects in terms of FIGO stages

Polymorphisms	Controls (*N*=260)	Early stage (*N*=88)	p value	OR (95 % CI)	Advanced Stage (*N*=42)	p value	OR (95 % CI)
IL1-α (+4845)							
G/G	50 (19.00 %)	12 (14.00 %)	–	Reference	6 (14.30 %)	–	Reference
G/T	124 (48.00 %)	46 (52.00 %)	0.151	1.54 (0.75–3.16)	27 (64.30 %)	0.149	1.81 (0.70–4.66)
T/T	86 (33.00 %)	30 (34.00 %)	0.216	1.45 (0.68–3.09)	9 (21.40 %)	0.506	0.87 (0.29–2.59)
G/T+T/T	192 (81.00 %)	76 (86.00 %)	0.097	1.64 (0.83–3.26)	36 (85.70 %)	0.231	1.56 (0.62–3.91)
G	224 (43.00 %)	70 (40.00 %)	–	Reference	39 (46.40 %)	–	Reference
T	296 (57.00 %)	106 (60.00 %)	0.249	1.14 (0.80–1.62)	45 (53.60 %)	0.323	0.87 (0.54–1.38)
IL1-β (-511)							
C/C	45 (17.00 %)	20 (22.70 %)	–	Reference	11 (26.20 %)	–	Reference
C/T	128 (49.00 %)	50 (56.80 %)	0.398	0.87 (0.47–1.63)	17 (40.50 %)	0.111	0.54 (0.23–1.24)
T/T	87 (34.00 %)	18 (20.50 %)	**0.030**	**0.46 (0.22–0.96)**	14 (33.30 %)	0.233	0.65 (0.27–1.56)
C/T+T/T	215 (83.00 %)	68 (77.30 %)	0.165	0.71 (0.39–1.28)	31 (73.80 %)	0.124	0.58 (0.27–1.26)
C	218 (42.00 %)	90 (51.00 %)	–	Reference	39 (46.40 %)	–	Reference
T	302 (58.00 %)	86 (49.00 %)	**0.020**	**0.68 (0.48–0.97)**	45 (53.60 %)	0.255	0.83 (0.52–1.32)
IL1-RN							
L/L	187 (72.00 %)	68 (77.30 %)	–	Reference	33 (78.55 %)	–	Reference
L/2*	58 (22.30 %)	12 (13.70 %)	0.066	0.56 (0.28–1.12)	3 (7.15 %)	**0.023**	**0.29 (0.08–0.99)**
2*/2*	15 (5.77 %)	8 (9.00 %)	0.270	1.46 (0.59–3.61)	6 (14.30 %)	0.100	2.26 (0.82–6.26)
L/2*+2*/2*	73 (28.00 %)	20 (22.70 %)	0.201	0.75 (0.42–1.32)	9 (21.45 %)	0.241	0.69 (0.31–1.95)
L	432 (83.07 %)	148 (84.00 %)	–	Reference	69 (82.20 %)	–	Reference
2*	88 (16.93 %)	28 (16.00 %)	0.427	0.92 (0.58–1.47)	15 (17.80 %)	0.468	1.06 (0.58–2.02)

N total number of cases (130), early FIGO stage (88), advanced FIGO stage (42) and controls (260); FIGO=International Federation of Gynecology and Obstetrics; OR odds ratio; p>0.05 no significant association; degree of freedom=1; Values in bold are statistically significant at the 5 % level

Our data have revealed that A1/A3 genotype of the IL1-RN VNTR may be a risk factor of CC (p=0.048; OR=2.92; 95 % CI=1.00–8.74). However, Singh et al. proved that A1/A2 and A2/A2 genotypes were significantly associated with an increased risk of CC in North India [35]. In contrast, Tamandani et al., showed high protective association between A1/A2 genotype and CC [33, 36]. The IL1-RN VNTR polymorphism showed no allelic association with CC.

Contrariwise Sehouli et al., and Mustea et al., have proved that carriers of allele A2 have a greater risk of CC [21, 26] and

Table 3 Association between IL-1α (4845G/T) [rs17561], IL-1β (-511C/T) [rs16944] and IL-1RN VNTR [rs2234663] haplotype combinations and cervical cancer cases in Tunisians

Haplotype	freq(case)	freq(ctrl)	chi2	Fisher's p	OR (95 % CI)
G C L	0.093	0.087	0.080	0.776	1.07 (0.63–1.83)
G C 2 *	0.099	0.066	2.556	0.109	1.55 (0.90–2.67)
G T L	**0.062**	**0.111**	**4.588**	**0.032**	**0.53 (0.29–0.95)**
GT 2*	0.150	0.163	0.201	0.653	0.90 (0.59–1.38)
T C L	0.128	0.134	0.065	0.797	0.94 (0.59–1.48)
T C 2*	0.109	0.120	0.192	0.661	0.89 (0.55–1.45)
T T L	0.126	0.157	1.265	0.260	0.77 (0.49–1.21)
T T 2 *	**0.230**	**0.160**	**5.575**	**0.018**	**1.57 (1.07–2.30)**

Values in bold are statistically significant at the 5 % level; Global chi2 is 2.7716 while ddf=3 (frequency<0.03 in both control & case has been dropped.); ferq frequency

OR odds ratio

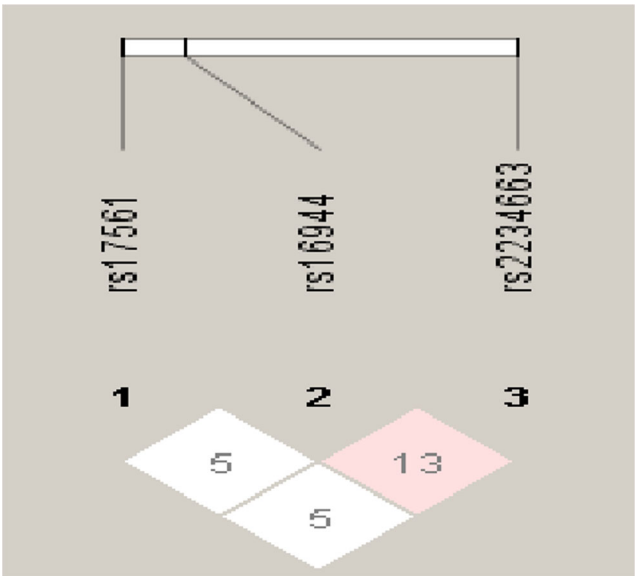

Fig. 1 Haploview analysis for LD (D′) measures between SNPs genotyped in IL1-A, IL1-B, IL1-RA genes

IL-1RN Allele 2 homozygosis may be determinant for reduced immune responses in the Portuguese population contributing thus for increased susceptibility for HPV infection and development of CC [37]. Moreover, Qian et al., showed no association between IL-1 RN (VNTR) polymorphism and CC development [37]. Recently, a Meta-analysis based on six studies showed that the IL-1RA polymorphism was associated with the risk of CC [38].

On the other hand, according to the same model used by Christoph et al., for the analysis of IL-1RA polymorphisms and CC, our results showed that the L/2* is more frequent in control group. Moreover, this genotype decreased the risk of the tumor developement (p=0.011; OR=0.47; 95 % CI= 0.25–0.88) and may be a protective factor against CC. While Christoph et al., did not find any associations between IL -1RA polymorphisms and CC [17]. Since few studies have implicated this model, our results cannot be wide discussed.

Subgroup analyses according to the FIGO stage, showed no association between early or advanced stages and controls for IL-1α (4845 G/T). However, a significant associations were found between IL1-β-511 T/T genotype and T allele and CC early FIGO stage (p=0.030; OR=0.46; 95 % CI= 0.22–0.96) (p=0.020; OR=0.68; 95 % CI=0.48–0.97) respectively, suggesting that TT genotype and T allele may be a protective factors against CC development among Tunisians. Similarly, the genotypic distribution for the advanced FIGO stage subgroup revealed that the IL-1RN (VNTR) L/2* genotype has a protective effect against CC development (p=0.023; OR=0.29; 95 % CI=0.08–0.99).

Hence, we had relatively lesser power to detect more associations with genetic variants.

Regarding the risk of CC, our study is the first evaluating the association between [rs17561], [rs16944], [rs2234663] polymorphisms and early/ advanced FIGO stages as subgroup.

Furthermore, the combined effect of the three gene polymorphisms on the risk for CC was investigated by haplotype analysis. We observed two significant associations; The (G-T-L) combination is a protective factor (p=0.032; OR=0.53; 95 % CI= 0.29–0.95) and the (T-T-2*) combination as a risk factor for the development of CC (p=0.018; OR=1.57; 95 % CI=1.07–2.30).

The three studied polymorphisms are in low linkage disequilibrium. However, the association of a gene polymorphism with altered protein production may also occur due to linkage with another gene directly affecting gene expression as IL-1b -511 was in total linkage with IL-1β-31T/C [39]. Therefore, there is still some possibility that -511 C/T polymorphism may affect CC susceptibility due to its linkage with some other polymorphism of IL-1 locus, which can directly influence the IL-1B gene expression.

To the best of our knowledge, this is the first study to investigate the association between [rs17561], [rs16944],[rs2234663] haplotypes and CC. Otherwise, due to its design our study has some shortcomings. Firstly, the study subjects enrolled were of similar ethnicity (both CC patients and controls were Tunisians), thereby minimizing the possibility of racial differences inherent in genetic association studies. Therefore the results are of limited generalizability. Secondly, the HPV status of the study population was not determined; biopsy immunochemical studies were not done. Thirdly, we did not aim to investigate the association between the three IL1polymorphisms and IL1 serum and tissue expressions. Serological levels of IL1 were not measured. Detailed stage-specific molecular and cellular expression studies in biopsy specimens of CC might help in determining the functional consequences of IL1 and its antagonist receptor gene polymorphisms. Thus, the exact mechanisms by which these three polymorphisms exert their effects on IL1 levels still remain unclear to date.

In conclusion, our results demonstrate that the IL1-RN (VNTR) A1/A3 genotype and (T-T-2*) haplotype appear as a CC risk factor. On the other side, IL1-RN (VNTR) L/2* and (G-T-L) combination may be a protective factor against CC. Also we showed a decreased risk for CC with the IL-1β-511T/T genotype and T allele distribution and CC early FIGO stage genotypes. Future studies using patients with different ethnic backgrounds should provide additional insights and improve our understanding of the IL1 gene variants in cervical carcinogenesis, which may in future lead to better prediction of individuals who are at risk of CC.

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Conflict of Interest None.

References

1. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420:860–867
2. International Agency for Research on Cancer (2008) GLOBOCAN v2.0: CancerIncidence and Mortality Worldwide; http://globocan.iarc.fr. Accessed 13.03.13
3. Maxwell PD, Bray F, Ferlay J, Jemal A (2014) Cancer in Africa 2012. Cancer Epidemiol Biomarkers Prev 23:953–966
4. Castle PE, Hillier SL, Rabe LK, Hildesheim A, Herrero R, Bratti MC, Sherman ME, Burk RD, Rodriguez AC, Alfaro M, Hutchinson ML, Morales J, Schiffman M (2001) An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papillomavirus (HPV). Cancer Epidemiol Biomarkers Prev 10:1021–1027
5. Smith JS, Herrero R, Bosetti C, Munoz N, Bosch FX, Eluf-Neto J, Castellsague X, Meijer CJ, Van den Brule AJ, Franceschi S, Ashley R (2002) Herpes simplex virus-2 as a human papillomavirus cofactor in the etiology of invasive cervical cancer. J Natl Cancer Inst 94: 1604–1613
6. van der Poll T, van Deventer SJ (1999) Cytokines and anticytokines in the pathogenesis of sepsis. Infect Dis Clin N Am 13:413–426
7. Cohen J (2002) The immunopathogenesis of sepsis. Nature 420: 885–891
8. Riedemann NC, Guo RF, Ward PA (2003) The enigma of sepsis. J Clin Invest 112:460–467

9. Dinarello CA (1996) Biologic bases for interleukin-1 in disease. Blood 87(6):2095–2147
10. Bird S, Zou J, Wang T, Munday B, Cunningham C, Secombes CJ (2002) Evolution of interleukin-1beta. Cytokine Growth Factor Rev 13:483–502
11. Nicklin MJH, Weith A, Duff GW (1994) A physical map of the region encompassing the human interleukin-1a, interleukin-1b, and interleukin-1 receptor antagonist genes. Genomics 19:382–384
12. Dinarello C (1998) Interleukin-1, interleukin-1 receptors and interleukin-1receptor antagonist. Int Rev Immunol 16:457–499
13. Majeed GS, Glew S, Bidwell J (1999) An association between LSIL and the high secretor phenotype of IL-1beta. Gynecol Oncol 73:359–361
14. Tarlow JK, Blakemore AI, Lennard A, Solari R, Hughes HN, Steinkasserer A et al (1993) Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 91(4):403–404
15. Kinane DF, Shiba H, Hart TC (2005) The genetic basis of periodontitis. Periodontol 2000(39):91–117
16. Marth C, Zeimet AG, Herold M, Brumm C, Windbichler G, Muller-Holzner E et al (1996) Different effects of interferons, interleukin-1ß and tumor necrosis factor- in normal (OSE) and malignant human ovarian epithelial cells. Int J Cancer 67:826–830
17. Grimm C, Watrowski R, Baumühlner K, Natter C, Tong D, Wolf A, Zeillinger R, Leodolter S, Reinthaller A, Hefler L (2011) Genetic variations of interleukin-1 and -6 genes and risk of cervical intraepithelial neoplasia. Gynecol Oncol 121:537–541
18. Tarlow JK, Blakemore AIF, Lennard A et al (1993) Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 91:403–404
19. Clay FE, Tarlow JK, Cork MJ, Cox A, Nicklin MJH, Duff GW (1996) Novel interleukin-1 receptor antagonist exon polymorphisms and their use in allele-specific mRNA assessment. Hum Genet 97:723–726
20. Carter MJ, di Giovine FS, Jones S et al (2001) Association of the interleukin-1 receptor antagonist gene with ulcerative colitis in Northern European Caucasians. Gut 48:461–467
21. Mustea A, Sehouli J, Konsgen D, Stengel D, Sofroni D, Lichtenegger W (2003) Interleukin 1 receptor antagonist (IL-1RA) polymorphism in women with cervical cancer. Anticancer Res 23:1099–1102
22. Sehouli J, Mustea A (2002) Interleukin-1 receptor antagonist gene polymorphism and cancer. Clin Infect Dis 34:1535–1536
23. Kang S, Kim JW, Park NH, Song YS, Park SY, Kang SB et al (2007) Interleukin-1 beta-511 polymorphism and risk of cervical cancer. J Korean Med Sci 22:110–113
24. Zidi S, Verdi H, Yilmaz-Yalcin Y et al (2014) Impact of toll-like receptors 2/3/4/9, IL-1-a/b and TNF-a Polymorphisms in cervical cancer susceptibility in Tunisia. Pathol Oncol Res. doi:10.1007/s12253-014-9793-7
25. Achyut BR, Srivastava A, Bhattacharya S, Mittal B (2007) Genetic association of interleukin-1beta (-511C/T) and interleukin-1 receptor antagonist (86 bp repeat) polymorphisms with Type 2 diabetes mellitus in North Indians. Clin Chim Acta 377:163–169
26. Eisenberg SP, Brewer MT, Verderber E, Heimdal P, Brandhuber BJ, Thompson RC (1991) Interleukin 1 receptor antagonist is a member of the interleukin 1 gene family: evolution of a cytokine control mechanism. Proc Natl Acad Sci U S A 88:5232–5236
27. Qian N, Chen X, Han S, Qiang F, Jin G, Zhou X, Dong J, Wang X, Shen H, Hu Z (2010) Circulating IL-1beta levels, polymorphisms of IL-1B, and risk of cervical cancer in Chinese women. J Cancer Res Clin Oncol 136(5):709–716
28. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA et al (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 404:398–402
29. He JF, Jia WH, Fan Q, Zhou XX, Qin HD, Shugart YY, Zeng YX (2007) Genetic polymorphisms of TLR3 are associated with nasopharyngeal carcinoma risk in Cantonese population. Bio Med Cent Cancer 7:194
30. Shin WG, Jang JS, Kim HS, Kim SJ, Kim KH, Jang MK, Lee JH, Kim HJ, Kim HY (2008) Polymorphisms of interleukin-1 and interleukin-2 genes in patients with gastric cancer in Korea. J Gastroenterol Hepatol 23(10):1567–1573
31. McIntyre KW, Stepan GJ, Kolinsky KD, Benjamin WR, Plocinski JM, Kaffka KL, Campen CA, Chizzonite RA, Kilian PL (1991) Inhibition of interleukin 1 (IL-1) binding and bioactivity in vitro and modulation of acute inflammation in vivo by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody. J Exp Med 173(4):931–939
32. Maruta Y, Okayama N, Hiura M, Suehiro Y, Hirai H, Hinoda Y (2008) Determination of ancestral allele for possible human cancer-associated polymorphisms. Cancer Genet Cytogenet 180(1):24–29
33. Kapoor S (2009) Role of polymorphisms of interleukin-1 receptor antagonist gene in systemic oncogenesis: significant etiopathologic connections besides in benign prostatic hyperplasia. Urology 73(1):215
34. Tamandani DM, Sobti RC, Shekari M, Kaur S, Huria A (2008) Impact of polymorphism in IL-1RA gene on the risk of cervical cancer. Arch Gynecol Obstet 277(6):527–533
35. Singh H, Sachan R, Goel H, Mittal B (2008) Genetic variants of interleukin-1RN and interleukin-1beta genes and risk of cervical cancer. BJOG 115(5):633–638
36. Shepperd JH (1995) FIGO staging of gynecologic cancers; cervical and vulva. Int J Gynecol Cancer 5:319–323
37. Hugo S, Santos AM, Catarino R, Pinto D, Moutinho J, Canedo P, Machado JC, Medeiros R (2012) IL-1RN VNTR polymorphism and genetic susceptibility to cervical cancer in Portugal. Mol Biol Rep 39:10837–10842
38. Sobti RC, Kordi Tamandani DM, Shekari M, Kaur P, Malekzadeh K, Suri V (2008) Interleukin 1 beta gene polymorphism and risk of cervical cancer. Int J Gynecol Obstet 101:47–52
39. Shimu W, Guiping H, Chen J, Xie G (2014) Interleukin 1β and interleukin 1 receptor antagonist gene polymorphisms and cervical cancer: a meta-analysis. Int J Gynecol Cancer 24(6):984–990