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ORIGINAL ARTICLE

Association of a variant in the tumor necrosis factor alpha gene with risk of cervical cancer

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Abstract

Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine involved in the regulation of the immune system and potentially the progression of cervical neoplastic lesions. In this study, we aimed to explore the possible relationship between polymorphisms of the TNF-α gene and susceptibility to cervical cancer. The relationship between a single nucleotide polymorphism (SNP) in the TNF-α gene (rs1800629) and the risk of cervical cancer was evaluated in a total of 445 subjects with (n = 153), or without (n = 292) cancer. Genotyping was performed using a Taq-Man based real time PCR method. Logistic regression analysis showed that individuals with AG/AA genotypes had an increased risk of cervical cancer compared to those with a GG genotype (OR 3.79, 95% CI 2.4–5.7, < 0.001). Our findings demonstrated that a genetic variant in the TNF-α gene (rs1800629) was associated with increased level and risk of developing cervical cancer, suggesting its potential use as a genetic risk factor for cervical neoplasia.

Keywords Cervical cancer · TNF-α · Cytokine · rs1800629

Introduction

Cytokines are molecular messengers that modulate several biological processes including cell proliferation, differentiation, inflammatory responses and cell death. Animal studies have demonstrated anti-tumour activity of several cytokines, and these are currently being investigated in clinical trials for the treatment of human malignancies [1]. So far, seven cytokine receptor families have been identified, that include tumor necrosis factor (TNF) receptors. TNF-α is an acute-response cytokine that can promote neutrophils and monocytes influx during the inflammatory process. Moreover, it has been shown that TNF-α can induce endothelial cells to express adhesion molecules which is considered to be a hallmark for inflammatory responses in endothelial cells [2, 3].

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Previous studies have shown that malignant cells produce small amounts of TNF that increase the permeability of blood vessels [4]. In normal states, TNF may be produced in lower quantities; however, aberrant expression of this cytokine may result in cell death [5]. There is increasing evidence that blood levels of TNF-α increase in the presence of solid tumors [6]. It has been reported that a genetic variant in the TNF-α gene (G-308A rs1800629) is associated with the expression of this gene [7]. TNF-α is regulated at the transcriptional level [8] and the rs1800629 polymorphisms is located in the TNF-α promoter region and has been shown to be related to the expression of TNF-α [9]. Several studies have examined the association of the rs1800629 SNP and cervical cancer but the results remain inconsistent [10, 11] and our previous study showed the potential value of the marker in cervical cancer patients [12]. Therefore, in the present study we aimed to investigate further and validate the potential value of the rs1800629, G-308A, in a large population sample of individuals with and without cervical cancer.

Material and methods

DNA extraction and genotyping

A total of 445 subjects (153 patients and 292 controls) were recruited from Mashhad University of Medical Sciences (MUMS). The patients with cervical cancer were diagnosed between 2016 and 2019. healthy individuals recruited from the Mashhad Stroke and Heart Atherosclerotic Disorders cohort study. our patients obtained based on diagnosis of histologically confirmed cervical cancer, by pathologist. Exclusion criteria for this group were: having family history of stroke, other cancer type, myocardial infarction, infectious disease, diabetes mellitus, chronic liver and/or renal diseases, pregnant women. The control group was having family history of stroke, cancer, myocardial infarction, infectious disease, and diabetes mellitus. Exclusion criteria were: medical illnesses such as chronic liver and/or renal diseases, pregnant women and alcohol consumption based on a questionnaire, taking any drugs including dietary supplements and anti-inflammatory drugs including aspirin and non-steroidal anti-inflammatory drugs. The consent form was approved by the Ethics Committee of the MUMS, informed consent was obtained from all participants. Our study was approved by Ethical Committee and Research Office of Mashhad University of Medical Sciences (IR.MUMS.fm.REC.1396.296; 960284).

Genomic DNA was extracted from peripheral blood leukocytes of control subjects and from tissue samples of patients using QIAamp® DNA Mini-Kit (Qiagen, San Diego, CA), according to the manufacturer's protocol. The concentrations of DNA were assessed by NanoDrop®-1000-Detector. Genotype analysis of tumor necrosis factor alpha -308 gene locus was carried out by TaqMan® Universal Master Mix with specific primers and probes (Assay ID C___7514879_10; Context Sequence [VIC/FAM]: GAGGCAATAGGTTTTGAGGGGCAT G[A/G]GGACGGGGTTCAGCCTCC AGGGTCC; Applied Biosystems Foster City, CA) [12, 13]. An ABI PRISM-7500 instrument was utilized for genotyping.[14].

TNF-α expression level

The expression of TNF-α was measured using ELISA kits (eBioscience, San Diego, CA) of 40 mg of 20 representative fresh tissue homogenates of patients, which was stored before use in -80 and expression was measured according to the manufacturer's protocol and standard control. patients obtained based on diagnosis of histologically confirmed cervical cancer, by pathologist. Exclusion criteria for this group were: having family history of stroke, other cancer type, myocardial infarction, infectious disease, diabetes mellitus, chronic liver and/or renal diseases, pregnant women.

Statistics

In this study, the Kolmogorov- Smirnov tests were performed to examine the distribution of data within the subgroups. Demographic and clinical information were assessed by Pearson's χ^2 tests. Continuous variables were measured by t-tests. The genotype frequencies were evaluated with χ^2 tests. The Hardy–Weinberg equilibrium assumption was determined. The association between the risk of a genetic variant in terms of the development of cervical cancer were explored by logistic regression. The data analysis was conducted by SPSS- 22 software. Statistical significance was considered as $P < 0.05$.

Result

The genotype frequencies of the variant in our studied population was in accord with the Hardy–Weinberg equilibrium (P = 0.054). The allelic frequencies of the minor A allele (MAF) in the total population was 0.4. However, the frequency of minor allele homozygote (AA) and AG in cancer group was statistically higher than for the healthy control group (p-value in different genetic models was < 0.001) (Table 1).

In further multivariate regression analysis, we found that the minor A allele was associated with an increased risk of cervical cancer. In particular using a co-dominant model, after adjustment, the risk of cancer in the holders of AG genotype was 3.15-fold higher than carriers of common GG genotype (OR 3.15, 95% CI (2.02–4.89)) (Table 2).

Moreover, we examined the result of pap-smear in association with rs1800269 polymorphism. Based on Table 3, it appeared that the A allele was associated with an increased risk of declining pap-smear test. The co-dominant model revealed that the risk of severity in pap-smear in carriers of AG and AA genotypes were (OR 2.89, 95% CI (1.84–4.5)) and (OR 8.1, 95% CI (3.4–19.3)) respectively. We further evaluated the protein expression level of TNF-α in tissue homogenates of cervical cancer patients. Our results indicate that the expression level of TNF-α in AA genotype was greatly increased in comparison with the GG genotype and healthy subjects. Additionally, the expression level of TNF-α in patients was considerably higher than the healthy individuals (Fig. 1).

Discussion

We investigated the value of the G-308A rs1800629 TNF alpha gene polymorphism in cervical cancer and its relationship with the PAP-smear test and pathological information of patients. Our finding demonstrated that G-308A variant in TNF-α was associated with increased tissue protein levels and an increased risk of cervical cancer. Although the influences of genetic factors in the development of cervical cancer need to be more elucidated, emerging epidemiological evidence proposes that genetic variants of cytokine genes could contribute to the pathology of cervical neoplasia. TNF-α considered as an important cytokine involving in inflammation, immune system responses, and various cellular functions like cell differentiation and cell death. Recent studies have reported that the pro-inflammatory function of this cytokine is an important risk factor for a number of epithelial carcinomas including cervical cancer [15].

Table 1 Allele and genotype frequencies of rs1800629 polymorphisms

Gene	SNP	Major/minor allele	Major allele homozygote (%)	Heterozygote (%)	Minor allele homozygote (%)	MAF	HWE p value
TNF	Rs1800629	G/A	200(47.8%)	190(45.5%)	28 (6.7%)	0.4	0.054
		Control (n = 295)	Cancer (n = 153)	Total (n = 418)	Genetic model		*P* value
GG		157 (59.2%)	43 (28.1%)	200 (47.8%)	Additive		< 0.001
AG		102 (38.5%)	88 (57.5%)	190 (45.4%)	Recessive		< 0.001
AA		6 (2.26%)	22 (14.4%)	28 (6.7%)	Dominant		< 0.001

SNP single nucleotide polymorphism, *MAF* minor allele frequency, *HWE* Hardy Weinberg Equilibrium

Table 2 Multivariable logistic regression analysis of rs1800629 polymorphism and cervix cancer under different genetic models

Co-dominant model		
GG	AG	AA
Reference (Common genotype)	OR (95% CI), *p* value	
1	3.15 (2.02–4.89), < 0.001	13.38 (5.1–35), **< 0.001**
Dominant model		
GG	AG/AA	
Reference	OR (95% CI), *p* value	
1	3.79 (2.4–5.7), < **0.001**	
Recessive model		
GG/AG	AA	
Reference	OR (95% CI), *p* value	
1	7.2 (2.86–18.3), < **0001**	

Data was adjusted for age, BMI.

Table 3 Multivariable logistic regression analysis of rs1800629 polymorphism and declining smear results under different genetic models

Co-dominant model		
GG	AG	AA
Reference (Common genotype)	OR (95% CI), *p* value	OR (95% CI)
1	2.89 (1.84–4.55), < 0.001	8.1 (3.4–19.3), < **0.001**
Dominant model		
GG		AG/AA
Reference		OR (95% CI), *p* value
1		3.3 (2.14–5.12), < **0.001**
Recessive model		
GG/AG		AA
Reference		OR (95% CI), *p* value
1		4.6 (2.03–10.5), < **0001**

Data was adjusted for age, BMI.

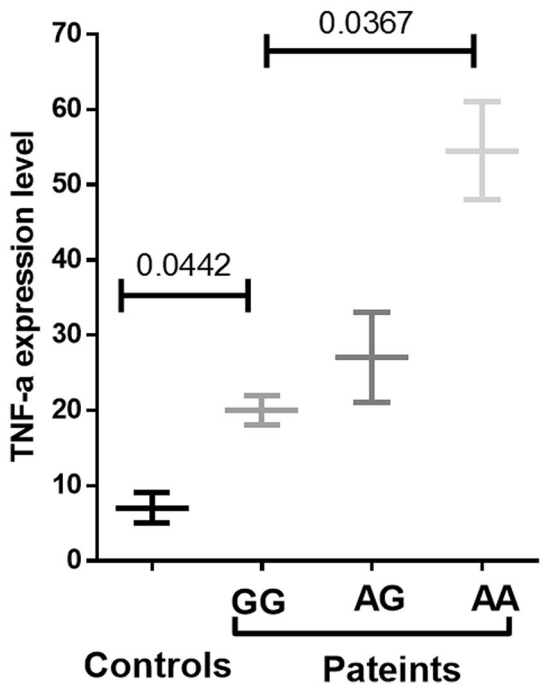

Fig. 1 The expression level of TNF-α in patients with cervical cancer and healthy controls

It appears that genetic variants on the promoter locus of TNF-α gene may have an effect on the TNF-α mRNA transcription. The -308A minor allele (TNF2), located within the promoter region, appears to induce transcription and subsequently up-regulation of TNF-α that stimulates angiogenesis process in cancer cells. It has been also shown that the over-expression of TNF-α may result in CIN lesions and high susceptibility for HPV infection and subsequently cervical carcinoma [7, 9, 16, 17].

Therefore, our results seem to be biologically relevant and a number of studies in other Asian populations such as in Korea have reported similar results [18]. Recent findings suggest that the association between -308AA and risk of cervical cancer was more significant in Asians [19]. Furthermore, a study conducted in the European population, different types of CIN were reported to be associated with minor AA genotype and in Portuguese population, A allele was related to higher risk of cervical carcinoma [17, 20]. Kirkpatrick et al. have reported that cervical neoplasia was affected by the -308G-rs1800629 genotype [20]. Inversely, some studies found no statistical association between this genetic variant with the risk of cervical carcinoma [11, 21, 22]. This inconsistency may be due to environmental exposure, ethnic background, geographic variations in HPV type, study design, and also a variety of analytic approaches. In aggregate our data illustrated that G-308A rs1800629 was associated with the risk of developing cervical cancer with respect to the cytological and pathological information of patients. Further multi center setting studies are needed to investigate the potential role of rs1800629 genotype as a risk stratification marker for cervical neoplasia.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to disclose.

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