Relationship between alpha-1 antitrypsin deficient genotypes S and Z and lung cancer in Jordanian lung cancer patients

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ABSTRACT

Objective: Alpha-1 antitrypsin (α1-AT) is a secretory glycoprotein produced mainly in the liver and monocytes. It is the most abundant serine protease inhibitor in human plasma. It predominantly inhibits neutrophil elastase thus, it prevents the breakdown of lung tissue. The deficiency of α1-AT is an inherited disorder characterized by reduced serum level of α1-AT. Protease inhibitors Z (PiZ) and protease inhibitors S (PiS) are the most common deficient genotypes of α1-AT. The aim of this study is to test the relationship between α1-AT deficient genotypes S and Z and lung cancer in Jordanian lung cancer patients.

Methods: We obtained the samples used in this study from 100 paraffin embedded tissue blocks of the lung cancer patients from Prince Iman Research Center and Laboratory Sciences at King Hussein Medical Center, Amman, Jordan. Analyses of the Z and S genotypes of α1-AT were performed by polymerase chain reaction and restriction fragment length polymorphism techniques at Jordan University of Science and Technology during 2003 and 2004.

Results: We demonstrated that all lung cancer patients were of M genotype, and no Z or S genotypes were detected.

Conclusion: There is no relationship between α1-AT deficient genotypes S and Z and lung cancer in patients involved in this study.

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Serine protease inhibitors are a group of glycoproteins that predominantly function as inhibitors of serine proteases. These glycoproteins constitute the third major protein component of blood plasma after albumin and immunoglobulins.[1] Alpha-1 antitrypsin (α1-AT) primarily binds neutrophil elastase (NE) and therefore, prevents the breakdown of the elastic tissues, mainly of the lung protecting the alveolar matrix from the destruction by NE.[2-4] Liver is the predominant site of α1-AT expression. This protein is also synthesized in blood monocytes and macrophages, alveolar macrophages and intestinal epithelial cells.[1]

Alpha-1 antitrypsin deficiency, which accompanied with a decrease in its plasma level is associated with 2 alleles, designated PiZ and PiS. Compared with the wild type PiM allele, the PiS genotype is characterized by AT substitution in exon III that codes for a change of glutamic acid to valine at position 264 of the protein. The PiZ genotype is caused by a Guanine to Adenine (GA) substitution in exon V that code for a change of glutamic acid to lysine at position 342 of the protein.[5-7] The α1-AT deficiency affects essentially all racial subgroups worldwide.[8] It has been demonstrated in laboratory and clinical

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research that a deficiency in α1-AT is associated with increased risk of liver cancer, bladder cancer, gall bladder cancer, malignant lymphoma and lung cancer.[9] Yang et al,[10] demonstrated that Caucasian patients with lung cancer, both smokers and nonsmokers, were more likely to carry α1-AT deficient alleles than other Caucasian population in the United States. Specifically, patients with squamous cell or bronchi-alveolar carcinoma were much more likely to be carriers than expected. On the other hand, high plasma levels of α1-AT were found to be associated with different types of carcinoma, respiratory infections, and smoking. It has been reported that lung cancer patients had significantly high mean plasma levels of α1-AT than those with other malignancies.[11-21] Marks et al,[22] reported that carrying α1-AT deficient genotypes might significantly associated with lung cancer risk. Based on the above stated facts, we studied the relationship between α1-AT deficient genotypes S and Z and lung cancer in Jordanian lung cancer patients. If a positive relationship was found, this will be very important in the screening as well as in the early diagnosis of lung cancer.

Methods. One hundred paraffin embedded tissue blocks from 83 patients with non small- and 17 patients with small- cell lung carcinoma were used in this study, conducted during 2003-2004. These samples were obtained from the Jordanian lung cancer patients admitted in King Hussein Medical Center, Amman, Jordan during 1999-2003. Tissue samples were taken by bronchial biopsies. The mean age of the patients was 64 years. The patients were consisted of 86 males and 14 females. For DNA isolation, at least 5 sections of 10 μm thickness from each specimen were cut using microtome equipped with steel knife. The section's thickness was maintained depending on the size of the tissue in the block. Sections were transferred to a 0.2 ml microtube. Genomic DNA was extracted using DNA extraction kit (EXTRAFFIN kit, Amplimedical, Canada, Torino) special for DNA extraction from paraffin embedded tissues. Detection of the PiZ allele was performed by a polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) methods. For PCR amplification of the region within exon V that contains the Z mutation, the following set of primers was used: 5'TAAGGCTG-TGCTGACCATCGTC3'as forward primer and 5'CAAAGGGTTTGTTGAACTTGACC 3'as reverse primer. The PCR was performed in 20 μl final volume containing, 1 μM forward primer, 1 μM reverse primer, 200 mM of each DNTP, 1.5 mM Mg^{2+}, 100 ng of DNA template, and 0.5 U of Taq DNA polymerase. The PCR amplification conditions were as follow: one cycle at 94°C for one minute as a first denaturation step followed by the following amplification profile, 40 cycles at 94°C for 30 seconds (denaturation step), 59°C for 30 seconds (annealing step), 72°C for 10 seconds (extension step), and one cycle at 72°C for 2 minutes (final extension step). The amplified product (110 bp) was digested at 65°C with 10 U of Taq1 restriction enzyme for 3 hours. The Taq1 enzyme is expected to cut the normal allele (M) but not the Z allele due to the disappearance of the restriction site in the result of the mutation that occurred in the M allele at the site of restriction. The M allele is expected to give 2 fragments (89 bp and 21 bp) but, Z allele must give only one fragment (110 bp) in the result of the treatment with Taq1 restriction enzyme. Detection of the S allele in exon III was performed by a modification of the PCR method described by Dahl et al[23] using a set of primers specific for S mutation: 5' TGAGGGGAAACTACAGCACCTCG 3' forward primer and 5' CGGTATCCATTGATTA-GACTGAA 3' reverse primer and the PCR was proceeded in 20 μl final volume containing, 0.5 μM of the forward primer, 0.5 μM of the reverse primer, 200 mM of each DNTP, 1.5 mM Mg^{2+}, 100 ng of DNA template, and 0.5 U of Taq DNA polymerase. The PCR amplification conditions were as stated above for the Z allele. The amplified product (121bp) was digested at 65°C with 10 U of Taq1 restriction enzyme for 3 hours. The Taq1 enzyme is expected to cut the normal allele (M) but not the S allele due to the disappearance of the restriction site in the result of the mutation that occurred in the M allele at the site of restriction. The M allele is expected to give 2 fragments (100 bp and 21 bp) but S allele must give only one fragment (121 bp) in the result of the treatment by Taq1 restriction enzyme.

Results. We determined the alpha-1 antitrypsin genotypes by PCR followed by RFLP methods. As demonstrated on the gel, the PCR product that generated using Z primers is 110 bp long and the treatment of this PCR product with Taq1 restriction enzyme resulted in 2 fragments with 89 bp and 21 bp long. This indicates that there is a restriction site for Taq1 enzyme within the amplified piece of exon V that presented only in the case of the wild type gene, M genotype, as the Z gene is the mutated form of the M gene in which the restriction site for Taq1 enzyme is vanished (**Figure 1**). The PCR product that generated using S primers is 121 bp long. Treatment of this PCR product with Taq1 restriction enzyme resulted in 2 fragments with 100 bp and 21 bp long. This again indicates that the restriction site that should be present in the PCR product in exon

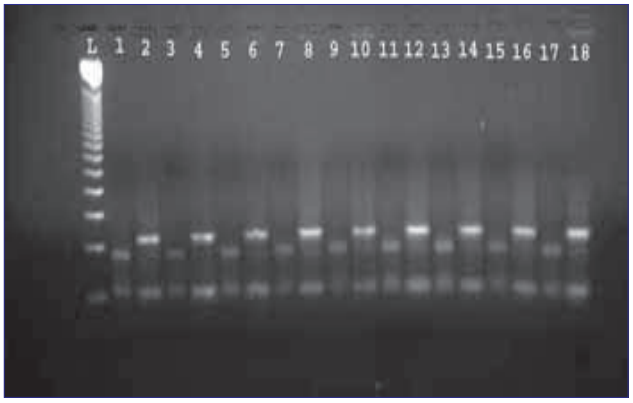

Figure 1 - Restriction fragment length polymorphism of polymerase chain reaction (PCR) product for the mutant Z genotype of α1-AT. Taq1 digest of the PCR product that was amplified from exon V of α1-AT gene. Lanes 2,4,6,8,10,12,14,16 and 18 are untreated PCR products. Lanes 1,3,5,7,9,11,13,15 and 17 are PCR products treated with Taq1 enzyme. L=50-bp DNA ladder. The yield products are 89-bp fragment from the M allele and 110-bp fragment from the Z allele.

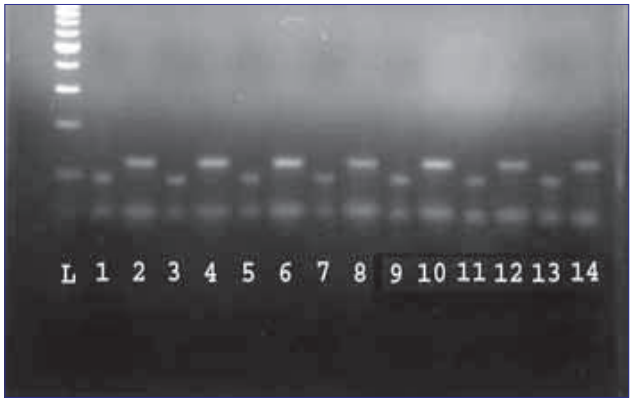

Figure 2 - Restriction fragment length polymorphism of polymerase chain reaction (PCR) product for the mutant S genotype of α1-AT. Taq1 digest of the PCR product that was amplified from exon III of α1-AT gene. Lanes 2,4,6,8,10,12 and 14 are untreated PCR product. Lanes 1,3,5,7,9,11 and 13 are PCR product treated with Taq1 enzyme. L=100-bp DNA ladder. The yield products are 100-bp fragment from the M allele and a 121-bp fragment from the S allele.

III of the wild type gene, M genotype, is there and no mutation has occurred (**Figure 2**). Concerning the small size product that resulted from the digestion process (21bp), it could not be visualized as it was washed out of the gel. Thus, all tested samples in the study were homozygous for the M allele and we detected no Z or S alleles.

Discussion. Alpha-1 antitrypsin is the most abundant serine protease inhibitor in human plasma. Its plasma levels were reported to be increased in many human tumors including lung carcinoma.[11-13] This fact stimulated many researchers to look into the importance of α1-AT in tumor biology and the role that this glycoprotein plays in tumorigenesis. A study performed by Yang et al,[10] where they reported the increase in the α1-AT deficient allele carrier's rate among newly diagnosed lung cancer patients in USA was a stimulant for this work. Yang et al,[10] suggested that the individuals who carry the α1-AT deficient alleles have an increase risk for developing lung cancer and particularly squamous cell and bronchi-alveolar carcinoma. Our results indicated that all studied cases with lung cancer have normal genotype (MM) and with no Z or S mutated genotypes. These results are in disagreement with what have been reported by Yang et al[10] This might be explained by the difference in the ethnic background of our studied group. De Serres,[24] reported that there are different gene frequencies of PiM, PiS, and PiZ among different ethnic background in healthy population worldwide. He found that the worldwide frequencies of these alleles were as follows: in USA, PiM was 0.9639, PiS was 0.0266, and PiZ was 0.0056; Canada, PiM was 0.9419, PiS was 0.0390 and PiZ was 0.0129; Sweden, 0.9663 for PiM, 0.0153 for PiS and 0.0151 for PiZ; Japan PiM was 0.9964, PiS was 0.0004 and PiZ was 0.0002; Australia 0.9407 for PiM, 0.0421 for PiS and 0.0122 for PiZ; in Saudi Arabia, PiM was 0.9254, PiS was 0.0333 and PiZ was 0.0220. In addition, he elicited that in Jordanian population this frequency is 0.9927 for PiM, 0.0083 for PiS, and 0.0000 for PiZ. These data came to confirm what we found in our work in term of MM genotype being the dominant one in lung cancer patients. As reported by Kamboh et al,[25] among 4,907 healthy individuals obtained from the Far East population, a 0% value of both S and Z alleles was found. Our results were in agreement with Christopher et al,[26] who reported that the Z and S alleles were very rare alleles among Asian population and the MM genotype of α1-AT is the dominant allele. He found out that 99.8% of Chinese have the MM genotype, 0.5% of MZ, and 0.5% of MS heterozygotes. The SS homozygotes were of 0.25% and ZZ homozygotes were 0.3%. The same author indicated in the same study that northern Europeans have a higher prevalence of the Z genotype than southern Europeans. He also showed that the percentage of Z mutations ranged from 1-4% and S mutations ranged from 5-10% in most Caucasians population. De la Roza et al,[27] reported that 0.37% of unselected patients with chronic obstructive pulmonary disease have a deficient Z allele and 0.3% have the SZ deficient genotype. Our findings are in

accordance with what have been reported earlier by Harris et al,[28] concerning the distribution of α1-AT alleles. His results indicated that there were no difference in the distribution of these alleles between lung cancer patients and a control group. It has been suggested by Harris et al,[28] that deficiency of α1-AT does not increase the risk of lung cancer, which is in accordance with what we found.

In conclusion, our results did not indicate any association between α1-AT deficient alleles (Z or S) and lung cancer in Jordanian lung cancer patients.

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