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Common variants in IL-1RN, IL-1β and TNF-α and the risk of ovarian cancer: a case control study

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Abstract

Aim of the study: *Several studies implicated altered inflammatory response in the susceptibility to ovarian cancer, and polymorphisms in inflammatory cytokines were shown to play an important role in the development of malignancies, including ovarian cancer (OC). Here we investigated the relationship between polymorphisms in IL-1β (-511C>T), IL-1RN VNTR, TNF-α (-308G>A), and TNF RII (-322 VNTR) and OC risk in Tunisian women.*

Methods and results: *Study subjects comprised 62 OC patients and 126 healthy women. Genotyping was done from genomic DNA obtained from blood simple by PCR. Positive association between IL-1RN (-VNTR) A1 allele (p = 0.0069; OR = 2.04; 95% CI:1.17-3.58) and OC risk, while negative association was seen with the A3 allele (P = 0.0034; OR = 0.09; 95% CI: 0.00-0.64), suggesting a protective role by the A3 allele. For IL-1β (-511C>T), homozygous C/C genotype was associated with significantly increased risk of OC (p = 0.0002; OR = 4.14; 95% CI: 1.77-9.76), while heterozygote C/T genotype was linked with reduced risk of OC (p = 0.0033; OR = 0.40; 95% CI: 0.20-0.78). Furthermore, TNF-α -308A allele was significantly associated with heightened risk of OC (p = 0.016; OR = 1.70; 95% CI: 1.08-2.69), and homozygote G/G genotype was associated with decreased risk of OC (p = 0.0018; OR = 0.25; 95% CI: 0.09-0.66). In contrast, TNFRII (-322 VNTR) polymorphism was not associated with altered OC risk in the studied group.*

Conclusions: *The significant association between IL-1RN VNTR, IL1-β (-511), TNF-α (-308) and OC susceptibility in Tunisian women confirms a role for altered inflammatory response in ovarian cancer pathogenesis.*

Key words: *ovarian cancer, tumor necrosis factor, interleukin-1, gene variants.*

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Introduction

Ovarian cancer (OC) is a significant malignant tumour among females and is the leading cause of death among gynaecological cancers. The high mortality rate associated with OC is due to the difficulty of its diagnosis in the early stages [1]. While it is immunogenic in nature, its malignancy is attributed to the establishment of a local and later systemic immunosuppressive microenvironment, which facilitates its escape from immune recognition and elimination [2]. Several studies have demonstrated strong correlation between the extent of chronic inflammatory changes and OC development [3]. Given their role in initiating and sustaining immunologic and inflammatory processes, cytokines were shown to play a decisive role in cancer immunity, and suppressed cytokine expression was associated with a state of tumour-specific immunosuppression and later antigen-nonspecific anergy, leading to the uncontrolled outgrowth of tumour cells [4].

Interleukin-1 (IL-1) and tumour necrosis factor α (TNF-α) are pleiotropic pro-inflammatory cytokines with overlapping biological activities, and are key inflammatory mediators. IL-1 and TNF-α produced by monocytes/macrophages or by the tumour itself seems to induce the growth of OC [4]. Insofar as OC is polygenic in nature, few genes were proposed as predictive and prognostic candidate genes for OC pathology. Many studies on populations of diverse ethnic backgrounds have suggested that

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specific functional gene variants in these and related cytokines might promote tumour growth, in part by dampening anti-tumour immune responses, and by precipitating a state of tumour-specific immune suppression [5]. Furthermore, increasing evidence implicates IL-1 and TNF-α in controlling carcinogenesis, through induction of invasion, proliferation, and metastasis [6, 7].

Polymorphisms in the promoter region of cytokine genes were shown to correlate with intra-individual variation in the expression of respective cytokine, and later expression of their high-affinity receptors, and intracellular signalling [8]. IL-1β (-511C>T), TNF-α (-308G>A), IL-1RN (-VNTR), and TNFRII (-322 VNTR) are functional polymorphisms that affect the secretion of IL-1β, IL-1Ra, and TNF-α, and expression of TNF receptor [9, 10]. These polymorphisms were demonstrated by many studies to be associated with various cancer types [5, 11-18, 24], of which IL-1RN (-VNTR) and IL-1β (-511C>T) were investigated for their possible association with altered OC risk, but with inconsistent findings [19-23]. The aim of this study was to evaluate the association of IL-1β (-511C>T), TNF-α (-308G>A), IL-1RN (-VNTR), and TNFRII (-322 VNTR) polymorphisms with OC in Tunisian women.

Material and methods

Study subjects

This case-control retrospective study comprised 62 cases of Tunisian women with OC, who were recruited from the outpatient oncology clinics of SAI, while the control group consisted of 126 healthy Tunisian women, who were recruited from the Military Hospital, Regional Hospital of Nefta, and Dispenser of Ettadhamen City. Clinical diagnosis/staging of OC patients was done according to FIGO (International Federation of Gynaecology and Obstetrics; www.figo.org) classification guidelines, and with histological types (serous-papillary, endometrioid, mucinous, and mixed/others). All subjects (cases and healthy controls) agreed to participate in the study. The study protocol followed the guidelines of the Local Ethnics Committee of the Salah Azeiz Institute (SAI) in Tunis, Tunisia.

Genotyping

Genomic DNA was extracted from peripheral venous blood, using the QIAamp® DNA blood Mini Kit (QiagenGmbH, Hilden, Germany). IL-1RN (VNTR) genotyping was done by PCR, using the following primers: forward, 5'- CTC CAG CAA CAC TCC TAT-3'; reverse, 5'-TCC TGG TCT GCA GGT AA-3'. Cycling conditions consisted of 30 cycles of denaturation (95°C for 20 s), annealing (51°C for 30 s), and extension (72°C for 50 s). Visualisation of the five alleles of 86-base pair repeat was done on agarose (1.5%) gel electrophoresis as follows: Allele 1 (4 repeats = 410 bp), Allele 2 (2 repeats = 240 bp), Allele 3 (5 repeats = 500 bp), Allele 4 (3 repeats = 325 bp), and Allele 5 (6 repeats = 595pb), as described elsewhere [19].

IL-1β (-511C>T) genotyping was done by PCR-restriction fragment length polymorphism (RFLP) using the following primers: forward, 5'-GCC TGA ACC CTG CAT ACC GT-3'; reverse, 5'-GCC AAT AGC CCT CCC TGT CT-3' [24]. PCR conditions comprised 30 cycles of denaturation (95°C for 1 min), annealing (59°C for 1 min), and extension (72°C for 1 min). The PCR product was digested by Ava I overnight at 37°C, and separated on 1.5% agarose gel; the T allele yielded the 155 bp band, and the C allele visualised as 92 bp and 63 bp bands.

Genotyping of TNFRII (-322 VNTR) insertion/deletion variant, which involves a 15 bp insertion/deletion, was done by PCR using the following primers: forward 5′-CAG GGA AGC CTG TGG GAG-3′, and reverse 5′-GGC CTT GGA CAC GCC T-3′ [26]. PCR conditions consisted of 30 cycles of denaturation (94°C for 30 sec), annealing (59°C for 30 sec), and extension at (72°C for 45 sec). The deletion and insertion alleles were visualised as 208 bp and 223 bp on 3% agarose gel, respectively. Furthermore, TNF-α (-308G>A) genotyping was done by PCR-amplification refractory mutation system (ARMS) method, as detailed elsewhere [25].

Statistical analysis

Statistical analyses were performed using EPI INFO 6 package program (http://wwwn.cdc.gov/epiinfo/html/downloads.htm). Allele and genotype frequencies were calculated by the gene counting method, and inter-group comparison was done by Pearson χ^2 test, or Fisher's exact test when the number of subjects in any of the cells in the 2×2 contingency table was less than five. Odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated as measures of the association of individual alleles or genotypes with OC risk; $p < 0.05$ was considered statistically significant.

Results

Study subjects

Clinical and demographic characteristics of OC cases ($n = 62$) and control women ($n = 126$) are described in Table 1. Median age (range) was comparable between OC cases [50.70 (18-78 years)] and control women [49.07 (30-80 years)]. Higher frequency of post-menopausal women was seen in cases (72.6%) than in healthy women (51.6%). Among OC cases, three histological OC types were identified: serious-Papillary (66.1%), endometrioid (21.0%), and mucinous-mixed/others (12.9%). According to International Federation of Gynaecology and Obstetrics (FIGO) staging, most cases were stage III (41.9%) and stage II (35.5%), followed by stage I (14.5%), and stage IV (8.1%).

Table 1. Characteristics of study participants

All women	Patients	Controls
	***n* = 62 (%)**	***n* = 126 (%)**
Age at diagnostic (mean ± SD)	50.66 ±14.21	49.07 ±11.40
≤ 40 years	13 (20.97%)	35 (27.78%)
41-50 years	18 (29.03%)	52 (41.27%)
51-60 years	17 (27.42%)	23 (18.25%)
≥ 61 years	14 (22.58%)	16 (12.70%)
Menopause status (*n*/%)		
Pre-menopausal	17 (27.40%)	61 (48.40%)
Post-menopausal	45 (72.60%)	65 (51.60%)
Histology (*n*/%)		
Serous-Papillary	41 (66.10%)	
Endometrioid	13 (21.00%)	
Mucinous – mixed/others	08 (12.90%)	
FIGO staging (*n*/%)		
Stage I	09 (14.50%)	
Stage II	22 (35.50%)	
Stage III	26 (41.90%)	
Stage IV	05 (08.10%)	

FIGO – Federation International of Gynaecology and Obstetrics; n – number of subjects; SD – standard deviation

Genotype analysis

The allele and genotype distribution of the tested IL-1RN polymorphisms in OC cases and control women are shown in Table 2. A significant positive association between A1 allele and OC risk was seen ($p = 0.007$; OR = 2.04; 95% CI: 1.17-3.58). In contrast, a negative association was seen with A3 allele ($p = 0.003$; OR = 0.09, 95% CI: 0.00-0.64), suggesting an OC-protective nature to this allele. No other associations between genotypic distributions of this polymorphism and OC were recorded.

Table 3 summarises the minor allele frequency (MAF) and genotype distribution of these SNPs in OC patients and control women. For IL-1β (-511C/T), while no significant association with OC was seen when analysed at the allelic level, significantly higher OC risk was associated with homozygote C/C genotype ($p = 0.0002$; OR = 4.14; 95% CI: 1.77-9.76), and heterozygous C/T ($p = 0.0033$; OR = 0.40; 95% CI: 0.20-0.78), and minor allele-carrying genotypes (C/T+ T/T) ($p = 0.0002$; OR = 0.24; 95% CI: 0.10-0.56) were significantly associated with decreased risk of OC.

The observed allele and genotype distribution of TNF-α (-308G>A) polymorphism revealed a positive association between minor (A) allele and the risk of OC ($p = 0.016$; OR = 1.70; 95% CI: 1.08-2.69)]. While homozygous TNF-α -308G/G genotype was negatively associated with OC, suggesting a protective nature of this genotype ($p = 0.002$; OR = 0.25; 95% CI: 0.09-0.66), TNF-α minor allele-carrying genotypes (G/A+A/A) was associated with increased risk of OC ($p = 0.002$; OR = 4.03; 95% CI: 1.50-11.39). Furthermore, no significant association with OC was noted for TNFRII (-322 VNTR) variant, both at the allele or genotype levels.

Discussion

OC is a leading cause of death among all gynaecological malignancies, which is aggravated by its asymptomatic nature, the lack of sensitive/specific screening tests, and predictive and prognostic factors [1, 27]. At present, CA-125 (Cancer Antigen 125) serum determination, and trans-vaginal ultrasound examination are utilised in the diagnosis of OC, but neither provide the required sensitivity and specificity, and thus positive and negative predictive values, for effective screening [27]. This highlights the need for identification of alternative predictive/prognostic risk factors for OC, which may improve clinical management. In this study, we evaluated the association of well-defined polymorphisms in the pro-inflammatory cytokines IL-1β and TNF-α, in TNF-α receptor, and in IL-1 receptor antagonist (IL-1RN) genes. The MAF of IL-1β, TNF-α, and TNFRII polymorphisms analysed in Tunisians were generally comparable to those of Caucasians (> 5%), and they were determined based on SNP arrays from the international HapMap consortium and the genetic variants detected in the 1000 Genomes project (http://www.1000genomes.org/category/dbsnp).

Our working hypothesis is that altered pro-inflammatory cytokine and receptor expression contribute to the tumour development, including OC, by modulating anti-tumour immunity [2, 3].

Previous studies implicated IL-1Ra in the host immunity to gynaecological cancers, in particular by decreasing tumour growth and angiogenesis [28, 29]. Insofar as IL-1RN and IL-1β genes regulate plasma IL-1Ra levels, polymorphisms in IL-1RN and IL-1β genes may affect OC susceptibility. Of the reported IL-β and IL-1RN gene variants, the promoter -511C/T variant and the 86-bp VNTR markedly affect the level of these cytokines, respectively [30]. In our hands, A1 allele, associated with high IL-1RN levels, was associated with OC susceptibility, in contrast to A3 allele, which appeared to be protective. This was reminiscent of earlier findings from our group, albeit with a lower number of subjects [19]. To the best of our knowledge, this is the first study documenting an association between A1 and A3 alleles and the risk of OC. An association between A2 allele and OC was reported earlier in German [23] but not in Austrian [21] women. These contradictory findings may be explained by differences in ethnic background, the contribution of additional genetic and non-genetic modulating factors, and sample size.

Table 2. Genotype and allele frequency distribution of IL-1 RN –VNTR polymorphism in OC cases and controls

Genotype Allele	Genotype /Allele frequencies				Association analysis Cases vs. Controls	
	Cases		Controls			
	***n* = 62**	**(%)**	***n* = 126**	**(%)**	**OR (95% CI)**	***P* value**
IL-1 RN 86 base pairs VNTR						
A1A1	45	72.60	75	59.50	1.73 (0.85-3.55)	0.10
A1A2	9	14.50	15	11.90	1.23 (0.46-3.24)	0.64
A1A3	–	–	1	0.80	0.00 (0.00-35.09)	0.66
A1A4	2	3.20	3	2.40	1.34 (0.15-10.24)	0.54
A2A2	2	3.20	7	5.60	0.56 (0.08-3.06)	0.37
A2A3	–	–	3	2.40	0.00 (0.00-4.51)	0.29
A2A4	1	1.60	4	3.20	0.49 (0.02-4.82)	0.45
A1A3	–	–	2	1.60	0.00 (0.00-8.24)	0.44
A3A2	–	–	1	0.80	0.00 (0.00-35.09)	0.66
A3A3	–	–	6	4.80	0.00 (0.00-1.85)	0.08
A4A2	–	–	3	2.40	0.00 (0.00-4.51)	0.29
A3A4	1	1.60	2	1.60	–	0.70
A2A5	1	1.60	–	–	–	0.33
A4A4	1	1.60	–	–	–	0.33
A1	**101**	**81.45**	**172**	**68.25**	**2.04 (1.17-3.58)**	**0.0069**
A2	15	12.09	40	15.87	0.73 (0.37-1.44)	0.32
A3	**1**	**0.81**	**21**	**8.34**	**0.09 (0.00-0.64)**	**0.0034**
A4	6	4.84	18	7.14	0.66 (0.23-1.83)	0.39
A5	1	0.81	1	0.40	2.04 (0.00-75.25)	0.55

VNTR – Variable Number of Tandem Repeats; n – number of subjects (62 cases and 126 controls); OR – odds ratio; nominal value of comparison, p > 0.05 no significant association, degree of freedom = 1. Values in bold are statistically significant at the 5% level

Carriers of IL-1β -511C/C genotype are at higher risk of OC, in contrast to -511C/T genotype carriers, in whom decreased OC risk was noted. Earlier studies on German [20] and Austrian [21] OC cases revealed a lack of association of this polymorphism with the risk of OC. Because only these two case-control studies investigated the association of the IL-1β -511C>T variant and OC susceptibility [20, 21], the comparison between our data and similar studies on other ethnic groups was limited. Additional studies are needed to confirm, or alternatively rule out, the contribution of this variant as a risk factor for OC.

TNF-α, a pro-inflammatory cytokine implicated in cancer pathogenesis, through modulation of the invasion, proliferation, and metastasis of tumour cells [7], and increased TNF-α expression is seen in solid tumours [31]. Because the TNF-α -308G/A variant influences TNF-α secretion, we investigated the association of this promoter polymorphism with OC risk. The minor (A) allele was found to constitute a risk factor for OC, while the major (G) allele and G/G genotype conferred protection from OC. This is the first study that investigated the association between this TNF-α variant and OC. Because the study involved a relatively small sample size and was limited to Tunisians, larger well-designed studies from different ethnic origins are needed to confirm its association of TNF-α -308G/A with OC.

TNFRII (-322 VNTR) is a promoter polymorphism, which modulates the expression of TNF receptor. The association of this variant with OC was not previously analysed. While no association between TNFRII (-322 VNTR) and OC risk was found here, this might be attributed to the relatively small sample size. As such, larger studies are needed to elucidate the possible contribution of this variant with OC risk.

In conclusion, IL-1RN (-VNTR), IL-1β (-511C>T), and TNF-α (-308G>A) polymorphisms may affect OC susceptibility. It is tempting to speculate that this association can be utilised as a predictive factor of OC pathogenesis, which in turn may improve clinical management of OC. However, it should be mentioned that these results are currently limited and thus need confirmation in larger studies in different ethnic groups. These studies should allow stratification

Table 3. Genotype and allele frequency distribution of IL-1β-511, TNF α -308 and TNF RII-322 polymorphisms and the risk of OC

Genotype Allele	Genotype /Allele frequencies				Association analysis	
	Patients		Controls		Patients vs. controls	
	n = 62	(%)	*n* = 126	(%)	OR (95% CI)	*P* value
IL-1β -511						
C/C	**20**	**32.30**	**13**	**10.30**	**4.14 (1.77-9.76)**	**0.0002**
C/T	**28**	**45.20**	**85**	**67.50**	**0.40 (0.20-0.78)**	**0.0033**
T/T	14	22.60	28	22.20	1.02 (0.46-2.24)	0.95
C/T + T/T	**42**	**67.80**	**113**	**89.70**	**0.24 (0.10-0.56)**	**0.0002**
*C	68	54.84	111	44.05	1.54 (0.98-2.43)	0.0488
TNF α -308						
G/G	**6**	**09.70**	**38**	**30.20**	**0.25 (0.09-0.66)**	**0.0018**
G/A	46	74.20	75	59.50	1.95 (0.95-4.05)	0.04
A/A	10	16.10	13	10.30	1.67 (0.63-4.40)	0.25
G/A+A/A	**56**	**90.30**	**88**	**69.80**	**4.03 (1.50-11.39)**	**0.0018**
***A**	**66**	**53.22**	**101**	**40.08**	**1.70 (1.08-2.69)**	**0.0158**
TNF R II -322 VNTR						
1/1	12	19.40	30	23.80	0.77 (0.34-1.72)	0.49
1/2	18	29.00	31	24.60	1.25 (0.60-2.61)	0.51
2/2	32	51.60	65	51.60	1.00 (0.52-1.92)	0.99
1/2+2/2	50	80.60	96	76.20	1.30 (0.58-2.96)	0.49
*1	42	33.87	91	36.11	0.91 (0.56-1.46)	0.66

** Minor allele frequency, MAF source: 1000 genomes phase 1 from dbSNP.*
n –number of subjects (62 patients and 126 controls); OR – odds ratio; nominal value of comparison, p > 0.05 no significant association, degree of freedom = 1. Values in bold are statistically significant at the 5 % level

analysis, based on tumour stage and related parameters, which in turn will validate the contribution of these, and probably other, variants as biomarkers of OC susceptibility, leading to better assessment of OC risk in Tunisia.

The authors declare no conflict of interest.

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