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TLR2 (-196 to -174 Ins/Del) and *TLR3* (1377C>T) as biomarkers for nasopharyngeal cancer in Tunisia

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Background/aim: We evaluated the association of *TLR2* (-196 to -174 Ins/Del) and *TLR3* (1377 C>T) as potential risk factors for nasopharyngeal carcinoma (NPC) in Tunisians.

Material and methods: The study subjects comprised 137 NPC patients and 164 cancer-free control subjects. *TLR2* genotyping was done by PCR and *TLR3* genotyping was performed by PCR-RFLP.

Results: Minor allele frequency (MAF) and genotypes of *TLR3* (1377 C>T) were comparable between NPC patients and controls. Significantly higher MAF and *TLR2*-containing Del allele genotypes of TLR2 (-196 to -174 Ins/Del) were seen in NPC patients compared to controls [OR (95% CI) = 2.10 (1.43–3.08), P < 0.001 and OR (95% CI) = 2.07 (1.27–3.37), P = 0.003]. In addition, higher increased NPC risk was associated with the *TLR2*-Del/Del genotype [OR (95% CI) = 2.74 (1.37–5.48), P = 0.004]. An increased frequency of the Del-T haplotype was seen in NPC cases compared to controls.

Conclusion: Our results demonstrate an increased risk of NPC with the *TLR2*-Del/Del genotype and Del-T *TLR2* and *TLR3* haplotype, suggesting their potential use as biomarkers to evaluate NPC risk in Tunisians.

Key words: Toll-like receptor, polymorphisms, nasopharyngeal carcinoma, Tunisia

1. Introduction

Nasopharyngeal carcinoma (NPC) is emerging as a global public health problem (1,2). NPC is a malignant tumor of the head and neck region, with a strong potential for metastasis at early stages of the disease. NPC is a multifactorial disease, and its development and progression is influenced by environmental, viral, and genetic factors (3,4). These include high intake of dietary salt, smoking, and Epstein–Barr virus infection (5). Altered proinflammatory and antiinflammatory mechanisms were also linked with cancer, including NPC (6,7).

Given their critical role in regulating innate and acquired immunity, and as biomarkers of infectious pathogens and cancer debris, toll-like receptors (TLRs) were described as key players in the pathogenesis and progression of NPC (8,9). Dysregulation of TLR signaling imparts a higher risk of the development of chronic inflammatory diseases and cancers (10), in which *TLR* gene polymorphisms contribute to this dysregulation and thus to disease susceptibility and progression, including cancers. Accordingly, *TLR* gene variants were proposed to serve as biomarkers of altered cancer risk (11,12). These included the -196 to -174 chromosome 4q32 22-bp insertion/deletion (Ins/Del) polymorphism in the promoter region, which alters *TLR2* promoter activity and thus its level of expression (13). In addition, a nonsynonymous 1377C>T polymorphism in exon 4 of the *TLR3* gene (chromosome 4) was shown to affect receptor-ligand interaction by altering the TLR3 ectodomain and thus functionally impairing the receptor (14). However, the relationship between the contribution of *TLR2* Ins/Del and *TLR3* 1377C>T polymorphisms and cancer risk remains unclear (15,16).

The present study aimed to study the association of *TLR2* (-196 to -174 Ins/Del) and *TLR3* (1377 C>T; rs3775290) polymorphisms with NPC in Tunisians, in view of their potential use as biomarkers to evaluate cancer risk.

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2. Materials and methods

2.1. Study subjects

Between November 2012 and October 2015, 137 NPC patients from North Tunisia were recruited from the Salah Azeiz Oncology Institute (Tunisia). NPC diagnosis was established by clinical examination and histopathology. Confirmation of undifferentiated carcinoma of nasopharyngeal type (UCNT) was based on the simplified World Health Organization (WHO) classification for NPC (17). Tumors were staged according to American Joint Committee on Cancer TNM staging system [T (tumor), size of the original primary tumor; N (nodes), regional lymph nodes, M = distant metastasis] (18).

The control group comprised 164 unrelated blood donors (102 males and 62 females, mean age 48.6 ± 11.2 years), who were free of chronic disease, history of malignancy, drug allergies, hypertension, diabetes, or cardiovascular disease and were matched for sex and age with NPC patients. Controls were recruited from the Military Hospital, Tunisian Center of Maternity and Neonatology, and the Dispenser of Ettadhamen City. Demographic and clinical data were collected from patients and controls using a unified questionnaire. All subjects were asked to sign a consent form, agreeing to participate in the study, after all institutional ethics requirements were met.

2.2. *TLR* genotyping

Total genomic DNA was extracted from the peripheral blood of study participants using the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). Determination of *TLR2* -196 to -174 Ins/Del polymorphism was performed by PCR as previously described (19), while *TLR3* (1377C>T; rs3775290) genotyping was determined by PCR-restriction fragment length polymorphism (RFLP) analysis, as previously described (20).

2.3. Statistical analysis

Statistical analysis was performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) and Vassar Stats (http://vassarstats.net/). Hardy–Weinberg equilibrium (HWE) was calculated for the tested variants in patients and controls. Student's t-test was used for variables with normal distribution (mean ± SD), while Pearson's chi-square test and Fisher's exact test (for low numbers of patients/controls) were used to analyze the association between *TLR2* Ins/Del and *TLR3* (1377C>T) and NPC development. Results were expressed as P-values, odds ratios (ORs), and 95% confidence intervals (CIs); $P < 0.05$ was considered statistically significant. Logistic regression was also performed to test the association between genotypes and NPC exposure, after adjusting for smoking and sex. Haplotype reconstruction was performed using SNPstats software (www.bioinfo.iconcologia.net/SNPstats/).

2.4. Ethics

The study protocol was approved by the Ethics Committee of Salah Azeiz Oncology Institute.

3. Results

3.1. Clinical parameters of NPC cases

The demographic and clinical characteristics of NPC patients and controls are summarized in Table 1. Study subjects comprised 137 patients (98 males and 39 females; mean age 46.3 ± 13.8 years) and 164 control subjects (102 males and 62 females; mean age 48.6 ± 11.2 years). All NPC patients underwent confirmation of UCNT, of whom 57 patients (41.6%) had T1–T2 tumor size, while the remaining 80 (58.4%) had T3–T4. In addition, 55 (40.1%) of NPC patients presented with either no regional lymph node metastasis or unilateral lymph node metastasis (N0–N1), while 82 (59.9%) had N2–N3. TNM staging identified 25 patients (18.2%) to be diagnosed at stage II and 112 (81.8%) at stages III–IV; distant metastasis was confirmed in only seven patients (5.1%).

3.2. Distribution of *TLR2* (-196 to -174 Del) polymorphism

The distribution of *TLR2* genotypes in controls was consistent with HWE. Table 2 displays *TLR2* Ins/Del genotype and minor allele frequencies among study subjects. The frequency of the *TLR2* Del/Del genotype was significantly higher in NPC patients compared to control subjects (19.7% vs. 9.1%, $P = 0.004$). After adjustment for smoking ($P < 0.001$) and sex ($P = 0.027$), data from logistic regression analysis showed that the *TLR2* Del/Del genotype is positively associated with NPC risk [adjusted OR = 2.40 (1.14–5.05), $P = 0.020$].

Positive association with NPC risk was seen when a comparison was made between patients and controls for the frequency of *TLR2* Del-containing genotypes (Ins/Del + Del/Del) versus noncarriers (Ins/Ins) [OR (95% CI) = 2.07 (1.27–3.37), $P = 0.003$] (Table 2). Logistic regression confirmed that *TLR2* Del allele carriers were more susceptible than noncarriers to develop NPC [adjusted OR = 1.83 (1.08–3.09), $P = 0.024$], which appeared to be independent of sex ($P = 0.054$). On the other hand, the carriage of the *TLR2* minor (Del) allele was positively associated with a risk of NPC [30.6% vs. 17.3%, $P < 0.001$, OR (95% CI) = 2.10 (1.43–3.08)].

3.3. Distribution of *TLR3* 1377 C>T polymorphism

The distribution of *TLR3* 1377 C>T genotypes was consistent with HWE in patients and controls ($P > 0.05$). The genotype and minor allele frequencies of *TLR3* 1377 C>T polymorphism in NPC patients and healthy controls are listed in Table 2. No significant differences were seen in frequencies between NPC patients and controls, indicating a lack of association with NPC.

Table 1. Characteristics of NPC patients and controls.

Characteristics	Patients n = 137	Controls n = 164	P-value	OR (95% CI)
Age (mean ± SD)	46.3 ± 13.8	48.6 ± 11.2	0.110	0.98 (0.96–1.00)
Sex, n (%) Male	98 (71.5)	102 (62.2)	0.088	1.52 (0.93–2.48)
Tobacco consumption, n (%) Yes	75 (54.7)	34 (20.7)	<0.001	4.62 (2.78–7.66)
Alcohol consumption, n (%) Yes	44 (32.1)	22 (13.4)	<0.001	3.05 (1.71–5.42)
Histology, n (%) UCNT	137 (100)	NA	-	-
Tumor size (T), n (%) T1–T2 T3–T4	57 (41.6) 80 (58.4)	NA	-	-
Lymph node (N), n (%) N0–N1 N2–N3	55 (40.1) 82 (59.9)	NA	-	-
Metastasis (M), n (%) M0 M1	130 (94.9) 7 (5.1)	NA	-	-
TNM stage, n (%) II III–IV	25 (18.2) 112 (81.8)	NA	-	-

NPC: Nasopharyngeal carcinoma; n: number of subjects; UCNT: undifferentiated carcinoma nasopharynx tumor; NA: not applicable; TNM: tumor/node/metastasis staging system; OR: odds ratio, CI: confidence interval.

Table 2. Distribution of *TLR2*/*TLR3* minor allele/genotypes in patients and controls.

SNP	Patients n = 137 (%)	Controls n = 164 (%)	Unadjusted odds ratio	P-value	Multiadjusted odds ratio [a]	P-value
TLR2 (-196 to -174 Del) genotype/minor allele						
Ins/Ins	80 (58.4)	122 (74.4)	1.00 (reference)	-	1.00 (reference)	-
Ins/Del	30 (21.8)	27 (16.5)	1.69 (0.93–3.06)	0.081	1.51 (0.80–2.85)	0.202
Del/Del	27 (19.7)	15 (9.1)	2.74 (1.37–5.48)	0.004	2.40 (1.14–5.05)	0.020
Ins/Del + Del/Del	57 (41.6)	42 (25.6)	1.81 (1.12–2.91)	0.003	1.83 (1.08–3.09)	0.024
Del	84 (30.6)	57(17.3)	1.67 (1.16–2.41)	<0.001	-	-
TLR3 1377 C>T genotype/minor allele						
CC	54 (39.4)	68 (41.5)	1.00 (reference)	-	1.00 (reference)	-
CT	59 (43.1)	79 (47.0)	0.96 (0.59–1.57)	0.887	1.05 (0.61–1.78)	0.859
TT	24 (17.5)	19 (11.6)	1.59 (0.79–3.20)	0.194	1.71 (0.79–3.71)	0.172
CT+TT	83 (60.5)	98 (58.6)	1.09 (0.68–1.72)	0.719	1.17 (0.71–1.93)	0.534
T	107 (39.0)	117 (35.6)	0.82 (0.60–1.14)	0.250	-	-

n: Number of subjects; [a] adjusted for sex (P = 0.027) and smoking (P < 0.001).

3.4. Association of *TLR2* and *TLR3* variants and clinical parameters

The association between *TLR2* Ins/Del and *TLR3* 1377 C>T polymorphisms and clinical parameters, including tumor size, lymph nodes, metastasis, and TNM stage, are listed in Tables 3 and 4. There was a lack of association of the studied polymorphisms with the examined clinical characteristics.

3.5. Linkage disequilibrium in *TLR2*/*TLR3* gene and association with NPC risk

Since both the *TLR2* and *TLR3* genes are located on chromosome 4, we calculated the pairwise linkage disequilibrium (LD) values (D' and r^2 values) for the analyzed polymorphisms. The low D' (<0.1) and r^2 (<0.01) values indicate that the *TLR2* Ins/Del polymorphism and *TLR3* rs3775290 were in low LD. Data from Table 5 indicate that of the four possible haplotypes, the frequency

Table 3. Stratified analysis of *TLR2* (-196 to -174 Ins/Del) polymorphism with clinical characteristics in NPC patients.

Characteristics	Patients		OR (95% CI)	P-value
Sex	Male	Female		
Ins/Ins	57	23	1.00 (reference)	-
Ins/Del	23	7	0.80 (0.31–3.51)	0.653
Del/Del	18	9	1.32 (0.50–3.51)	0.571
Ins/Del + Del/Del	41	16	1.30 (0.46–2.35)	0.930
Del	59	23	1.03 (0.56–1.91)	0.920
Tumor size	T1–T2	T3–T4		
Ins/Ins	34	46	1.00 (reference)	-
Ins/Del	12	18	1.10 (0.47–2.60)	0.813
Del/Del	11	16	1.07 (0.44–2.60)	0.873
Ins/Del + Del/Del	23	34	1.09 (0.52–2.31)	0.801
Del	34	50	1.07 (0.61–1.86)	0.800
Lymph nodes	N0–N1	N2–N3		
Ins/Ins	36	44	1.00 (reference)	-
Ins/Del	10	20	1.63 (0.68–3.93)	0.271
Del/Del	9	18	1.63 (0.65–4.08)	0.291
Ins/Del + Del/Del	19	38	1.63 (0.80–3.31)	0.171
Del	28	56	1.52 (0.86–2.69)	0.126
Metastasis	M0	M1		
Ins/Ins	76	4	1.00 (reference)	-
Ins/Del	30	0	-	0.998
Del/Del	24	3	2.37 (0.49–2.60)	0.279
Ins/Del + Del/Del	54	3	1.06 (0.18–5.89)	1.000
Del	78	6	1.75 (0.52–5.79)	0.373
TNM stage	II	III–IV		
Ins/Ins	15	65	1.00 (reference)	-
Ins/Del	4	26	1.50 (0.45–4.94)	0.505
Del/Del	6	21	0.80 (0.27–2.34)	0.695
Ins/Del + Del/Del	10	47	1.08 (0.41–2.87)	0.857
Del	16	68	0.93 (0.46–1.89)	0.819

TNM: Tumor/node/metastasis staging system; OR: odds ratio, CI: confidence interval.

Table 4. Stratified analysis of *TLR3* 1377 C>T polymorphism with clinical characteristics in NPC patients.

Characteristics	Patients		OR (95% CI)	P-value
Sex	Male	Female		
CC	38	16	1.00 (reference)	-
CT	41	18	0.95 (0.42–2.14)	0.919
TT	19	5	1.60 (0.50–5.03)	0.421
CT+TT	60	23	1.10 (0.48–2.50)	0.807
T	79	28	1.21 (0.68–2.15)	0.499
Tumor size	T1–T2	T3–T4		
CC	24	30	1.00 (reference)	-
CT	22	37	1.34 (0.63–2.85)	0.440
TT	11	13	0.94 (0.36–2.48)	0.909
CT+TT	33	50	1.21 (0.57–2.57)	0.412
T	44	63	0.97 (0.57–1.63)	0.896
Lymph nodes	N0–N1	N2–N3		
CC	23	31	1.00 (reference)	-
CT	21	38	1.34 (0.62–2.86)	0.446
TT	11	13	0.87 (0.33–2.30)	0.790
CT+TT	32	51	1.18 (0.55–2.52)	0.637
T	43	64	1.00 (0.59–1.70)	0.991
Metastasis	M0	M1		
CC	53	1	1.00 (reference)	
CT	55	4	3.85 (0.41–35.61)	0.234
TT	22	2	4.81 (0.41–55.91)	0.209
CT+TT	77	6	0.24 (0.01–2.13)	0.162
T	99	8	0.46 (0.14–1.52)	0.154
TNM stage	II	III–IV		
CC	13	41	1.00 (reference)	-
CT	9	50	1.76 (0.68–4.53)	0.240
TT	3	21	2.22 (0.56–8.65)	0.251
CT+TT	12	71	1.88 (0.72–4.90)	0.154
T	15	92	1.63 (0.80–3.32)	0.146

TNM: Tumor/node/metastasis staging system; OR: odds ratio, CI: confidence interval.

of the Del-T haplotype was significantly higher in NPC patients than healthy controls (13.6% vs. 5.7%) [P = 0.0081, OR (95% CI) = 2.14 (1.22–3.75)].

4. Discussion

The study of candidate gene polymorphisms as potential biomarkers of cancer risk has facilitated understanding the association between genotype/haplotype and disease, thus underscoring the contribution of specific gene variants to susceptibility to various cancers, including NPC. TLRs are potent immune modulators, modulate inflammatory responses, and are involved in regulating cell proliferation, survival, and removal of cancer debris (8,9). However, inconsistencies in the exact role of TLRs in cancer pathogenesis were reported. The present study examined the association of two common polymorphisms in *TLR2* and *TLR3* with the susceptibility to NPC.

Table 5. Possible haplotypes of *TLR2* (-196 to -174 Ins/Del) and *TLR3* (rs3775290) gene polymorphisms and the association with NPC risk.

Haplotypes	Frequency	Patients n = 137	Controls n = 164	OR (95% CI)	P-value
Ins-C	0.481	0.439	0.532	1.00 (ref.)	-
Ins-T	0.272	0.254	0.293	1.06 (0.71–1.60)	0.77
Del-C	0.145	0.170	0.116	1.46 (0.91–2.35)	0.12
Del-T	0.100	0.136	0.057	2.14 (1.22–3.75)	0.0081

OR: Odds ratio, CI: confidence interval.

The *TLR2* Ins/Del polymorphism alters *TLR2* promoter activity, leading to decreased transcription of the *TLR2* gene. No previous studies have investigated the likely association between this variant and NPC risk. We found that the *TLR2* Del allele and *TLR2* Del/Del genotype were significantly associated with increased risk of NPC and thus may represent candidate biomarkers for evaluating NPC risk. Consistent with our findings, it was previously shown that the *TLR2* Ins/Del polymorphism was associated with a heightened risk of some cancers, such as breast cancer, gastric cancer, prostate cancer, hepatocellular cancer, and cervical cancer (21–25).

The distribution of the *TLR3* 1377 C>T allele and genotypes was comparable between NPC patients and controls, thus challenging the role (if any) for this *TLR3* variant in NPC pathogenesis. Mixed findings on the association of *TLR3* 1377C>T with different cancer types were reported. This was highlighted by the lack of association of rs3775290 with NPC in China (26), bladder cancer in India (27), prostate cancer in India (23), and breast cancer in Croatia (28). In contrast, an earlier study documented the association of the *TLR3* 1377C/C genotype with increased susceptibility to cervical cancer in Tunisian women (29). Further controlled studies involving larger sample sizes and molecular approaches are required to clarify the exact contribution of this variant (if any) to the development and/or evolution of different cancer types, including NPC.

Analysis of LD patterns between *TLR2* and *TLR3* polymorphisms identified the Del-T haplotype to be positively associated with NPC. To the best of our knowledge, this is the first study that investigated the possible linkage between these two polymorphisms and the risk of NPC, and as such we cannot compare our results to related studies. Additional studies investigating the linkage of these two variants, and possibly others in the *TLR2* and *TLR3* genes, with NPC and other tumors are needed to clarify the implication of *TLR2* and *TLR3* polymorphic loci as biomarkers to evaluate cancer risk.

Our study has some strengths, namely that the NPC patients and controls have a similar ethnic background, as they originated from North Tunisia, thus minimizing the contribution of racial/ethnic differences inherent in genetic association studies. In addition, NPC assessment involved questionnaire-based interviews and laboratory assessment, including histology screening, and hence cancer was ascertained. However, our study also had a few limitations, namely that the sample size was relatively low, thereby necessitating future studies involving a larger number of cases and controls so as to fully understand the contribution of *TLR2* and *TLR3* gene polymorphisms in NPC, as well as related malignancies.

In conclusion, our results demonstrate an increased risk of NPC with the *TLR2* Del/Del genotype and Del-T *TLR2* and *TLR3* haplotype, suggesting their potential use as biomarkers to evaluate NPC risk in Tunisians. Future studies using a larger number of patients and controls with different backgrounds should improve the evaluation of *TLR2* and *TLR3* variants as biomarkers for NPC risk.

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References

1. Schottenfeld D, Fraumeni JF. Cancer Epidemiology and Prevention. Oxford, UK: Oxford University Press; 2006.

2. Chang ET, Adami HO. The enigmatic epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev 2006; 15: 1765-1777.

3. Razak AR, Siu LL, Liu FF, Ito E, O'Sullivan B, Chan K. Nasopharyngeal carcinoma: the next challenges. Eur J Cancer 2010; 46: 1967-1978.
4. Yoshizaki T, Ito M, Murono S, Wakisaka N, Kondo S, Endo K. Current understanding and management of nasopharyngeal carcinoma. Auris Nasus Larynx 2012; 9: 137-144.
5. Busson P, Keryer C, Ooka T, Corbex M. EBV-associated nasopharyngeal carcinomas: from epidemiology to virus-targeting strategies. Trends Microbiol 2004; 12: 356-366.
6. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002; 420: 860-886.
7. Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C et al. Cytokine gene polymorphism in human disease: on-line databases, supplement 1. Genes Immun 2001; 2: 61-70.
8. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2001; 2: 675-680.
9. Wang RF, Miyahara Y, Wang HY. Toll-like receptors and immune regulation: implications for cancer therapy. Oncogene 2008; 27: 181-189.
10. Lawrence T. Inflammation and cancer: a failure of resolution? Trends Pharmacol Sci 2007; 28: 162-165.
11. Misch EA, Hawn TR. Toll-like receptor polymorphisms and susceptibility to human disease. Clin Sci 2008; 114: 347-360.
12. Netea MG, Wijmenga C, O'Neill LA. Genetic variation in Toll-like receptors and disease susceptibility. Nat Immunol 2012; 13: 535-542.
13. Noguchi E, Nishimura F, Fukai H, Kim J, Ichikawa K, Shibasaki M, Arinami T. An association study of asthma and total serum IgE levels for toll-like receptor polymorphisms in a Japan population. Clin Exp Allergy 2004; 34: 177-183.
14. Dick DM. Gene-environment interaction in psychological traits and disorders. Annu Rev Clin Psychol 2011; 7: 383-409.
15. Wang BG, Yi DH, Liu YF. *TLR3* gene polymorphisms in cancer: a systematic review and meta-analysis. Chin J Cancer 2015; 34: 272-284.
16. Zhu L, Yuan H, Jiang T, Wang R, Ma H, Zhang S. Association of *TLR2* and *TLR4* polymorphisms with risk of cancer: a meta-analysis. PLoS One 2013; 8: e82858.
17. Shanmugaratnam K, Sobin LH. Histological Typing of Tumors of the Upper Respiratory Tract and Ear. World Health Organization: International Histological Classification of Tumors. Berlin, Germany: Springer; 1991.
18. Beahrs OH, Henson DE, Hutter RVP, Kennedy BJ. American Joint Committee on Cancer Manual for Staging of Cancer. 4th ed. Philadelphia, PA, USA: J.B. Lippincott; 1992.
19. Tahara T, Arisawa T, Wang F, Shibata T, Nakamura M, Sakata M, Hirata I, Nakano H. Toll-like receptor 2 -196 to -174 del polymorphism influences the susceptibility of Japanese people to gastric cancer. Cancer Sci 2007; 98: 1790-1794.
20. Cheng PL, Eng HL, Chou MH, You HL, Lin TM. Genetic polymorphisms of viral infection-associated Toll-like receptors in Chinese population. Transl Res 2007; 150: 311-318.
21. Theodoropoulos GE, Saridakis V, Karantanos T, Michalopoulos NV, Zagouri F, Kontogianni P, Lymperi M, Gazouli M, Zografos GC. Toll-like receptors gene polymorphisms may confer increased susceptibility to breast cancer development. Breast 2012; 21: 534-538.
22. De Oliveira JG, Silva AE. Polymorphisms of the *TLR2* and *TLR4* genes are associated with risk of gastric cancer in a Brazilian population. World J Gastroenterol 2012; 18: 1235-1242.
23. Mandal RK, George GP, Mittal RD. Association of Toll-like receptor (TLR) 2, 3 and 9 genes polymorphism with prostate cancer risk in North Indian population. Mol Biol Rep 2012; 39: 7263-7269.
24. Nischalke HD, Coenen M, Berger C, Aldenhoff K, Muller T. The toll-like receptor 2 (TLR2) -196 to -174 del/ins polymorphism affects viral loads and susceptibility to hepatocellular carcinoma in chronic hepatitis C. Int J Cancer 2012; 130: 1470-1475.
25. Pandey S, Mittal RD, Srivastava M, Srivastava K, Singh S, Punita L. Impact of Toll like receptors [TLR] 2 (-196 to -174 del) and TLR 4 (Asp299Gly, Thr399Ile) in cervical cancer susceptibility in North Indian women. Gynecol Oncol 2009; 114: 501-505.
26. He JF, Jia WH, Fan Q, Zhou XX, Qin HD, Shugart YY, Zeng YX. Genetic polymorphisms of TLR3 are associated with nasopharyngeal carcinoma risk in Cantonese population. BMC Cancer 2007; 7: 194.
27. Singh V, Srivastava N, Kapoor R, Mittal RD. Single-nucleotide polymorphisms in genes encoding Toll-like receptor-2, -3, -4, and -9 in a case-control study with bladder cancer susceptibility in a North Indian population. Arch Med Res 2013; 44: 54-61.
28. Etokebe GE, Knezevic J, Petricevic B, Pavelic J, Vrbanec D, Dembic Z. Single nucleotide polymorphisms in genes encoding toll-like receptor-2, -3, -4, and -9 in case-control study with breast cancer. Genet Test Mol Biomarkers 2009; 13: 729-734.
29. Zidi S, Verdi H, Yilmaz-Yalcin Y, Yazici AC, Gazouani E, Mezlini A, Atac FB, Yacoubi-Loueslati B. Involvement of Toll-like receptors in cervical cancer susceptibility among Tunisian women. Bull Cancer 2014; 101: 31-35.