RESEARCH ARTICLE

Editorial Process: Submission:02/15/2018 Acceptance:05/13/2018

An Interleukin-6 Single Nucleotide Polymorphism and Susceptibility to Prostate Adenocarcinoma and Bone Metastasis in an Iranian Population

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Abstract

Objective: Interleukin-6 (IL-6) is an inflammatory cytokine shown to be a strong factor for growth, proliferation and metastasis with many malignancies. The promoter single nucleotide polymorphism (SNPs) -174G>C (rs1800795) can alter the transcriptional pattern of this gene. The present study was aimed at assessing effects of the IL-6 (rs1800795) SNP on risk of benign prostate hyperplasia (BPH) and prostatic adenocarcinoma (PCa). **Methods:** The project was performed on 112 men with PCa, 118 with BPH and 250 healthy controls. After DNA extraction, genotyping of IL-6 (rs1800795) was performed using PCR TaqMan Allelic Discrimination (ABI MGB). **Results:** The G allele frequency for rs1800795 of the IL-6 gene was 74.1%, 68.6% and 67% in PCa patients, BPH patients and healthy men, respectively. PCa and control groups showed significant differences (P =0.030, OR = 1.73, 95% CI: 1.05-2.21). The GG genotype was more frequent in the PCa group, whereas the GC genotype was more common in the BPH in comparison to other groups. **Conclusion:** The current study identified IL-6 -174G>C (rs1800795) as a significant predictor of susceptibility for prostate cancer and bone metastasis in a northwest Iranian population.

Keywords: Interleukin-6- IL-6- prostate- adenocarcinoma- hyperplasia- BPH- PCa

Asian Pac J Cancer Prev, **19 (6)**, 1717-1720

Introduction

Prostate disorders have been placed into the most prevalently diagnosed and still growing male malignancies aged over 60 during the recent decades (Bull et al., 2016). It remains among 4 major lethal cancers (lung, breast, prostate, and colorectum) and accounts for almost 1 in 5 new diagnoses, according to the most updated cancer statistic study (Siegel et al., 2017).

Progression of Prostate carcinoma is triggered by many important risk factors. Inflammation has a very deep relation with cancer pathology. High proliferation rate is a well-known characteristic of malignant cells, and the inflammatory molecules which continuously secreted by other immune cells and/or tumour cells facilitate this process. Interleukin 6 (IL 6) is one of the important inflammatory molecules produced and released by most of cancerous cells. This cytokine contributes to the proliferation, differentiation and metastasis of many malignant tumours and has been shown to be higher in serum and tumour tissues of a majority of cancers (Culig and Puhr, 2012). Studies indicate that IL-6 expression is increased in both human benign prostate hyperplasia and prostate adenocarcinoma (Giri et al., 2001; Royuela et al., 2004). Adrel et al., (1999) showed that increased IL-6 could be a sign for metastatic prostate carcinoma.

Based on human genome mapping, 7p21-14 locus is the chromosomal location of IL-6 gene. Three promoter SNPs including -597G>A, -572G>C and -174G>C are very common and -174G>C (rs1800795) has been indicated to affect the gene transcription, and therefore alter the cytokine production (Zhou et al., 2015). According to several studies, there might be statistical association between IL-6 SNPs and susceptibility to various cancers specially prostate cancer (Giannitrapani et al., 2013; Gomes et al., 2015; Zhang et al., 2016). However, considering some meta-analysis, the results are inconclusive and more studies on various races and groups are required to achieve comprehensive reliable conclusions (Zhang et al., 2012; Yang et al., 2014; Liu et al., 2017). It is believed that 174G>C (rs1800795) gene polymorphisms play role in its transcription level, and different populations show various single nucleotide

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polymorphism. We hypothesized that this polymorphism might affect the susceptibility and risk of prostatic adenocarcinoma and/or benign prostate hyperplasia in an Iranian population.

Materials and Methods

Participants

We prepared a vast exclusion criteria for the study samples. At the beginning, 150 men with prostate adenocarcinoma and 170 men with benign prostate hyperplasia attending public hospitals of northwestern region of Iran (East and West Azerbaijan provinces, Iran) were selected for the case group. However, according to the exclusion criteria, 112 patients for PCa (Mean age: 67.8±10.3) and 118 patients for BPH (Mean age: age 68±9.3) were selected for the present study. A total of 250 unrelated healthy males (ethnicity and age matched, Mean age: 63±7.8), attending same hospitals for routine checkup or health awareness were selected as control group. Determination of the sample size was based on similar studies and the availability of the disorder in the population.

Inclusion criteria for the PCa was based on precise pathological and histopathological records, Gleason score and PSA levels. Moreover, participants had no other cancers, previous surgery, radiotherapy, or chemotherapy, or any serious immunological disorder and taking any kind of special drug. For the BPH group, the statement of an urologist, PSA levels, negative history for any cancer, receiving no treatments such as surgery, radiotherapy, and chemotherapy. Lack of any serious immunological disorder and taking any kind of special drug. Normal PSA level (<4.0 ng/ml), lack of any history of urinary tract disorders and any personal or familial history of cancer were the inclusion criteria for control group.

Blood sample collection was performed between 2014 and 2016. Written informed consent was provided by each participated subject in the study. The Vice-Chancellor for the Research Ethics of the University of Tabriz approved the study.

DNA extraction

EDTA coated tubes used for collecting subjects peripheral venous blood samples. Blood Genomic DNA Miniprep Kit (Axygen, USA) was used for genomic DNA extraction. DNA was stored at -20 °C.

Genotyping

IL-6 (rs1800795) was genotyped using validated TaqMan Allelic Discrimination kit (Applied Biosystems, Foster City, CA, USA). The assay includes two probes with distinct fluorescent dyes specific for each allele, and a pair of PCR primer to identify specific SNPs. This genotyping methodology is more efficient, accurate and sensitive. Genotyping assay was performed by StepOnePlus Real-Time PCR System (Applied Biosystems).

Statistical analysis

Mean ± standard deviation (SD) and frequencies (number of cases) of alleles and genotypes in all groups were statistically described depending on their distribution. Chi square (χ^2) analysis were used to calculate the deviation of distribution of genotypes from Hardy–Weinberg Equilibrium (HWE). Social Sciences Package (SPSS) version 18 was used for statistical analysis. To compare alleles/genotypes frequencies in case and control groups, Fisher's exact test and Chi square test were performed. Determination of the relation between genotypes and age, BMI, PSA and smoker categories was done by ANOVA test. Odds ratios and 95% confidence intervals (CIs) were calculated and a probability value of $P < 0.05$ (two-tailed) was considered as statistical significance. Multivariate logistic regression was used to analyze the association between -174G>C (rs1800795) single nucleotide polymorphism and the PCa or BPH occurrence. We found no significant differences comparing some important indexes such as age, BMI, PSA, smoking status and Gleason grade between cases and healthy controls (all $P > 0.05$) (Table 1).

Results

Allele and genotype frequencies

Polymorphisms and clinicopathological variables of IL-6 in pateint groups and healthy control are presented in Table 2 and 3. The frequency of the G allele for polymorphism -174G>C (rs1800795) in PCa, BPH and control group was calculated 74.1%, 68.6% and 67 % respectively. Therefore, only PCa and healthy showed significant differences group (P =0.030, OR = 1.73, 95% CI: 1.05-2.21). Albeit being less frequent, the GG genotype was more in PCa group in comparison to BPH and healthy individuals (51.6% vs. 51%, respectively) and significantly different (P = 0.035, OR = 1.85, 95% CI: 1.12-2.63). On the other hand, BPH group (33.8%) compared with PCa (32.1%) and healthy group (32%) showed more frequency in GC genotype, without significant difference. The CC genotype frequency was shown to be low in PCa patients (9.8%) in comparison to BPH and healthy groups (14.6% and 16%, respectively), with no significant results.

Table 1. Demographic Characteristics of Study Population

Characteristic	Control (n=200) (mean±SD)	BPH (n=118) (mean±SD)	PCa (n=112) (mean±SD)	P value
Average Age	63±7.8	68±9.3	67.8±10.3	0.313
Average BMI	25.6±4.8	24.7±3.9	24.3±1.4	0.217
PSA (ng/mL)	2.1 (1.2)	7.51 ± 7.1	8.96 ±10.11	0.098
Smoker	32 (16%)	17 (14.6%)	11 (9.8%)	0.262
Alcohol Consumption	Not available	----	----	----

SD, Standard deviation

Table 2. -174G>C (rs1800795) Polymorphisms and Clinicopathological Variables

Variables	GG N (%)	GC+ CC N (%)	OR (95% CI) P value
High Gleason grade ≥7	34 (53.1)	30 (46.9)	1.01 (0.49-2.01) 0.83
Low Gleason grade <7	25 (52)	23 (48)	-------
Bone Metastasis	26 (44.5)	33 (55.5)	2.02 (1.16-3.15) 0.04
No Bone Metastasis	31 (58.4)	22 (41.5)	------
High PSA >10	24 (52.2)	22 (47.8)	0.7 (0.41-1.23) 0.10

OR; Odds ratio, CI; Confidence interval

Table 3. Distribution of the IL-6 174G>C (rs1800795) Genotypes and Alleles in PCa Patients, BPH Patients and Healthy Controls

Alleles/ Genotyps	Control (n=200) N (%)	BPH (n=118) N (%)	PCa (n=112) N (%)	BPH vs Control OR (95% CI) P value Adj. P[a]	PCa vs Control OR (95%CI) P value Adj. P[a]	PCa vs BPH OR (95%CI) P value Adj. P[a]
G	268 (67)	162 (68.6)	166 (74.1)	1.21 (0.53-1.92) 0.64 0.73	1.73 (1.05-2.21) 0.030 0.038	0.82 (039-2.02) 0.21 0.82
C	132 (33)	74 (31.4)	58 (25.9)	--------	--------	--------
GG	102 (51)	61 (51.6)	65 (58)	1.09 (0.64-2.02) 0.73	1.85 (1.12-2.63) 0.035	0.91 (041-1.88) 0.112
GC	64 (32)	40 (33.8)	36 (32.1)	0.83 (0.42-1.21) 0.49	0.90 (0.61-1.29) 0.63	0.83 (0.31-1.42) 0.512
CC	32 (16)	17 (14.6)	11 (9.8)	0.74 (0.32-1.31) 0.52	0.42 (0.24-1.09) 0.09	0.94 (051-2.48) 0.112
HWE	0.25					

P[a], adjusted p value for multiple testing using Bonferroni correction method; HWE, Hardy–Weinberg Equilibrium; OR, Odds ratio; CI, Confidence interval; Adjusted for age, cigarette smoking, p value ≤ 0.05

The GG genotype frequency in the PCa patients with bone metastasis was less than non-metastasis group (58.4% vs 41.5%, P=0.04, OR = 2.02, 95% CI: 1.16-3.15) (Table 2). PCa patients with high Gleason grade and high serum PSA levels had more GG genotype frequency (53.1% and 52.2%, respectively) and insignificant (P =0.83, OR = 1.01 95% CI: 0.49-2.01, and (P =0.10, OR = 0.7, 95% CI: 0.41-1.23) (Table 2).

Discussion

Many factors such as genetic, age, life style, diet, family history and environment may play role in etiology of the prostate carcinoma (Giovannucci, 2001). Gene polymorphism studies about susceptibility to the prostate cancer have been of growing interest. Indeed, the gene polymorphisms as an inherent mechanisms of human diseases, have shown to affect tumor incidence, manifestations, and response to treatment, including prostate cancer (Bao et al., 2008).

Multiple investigations have indicated that there might be a correlation between increased serum IL-6 levels, with metastatic prostate cancer, tumor burden and serum prostate specific antigen (PSA) (Michalaki et al., 2004). According to previous studies, plasma concentration and bioactivity of inflammatory mediators could be influenced by their gene polymorphisms (Howell and Rose-Zerilli, 2007). It has been reported that rs1800795 IL-6 gene promoter polymorphism may alter its gene transcription and thus change the serum levels of IL-6 and the disease hormone-dependency/independency status (Sakai et al., 2011). Therefore, it is hypothesized that the cytokine gene polymorphisms could affect the susceptibility to and severity of prostate cancer (Yang et al., 2014; Liu et al., 2017). Our data in current study show that patients with prostate cancer and its metastasized form, have statistically significant differences in IL-6 -174G>C (rs1800795) GG genotype and G allele, compared with healthy group.

Several studies have indicated that prostate cancer risk could statistically be associated with IL-6 (rs1800795) gene polymorphisms (Kesarwani et al., 2008; Mandal et al., 2014). Amirzargar et al., (2008) showed that -174 G allele is the most frequent allele in the general population in almost all studied ethnic groups selected from central and east regions of Iran (Tehran, Yazd, Sistan and Balochistan) which is in parallel with our findings (Amirzargar et al., 2008). Mandal et al., (2014) and Bao et al., (2008) found significant associations between IL-6 174G>C gene polymorphisms and susceptibility to prostate cancer (Yang et al., 2014). However, Magalhães et al., (2013) found no association. Additionally, in the most updated meta-analysis conducted by Tan et al., (2005) African-American group was the only showing an statistical association, which indicates that IL-6 -174G>C polymorphism might play totally opposite effects in different races (Liu et al., 2017).

IL-6 (-174 C) allele polymorphisms indicated a strong association with severity and recurrence of PCa in Chinese population (Tan et al., 2005), and also found to be associated with bone metastasis of PCa in Indian population (Kesarwani et al., 2008). Our data indicate that IL-6 (-174 C) allele is more frequent and statistically significant in PCa bone metastasis.

BMI and smoking has been shown to have associations with prostate disorders (Rodriguez et al., 2007; Kenfield, et al., 2011), however, the present data did not present such statistical associations neither in PCa nor BPH groups. Selection of samples from the northwestern region of the

country which may not represent the general population, as well as small sample size, could be considered as main limitations of the study. Moreover, causal polymorphism information and its functional role(s), and the influence of many other genetic factors in development of prostate cancer are unknown. Due to some cultural issues, data regarding alcohol consumption seemed to be unreliable and excluded from the study.

In conclusion, in summary, our results identified IL-6 -174G>C (rs1800795) as a statistically disease-susceptible SNP in prostate cancer and its bone metastasis in a new population. Owing to the limitations of our research, further investigations in cellular and molecular-level with larger sample size in more ethnical groups and races are required in order to study any possible regulative role of this SNP in IL-6 production and subsequently, the initiation and/or progression of prostate cancer and metastasis.

Ethical approval

The ethics committee of the University of Tabriz approved all practical procedures of the study being in accordance with 1964 Helsinki declaration. All the individual participants provided written consent.

Acknowledgments

Authors would like to thank to Department of Animal Biology, University of Tabriz and also faculty of Medicine, Tabriz University of Medical Sciences for their kind assistance.

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