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Source / Izvornik: **Collegium antropologicum, 2013, 37, 907 - 911**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:239:509162

Coll. Antropol. **37** (2013) 3: 907–911
Original scientific paper

Interleukin-6 Polymorphism and Prostate Cancer Risk in Population of Eastern Croatia

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A B S T R A C T

Recent studies suggest that chronic inflammation is crucial in the development and progression of prostate cancer (CaP). Interleukin-6 (IL-6) is a proinflammatory cytokine that plays an important role in intraprostatic inflammation and thus carcinogenesis. The –174G>C polymorphism of IL-6 gene has been associated with high IL-6 producer phenotype and an increased risk for CaP. The aim of this study was to evaluate the association between the mentioned IL-6 polymorphism and CaP risk, as well as to compare the genotype frequency between the different tumour grades of CaP, in population of Eastern Croatia. We analyzed the IL-6 polymorphism in 120 CaP patients and 120 controls with benign prostatic hyperplasia (BPH). CaP patients and BPH controls did not statistically differ in studied IL-6 polymorphism. Furthermore, high IL-6 producer genotypes (GG or GC) were more frequent in controls than in CaP group (86.7% vs 80.8%, respectively, p=0.147). Also, no statistically significant difference in IL-6 high and low producer genotype frequency was noticed between well, moderately and poorly differentiated tumours. Our results, taken together with other studies on the subject, suggest that IL-6 – 174 single nucleotide polymorphism (SNP) distribution may differ between various ethnic groups and that a single cytokine gene polymorphism has probably just a minor effect on CaP susceptibility. Further studies should be performed to clarify the link between SNPs of different cytokines and the risk for CaP.

***Key words**: prostate cancer, IL-6, SNPs, Croatia*

Introduction

Although the etiology of prostate cancer (CaP) has not yet been established, both genetic and environmental factors appear to be involved. The lack of high-penetrant susceptibility genes in CaP development[1–3] and immigration studies[4–7], which explain the rise in CaP risk when immigrating from low to high incidence countries, all support the role of environmental factors in CaP occurence. Recent studies recognize infection, dietary derived toxins, trauma and hormonal changes as prominent environmental factors relevant for the development of the disease[8–10]. In response to all these events, intraprostatic inflammation mostly develops as a necessary step to remove generated necrotic debris[11]. On the other hand, reactive oxygen and nitrogen species, produced by phagocytic cells, cause collateral damage in DNA, thus promoting tumourigenesis[9,12]. Several works based on epidemiological, histopathological and molecular pathological studies have proposed a causative role of chronic inflammation in the development and progression of many types of human cancers, approximately 20% of all cancers[8,9,13], including CaP[9,13–15]. Inflammatory infiltrates and proliferative inflammatory atrophy (PIA) detected in CaP and benign prostatic hyperplasia (BPH) specimens are histological manifestation of inflammation and have been proposed as a precursor in the development of CaP, highlighting an inflammatory contribution to cancer occurence[8,9].

Received for publication September 9, 2010

Entire process of inflammation is controlled by various mediators, primarily cytokines and chemokines, which are mostly produced and released locally, where they mediate by autocrine and paracrine mechanisms[11]. Among them, the strongest evidence for a role in prostate carcinogenesis is found for Interleukin-6 (IL-6)[16]. IL-6 is a pleiotropic proinflammatory cytokine synthesized by many cell types involved in diverse biological areas, including regulation of T- and B-cell function, Ig secretion, acute phase inflammatory reactions and hematopoiesis[11,17,18]. It also acts as a paracrine growth factor for some cancers[14,17]. Its uncontrolled production may lead to chronic inflammation and thus could play an important role in cancer risk[11]. There are several single nucleotide polymorphisms (SNPs) in the IL-6 gene that have been identified. It has been shown that the G>C transition at position –174 in the promoter region of the IL-6 gene appears to affect IL-6 transcription[19] and therefore is associated with altered production of IL-6[20]. Accordingly, high producer genotypes (GG and GC) and low producer genotype (CC) of IL-6 could be distinguished[19]. Abovementioned polymorphism was found to be associated with increased risk for several cancers[21–23], including CaP[20,24]. However, contoversial results concerning association between IL-6 G-174C SNP and CaP are thought to be influenced by ethnic differences.

The purpose of this study was to determine the IL-6 high and low producer genotype distribution in CaP cases and BPH controls and to examine the association of tested polymorphism with different tumour grades of CaP.

Patients and Methods

120 CaP patients and 120 BPH controls treated at the Clinic for Urology, University Hospital Centre Osijek were included in this study, after giving informed consent. Groups were age-matched. Detailed urological examination which included digital rectal examination (DRE), prostate-specific antigen (PSA) testing and transrectal or suprapubic ultrasonography was performed. Clinical patient characteristics including family history were obtained by a questionnaire. Furthermore, all subjects who had serum PSA value greater than 4 ng/mL and/or abnormal DRE were submitted to transrectal ultrasound-guided prostate needle biopsy, using a 12-core protocol. Patients with histologically confirmed CaP on prostate biopsy were divided into three subgroups according to the Gleason score (GS) (well differentiated (GS<6), moderately differentiated (GS 6 and 3+4=7) and poorly differentiated tumors with GS ≥ 4+3=7). Patients in the Control group were selected according to the histological finding of BPH, obtained by prostate biopsy, transuretral resection of the prostate or by open prostatectomy. Those patients who had pathological finding of atypical small acinar proliferation (ASAP) or (before 2009.) high-grade prostatic intraepithelial neoplasia (high-grade PIN) on prostate biopsy were subjected to another biopsy and later classified, according to the results, to either CaP or BPH group.

To determine the IL-6 –174 SNP, genomic DNA was extracted from 200 μl ethylenediaminetetraacetic acid (EDTA) whole blood using High Pure polymerase chain reaction (PCR) Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions. IL-6-174 G/C gene polymorphism was assessed by real-time PCR method with melting curve analysis. In the reaction volume of 10 μL approximately 40 ng of template DNA were amplified in the presence of 0.5 μM primers each (sense 5'-TTA CTC TTT GTC AAG ACA TGC CA-3', antisense 5'-ATG AGC CTC AGA CAT CTC CAG-3'), 0.2 μM probes each (anchor 5'-CTA AGC TGC ACT TTT CCC CCT AGT-fluorescein-3', sensor 5'-LCRed640-GTG TCT TGC GAT GCT AAA GGA-P-3'), 2.5 mM $MgCl_2$ and 1x LC DNA Master Hybridization Probes buffer (Roche Diagnostics). PCR reactions were performed using LightCycler Instrument (Roche Diagnostics) according to the conditions described previously[25]. To confirm the results of genotyping, 20% of the study samples were randomly selected for genotyping again and the results were 100% congruent.

Descriptive statistical analysis was performed with SPSS 10.0 statistical program (SPSS Inc., Chicago, Ill, USA); Kolmogorov-Smirnov test was used to determine the differences between the groups and Fisher Exact test for comparing different variables between the groups. P <0.05 was considered statistically significant.

Results

There were 120 CaP patients and 120 BPH controls included in this study. Median age of all patients was 68 years (range 42–84). Two studied groups were age-

TABLE 1
PATIENT CHARACTERISTICS

	All patients	CaP group	BPH group	CaP *vs* BPH
No. of patients	240	120	120	
Median (range) age in years	68 (42–84)	68 (46–84)	68 (42–79)	p=0.564
Median (range) PSA in ng/mL	8.1 (0.57–3346)	12.4 (2.10–3346)	6.81 (0.57–28)	p=0.0001
Positive family history (%)	17 (7.1)	10 (8.3)	7 (5.8)	p=0.308

CaP – prostate cancer; BPH – benign prostatic hyperplasia; PSA – prostate-specific antigen; p<0.05 considered statistically significant

TABLE 2
CHARACTERISTICS AND COMPARISON OF CaP SUBGROUPS

	Subgroup A	Subgroup B	Subgroup C	Subgroup A *vs* subgroup B	Subgroup A *vs* subgroup C	Subgroup B *vs* subgroup C
No. (%) of patients	10 (8.3)	69 (57.5)	41 (34.2)			
Median (range) age in years	65 (52–74)	67 (46–83)	72 (48–84)	p=0.779	p=0.154	p=0.047
Median (range) PSA in ng/mL	8.05 (4–41.6)	10.3 (2.4–112)	28 (2.1–3346)	p=0.188	p=0.0001	p=0.0001
Positive family history (%)	1 (10)	7 (10.14)	2 (4.88)	p=0.680	p=0.488	p=0.277

Subgroup A – Well differentiated tumors (GS<6); Subgroup B – Moderately differentiated tumors (GS 6 and 3+4=7); Subgroup C – Poorly differentiated tumors (GS ≥ 4+3=7); PSA – prostate-specific antigen; p<0.05 considered statistically significant

TABLE 3
IL-6 HIGH PRODUCER GENOTYPE FREQUENCY FOR ALL GROUPS

	BPH group	CaP group	BPH vs CaP			
No (%) of patients	104/120 (86.7)	97/120 (80.8)	p=0.147			
	Subgroup A	Subgroup B	Subgroup C	A *vs* B	A *vs* C	B *vs* C
No (%) of patients	8/10 (80)	54/69 (78.3)	35/41 (85.4)	p=0.633	p=0.497	p=0.256

BPH – benign prostatic hyperplasia; CaP – prostate cancer; Subgroup A – Well differentiated tumors (GS<6); Subgroup B – Moderately differentiated tumors (GS 6 and 3+4=7); Subgroup C – Poorly differentiated tumors (GS ≥ 4+3=7); p<0.05 considered statistically significant

-matched (p=0.564). CaP group had, as expected, significantly higher median PSA values than control group (12.40 vs 6.81 ng/mL, with p=0.0001). Positive family history of CaP was more common in CaP (8.3%) than in control group (5.8%), but this was not statistically significant (p=0.308). Patient characteristics for CaP and BPH groups are listed in Table 1.

Out of 120 CaP subjects 10 had well, 69 moderately and 41 poorly differentiated tumors. Most common GS was 6(3+3) in 37.5% of the patients. The three subgroups of CaP patients did not differ in family history, but we did find significant difference in age and PSA value. The characteristics and comparison of the three CaP subgroups are listed in Table 2.

The IL-6 high producer genotypes (GG or GC) were more frequent in patients with BPH than in CaP group (86.7 vs 80.8%, respectively), but the difference was not statistically significant (p=0.147) and odds ratio (OR) for BPH was 1.54 (95% CI 0.77–3.09). Analysis of genotype frequency within CaP subgroups showed no statistically significant difference between IL-6 high and low producer genotypes (p=0.633, p=0.497 and p=0.256 for well vs moderately, well *vs* poorly and moderately vs poorly differentiated tumors). IL-6 high producer genotype frequency for all groups is listed in Table 3.

Discussion

The objective of our research was to investigate whether there was difference in IL-6 cytokine gene polymorphism between CaP patients and controls with BPH, as well as between the CaP subgroups, which were divided according to tumour differentiation.

It was previously shown that IL-6 has a role in the development and progression of several types of human cancers and inflammatory diseases[26,27]. Because of the various functions of IL-6 it may play different roles in CaP natural history[28], acting as a paracrine growth inhibitor in hormone dependent cell lines[29] and an autocrine and paracrine growth factor in hormone refractory human prostate cancer cell lines[30]. The G>C polymorphism at position –174 in the promoter of the IL-6 gene has been associated with the aggressiveness and recurrence of CaP[20] and serum IL-6 levels were significantly elevated in hormone-refractory prostate cancer patients compared to controls[31,32], as well as in patients with clinically evident CaP metastases[33,34].

As it was mentioned before, the G>C transition polymorphism at position –174 in the promoter region of the IL-6 gene is associated with the difference in the production of IL-6. Individuals can accordingly be classified into high (genotypes GG and GC) and low (genotype CC) IL-6 producers[19]. The reason we included the abovementioned classification in our study was because we wanted to correlate genotype (GG/GC and CC) with phenotype (high and low producer) of IL-6 cytokine.

In our analysis, CaP patients and age-matched controls with BPH did not statistically differ in studied IL-6 polymorphism. Furthermore, high IL-6 producer genotypes (GG or GC) were more frequent in controls than in CaP group (86.7% vs 80.8%, respectively, Table 3). Odds ratio for BPH patient to be high IL-6 producer was 1.54, with 95% CI 0.77–3.09.

In dividing CaP patients into three subgroups based on the tumour differentiation we analyzed and compared the studied IL-6 polymorphism between more and less aggressive prostate tumours. Again, no statistically significant difference in IL-6 high and low producer genotype frequency was noticed between well, moderately and poorly differentiated tumors (Table 3).

In a study by Tan and coworkers[20] a strong association between –174 G>C polymorphism and GS of the CaP patients was found. The authors also found different distributions of these genotypes between stages T3–T4 and T1–T2 of the tumours. In that retrospective study the analysis included patients treated with radical prostatectomy only and the comparison was made between GC/CC and GG genotypes. This is contrary to our analysis where GG/GC (high producer) and CC (low producer) were compared. Furthermore, the definition of well, moderately and poorly differentiated tumors differed in the two studies, so the comparison with the abovementioned study can not be made.

Kesarwani[24] and others analyzed Interleukin-4 (IL-4) and IL-6 (–174 G/C) gene polymorphisms in 200 controls and 200 cases of CaP in North Indian population and found no significant association with the risk of CaP. However, they noticed a twofold risk for progression to bone metastasis in CaP patients, but no association with GS was seen.

When studying the association between the SNPs in –174G/C and –634C/G of interleukin-6 promoter region and CaP in the population of Han people in Hubei region, Bao and coworkers[35] concluded that no SNP at position –174 was found (no CG and CC genotypes were observed).

The aforementioned studies and contradictory results clearly imply the ethnic background and distribution of IL-6 polymorphisms. Ethnicity appears to also influence the frequency and distribution of the polymorphisms of other cytokines, which altogether makes it almost impossible to make a global cytokine gene pattern. Our study, a part of the project »Immunological factors in development and progression of prostate cancer«, analyzed the difference in IL-6 cytokine gene polymorphism between CaP patients and controls with BPH, with final goal to map polymorphisms of genes for IL-6, Tumor necrosis factor-alpha (TNFα), Interleukin-10 (IL-10), Transforming growth factor beta (TGF-β), Interferon-gamma (IFN-γ) and Toll-like receptor 4 (TLR 4) in CaP patients of our population. So far such results have not been published for Croatian population.

In conclusion, our study showed that there were no significant differences in the distribution of the IL-6-174 SNP genotypes between CaP and BPH control subjects in population of Eastern Croatia. These findings, taken together with contradictory results of other studies imply the ethnical dependency of the IL-6-174 polymorphism. Other possible explanation is that a single cytokine gene polymorphism has probably just a minor effect on CaP susceptibility. Combinations and interactions of SNPs of different cytokines have a greater impact on CaP development and they might modify the risk for CaP. Further studies concerning SNPs of different cytokines and susceptibility to CaP should be performed in order to define their true importance in the development and progression of the disease.

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POLIMORFIZAM INTERLEUKINA-6 I RIZIK OD KARCINOMA PROSTATE U POPULACIJI ISTOČNE HRVATSKE

S A Ž E T A K

Novija istraživanja navode kroničnu upalu kao ključni čimbenik u nastanku i progresiji karcinoma prostate (CaP). Interleukin-6 (IL-6) je proinflamatorni citokin koji ima važnu ulogu u upali prostate, a prema tome i u karcinogenezi. –147G>C polimorfizam IL-6 gena se povezuje s fenotipom visoke produkcije IL-6 i povećanim rizikom za CaP. Cilj ovog istraživanja bio je ispitati povezanost između navedenog IL-6 polimorfizma i rizika za razvoj CaP, kao i usporediti frekvenciju genotipova između različitih stadija karcinoma prostate, u populaciji Istočne Hrvatske. Ispitali smo polimorfizam IL-6 u 120 pacijenata s CaP i 120 kontrolnih subjekata s benignom hiperplazijom prostate (BPH). Pacijenti s CaP se u ispitivanom polimorfizmu IL-6 nisu statistički razlikovali od kontrola s BPH. Štoviše, genotipovi visoke produkcije IL-6 (GG ili GC) bili su učestaliji u kontrolnoj grupi nego u grupi s CaP (86,7% naspram 80,8%, p=0,147). Osim toga, značajna razlika u raspodjeli genotipova visoke i niske produkcije nije nađena niti između dobro, srednje i slabo diferenciranih tumora. Naši rezultati, ako se uzmu u obzir i ostale studije o istoj temi, navode na zaključak da bi se raspodjela polimorfizma IL-6-174 mogla razlikovati među različitim etničkim skupinama i da polimorfizam jednog nukleotida gena za citokine vjerojatno ima samo manji utjecaj na podložnost razvoja CaP. Potrebna su daljnja istraživanja kako bi se pojasnila poveznica između polimorfizama gena različitih citokina i rizika za CaP.