p53 Variation Predisposition to Benign Prostate Hyperplasia

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Abstract: Benign Prostatic Hyperplasia (BPH) is the most common clinical condition related to aging in males over 40 years. The current study is a Case control includes 20 patients diagnosed with BPH by specialist in Al Kindy Teaching Hospital who theirs PSA under 4ng/ ml, and 20 healthy individuals as a control group. Blood samples were collected from both patients and healthy groups and DNA was extracted and subjected into PCR techniques then the products were digested by BstUI to identify codon 72 polymorphism, samples PCR products also subjected into direct sequencing to confirm the polymorphism and detect any further variation in intron 3. polymorphism at the codon 72 in 17 of 20 patients, 8 of the patients had the common homozygous alleles, 9 had heterozygous genotype and 3 had the recessive, while this polymorphism in codon 72 was detected only in 3 of 20 in control group. Complex duplication in intron 3 composed of 16 bp were detected in 17 patients of 20, eight of them had duplication of both alleles and 9 of them one allele duplicated. and one individual in control group had homozygous to the duplicated 16 bp, 2 had heterozygous and 17 were homozygous wild type.

Conclusion: P53 polymorphism in codon 72 and intron 3 sixteen bp duplication may have a significant role in promote hyperplasia in prostatic cells.

Key words: Benign Prostatic Hyperplasia (BPH), P53, codon 72 polymorphism.

I. Introduction

One of the most common clinical conditions related to ageing in men, is the Benign Prostatic Hyperplasia (BPH). It's characterized by highly proliferative epithelial and stromal cells leading to enlargement of prostate gland in one of four men over 40 years. The benign prostatic hyperplasia is considered as a heterogeneous disease and the etiology of it still not fully understood but several risk factors have a strong association with disease occurrence such as: the race, age of patient, androgens level, genetic factors, and overexpression of growth factors, obesity, metabolic syndromes and life style. The pathological process starts when the balance between proliferative and apoptotic cells is disrupted, as a response to elevated levels of circulating testosterone and underneath steroid intracellular signaling pathway via activation of androgen receptor (AR). In prostate cells, testosterone is converted into dihydrotestosterone the potent stimulator to prostatic stromal cells by 5α- reductase type 1 and type 2 leading to increase cellular proliferation. Other mechanisms elucidate the hyperplasia are androgen independents mechanisms include amplification of Androgen Receptor (AR) gene in prostate cells, stimulation of AR by nonsteroid ligand or blocking apoptosis by corruption of the normal function of tumor suppression gene such as P53. Cell cycle control is the main function of p53 protein which play crucial role in tumor suppression by leading DNA damaged cell into programmed cell death, this gene was found mutated in 50% of all cancer types. It's located on chromosome 17p13 and composed of 11 exons encode for 393 amino acids precisely distributed on five domains: a N-terminal domain transactivation domain, a proline-rich domain, a core DNA binding domain, a tetramerization domain and C-terminal regulatory domain. Several single nucleotide polymorphisms in different gene exons and mutations reflect both the genetic inherited lineage and mutability of specific codon under the stress of endogenous metabolites or exogenous mutagens. More than 10000 somatic mutations were
Variation in exon 4 of P53 were investigated by PCR and PCR-RFLP Technique as well as direct sequencing of PCR products to confirm codon 72 polymorphism and to detect other variation in exon 4. Exon 4 had polymorphism at the codon 72 in 17 of 20 patients. 8 of the patients had the common homozygous alleles (CCC) at the codon 72 that sequence encoded for Proline/Proline amino acids, 9 had heterozygous (C/C G/C) genotype which encode for Proline/Arginine and 3 had the recessive (CGC) genotype which encode for Arginine/Arginine amino acids. The polymorphism in codon 72 was detected only in 3 of 20 in control group as shown in figure(1) and table(1).

Exon 4 sequencing also showed a complex duplication in intron 3 (which was included in the amplified fragment) composed of 16 bp (CTGGGGACCTTGAGGG) were detected in 17 patients of 20, eight of them had duplication of both alleles and 9 of them one allele duplicated. and one individual in control group had homozygous to the duplicated 16 bp, 2 had heterozygous and 17 were homozygous wild type. as shown in figure (2) and table (4).
Figure 1: A Genotyping of exon 4 codon 72 polymorphism by PCR-RFLP, 415bp band represented the wild type, the heterozygous genotype represented by 3 bands 415,306bp and 109 bp bands, and the recessive genotype represented by 306 and 109 bands on agaros gel at 80v for 60minutes . B:is chromatogram of exon4 codon 72 , wild type (CCC),heterozygous(C C/G C) and recessive genotype (CGC).

Figure (2): A: duplication of 16 bp(CTGGGGACCTGGAGGG) in 3 different patients, B: the normal alleles.

Table(1): Numbers of different genotypes in studied groups studied samples.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotype Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample No.</td>
</tr>
<tr>
<td>Patients</td>
<td>20</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
</tr>
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</table>

*significant at P≤0.05.

Table(2): P53 Alleles frequencies in studied samples.

<table>
<thead>
<tr>
<th>groups</th>
<th>Allele Frequency</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sample No.</td>
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<tr>
<td>Patients</td>
<td>20</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
</tr>
</tbody>
</table>

NS: Not Significant at  P ≤0.05.

Table (3): P53 genotype frequencies in studied samples.

<table>
<thead>
<tr>
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</tr>
<tr>
<td>Controls</td>
<td>20</td>
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Table(4): percentage of patients with complex duplication in intron 3.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotype Frequency</th>
<th>Percentage of Patients with Complex Duplication in Intron 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heterozygous</td>
<td>Normal Both Alleles (%) For Duplication (%) P Value</td>
</tr>
<tr>
<td>Sample No.</td>
<td>Heterozygous</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Patients</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Controls</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

IV. Discussion

Benign prostate hyperplasia is the most common disease in males over 40 years. It had been proved that BHP is initiated by proliferation of epithelial cells in the prostate transition zone. The proliferation of prostate cells as a response to two major mechanisms which are: androgen dependent and androgen independent. Losing of cell control lead to continuous cell division in an associated to mutation in the P53 gene. Mutations in P53 gene affect p53 protein configuration which alter its ability to stimulate other genes located under its control to arrest cell cycle or induce apoptosis. Alterations in P53 always linked to poor prognosis in patients with prostate cancer or any other type of cancer. Proline rich domain (encoded by exon4) in p53 protein is a critical domain required for apoptosis promotion. In this study, patients and control individuals has a tendency to have Proline allele at the codon 72 rather than Arginine allele. Evolutionary, it was found that, Proline allele characterized by its higher activity in transactivation of Leukemia inhibitory factor expression which is important for blastocyst implantation in cold climates, while the Arginine allele is more effective in inducing apoptosis rather than Proline allele. Codon 72 also showed sharp ethnic distribution among populations, with north to south gradient, the lowest Proline allele frequency in Swedish saamis 0.17 to the highest in African 0.63. Codon 72 polymorphism has a controversial impact in different diseases and cancers in different populations. In an Iranian study on codon 72 polymorphism association in prognosis to prostate cancer patients it was found that 56.6% of patients carried the Proline/Proline alleles. Another Iranian study found that Iranian prostate cancer, had 9.9 fold increased risk associated with codon72 polymorphism in patients over 65 who had Proline/Proline alleles comparing with younger patients. While in Pakistani study on patients with prostate adenocarcinoma it was found that the Arginine allele at codon72 polymorphism was significantly associated with disease. In this study, patients and control individuals has a tendency to have Proline allele at the codon 72 rather than Arginine allele. Evolutionary, it was found that, Proline allele characterized by its higher activity in transactivation of Leukemia inhibitory factor expression which is important for blastocyst implantation in cold climates, while the Arginine allele is more effective in inducing apoptosis rather than Proline allele. Codon 72 also showed sharp ethnic distribution among populations, with north to south gradient, the lowest Proline allele frequency in Swedish saamis 0.17 to the highest in African 0.63. Codon 72 polymorphism has a controversial impact in different diseases and cancers in different populations. In an Iranian study on codon 72 polymorphism association in prognosis to prostate cancer patients it was found that 56.6% of patients carried the Proline/Proline alleles. Another Iranian study found that Iranian prostate cancer, had 9.9 fold increased risk associated with codon72 polymorphism in patients over 65 who had Proline/Proline alleles comparing with younger patients. While in Pakistani study on patients with prostate adenocarcinoma it was found that the Arginine allele at codon72 polymorphism was significantly associated with disease.

Also, in this study P53 intron 3 (PIN3) duplication of 16 bp was detected in 17(85%) of benign prostate hyperplasia patients, in 8 as homozygous for the duplication and 9 as a heterozygous for the duplication while it was found only in 3 individuals of the control groups, one as homozygous for the duplication and 2 as a heterozygous. These findings showed a significant association to the benign prostate hyperplasia in Iraqi patients. P53 intron 3 duplication of 16 bp (CTGGGGACCTGGAGGG) was found associated to esophagus, gastric cancer and breast cancer as it leads to lower level of P53 transcription.

V. Conclusion

P53 polymorphism in codon 72 and intron 3 sixteen bp duplication may have a significant role in promote hyperplasia in prostatic cells as intron 53 duplication lowering P53 transcription and leading to its loosing of its normal function in arrest cell cycle in DNA damaged cells. And as the dominant of Proline/Proline genotype in benign prostatic hyperplasia as this genotype less active in promote apoptosis in DNA damaged cells than Arginine allele, leading to accumulation of mutation in the genome and reduce its stability. These variations impact may be prognostic marker to BPH.

References


