RESEARCH PAPER | Medical Science | Volume : 5 | Issue : 12 | December 2015 | ISSN - 2249-555X

Incidence of Polymorphism of GSTT1 Gene in Benign Prostatic Hyperplasia in Basra Province

KEYWORDS: Benign prostatic hyperplasia, Polymorphism, GSTT1 gene.

ASAAD MOHAMMED KADHIM — Surgery Dept. Uni. of Basra, Iraq.

Jihad Abdulameer Ahmed — Pathology Dept., College of Veterinary medicine, Uni. of Basra, Iraq.

ABSTRACT *Prostate tumor has become a major public health problem concern worldwide for its high morbidity and mortality levels, therefore It is the second leading cause of cancer related to death in the world, on the other hand the prostate tumor is a multiphase process that depend on various risk factors included genetic factors which play an important role in it initiation and progression, so that the GSTT1 gene polymorphisms might be involved in inactivation of procarcinogens that contribute to the genesis and2 progression of cancers. The methodology consist of DNA extraction from 40 patient of benign prostatic hyperplasia and 40 healthy served as controls, then done the amplification of GSTT1 gene to study the polymorphism of it. The results showed that 77.5% of patients of benign prostatic hyperplasia have polymorphism with GSTT1 null genotype when compared with controls. We concluded that the polymorphism of GSTT1 gene may considered as risk factor to initiate prostate tumor.*

INTRODUCTION

Prostate cancer is the most commonly diagnosed type of cancer among men and it remains the second leading cause of cancer deaths worldwide (Siegel et al., 2012). Both genetic and environmental factors are considered to play important roles in the occurrence of 2

prostate cancer, moreover, the etiology of prostate cancer remains unresolved, although genetic polymorphisms may play important roles in the genesis of prostate cancer (Wang et al., 2012). Therefore, prostate cancer is the most common cancer among men in industrialized countries with the main risk factor being the age of over 50. Prostate cancer is uncommon in men younger than 45, but becomes more common with increasing age. The average age at the time of diagnosis is 65 (Tewari, et al., 2004 ; Jang, et al., 2007).

Glutathione-S-transferases (GST) are phase II enzymes which are responsible for catalyzing the biotransformation of a variety of electrophilic compounds, and have therefore a central role in the detoxification of activated metabolites of procarcinogens produced by phase I reactions (Nebert and Vasiliou, 2004). The GSTT1 member of the multigene family are candidate cancer-predisposing genes. The relation of polymorphism in this gene to chemical carcinogenesis has been extensively studied in various populations. Several population-based studies have reported prevalence ranging from 13% to 25% for the GSTT1-null genotype among white Europeans (Rebbeck, 1997 ; Garte et al., 2001).

GSTs are generally involved in detoxification, but they are also important in activating and inactivating oxidative metabolites of carcinogenic compounds associated with causing prostate cancer (Gibbs et al., 1996). Combinations of various unfavorable deletion genotypes theoretically confer an even higher risk to patients with prostate cancer. An increased frequency of GST genotypes has been associated with several malignancies (Lee et al., 1998 ; Sreelekha et al., 2001). Some studies indicate that GST 3

polymorphisms are associated with prostate cancer (Gsur et al., 2001 ; Nakazato et al., 2003).

Therefore, in the present study, we attempt to determined the genotypic frequency of the genotype GSTT1 null polymorphism, to understand whether the GST polymorphisms are associated with the risk of benign prostatic hyperplasia in Basra province.

MATERIALS & METHODS

A total 40 patients of benign prostatic hyperplasia and equal number of healthy controls were studied from the periods between 15th January 2015 to 15th June 2015. The patients was confirmed diagnosis by clinical examination and histopathology which had a benign prostatic hyperplasia, then we take the history of smoking status, age and geographical location of the patients.

A 1.5 ml of blood was taken from the patients as well as controls and put in anticoagulant tube which immediately transferred to the central research unit in the college of Vet. Medicine / Uni. of Basra to extract the DNA from whole blood was performed by using Geneaid mini kit (Geneaid kit, USA), the steps of the method was according to the manufacturing company, and genes profile was carried out by electrophoresis according to Green and Sambrook, (2012). The genomic DNA was measured by using Nano drop spectrophotometer (Optizen / Korea) and the DNA purity measured depending on the sample absorbance at wavelengths 260nm. A ratio equal to 100µg/1µl considered a pure DNA according to (Ahmed, 2014).Then DNA stored at -20C° until genotype analysis.

A dultiplex polymerase chain reaction (dPCR) method was used to detect either the presence or absence of GSTT1 gene in the 4

genomic DNA samples and β- globin gene was co-amplified and used as an internal control in the same PCR tubes (Chen et al., 1996).

Genomic DNA (100 ng) was amplified in a total volume of 25 µl reaction mixture containing 25 pmol of each (GSTT1 gene: forward 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' and reverse 5'- TCA CCG GAT CAT GGC CAG CA-3', according to GenBank accession no. NM_000853) and 25 pmol β-globin gene primers (forward 5'-CAA CTT

Volume : 5 | Issue : 12 | December 2015 | ISSN - 2249-555X

CAT CCA CGT TCA CC-3′ and reverse 5′- GAA GAG CCA AGG ACA GGT AC-3′); 200 µmol/l deoxynucleoside triphosphates; 1 U of Taq polymerase in 10 × PCR buffer composed of 16.6 mmol/l (NH4)2SO4 and 20.0 mmol/l MgCl2, pH 8.8. After initial denaturation for 3 min at 94°C, 39 cycles were performed for 1 min at 94°C (denaturation), for 1 min at 60°C (annealing) and for 1 min at 72°C (extension), followed by a final step for 5 min at 72°C. The GSTT1 gene has (480 base pair) and β-globin gene has (268 base pair), the amplified products were visualized by electrophoresis on ethidium bromide-stained 2% agarose gel (Figure 1).

For deletions of GSTT1 gene, the no amplified products can be observed, whereas the β-globin gene specific fragment confirms the presence of amplifiable DNA in the reaction mixture.

RESULT & DISCUSSION

The study included 40 patients of benign prostatic hyperplasia and 40 controls. The distribution of the genotype of GSTT1 in control and benign prostatic patients is shown in Table (1).

Detection of GSTT1 null allele homozygote was carried out by sequence specific amplification using β-globin gene as an internal control. 5

Among patients with benign prostate hyperplasia were 31 (77.5%) had homozygous for the GSTT1 null allele as compared to the controls (Figure 1).

Figure (1): Show 2% Agarose gel which the PCR amplification of GSTT1 gene and β- globin gene, lane 1: DNA Marker, lane 2 and 3: Normal GSTT1 and β- globin genes of controls, Lane 4,5,6,7,8 and 9 null GSTT1 genes (Absent) in the benign prostatic hyperplasia.

Patients	Control	Table (1): Freguency distribution of GSTT1 genotypes and risk of prostate cancer. Genotype
40 case	40 healthy	GSTT1
4 (10%)	40 (100%)	Present
31(77.5%)	0 (0%)	Null
62 average	59 average	Age
27(67.5%)	2 (5%)	Smoking