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Research Article

STUDY OF ASSOCIATION OF LYSOSOMAL ASSOCIATED PROTEIN TRANS MEMBRANE 4 BETA (LAPTM4B) GENE POLYMORPHISM WITH PROSTATE CANCER

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ABSTRACT

Background: According to Annual Report Iraqi Cancer Registry, prostate cancer were classified within top ten cancers in Iraqi male and composed 6.5 % of all cancer cases. Worldwide studies have shown relationship between LAPTM4B gene polymorphism and risk of prostate cancer. Aim: To study the association of LAPTM4B gene polymorphism with prostate cancer in Iraqi population. Methods: This case control study consisted of 80 prostate cancer patients and 80 healthy individuals as control group. Genotyping of LAPTM4B gene polymorphism is carried out by PCR. DNA was extracted from whole blood and genotyping was achieved with specific primers to amplify gene fragments followed by electrophoresis on agarose gel. Various statistical analyses were applied to analyze the data. Results: The LAPTM4B gene polymorphism was associated with decreased risk of Pca in co-dominant (OR= 49 %, CI = 0.24 _0.99, P = 0.049, *1/2 versus *1/1) and dominant (OR = 47 %, CI = 0.24 _0.90, P = 0.024, *1/2+*2/2 versus *1/1) inheritance models. Conclusion: Our findings suggest that The LAPTM4B*2 allele significantly decreased the risk of Pca compared to LAPTM4B*1 and consider as protective factor in Iraqi population.

Keywords: prostate cancer, lysosome

INTRODUCTION

Prostate cancer (Pca) is the most common form of malignancy and the second leading cause of cancer death among men[1]. It is second only to lung cancer and the key to its successful treatment is in its early detection[2]. It is one of the most common cancers affecting men with more than 1,100,000 new cases and 300,000 deaths world- wide each year[3]. According to Annual Report Iraqi Cancer Registry 2015, prostate cancer was, classified within top ten cancer in Iraqi male and composed 6.5 % of all cancer cases[4]. Although many factors may contribute to the underlying biology and clinical causes of Pca, it is thought that genetic variation in androgen biosynthesis and signaling genes most likely influence the eventual outcome of the disease[5]. The factors that determine the risk of developing clinical Pca are not well known, although three well-established risk factors have been identified: increasing age, ethnic origin, and heredity[6]. The most significant of these is age with an increased incidence of Pca in men older than 50 years[2]. Prostate cancer symptoms can include erectile dysfunction, blood in the semen, pain in the lower back, hips, and/or upper thighs, urinary problems, or enlargement of the prostate[7]. The main tools to diagnose Pca include digital rectal examination (DRE), serum concentration of prostate specific antigen (PSA) and trans rectal ultrasound (TRUS)- guided biopsy[6]. In order to assess the prognosis of Pca, the cancers are graded based on a scoring system called the Gleason scale (GS)[2]. To date, conventional anatomic imaging techniques of computed tomography (CT), ultrasound, magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT) and positron emission tomography (PET) are currently used in the common clinical practice to stage men suffering from Pca[8]. The American Joint Committee on Cancer (AJCC) methodology uses the T (tumor extent), N (lymph node invasion), and M (presence or absence of metastasis) classifications to group patients[9]. Considering that the disease progression of Pca varies among individuals and as the disease is slow and not painful, approaches towards definitive treatment may also differ[3]. The first decision to be made in managing Pca is whether treatment is needed[10].

Lysosome associated protein trans membrane 4 β (LAPTM4B) is an oncogene associated with many human cancers[11]. LAPTM4B is a newly identified oncogene (NM_018407, Gene ID: 55353) and was first cloned in human hepatocellular carcinoma (HCC) in 2000[12]. It is a recently discovered gene that has been mapped to chromosome 8q22.1; it contains seven exons and six introns, spans ~50 kb, and is an essential factor in maintaining cellular homeostasis[13]. LAPTM4B exists as two alleles: LAPTM4B*1 with one 19 bp segment (GenBank accession no. AY219176) and LAPTM4B*2 with two tandem repeat segments (GenBank accession no. AY219177) in the 5' untranslated region of exon one[14]. Previous studies have demonstrated that LAPTM4B polymorphisms were associated with susceptibility to multiple types of cancer, including lung, breast, gastric, colon, ovarian and primary liver cancer, which suggested that LAPTM4B*2 may be associated with a significantly increased risk of developing these types of cancer[14]. Studies have illustrated that LAPTM4B promotes tumorigenesis by inhibiting apoptosis, up regulating autophagy and rendering resistance to chemotherapy[13]. Also overexpression of LAPTM4B was significantly correlated with poor prognosis in breast cancer, gallbladder cancer, ovarian cancer, HCC, gastric cancer and cervical cancer etc[12].

This study is aimed to detect the association of LAPTM4B gene polymorphism with prostate cancer in Iraqi population.

MATERIALS AND METHODS

This study is case-control study included 160 subjects divided into two groups; 80 patients with prostate cancer who visit Middle Euphrates Oncology Center (MEOC) for their routinely visiting periods for clinical examination, and for receiving chemotherapy and radiotherapy. The ages of patients ranged between 45_96 year.

The control group consisted of 80 obviously healthy subjects (without a history of any types of cancer). The ages of the control individuals ranged between 42_83 year.

The study was in accordance with ICH GCP Guidelines and ethical committee of University of Kufa approved the project protocol.

The practical part of the study was carried out in laboratory of Clinical Laboratory Sciences department / College of Pharmacy / University of Kufa.

Peripheral blood samples of prostate cancer patients and control groups were collected in EDTA-anticoagulated tubes, and then DNA was extracted from whole-blood samples using the genomic DNA extraction kit (Promega). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (Bio Drop, U.K).

Genotyping was performed by polymerase chain reaction (PCR) for LAPTM4B gene using thermo cycler (Bio metra, Germany). The sequence of primers used was: forward 5′′5′-GAGTTACACGAACGGCCAGA-3′ and reverse 5′ ATGTGACCCGAGTCCGTGA-3'.

Different trials of the reaction conditions revealed the optimal conditions that were used in the next steps of amplification reactions. Amplification was performed in a total volume of 25 µl contained 12.5 µl Go Taq Green Master Mix, (Promega Corporation, Madison, WI), 1.5 µl of each primer (1 Mm final concentration) (One Alpha, U.S.A), 3.5 µl nuclease free water, and 6 µl of DNA template. The reaction volume of 25 µl was putin 0.5 ml PCR tube at room temperature, then centrifuged in a micro centrifuge at 2000 xg for 30 seconds in order to mix the solutions well. After that, tubes were transferred to the thermo cycler. Cycling condition was 95°C for 5 min followed by 37 cycles of 94°C for 30s, 65.7°C for 30s, 72°C for 30s, and a final extension of 72°C for 10 min. The product was run on 3 % agarose gel. To determine genotyping error rate, we performed random duplication in 20 % of the samples.

Statistical analysis

Genotype and allele frequencies were compared using the $\chi 2$ statistics or the fisher's exact test. The Hardy Weinberg equilibrium was tested using the goodness-of-fit chi-square. Odds ratios were calculated by logistic regression. A p value less than 0.05 was considered statistically significant.

RESULTS

The PCR products for LAPTM4B gene were analyzed by 3 % agarose gel electrophoresis. Results revealed one band for LAPTM4B allele *1 at (162 bp) which is considered as wild type (*1/1) and another band, LAPTM4B allele *2 at (181bp) which is considered homozygous (*2/2). Also heterozygous LAPTM4B genotype (*1/2) was shown by UV documentation.

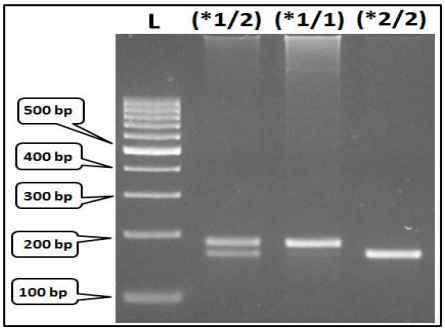

Figure 1: Genotyping of LAPTM4B gene

PCR products were analyzed by 3% agarose gel and there sizes were 162_bp for LAPTM4B*1 allele (deletion allele) and 181_bp for LAPTM4B*2 allele (insertion allele). UV documentation shows; L: DNA ladder ; (*1/1) : wild type allele,(*2/2) homozygous allele and (*1/2): heterozygous genotype.

The genotype and allele frequencies of LAPTM4B gene polymorphism are shown in Table 1. The frequency distributions of LAPTM4B genotypes were significantly different between Pca patients (47.5 % for *1/1, 35 % for *1/2, and 17.5 % for *2/2) and controls (30 % for *1/1, 45 % for *1/2, and 25 % for *2/2). The LAPTM4B genotype was associated with decreased risk of Pca in co-dominant (OR= 49 %, CI = 0.24 0.99, P = 0.049, *1/2 versus *1/1) and dominant OR = 47 %, CI = 0.24 _0.90, P = 0.024, *1/2 + *2/2 versus *1/1) inheritance models tested. The minor allele frequency (MAF) in cases and controls was 0.35 and 0.475, respectively Table 1. The LAPTM4B*2 allele significantly decreased the risk of Pca compared to LAPTM4B*1 (OR = 0.59 %, CI = 0.37_0.93, p = 0.023). In addition the LAPTM4B genotype was not associated with clinic pathological characteristics of Pca patients such as age, stage, prostate-specific antigen (PSA), dihydrotetosterone (DHT) and Gleason score.

Table 1: Genotype and allelic frequencies of LAPTM4B gene polymorphism among prostate cancer patients and controls

LAPTM4 B genotype	Cases n (%)	Controls n (%)	OR (95%CI)	p-value
Co-dominant				
LAPTM4B*1/1	38 (47.5)	24 (30)	————	———
LAPTM4B*1/2	28 (35)	36 (45)	0.49(0.24 0.99)	0.049
LAPTM4B*2/2	14 (17.5)	20 (25)	0.44(0.18 1.03)	0.060
Dominant				
LAPTM4B*1/1	38 (47.5)	24 (30)	————	———
LAPTM4B*1/2+*2/2	42 (52.5)	56 (70)	0.47(0.24 0.90)	0.024
Recessive				
LAPTM4B*1/1+*1/2	66 (82.5)	60 (75)	————	———
LAPTM4B*2/2	14 (17.5)	20 (25)	0.63(0.29 1.37)	0.248
Allele				
LAPTM4B*1	104 (65)	84 (52.5)	————	———
LAPTM4B*2	56 (35)	76 (47.5)	0.59(0.37_0.93)	0.023

DISCUSSION

Prostate cancer is more common in the western countries, least common in Asia, and the leading cause of cancer deaths in males' worldwide[10]. It is a growing concern in global epidemiology, where more than one million cases are diagnosed annually and the mortality burden has risen to over 300,000 deaths per year[15].

In the present study we examined the impact of LAPTM4B polymorphism on risk of Pca in a sample of the Iraqi population. Our findings revealed that LAPTM4B*2 significantly decreased the risk of Pca in our study population. To the best of our knowledge this is the first report describing LAPTM4B polymorphism and risk/protection of Pca in Iraqi population. Lysosome-associated protein transmembrane-4β (LAPTM4B) is a novel oncogene and LAPTM4B-35 protein was found to be overexpressed in various malignant tumors[16]. It can play critical roles in various solid tumors, including proliferation, migration, invasion, apoptosis, angiogenesis and motivated multidrug resistance through promoting drug efflux by interacting with P-glycoprotein and activating PI3K/AKT signaling pathway. In addition, new evidence has also revealed that LAPTM4B can participate in the autophagy initiation through binding with inactive epidermal growth factor receptor (EGFR)[17]. Levels of up regulated mRNA and LAPTM4B-35 protein were revealed to correlate significantly with pathological grades/differentiation of cancers as well as the outcomes of patients with hepatocellular, lung, breast, gall bladder, ovarian and prostate carcinomas[18]. Hashemi *et al.*, 2016 found that LAPTM4B gene polymorphism was not associated with the risk of Pca in Iranian male[11]. They found that the distribution of LAPTM4B*2/2 genotype as well as LAPTM4B*2 allele was significantly lower in the Pca patients compared to the normal subjects in their study. Also they revealed that LAPTM4B*2 significantly decreased the risk of Pca. Zhang *et al* 2014; showed that LAPTM4B-35 is over expressed in Pca and that high LAPTM4B-35 expression correlated with Pca progression and poor prognosis[19]. They concluded that overexpression of LAPTM4B-35 may serve as a new molecular marker to predict the prognosis of Pca patients. Previous studies have shown the activity of LAPTM4B-35 to be up regulated in a wide variety of cancers, including hepatocellular, lung, breast, ovarian, gallbladder, prostate and colorectal carcinomas[18]. Other studies showed no statistical differences between alleles for nasopharyngeal carcinoma, lung cancer, breast cancer, rectal or esophageal cancers, melanoma and pancreatic cancer[11]. It has beenshown that miR-188-5p, which acts as a tumor suppressor, inhibits Pca cell proliferation, invasion and migration through down regulation of LAPTM4B by directly binding to its 3"-UTR and subsequent inhibition of the PI3K/AKT signaling pathway, where decreased expression of miR-188-5p is associated with poor prognosis in patients with Pca, which strongly suggests a potential role of miR-188-5p in suppression of Pca[11].

Finally, our findings are the first to show an association between LAPTM4B polymorphism and risk of Pca in a sample of the Iraqi population. Further studies with larger samplesizes and different ethnicities are required to validate our findings.

CONCLUSION

Our findings suggest that The LAPTM4B*2 allele significantly decreased the risk of Pca compared to LAPTM4B*1 and my consider as protective factor in a sample of Iraqi population.

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