Alpha-1 Antitrypsin Genotypes in Breast Cancer Patients

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Proteolytic enzymes play a significant role in malignancy including, loss of growth regulation, invasiveness and metastases formation. Alpha-1 antitrypsin (A1AT) is a secretory glycoprotein produced mainly in liver and monocytes. It is the most abundant serine protease inhibitor in human plasma. Deficiency of A1AT is an inherited disorder characterized by reduced serum level of A1AT. Protease inhibitor Z (PiZ) and protease inhibitor S (PiS) are the most common deficient genotypes of A1AT. The association of deficient A1AT subtypes with several tumors such as primary liver carcinoma, lung cancer, bladder cancer and malignant hepatoma was reported. This study was aimed to test the relationship between A1AT genotypes Z and S and breast cancer in Jordanian female patients. Blood samples were collected from 111 patients. DNA was isolated and polymerase chain reaction (PCR) was performed to amplify the regions contain the Z and S mutations in exon V, and III, respectively. Genotyping of Z and S alleles was performed by restriction fragment length polymorphism technique using *Taq*1 restriction enzyme. Our results demonstrated that 100% of the breast cancer patients were homozygous for the normal allele Protease inhibitor MM (PiMM) and no PiZ and PiS genotypes were found. In conclusion, there is no relationship between A1AT deficient genotypes Z and S and breast cancer in Jordanian female patients.

Key words —— alpha-1 antitrypsin, cancer, breast, genotypes

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INTRODUCTION

Alpha-1 antitrypsin (A1AT) is one of the serine protease inhibitors (serpins), with a molecular weight of 52 kDa. Therefore, it is relatively small and can diffuse into tissue fluids. Its primary physiological target is neutrophil elastase, a powerful protease capable of cleaving the elastin of the alveolar walls and many other structural proteins.[1)] A1AT is the most abundant of the anti-proteases in human serum. A1AT gene is a single gene of 12.2 kb located on chromosome 14 at q31-32.3. The two major sites of A1AT gene expression are hepatocytes and the mononuclear phagocytes. The A1AT gene is highly polymorphic. Using molecular biology techniques a number of A1AT alleles have been identified. These alleles are categorized into four groups based on the status of A1AT protein levels in serum, including; normal alleles, produces A1AT molecules that function normally and have a normal serum level (150 to 350 mg/dl), deficient alleles, associated with serum level <35% of normal (A1AT protein may or may not function normally), null alleles, with no detectable A1AT protein in serum, dysfunctional alleles, coding for A1AT molecules present in normal amount but do not functioning normally.[2, 3)]

Normal allele of A1AT (M) is the most common. The deficient alleles Z and S represent much smaller portion of A1AT variants.[4)] A1AT serum levels of individuals, homozygous for a Z gene, are reduced to 15 to 50 mg/dl. The exons sequence of Z gene is the same as that of the M1(Ala213) gene, except for the substitution of a lysine for a glutamate at position 342 due to a single base substitution in the gene sequence.[5)] The Z allele is not only associated with reduced serum levels of A1AT, but generated protein does not function normally as an inhibitor for neutrophil elastase.[1, 2)] The S variant is more common than the Z variant. In its homozygous form it is associated with A1AT serum level of 100 to 200 mg/dl. However, when inherited with the Z variant or with a null variant, the A1AT serum levels are sufficiently low which might increase the individual risk for a disease.[2, 3)] The sequence of the exons of the S genes differs from the M1(Val213) gene at a single base in exon III, resulting in the amino acid substitution Glu264 to Val. A1AT synthesizing cells of Protease inhibitor SS (PiSS) individuals, secret approximately 40 percent of the amount of A1AT that secreted by normal cells. Unlike the Z mutation, the S mutation is not

associated with accumulation of A1AT in the synthesizing cells.[2, 3] The serum deficiency occurs in S homozygotes because the newly synthesized protein is unstable and destroyed intracellular shortly after its synthesis. Unlike the Z protein, the S protein once secreted it functions normally as a neutrophil elastase inhibitor.[1, 2] Mutations in A1AT gene have been associated with a number of diseases including renal disease, arthritis and malignancies, but the major associations are with lung and liver diseases.

Proteolytic enzymes were shown to have a significant role in the expression of the malignant phenotypes including, loss of growth regulation, invasiveness and formation of metastases. Tumor cell-derived proteases have multiple activities and have been shown to degrade basement membrane components, stimulate angiogenesis and promote cell proliferation and migration.[6] Serum level of A1AT was found to be increased in malignant diseases such as: Gastrointestinal cancer, prostate cancer, brain tumors, biliary tract cancer, pancreatic adenocarcinoma and breast cancer.[7–12] A significant correlation between A1AT serum levels and cancer stages was also observed.[7, 13] An imbalance in the ratio between the levels of particular protease and protease inhibitor found to be responsible for the increase in tumor formation potential.[6, 7, 14] Consequently, individuals with A1AT deficient alleles (Z, S and null) may be at greater risk of tumor invasion.[15] Some reports have shown the association between deficient A1AT subtypes and several tumors such as primary liver carcinoma, lung cancer and bladder cancer.[14, 16, 17] Homozygotes with A1AT deficiency type protease inhibitor Z (PiZ) were shown to be associated with an increased risk of chronic liver disease and liver carcinoma.[14] Zhou *et al.*, have observed that PiZ heterozygotes are associated with an increased risk of primary liver carcinoma.[14] Scheele *et al.*, have presented a case of bile duct adenomas in association with heterozygous (MZ) deficiency of A1AT.[15] Benkmann *et al.*, have shown a significant increase of the PiZ allele in bladder cancer patients. Furthermore, a distinct association between a lowered M3 allele and bladder carcinoma was observed.[17] Yang *et al.*, have studied A1AT deficiency allele carrier among lung cancer patients. They found that 32 out of 260 lung cancer patients are carriers for A1AT deficient allele. Twenty-four of the 32 carriers have allele S, 6 have allele Z and 2 have allele I.[16] The main goal of this study is to test the relationship between A1AT genotypes Z and S and breast cancer in Jordanian female patients.

MATERIALS AND METHODS

One hundred and eleven breast cancer patients were included in this study. These patients were attending King Hussein Medical Center (K. H. M. C.), Princess Basma and King Abdullah teaching hospitals during 2003–2004. Blood samples were collected from the median vein in EDTA tubes and transported to our lab in icebox. Blood samples were processed immediately or were stored in the refrigerator at 4°C for no longer than 24 hr then used to extract DNA. Genomic DNA was isolated from the whole blood using a commercially available Promega kit (Promega, Madison, Wisconsin U.S.A.) following the manufacturer instructions and stored at −60°C until used. Oligonucleotide primers that used for the polymerase chain reaction (PCR) amplification of S and Z genes were purchased from Alpha DNA (Montreal, Canada). To identify Z allele the following pairs of primers were used; forward 5′-TAAGGCTGTGCTGACCATCGTC-3′, reverse 5′-CAAAGGGTTTGTTGAACTTGACC-3′ and to identify S allele; forward 5′-TGAGGGGAAACTACAGCACCTCG-3′, reverse 5′-AGGTGTGGGCAGCTTCTTGGTCA-3′. The following PCR amplification profile was applied, initial denaturation step, one cycle, at 94°C for 1 min followed by 39 cycles, each cycle was consisting of denaturation step at 94°C for 30 sec, Annealing step at 59°C for 30 sec, extension step at 72°C for 30 sec and the final extension step at 72°C for 2 min.

A1AT genotyping for S and Z alleles was performed by restriction fragment length polymorphism (RFLP) using *Taq*1 restriction enzyme (Promega). Restriction enzyme digest was performed following the manufacturer instructions. Three percent agarose gel contained the ethidium bromide was used to separate the *Taq*1 treated PCR product. Images for the gels were taken using UV scanner connected to a computer system. This work was approved by the University Review Committee for Research on Humans.

RESULTS AND DISCUSSION

PCR product that was generated using Z primers is 110 bp long. The treatment of this product with

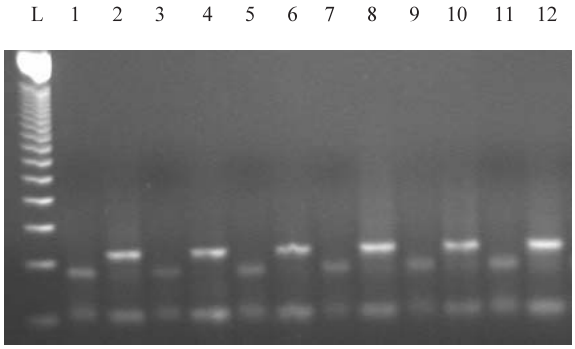

Fig. 1. A Representative Agarose Gel, Shows the *Taq*1 Digest of the PCR Product That was Amplified from Exon V of A1AT Gene Using Z Specific Primers

Lanes 2, 4, 6, 8, 10, and 12 are untreated PCR products with the full length of 110 bp. Lanes 1, 3, 5, 7, 9, and 11 are treated PCR products with *Taq*1 enzyme. L: is a 50-bp DNA ladder.

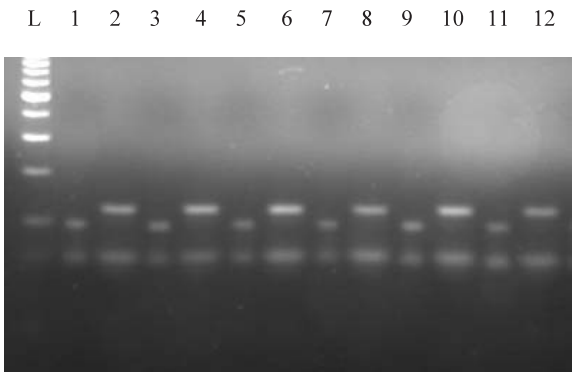

Fig. 2. A Representative Agarose Gel, Shows the *Taq*1 Digest of the PCR Product That was Amplified from Exon III of A1-AT Gene

Lanes 2, 4, 6, 8, 10, and 12 are untreated PCR product with the full length of 121 bp. Lanes 1, 3, 5, 7, 9, and 11 are PCR product treated with *Taq*1 enzyme. L: is a 50-bp DNA ladder.

*Taq*1 restriction enzyme might result in either one uncut fragment with 110 bp or in two fragments with 89 bp and 21 bp. The presence of two fragments indicates that there is a restriction site for *Taq*1 within the amplified piece of exon V that presented only in the case of the wild type gene, the M genotype. The Z gene is the mutated form of the M gene and this mutation results in the disappearance of the restriction site for the *Taq*1 enzyme. In this case, the treatment of the PCR product with the restriction enzyme will give one uncut fragment. Visualizing the gels of the patient's samples we saw only the fragmented PCR product with no single sample with the full length. This indicated that all our samples are of M genotype and no Z alleles (Fig. 1).

PCR product that was generated using S primers is 121 bp long. The treatment of this product with *Taq*1 restriction enzyme might result in either one uncut fragment with 121 bp or in two fragments with 100 bp and 21 bp. The appearance of two fragments in the gel indicates that there is a restriction site for *Taq*1 within the amplified piece of exon III that presented only in the case of the wild type gene, the M genotype. The S gene is the mutated form of the M gene. This mutation results in the disappearance of the restriction site for the *Taq*1 enzyme and the treatment of this PCR product will give only the full length polynucleotide (Fig. 2). Thus, obtained data demonstrated that normal M genotype is present in all breast cancer patients and no Z or S deficient genotypes. These results came to confirm what was reported by Hitzeroth *et al.*, in South Africa. They found that A1AT phenotypes and gene frequency distributions in breast cancer patients were similar to those of healthy controls.[18] Gracia-Orad *et al.*, also showed that A1AT deficient phenotypes in breast cancer patients appeared in similar frequencies compared to those of the control samples.[19] In conclusion, there is no relationship between A1AT deficient genotypes Z and S and breast cancer in Jordanian female patients.

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