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ORIGINAL ARTICLE

Evaluation of Toll-Like Receptors 2/3/4/9 Gene Polymorphisms in Cervical Cancer Evolution

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Abstract Accumulative epidemiological evidence suggests that polymorphisms of Toll-like receptors signaling pathway elucidated the cellular and molecular mechanisms of human diseases whose gaining a primordial importance. The aim of our study is to identify the role of TLR 2 (−196 to −174 del), TLR 3 (1377 C>T), TLR 4 (Asp299Gly) and TLR 9 (G2848A) gene polymorphisms with the evolution of cervical cancer in Tunisian women. Blood samples were collected from histopathologically confirmed patients with cervical cancer and unrelated healthy female controls of similar ethnicity. Genotyping of the analyzed polymorphisms were done using Polymerase Chain Reaction and Restriction Fragment Length Polymorphism. For the TLR 2, Ins/Ins genotype is a protector factor [p = 0.006; OR: 0.35(0.16–0.73)] and the dominant genotype of TLR 3 increased the risk of CC in stage (III+IV); C/C versuss C/T [p = 0.033; OR: 2.03(1.00–4.13)] and C/C versus C/T+T/T [p = 0.036; OR: 1.93(1.00–3.74)]. For TLR 4, the dominant genotype Asp/Asp is implicated in the occurrence of CC in stage (I+II) [p = 0.000; OR: 4.55(1.58–13.06)], [p = 0.001; OR: 3.49(1.44–8.45)] and in stage (III+IV) [p = 0.038; OR: 3.77(0.87–16.29)], [p = 0.007; OR: 5.21(1.65–16.46)] and the major allele Asp is a risk factor for the development of tumor in stage (I+II). The TLR2 Ins/Del genotype is associated with tumor evolution to stage (III+IV) [p = 0.003; OR: 3.00 (1.22–7.35)] and the genotypes Gly/Gly and Asp/Gly+Gly/Gly and Gly allele of TLR 4 are implicated in tumor evolution to the advanced stages. Further, TLR 2, TLR 3, TLR 4 and TLR 9 gene polymorphisms are implicated in the modulation of CC risk due to tobacco usage and statue of menopause among cases. Our study suggests a relationship between the incidence of the TLR2, TLR 3, TLR 4 and TLR9 mutations and the clinical progression of CC according to the FIGO classification. However, future studies with different demographic and clinical characteristics in ethnically diverse populations may provide a more comprehensive involvement of innate immunity in cervical cancer etiology in women worldwide.

Keywords Toll-like receptor · Cervical cancer · Gene polymorphism · FIGO stage

Introduction

Cervical cancer (CC) rates as the third most common malignancy and the fourth leading cause of cancer-related deaths in women worldwide [1]. Deciphering the cellular and molecular mechanisms involved in the etiology of CC has been a major thrust area in medical research.

Several causative agents contribute to the development of CC of which infection Human papillomavirus (HPV) is recognized as it's the principal causative agent [2]. However, only a minority of HPV infected women will develop CC, suggesting that HPV is not a sufficient separate factor responsible for tumorigenesis of the cervix [3]. TLRs play pivotal roles in the immune system and initiate inflammatory response to foreign pathogens including viruses and are emerging as plausible susceptibility markers in diverse human cancers [4]. HPV can directly inhibit the function of TLR

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downstream molecules involved in the interferon pathway and accumulating evidence supports an interaction between TLRs and HPV [5]. Single nucleotide polymorphisms are the most common form of genetic variants in human genome and have a potential functional influence on the susceptibility to human diseases including cancer. Several studies has reported the marginal role of TLR 2,3,4 and 9 gene polymorphisms in CC susceptibility [6–9].

The present study aimed to identify the association of TLR2 (−196 to −174 del), TLR3 (c.1377 C>T; rs3775290), TLR4 (Asp299Gly; rs4986790) and TLR9 (2848G>A; rs352140) SNPs with cervical cancer evolution in Tunisian women, in view of the potential role of these polymorphisms as host immune modifiers.

Materials and Methods

Study Setting and Participants

In the present case–control study, we recruited a total of 390 study subjects; 130 cervical cancer patients and 260 controls from October 2010 to August 2012. The cases and controls in the present study were unrelated and were of similar ethnicity. Clinical diagnosis of cervical cancer patients was performed by cervical biopsy were consecutively recruited from Salah Azeiz Oncology Institute (SAI, Tunisia). Cancer diagnosis was established by clinical examination and biopsy findings, confirmed by two senior SAI pathologists. Clinical data were obtained from questionnaires, review of case records, and from personal interviews. Tumors were staged according to International Federation of Gynecology and Obstetrics (FIGO) classification (www.figo.org) [10]. The Control group was free from chronic clinical problem history of malignancy, drug allergy, hypertension, diabetes, or cardiovascular disease. Written informed consent was taken from all study subjects prior to study enrollment and the protocol was approved by local ethics review committee.

Blood Collection and DNA Extraction

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

Genotyping

The TLR 2(−196 to −174 del), genotype was performed by PCR and size separation on agarose gel, while genotyping of TLR3 (1377C>T) [rs3775290], TLR4 (Asp299Gly; rs4986790) and TLR9 (2848 G>A) [rs352140] were determined by PCR-restriction fragment length polymorphism analyses, with appropriate primers and restriction enzymes, as previously described [11–15].

Statistical Analysis

Shapiro-Wilk's test was used to assess the normality of distributions of the age variable, and Levene's test was used to assess the homogeneity of variances in the control and patient groups. Parametric test assumptions were not available for the age variable, so the comparisons of two independent groups were performed with the Mann-Whitney U test. The results of tests were expressed as the number of observations (n), the mean ± the standard deviation and the median. The allele and genotype frequencies were calculated for all investigated polymorphisms by direct counting. Distribution of genotypes for all polymorphisms was tested for conformity with Hardy-Weinberg equilibrium. Categorical data were analyzed by Pearson chi-square analysis and Fisher's Exact test. Binary logistic regression analysis and multinomial regression analysis were used and Odd's ratios and 95 % confidence intervals were calculated in order to measure the strength of the association of individual alleles or genotypes with risk of cervical cancer. A p value of <0.05 was considered statistically significant. Statistical analyses were performed by Epi info 7 (http://wwwn.cdc.gov/epiinfo/html/downloads.htm).

Results

Table 1 lists demographic and clinical characteristics of the study populations. The median (range) age, was 52 ± 0.9 years for patients, and 53 ± 1.2 years for controls. Among the 130 patients, 124 (95.40 %) are married, 115 (88.50 %) have used hormonal contraceptives and 32 (25 %) are tobbaco users. According to the menopausal status of women with CC, our sample was divided into two groups; 38 (29.25 %) were premenopausal 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 %).

Table 1 Demographic and clinical characteristics of the study populations

	Patients n (%)	Controls n (%)
All women	130	260
Age (mean ± SD)	52 ± 0.9	53 ± 1.2
30–40 years	17 (13.08 %)	87 (33.46 %)
41–50 years	33 (25.38 %)	93 (35.77 %)
51–60 years	34 (26.15 %)	53 (20.38 %)
61–70 years	24 (18.46 %)	24 (9.23 %)
71–81 years	22 (16.93 %)	3 (1.16 %)
Married status		
+/−[1]	124 (95.40 %)/6 (4.60 %)	257 (98.00 %)/3 (2.00 %)
Hormonal contraception		
+/−[2]	115 (88.50 %)/15 (11.50 %)	220 (84.00 %)/40 (16.00 %)
Status of menopause		
Premenopausal	38 (29.25 %)	67 (26 %)
Postmenopausal	92 (70.75 %)	193 (74 %)
Tobcco users		
+/−[3]	32 (25 %)/98(75 %)	18 (7 %)/242 (93 %)
FIGO staging		
Stage I	39 (30.00 %)	NA
Stage II	49 (37.70 %)	NA
Stage III	32 (24.60 %)	NA
Stage IV	10 (7.7 %)	NA
Histological type		
Squamous cell carcinoma	108 (83.08 %)	NA
Adenocarcinoma	19 (14.61 %)	NA
Sarcoma	3 (2.31 %)	NA

+/−1; (+) Married; (−) Single, +/−2; (+) Yes; (−) No, +/−3; (+) Yes; (−) No; *FIGO* International Federation of Gynecology and Obstetrics; *n* Number of women; *NA* not applicable

Association of TLR2 (−196 to −174 del), TLR3 (c.1377 C>T; rs3775290), TLR4 (Asp299Gly; rs4986790) and TLR9 (2848G>A; rs352140) Gene Polymorphisms with Early and Advanced Tumor Stage

The observed genotype and allele frequency distribution of TLR2 (−196 to −174 del), TLR3 (c.1377 C>T; rs3775290), TLR4 (Asp299Gly; rs4986790) and TLR9 (2848G>A; rs352140) gene polymorphisms between cases with a tumor in stage (I+II) and (III+IV) versus controls are depicted in Table 2. For the TLR 2, Ins/Ins genotype is a protector factor [$p = 0.006$; OR: 0.35(0.16–0.73)]. The dominant genotype of TLR 3 was significantly associated with an increased risk of CC in stage (III+IV); C/C vs C/T [$p = 0.033$; OR: 2.03(1.00–4.13)] and C/C vs C/T+T/T [$p = 0.036$; OR: 1.93(1.00–3.74)]. Our results showed that for TLR 4, the dominant genotype Asp/Asp is more common among cases with tumor in stage (I+II) [$p = 0.000$; OR: 4.55(1.58–13.06)], [$p = 0.001$; OR: 3.49(1.44–8.45)] and in stage (III+IV) [$p = 0.038$; OR: 3.77(0.87–16.29)], [$p = 0.007$; OR: 5.21(1.65–16.46)] then controls. Furthermore, the major allele Asp is a risk factor for the development of tumor in stage (I+II). The TLR 9 genotypic and allelic frequency distributions between cases and healthy controls were similar and no significant association was observed for CC development in stage (I+II) and in stage (III+IV).

Association of TLR2 (−196 to −174 del), TLR3 (c.1377 C>T; rs3775290), TLR4 (Asp299Gly; rs4986790) and TLR9 (2848G>A; rs352140) Gene Polymorphisms with Cervical Cancer Evolution

A case-only, analysis was carried out to investigate the implication of gene polymorphisms in CC evolution by combining stages I+II into early stage ($n = 88$ cases) versus stages III+IV into advanced stage ($n = 42$ cases). There was statistically significant relationship between the incidence of the TLR2 and TLR4 mutations and the clinical progression of CC according to the FIGO classification. Our data demonstrated that; the heterozygous genotype TLR2 Ins/Del is more common among women with advanced tumor stage [$p = 0.003$; OR: 3.00 (1.22–7.35)] and the homozygous genotype Gly/

Table 2 Genotype and allele frequency distribution of TLR 2/3/4 and 9 polymorphisms in the study subjects

TLR gene polymorphisms	Controls (N = 260) (%)	Stage I+II (N = 88) (%)	p value	OR (CI 95 %)	Stage III+IV (N = 42) (%)	p value	OR (IC 95 %)
TLR2 (del −196 −174)							
Ins/Ins	196(75.4)	67(76)	–	Reference	26(62)	–	Reference
Ins/Del	37(14.2)	12(13.5)	0.521	1.05(0.51–2.13)	14(33)	0.006	0.35(0.16–0.73)
Del/Del	27(10.4)	9(10.5)	0.566	1.02(0.45–2.29)	2(5)	0.342	1.79(0.40–7.97)
Ins/Del+ Del/Del	64(24.6)	21(24)	0.505	1.04(0.59–1.83)	16(38)	0.052	0.53(0.26–1.05)
Ins	429(82.5)	146(83)	–	Reference	66(78.5)	–	Reference
Del	91(17.5)	30(17)	0.495	1.03(0.65–1.62)	18(21.5)	0.233	0.77(0.44–1.37)
TLR3 +1377 (C>T)							
C/C	106(40.8)	45(51)	–	Reference	24(57)	–	Reference
C/T	126(48.5)	34(38)	0.054	1.57(0.94–2.63)	14(33)	0.033	2.03(1.00–4.13)
T/T	28(10.8)	9(11)	0.329	1.32(0.57–3.02)	4(10)	0.304	1.58(0.50–4.94)
C/T+T/T	154(59.3)	43(49)	0.058	1.52(0.93–2.47)	18(43)	0.034	1.93(1.00–3.74)
C	338(65.0)	124(70)	–	Reference	62(73.8)	–	Reference
T	182(35)	52(30)	0.108	1.28(0.88–1.86)	22(26.2)	0.070	1.51(0.90–2.54)
TLR4 Asp299Gly							
Asp/Asp	207(79.6)	82(93)	–	Reference	34(81)	–	Reference
Asp/Gly	46(17.7)	4(4.5)	0.000	4.55(1.58–13.06)	2(4.7)	0.038	3.77(0.87–16.29)
Gly/Gly	7(2.7)	2(2.5)	0.510	1.38(0.28–6.81)	6(14.2)	0.007	5.21(1.65–16.46)
Asp/Gly+Gly/Gly	53(20.4)	6(7)	0.001	3.49(1.44–8.45)	8(18.9)	0.514	1.08(0.47–2.48)
Asp	460(88.5)	168(95.45)	–	Reference	70(83.3)	–	Reference
Gly	60(11.5)	8(4.55)	0.003	2.73(1.28–5.84)	14(16.7)	0.126	0.65(0.34–1.22)
TLR9 +2848 (G>A)							
G/G	83(31.9)	31(35.2)	–	Reference	11(26.2)	–	Reference
G/A	117(45.0)	29(32.9)	0.107	1.54(0.84–2.68)	19(45.2)	0.384	0.81(0.36–1.80)
A/A	60(23.1)	28(31.9)	0.286	0.80(0.43–1.47)	12(28.6)	0.243	0.66(0.27–1.60)
G/A+A/A	177(58.1)	57(64.8)	0.328	0.86(0.51–1.43)	31(73.8)	0.290	1.32(0.63–2.75)
G	283(54.4)	91(51.7)	–	Reference	41(49)	–	Reference
A	237(45.6)	85(48.3)	0.295	0.89(0.63–1.26)	43(51)	0.200	0.79(0.50–1.26)

Total number of cervical cancer cases of early stages (I + II) = 88 and of advanced stages (III + IV) = 42

OR age-adjusted odds ratio, *CI* confidence interval

Gly, the combined genotype Asp/Gly+Gly/Gly and Gly allele of TLR 4 increased the risk for tumor evolution to the advanced stages of CC when compared to the early stages (I+ II) successively [p = 0.013; OR: 7.23(1.39–37.65)] [p = 0.039; OR: 3.21(1.03–9.96)] [p = 0.001; OR: 4.20(1.68–10.45)]. However, no significant association of TLR3 and TLR9 was identified with clinical stages of CC (Table 3).

Association of TLR2 (−196 to −174 del), TLR3 (c.1377 C>T; rs3775290), TLR4 (Asp299Gly; rs4986790) and TLR9 (2848G>A; rs352140) Gene Polymorphisms with Demographic Characteristics

In our study group comprising 130 CC cases; 32 (25 %) were tobacco users and 98(75 %) were non-users and 38 (29.25 %) were premenopausal and 92 (70.75 %) were postmenopausal. Using a case-only study approach, our results showed that; C/C genotype of TLR 3 1377 C>T is more common among tobacco users [p = 0.001; OR: 4.8(1.7–13.93)], the homozygous genotype Asp/Asp of TLR 4 polymorphism is more common among non tobacco users [p = 0.002; OR: 0.04(0.01–0.4)] and the major genotype of TLR 9 is more frequent among tobacco users [p = 0.000; OR: 5.5 (2.08–14.5)] [p = 0.000; OR: 20.9(4.45–98)]. We observed that TLR 3, TLR 4, TLR 9 and not TLR 2 gene polymorphisms are associated with modulation of CC risk due to tobacco usage in the study population (Table 4).

In the second hand, we observed that; the genotype Ins/Isn of TLR 2 polymorphism is more frequent among postmenopausal patients [p = 0.000; OR: 0.16(0.06–0.40)]. However, the major genotype G/G of TLR 9 polymorphism is more common among premenopausal cases [p = 0.000; OR:

Table 3 Implication of TLR 2/3/4 and 9 gene polymorphisms in cervical cancer evolution

Polymorphisms	Stage I+II (N = 88) (%)	Stage III+IV (N = 42) (%)	p value	OR (IC-95 %)
TLR2 (del −196 −174)				
Ins/Ins	67(76)	26(62)	−	Reference
Ins/Del	12(13.5)	14(33)	0.013	3.00(1.22–7.35)
Del/Del	9(10.5)	2(5)	0.386	0.57(0.11–2.82)
Ins/Del+ Del/Del	21(24)	16(38)	0.071	1.96(0.88–4.33)
Ins	146(83)	66(78.5)	−	Reference
Del	30(17)	18(21.5)	0.245	1.32(0.69–2.54)
TLR3 +1377 (C>T)				
C/C	45(51)	24(57)	−	Reference
C/T	34(38)	14(33)	0.332	0.77(0.34–1.71)
T/T	9(11)	4(10)	0.524	0.83(0.23–2.99)
C/T+T/T	43(49)	18(43)	0.325	0.78(0.37–1.64)
C	124(70)	62(73.8)	−	Réference
T	52(30)	22(26.2)	0.341	0.84(0.47–1.51)
TLR4 Asp299Gly				
Asp/Asp	82(93)	34(81)	−	Reference
Asp/Gly	4(4.5)	2(4.7)	0.573	1.20(0.21–6.89)
Gly/Gly	2(2.5)	6(14.2)	0.013	7.23(1.39–37.65)
Asp/Gly+ Gly/Gly	6(7)	8(18.9)	0.039	3.21(1.03–9.96)
Asp	168(95.45)	70(83.3)	−	Reference
Gly	8(4.55)	14(16.7)	0.001	4.20(1.68–10.45)
TLR9 +2848 (G>A)				
G/G	31(35.2)	11(26.2)	−	Reference
G/A	29(32.9)	19(45.2)	0.131	1.84(0.75–4.53)
A/A	28(31.9)	12(28.6)	0.444	1.20(0.46–3.16)
G/A+A/A	57(64.8)	31(73.8)	0.204	1.53(0.67–3.46)
G	91(51.7)	41(49)	−	Reference
A	85(48.3)	43(51)	0.380	1.12(0.66–1.88)

Total number of cervical cancer cases of early stages (I + II) = 88 and of advanced stages (III + IV) = 42

OR age-adjusted odds ratio, *CI* confidence interval

6.37(2.46–16.4)] [p = 0.000; OR: 13.2(3.9–44.1)]. we observed that there was no significant association between TLR 3 and TLR 4 gene polymorphisms and modulation of cervical cancer risk due to the status of menopause in the study population (Table 5).

Discussion

Analysis of potentially functional polymorphisms in many candidate genes has emerged as an approach in order to understand the complex relationship between genotype and phenotype. It was established that Toll-Like receptors have a pivotal role in tumors development and progression. Previous studies have reported that TLRs play pivotal roles in the immune system and initiate inflammatory response to foreign pathogens including viruses, bacteria and fungi [4]. In this context, association analyses can be used to explore the role of genetic polymorphisms in susceptibility to various cancers, including cervical cancer. We therefore assessed whether TLR2 (−196 to −174 del), TLR3 (c.1377 C>T; rs3775290), TLR4 (Asp299Gly; rs4986790) and TLR9 (2848G>A; rs352140) polymorphisms had a clinically relevant impact on CC evolution among Tunisian women.

Our finding is the first to evaluate the involvement of TLRs polymorphisms with the incidence of cervical cancer according to the statue of tumor evolution. This study demonstrated that Ins/Ins genotype of TLR 2 is a protector factor from the incidence of CC in stage (III+IV). A lack of association between this polymorphism and CC development was revealed in Tunisian women [16] controversy to results among Indian population [7]. The TLR 3 polymorphism was significantly associated with an increased risk of CC in stage (III+IV) in agreement with our previous study who showed that TLR3 C/

Table 4 Genotype and allele frequency distribution of TLR 2/3/4 and 9 gene polymorphisms with tobbaco users

TLR gene polymorphisms	Patients		p value	OR (IC-95 %)
	Tobbaco users			
	Users N(%)	Non-users N(%)		
TLR2 (del −196 −174)				
Ins/Ins	21(65)	72(73)	-	Reference
Ins/Del	9(28)	17(17.5)	0.159	0.5(0.21–1.41)
Del/Del	2(7)	9(9.5)	0.543	1.3(0.26–6.54)
TLR3 +1377 (C>T)				
C/C	25(78)	44(45)	-	Reference
C/T	5(16)	43(43.8)	0.001	4.8(1.7–13.93)
T/T	2(6)	11(11.2)	0.123	3.1(0.64–15.24)
TLR4 Asp299Gly				
Asp/Asp	23(72)	93(94)	-	Reference
Asp/Gly	5(15)	1(1)	0.002	0.04(0.01–0.4)
Gly/Gly	4(13)	4(4)	0.067	0.2(0.05–1.06)
TLR9 +2848 (G>A)				
G/G	22(69)	20(20)	-	Reference
G/A	8(25)	40(41)	0.000	5.5(2.08–14.5)
A/A	2(6)	38(39)	0.000	20.9(4.45–98)

Total number of tobacco users among cervical cancer cases =32 and total number of non-users =98 cases

OR age-adjusted odds ratio, *CI* confidence interval

C genotype was associated with increased cervical cancer susceptibility in Tunisia [16]. Other studies reported no significant association between the TLR3 (c.1377 C>T) variant and rheumatoid arthritis [17], nasopharyngeal carcinoma [18] and breast cancer [19]. The TLR4 gene consists of three exons mapped to chromosome 9q32–33. The SNP A896G [rs4986790] in exon 3 lead to Asp299Gly amino acid substitution. This polymorphism has been shown to belly positive correlated to increased susceptibility to infection [20]. Our results showed that for TLR 4, the dominant genotype Asp/Asp is more common among cases with tumor in stage (I+II) and in stage (III+IV) then controls and the major allele Asp is a risk factor for the development of tumor in stage (I+II). An earlier meta-analysis involving 8623 cancer cases and 9654 controls from21 studies investigating the association between Asp299Gly TLR4 variant and cancer risk, found no correlation of this polymorphism with cancer under any genetic models [21]. However, cancer-subgroup analyses suggested that the contribution of Asp299Gly to the risk of cancer depends on the cancer type, since it is a risk factor for gastrointestinal cancer, but is protective of prostate cancer [21]. For TLR 9, no genotypic and allelic association were observed for CC development in stage (I+II) and in stage (III+IV). However, this polymorphisms was associated with CC development among Polish women [8]. Previous studies evaluating the association of TLR 9 (G2848A) gene polymorphisms with disease susceptibility in Japanese, Chinese, Germans and European Americans have reported minor allele frequency as 44, 34, 55.4 and 57 % respectively, suggesting the relevance of host genetic factors in inter individual differences to disease susceptibility [22, 23].

For the CC evolution, our results showed that there was statistically significant relationship between the incidence of the TLR2 and TLR4 mutations and the clinical progression of CC according to the FIGO classification. The heterozygous genotype TLR2 Ins/Del is implicated in the evolution of cervical tumor to the advanced stages. The homozygous genotype Gly/Gly and the combined genotype Asp/Gly+Gly/Gly and Gly allele are associated with the evolution of tumor to stage (III+IV) of TLR 4. However, no significant association of TLR3 and TLR9 was identified with clinical stages of CC. Similar results was revealed in India [6]. Previous studies revealed that, in the stroma, a trend of increasing TLR 1, 2, 5, 6, and 9 mRNA levels with disease severity. These findings implicate the involvement of TLRs in early and late cervical carcinogenesis, respectively, suggesting that stromal upregulation of TLRs may play a role in cervical disease progression [24]. A lack of studies have reported the implication of gene polymorphisms in CC evolution among FIGO stage. Thus, new studies are needed for comparison of ours.

Table 5 Genotype and allele frequency distribution of TLR 2/3/4 and 9 gene polymorphisms with Status of menopause

TLR gene polymorphisms	Patients		p value	OR (IC-95 %)
	Status of menopause			
	Premenopausal N(%)	Postmenopausal N(%)		
TLR2 (del −196 −174)				
Ins/Ins	19(50)	74(80)	-	Reference
Ins/Del	16(42)	10(11)	0.000	0.16(0.06–0.40)
Del/Del	3(8)	8(9)	0.422	0.68(0.16–2.83)
TLR3 +1377 (C>T)				
C/C	20(52)	49(53)	-	Reference
C/T	11(30)	37(40)	0.303	1.37(0.58–3.21)
T/T	7(18)	6(7)	0.079	0.34(0.10–1.17)
TLR4 Asp299Gly				
Asp/Asp	34(89)	82(89)	-	Reference
Asp/Gly	2(5.5)	4(4)	0.573	0.82(0.14–4.74)
Gly/Gly	2(5.5)	6(7)	0.071	0.35(0.11–1.13)
TLR9 +2848 (G>A)				
G/G	25(65)	17(18)	-	Reference
G/A	9(23)	39(42)	0.000	6.37(2.46–16.4)
A/A	4(12)	36(40)	0.000	13.2(3.9–44.1)

Total number premenopausal cervical cancer cases = 38 and total number of postmenopausal cervical cancer cases = 92 cases

OR age-adjusted odds ratio, *CI* confidence interval

According demographic characteristics, our results showed that TLR 3, TLR 4 and TLR 9 gene polymorphisms are associated with modulation of CC risk due to tobacco usage and TLR 2 and TLR 9 are implicated in CC risk due to the statue of menopause in the study population. However, Pendy et al. did not found any significant association between TLR 3 and TLR 9 and the modification of CC risk due to tobacco use among Indian women [6]. Additional studies in larger sample size may be required in understanding the role of TLRs gene polymorphism and tobacco usage and statue of menopause in CC patients. There is a paucity of data regarding the associational and functional implications of common TLRs gene variants in human cancers.

This is the first study investigating the role of TLR2 (−196 to −174 del), TLR3 (c.1377 C>T; rs3775290), TLR4 (Asp299Gly; rs4986790) and TLR9 (2848G>A; rs352140) gene polymorphisms in CC evolution according FIGO stage among Tunisians. The present study has some strengths as well as limitations. The women includes in this study were of similar ethnicity and the possibility of admixture was ruled out. Only de novo cases of CC prior to chemo/radiotherapy were recruited and the clinical diagnosis, histological type of tumor and staging (FIGO) of cases with CC was accurate. Questionnaire-based personal interviews were conducted for assessment of demographic data; married status, status of menopause, hormonal contraception use and tobacco status. About its limitations, the study was conducted with limited sample size that might lead to a relatively lower statistical power. The HPV status of the study population was not known. In addition, detailed stage-specific molecular and cellular expression studies in tissue biopsy specimens of CC might help in determining the precise functional effects of TLRs gene polymorphisms and their implication in tumor evolution.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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