Impact of toll-like receptor 4 variations on nasopharyngeal carcinoma risk and survival in tunisian population

Impact des variantes de toll-like receptor 4 sur le risque et la survie au cours du carcinome de nasopharynx chez la population tunisienne

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Abstract

Introduction: The Toll-like receptor 4 (TLR4), an important member of the host's innate immune response, is coded by a polymorphic gene. This polymorphism could be a predisposing factor for NasoPharyngeal Carcinoma (NPC).
Aim: To determine the association between TLR4 gene polymorphisms and the susceptibility to NPC in a cohort of Tunisian affected patients.
Methods: Genomic DNAs from 245 unrelated patients affected by undifferentiated carcinoma type (UCNT) and 264 unrelated healthy controls were genotyped for the five single nucleotides polymorphisms (SNPs) of TLR4 locus (4434 A>G (rs1927914),7263 G>C (rs10759932), 6134 A>G(rs4986790), 8851C>T (rs 4986791), 5272 T>C(rs11536889), +8469 T>C (rs11536891)) by Taqman® 5'-nuclease assay.
Results: Among all polymorphisms studied, only the rs4986790 G and rs4986791 T alleles were significantly more prevalent in patients' group than controls (45% vs. 38%; p=0.03; pc=0.06) and increased the risk of the NPC (OR=1.3, 95% CI=1.01-1.69). Also, we found that the frequency of the rs4986790 AA and rs4986791 TT genotypes was significantly higher in controls than in patients (25.7% vs 37%; p=0.006, pc=0.02) and conferred a protector factor in NPC (OR= 0.59, 95% CI= 0.39-0.87). Further, based on the Kaplan-Meier survival curve we observed also the positive effect ofrs1927914 AA genotype on a prognostic of NPC (p=0.006; pc=0.01).
Conclusion: Our study demonstrated that impaired production of TLR4 seems to be a risk factor of NPC development but functional studies are needed to confirm these findings. As to rs1927914 AA appears to be a good biomarker for better survival in a patient with NPC.

Key words: Nasopharyngeal carcinoma, TLR4, gene, polymorphism, risk

Résumé

Introduction: Le Toll-like receptor 4 (TLR4), joue un rôle clé dans la réponse immune innée et la détection des microorganismes. La variation génétique du gène codant pour ce récepteur pourrait constituer un facteur prédisposant pour le carcinome du nasopharynx (CNP).
Objectif: L'objectif de cette étude était de déterminer l'association entre les variations du gène de ce récepteur et la susceptibilité au CNP dans une cohorte de patients tunisiens.
Méthodes: L'ADN génomique de 245 patients non apparentés atteints d'un carcinome de type indifférencié (UCNT) et de 264 témoins sains ont été génotypés pour les cinq polymorphismes nucléotidiques simples (SNP) du locus TLR4 (4434 A>G (rs1927914),7263 G>C (rs10759932), 6134 A>G(rs4986790), 8851C>T (rs 4986791), 5272 T>C(rs11536889), +8469 T>C (rs11536891)) par la technique Taqman® 5'-nuclease. Parmi toutes les variations étudiées, seuls les allèles rs4986790G et 4986791 T étaient significativement plus fréquent chez les patients que chez les témoins (45% vs 38% ; p=0,03) et augmenterait le risque de NPC (OR=1,3, IC à 95%=1,01-1,69).
Résultats: En revanche, la fréquence des génotypes rs4986790 AA et rs4986791 TT était significativement plus élevée chez les témoins que chez les patients (25,7% vs 37% ; p=0,006) et conférerait un facteur protecteur contre le NPC (OR=0,59, IC à 95%=0,39-0,87). En outre, sur la base de la courbe de survie de Kaplan-Meier, l'effet positif du génotype AA rs1927914 a été observé sur le pronostic de NPC (p=0, 006).
Conclusion: Notre étude suggère que lers1927914 AA semble être un bon biomarqueur pour une meilleure survie chez un patient atteint de NPC.

Mots clés: carcinome du nasopharynx, TLR4, gène, polymorphisme, risque

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Introduction

Nasopharyngeal carcinoma is an epithelial carcinoma arising from the nasopharyngeal mucosal lining. In the nasopharynx, the tumour is often observed at the pharyngeal recess. In comparison with other cancers, nasopharyngeal carcinoma is relatively uncommon. According to the International Agency for Research on Cancer, in 2018, there were about 129 000 new cases of nasopharyngeal carcinoma, accounting for only 0.7% of all cancers diagnosed in 2018 [1,2]. Nevertheless, its geographical global distribution is extremely unbalanced; >70% of new cases are in east and southeast Asia, with an age-standardized rate (world) of 3.0 per 100 000 in China to 0.4 per 100 000 in populations that are mainly white [1,2]. In Tunisia, a Northern African country, is an intermediate risk area with an incidence rate of 3,4 and 1,6 / 100 000 person-years respectively in men and female. NPC is the second neoplasm of head and neck cancer after laryngeal cancer with a bimodal distribution characterized by 2 peaks at 15-20 years old and a second at 50 years old [3].

The remarkable geographical distribution of nasopharyngeal carcinoma incidence has spurred studies on its risk factors, and it is suggested that multiple factors, including EBV infection, host genetics, and environmental factors are contributors in the development of nasopharyngeal carcinoma [4].

Linkage and association studies have identified that genetic susceptibility genes increase nasopharyngeal carcinoma risk. HLA genes residing at the MHC region on chromosome 6p21 have been widely recognized as major risk loci conferring nasopharyngeal carcinoma risk [5, 6]. Besides host genetics, EBV infection is perhaps the most common causal agent of nasopharyngeal carcinoma [7]. This interaction could cause a chronic inflammation. This chronic infection that may lead to the tumorigenesis in nasopharyngeal epithelial cells is mediated in part through recognition of EBV stimuli by TLRs. Disrupted regulation or altered TLR4 function may contribute to NPC [8]. Toll-like receptors (TLRs) are important components of inflammatory response which induce activation of TLR, MAPKs, and NF-κB and in turn activation of NF-κB results in synthesis of proinflammatory cytokines as TNF-α, IL-6, and IL-1β and inflammatory enzymes including inducible nitricoxide synthase and cyclooxygenaase-2 [9, 10].

TLRs belong to the pattern recognition receptor (PRR) family, a group of transmembrane glycoproteins, which ensures formation of natural immune response against many pathogens. In human beings, it is a homologue of interleukin-1 receptor (IL-IR). It has also avery important role in host immunity by activating adaptive immune response. TLRs recognize pathogen- associated molecular patterns (PAMPs) produced by microbial agents [11,12]. Among this family of receptor there is TLR4. Its activation leads to an intracellular signaling pathway NF-κB and inflammatory cytokine production which is responsible for activating the innate immune system [13].

TLR4 expressing cells are myeloid (erythrocytes, granulocytes, macrophages) rather than lymphoid (T-cells, B-cells, NK cells)[13]. Most myeloid cells also express high levels of CD14, which facilitates activation of TLR4 by LPS [14].

TLR4 gene is located on chromosomal region 9 (9q32-q33); the altered expression induced by 3'UTR SNP modulates the immune response to EBV and consequently induced NPC [8]. Genetic variations of TLR4 directly affect the risks of allergies, cardiovascular disease, and cancer [15]. Two missense mutations were identified in the TLR4 gene which results in an amino acid substitution Asp299Gly (D299G) and Thr399Ile (T399I) in the third exon of the gene, in fact, many investigations demonstrated that Asp299Gly polymorphism (D299G) and Thr399Ile (T399I) affect the extracellular domain of the TLR4 protein [16].

It has been shown that polymorphisms of the TLR4 gene and more specifically the mutated alleles increase the risk of certain cancers such as breast cancer, gastric cancer and colorectal cancer [17-20], in addition It has been reported that these two polymorphisms are associated with an increased risk of NCP [8], in fact this modification resulted in improper transcription regulation in function of TLR4.

The aim of our study was to investigate the possible association between polymorphisms of the TLR4 gene and the occurrence of NCP on the hand then to prove the impact of TLR polymorphisms on survival in the hand.

Methods

Patients and controls

Two hundred and forty-five NPC patients were recruited from the clinical biology department of “Salah Azaiez Institute”, Tunis, Tunisia. All patients presented with undifferentiated carcinoma type (UCNT) (confirmed by histopathological analysis). The clinical stages ranged from I to IV (according to the 1997 classification) (Sobin, L.H). The sex ratio was 1.5 (147 men, 98 women). Age ranged from 13 to 84 years with a mean age of 45±16 years. The distribution of age-specific rates for NPC showed a bimodal age distribution. Young patients were defined as being between 13 to 35 years of age, whereas adult patients were over 35 years old. The proportion of NPC patients under 35 represented 26% of all affected individuals. A set of demographic and clinical variables (Table 1) were assessed and recorded. Two hundred and two unrelated healthy controls were recruited, who were free of any personal or familial cancer antecedents and originating from the same area (north and central regions of Tunisia). The sex ratio of the group was 1.64 (164 men, 100 women) and the mean age was 51±88 years ranged to 12 to 75 years. Written informed consent was obtained from all subjects.

TLR4-genotyping

Genomic DNA was extracted from peripheral blood leukocytes by salting out procedure. The genotyping of the five single nucleotide polymorphisms (SNPs) herein studied: promoter rs1927914 (+4434) A/G, rs 10759932 (+7263) C/T; Exon3 rs4986790 (+6143, Asp 299 Gly)

A/G, rs4986791 (+8851, Thre 399 Ile) G/C and 3'-UTR rs 11536889 (+5724) C/G, rs11536891 (+8469) C/T by a TaqMan® 5'-nuclease assay (AB Applied Biosystems, (Foster City, CA, USA) using probes and fluorogenic oligonucleotide probes (Table 2).

Table 1. demographic and clinical characteristics of patients and healthy controls

Clinical characteristics	*Patients n=245*		*Healthy controls n=264*	
Gender	**Number**	**Frequencies%**	**Number**	**Frequencies%**
Men	147	60	164	62
Women	98	40	100	38
Age				
Mean (Years)	46 (range:13-84)		52 (range: 12-75)	
>35 years	179	73	255	97
< 35 years	66	26	9	3
TNM				
Tumor size (T)	**234**	**100%**		
T1-T2	64	27	-	-
T3-T4	170	73	-	-
Lymph Node status (N)	**237**	**100%**		
N0	56	24	-	-
N+	181	76	-	-
Distant metastasis (M)	**229**	**100%**		
M0	221	97		
M+	8	3		
Disease Stage	**157**	**100%**		
I	4	2		
II	19	12		
III	20	13		
VI	109	70		

Table 2. Primers and probes sequences of SNP genotyping

TLR4 T-5724C	F : 5' GTG ATT ACC ACA TTT TAC AGA CCA GAA 3'	VIC – TTC ACC AAC **A**CT TAT T (allele T)
	R : 5' CCA CAA ATG GTG TAC AGG AGT TCT C 3'	FAM – CAC CAA C**G**C TTA TT (allele C)
TLR4 C+4434T	F : 5' GGC CTG TGC AAT TTG ACC AT 3'	VIC – TCG ATG **G**TA TTA TTG (allele C)
	R : 5' AGT CAC ACT CAC CAG GGA AAA TG 3'	FAM – CTC GAT G**A**T ATT ATT G (allele T)
TLR4 T+6143C	F:5'AAG TGC TTG GAG GAT ATT ACA GTA GAA CTA 3'	VIC–ACTTAGCAT **A**CA TAA TAT T (allele T)
	R:5'GGA AAG TAG CAA GTG CAA TGT AAG TTT3'	FAM – GCT TAG CAT **G**CA TAA TA (allele C)
TLR4 G+7263C	F : 5' GTT TCC TGT TGG GCA ATG CT 3'	VIC – CAT CCA CT**C** TTC C (allele G)
	R : 5' CAT TAA TTC CAG ACA CAT TGT TTT CTC 3'	FAM – AAC ATC CAC T**G**T TCC (allele C)
TLR4 T+8469C	F : 5' GGT GTT TCC ATG TCT CAT GTA CTA GTG 3'	VIC – CAA ATG C**A**C ACA TCT (allele T)
	R: 5' CCT GAT AGG GAT ACA TAG GGA TAT GTG 3'	FAM – AAA TGC **G**CA CAT CT (allele C)

This reaction was comprised of 20 µM of each primer and probe with 10 µl de Master Mix (Universal Master Mix, Applied Biosystems, (Foster City, CA, USA) and 50ng of DNA. PCR reactions were performed on a Gene Amp 7000 sequence detection System (AB Applied Biosystems, (Foster City, CA, USA). An initial incubation step of 2 min at 50°Cand an enzyme heat activation step of 10 min at 95°C were followed by 50 two-step amplification cycles of 15 s at 95°C for denaturing and 1min 30 s for 60°C for annealing and extension.

Statistical analyses

Alleles and genotype frequencies were determined and compared using a standard chi-square testing or Fisher exact test as appropriate by Win Pepi software V11.15. P values were corrected (Pc) and obtained by Bonferroni correction and considered statistically significant at values less than 0.05 (P< 0.05). Both odds ratio (OR) and confidence interval 95% (CI 95%) were calculated to assess the relative risk conferred by a specific allele or genotype. Hardy-Weinberg equilibrium was calculated using $\chi 2$ tests for genotype frequencies in the healthy controls group. Haplotype was reconstructed by haploview 4.2 software and was used to compute pairwise linkage disequilibrium (LD) statistics (Barrett JC). D' and r2 were plotted. LD blocks were defined according to the criteria of Gabriel et al (Gabriel SB).

Survival curves in accordance with various potential prognostic indicators were produced. Survival was defined as the time between the date of diagnostic and date of death. Patients still alive at the end of the study were censored at the date of the last follow-up. Survival rate was computed using standard Kaplan-Meir methods, and the difference in survival curves was analyzed by the log-rank test. A P value of less than 0.05 was considered the statistical significance.

Results

The phenotypic characteristics of patients are summarized in Table 1. Tunisian NPC cohort has a particular age distribution, the mean of the age of NPC patients at inclusion was 45 years (range: 13-84 years) and that they exhibit two peaks of incidence, the first one that around 15-25 years and the second one is around 45-55 years. The majority of patients (83%), at the time of diagnostic, showed an advanced tumour stage (III-IV), with lymph node metastasis (N+).

Effect of polymorphism of TLR4 on the risk of NPC

Genotypic characteristics of patients and control groups are summarized in Table 3. To determine the polymorphisms in the TLR4 gene, genomic DNA from 245 NPC patients and 264 healthy controls were amplified by Real-Time PCR. The observed genotype distribution stratified the excepted Hardy-Weinberg proportions for both patient and control samples and the overall frequencies were comparable to those previously published in public database (http://www.ncbi.nlm.nhi.gov/).

Table 3. Genotypes and alleles distribution of theSNPs of TLR4 gene in NPC group and in a healthy group.

TLR4 Polymorphisms	*Patients n= 245*		*Healthy controls n= 264*		*P value*	*Pc*	*OR*	*95%CI*
TLR4 rs 1927914	**Number**	**Frequencies%**	**Number**	**Frequencies%**				
Alleles	**490**	**100%**	**528**	**100%**				
4434 A	462	94	497	94	1	2	1.03	0.59-1.81
4434 G	28	6	31	6	1	2	0.97	0.59-1.6
Genotypes*	**245**	**100%**	**264**	**100%**				
4434 AA	223	91	241	91	0.91	2.7	0.97	0.5-1.87
4434 AG	16	7	15	6	0.83	2.4	1.4	0.52-2.58
4434 GG	6	2	8	3	0.79	2.4	0.8	0.22-2.68
TLR4 rs10759932	**Number**	**Frequencies%**	**Number**	**Frequencies%**				
Alleles	**490**	**100%**	**528**	**100%**				
7263 T	414	84.5	457	86.5	0.35	0.7	0.85	0.59-1.22
7263 C	76	15.5	71	13.5	0.35	0.7	1.18	0.82-1.7
Genotypes*	**245**	**100%**	**264**	**100%**				
7263 CC	182	74	203	77	0.56	1.8	0.87	0.56-1.32
7263 TC	50	21	51	19	0.52	1.8	0.84	0.67-1.69
7263 CC	13	5	10	4	0.52	1.8	1.42	0.56-3.69
TLR4 rs4986790	**Number**	**Frequencies%**	**Number**	**Frequencies%**				
Alleles	**490**	**100%**	**528**	**100%**				
6134A	270	55	325	62	0.03	0.06	0.7	0.59-0.99
6134G	220	45	203	38	0.03	0.06	1.3	1.01-1.69
Genotypes*	**245**	**100%**	**264**	**100%**				
6134 AA	**63**	**25**	**98**	**37**	**0.006**	**0.02**	**0.59**	**0.39-0.87**
6134 AG	**144**	**59**	**129**	**49**	**0.02**	**0.06**	**1.49**	**1.03-2.15**
6134 GG	**38**	**16**	**37**	**14**	**0.7**	**2.1**	**1.13**	**0.67-1.9**
TLR4 rs4986791	**Number**	**Frequencies%**	**Number**	**Frequencies%**				
Alleles	**490**	**100%**	**528**	**100%**				
8851 G	270	55	325	62	0.03	0.06	0.7	0.59-0.99
8851 C	220	45	203	38	0.03	0.06	1.3	1.01-1.69
Genotypes*	245	100%	264	100%				
8851 GG	63	25	98	37	0.006	0.02	0.59	0.39-0.87
8851 GC	144	59	129	49	0.02	0.06	1.49	1.03-2.15
8851CC	38	16	37	14	0.7	2.1	1.13	0.67-1.9
TLR4 rs11536889	**Number**	**Frequencies%**	**Number**	**Frequencies%**				
Alleles	**490**	**100%**	**528**	**100%**				
5724 C	413	84.3	453	85.79	0.5	1	0.89	0.62-1.27
5724 G	77	15.7	75	14.20	0.5	1	1.13	0.8-1.59
Genotypes*	**245**	**100%**	**264**	**100%**				
5724 CC	163	66	198	75	0.3	0.9	1.25	0.85-1.83
5724CG	75	31	57	21.6	0.3	0.9	0.81	0.55-1.19
5724GG	7	3	9	3.4	1	3	0.94	0.286-3.021
TLR4 rs11536891	**Number**	**Frequencies%**	**Number**	**Frequencies%**				
Alleles	**490**	**100%**	**528**	**100%**				
8469 C	413	84.3	453	85.79	0.5	1	0.89	0.62-1.27
8469 T	77	15.7	75	14.20	0.5	1	1.13	0.8-1.59
Genotypes*	**245**	**100%**	**264**	**100%**				
8469 CC	176	72	198	75	0.47	1.2	0.85	0.562-1.285
8469 CT	61	25	57	22	0.43	1.2	1.2	0.781-1.858
8469TT	8	3	9	3	1	3	0.96	0.315-2.844

P<0.05, Pc with Bonferonni test; OR: odds ratio; 95% CI: 95% confidence interval,*: One versus Other

In terms of TLR4-genotype distribution, we found that the homozygous state for TLR4 - rs4986790 A (Asp 299) and rs4986791 C (Thre 399) alleles were significantly higher in healthy controls than in patients (37% vs 25%; p= 0.006 pc= 0.02) and confer protection for NPC (OR= 0.59, 95% CI=0.39-0.87) (Table 3). However, rs4986790 AG and rs4986791 CT genotypes are significantly more prevalent in patients than in healthy controls (59% vs 49%; p= 0.02 pc= 0.06; OR= 1.49, 95% CI=1.03-2.15).

Moreover, after the analysis of allelic frequencies we revealed that rs4986790 G (Gly 299) and rs4986791 T (Ile 399) alleles were significantly highest in patients more than controls and increased the risk of NPC 45% vs. 38%; p=0.03; pc =0.06; OR= 1.3, 95% CI= 1.01-1.69)
Concerning of the others polymorphisms of TLR4 gene, they did not show a difference between NPC patients and controlswith P>0.05(Table 3).
Further, after stratification of patients based on tumour extension (T), lymph node metastasis (N), distant metastasis (M), stage of the disease and TLR4 polymorphisms, we don't find any difference between NPC patients and controls (data not shown).
Concerning haplotype, we found that only AGAGCT haplotype was significantly higher in NPC group (p=0.01) and increased the risk (OR=2.42; %95 CI=1.21-5.09) (Table4) (Figure1). Whereas we do not find any significant association between the other of haplotypes and NCP in our study.

Table 4. Haplotypes frequencies in NPC and healthy groups

Haplotype	Frequency,%		P value	OR (95%CI)
	Patients (n=245)	Controls (n=264)		
AGAGTT	42	38	0.18	
AGGCTT	13.5	14.3	0.71	
AGGCCT	9.8	10.8	0.61	
ACAGTT	8.5	9.1	0.76	
AGGCTC	6.6	8.4	0.27	
AGAGCT	6	2.7	0.01	2.42 (1.21-5.09)
GGGCTT	2.5	4.1	0.15	
AGAGTC	2.2	2.5	0.77	
AGGCCC	1.7	2.3	0.45	
ACGCTT	1.8	1.1	0.35	
GGAGTT	1	1.2	0.73	
ACGCTC	0.7	1.3	0.37	

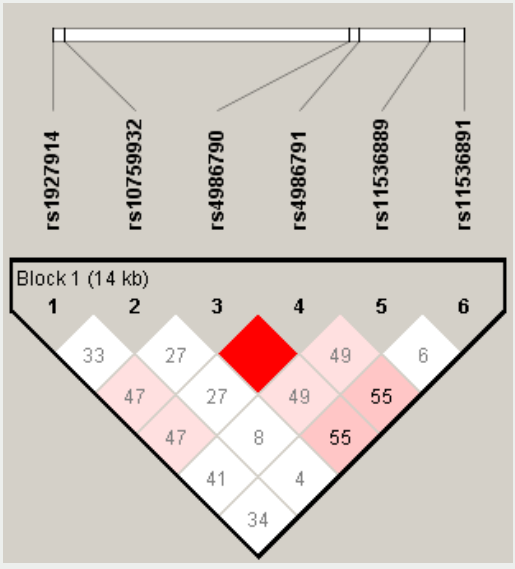

Figure 1. Linkage disequilibrium plot of six SNPs of the TLR4 gene in 509 study subjects. D' and r^2 corresponding to each SNP pair are expressed as a percentage and shown within the respective square. Higher D' is indicated by a brighter red. The six SNPs constitute a haplotype block spanning 14 Kb of the TLR4 gene.

Combined polymorphism of TLR4 gene with better survival patients with NPC

Using all polymorphisms of TLR4 studied and the number of month survival patients with a good response to treatment variable in a Kaplan-Meier survival curve, we revealed that the rs1927914 AA "wild type" and a protector genotype correlate with positive patients with a high number of months' survival (p=0.006, pc=0.01) (Figure 2).

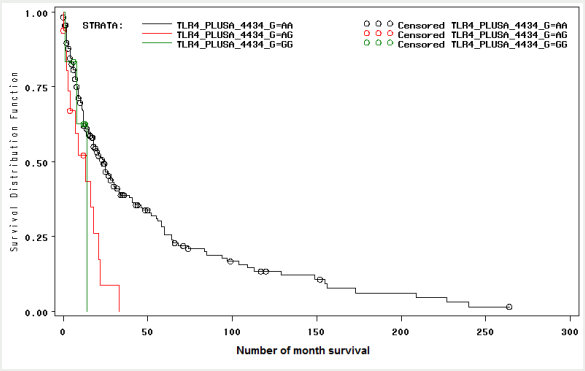

Figure 2. Kaplan-Meir survival curves based on correlation between rs1927914 and NPC

Discussion

It has been demonstrated that chronic inflammatory diseases contribute to the development of cancer via TLR signaling. In many studies, increased TLR expression has been reported in different types of tumor cells. Various endogenous TLR ligands released from inflammatory cells activate TLR signaling pathways in precancerous cells with resultant expression of cytokines, growth factors, angiogenic factors, and proteases which degrade extracellular matrix. Thus, TLR induces micro environmental circumstances, which support development and progression of cancer [21-23]. In our whole population-based study of NPC in Tunisia, we examined the potential relationship between TLR4 polymorphisms and susceptibility to NPC in Tunisian sample. To our knowledge, this is the first study on TLR4 in NPC published to date.
The results of the current study showed that TLR4 - rs4986790 A (Asp 299) and rs4986791 C (Thre 399) alleles were significantly higher in healthy group than in patients' group (37% vs 25%; p= 0.006 pc= 0.02) and confer protection for NPC (OR= 0.59, 95% CI=0.39-0.87), howeverrs4986790 AG and rs4986791 CT genotypes are significantly more prevalent in patients than in healthy controls. These results were in concordance with other study demonstrating that T399I TLR4 heterozygous polymorphism was associated with NPC, this is related to that T399I TLR4 SNP can increase expression of IL-1a, TNF-a, and IL-10 expression, suggesting that this polymorphism influence cytokine and plays a role in tumor-genesis pathway [24].According to literature data, SNP rs4986791 which is situated on TLR4 gene isoforme by cytosine/thymine substitution at nucleotide1196. position, and studies performed have detected the

presence of an association between this genetic variant andrisks of others types gall bladder cancer, precancerous gastric lesionand gastric cancer [25–28]. Another genetic variant (rs4986790), on TLR4 gene, is formed via Adenine/Guanine substitution at nucleotide 896. position and a correlation between this genetic variant and risks of gastric cancer, prostate cancer, colorectal carcinoma and non-Hodgkin lymphoma [29-31].In our study, TLR4 is associated with susceptibility to nasopharynx carcinoma and therefore it is involved in the initiation and development of this cancer in the Tunisian population.

The SNPs disrupt the normal structure of the extracellular region of theTLR4 and are therefore hypothesized to decrease responsiveness to lipopolysaccharide (LPS) through alterations in binding. Furthermore, mutant TLR4-transfected THP-1 cell lines have been shown to elicit a decreased LPS-induced immune response resulting in lower levels of cytokine production, which subsequently increases the susceptibility of host to bacterial infection [32]. To date, 44 TLR4SNPs have been identified; however, both polymorphisms Asp299Gly (AG) and Thr399Ile (CT) remain the best described and studied in relation to identify the association of developing cancer and altered functionality of the gene [15,26].

In our case, 73% of cases have an advanced stage and treated with chemotherapy, genetic factors influence the inflammatory response and outcomes after therapy. In fact, in term of survival we have shown that TLR4 rs1927914 AA “wild type” genotypes are associated with longer survival OS (p=0.006, pc=0.01). In fact, D299G and T399I polymorphisms had a significantly reduced disease-free and overall survival and poor responses to adjuvant therapy and chemotherapy [33]. In other studies of association of patients with head and neck small cell carcinoma, Tina Bagratuni et al. found the same accordance [34]. In term of functional effect, these results could be explained by the activation of TLR4 by host protein (HMGB-1) released following conventional chemotherapy or radiotherapy previously shown [35].

Concerning haplotype, in our investigation we found that only AGAGCT haplotype was associated with NPC (p=0.01) and present a risk (OR=2.42;%95 CI=1.21-5.09). In this context, larger studies find no correlation between the TLR4 Asp299gly/Thr399ILe haplotype and cancer [33]. However, the study of Arbour et al showed that transfected cells with each type of TLR4 haplotype led to reduction of cytokine production by the innate immune response [36].

Taken together, our data showed an association betweenrs4986790 (Asp 299) and rs4986791 (Thre 399) variations of TLR4 gene and the risk of NPC development and prognostic. These results indicate that these variations could be used as classification biomarkers for better therapeutic management in NPC.

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