Alpha–1 antitrypsin phenotypes in patients with lung, prostate and breast cancer

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ABSTRACT

Objectives: Determination of Alpha$_1$-antitrypsin (α_1-AT) phenotypes in Jordanian patients with lung, prostate and breast cancer to find a prevalent phenotype that could be recommended for the early diagnosis of cancer.

Methods: This study was conducted at Jordan University of Science and Technology, Irbid, Jordan, during the period May 2001 to May 2002. Alpha$_1$-AT phenotypes for 83 Jordanian cancer patients distributed as follows, 25 lung cancer, 25 prostate cancer and 33 with breast cancer, were tested using isoelectric focusing gel electrophoresis and immunofixation techniques.

Results: Isoelectric focusing results demonstrated that 96% of lung cancer patients were of PiMM phenotype and 4% of PiFM phenotype. All prostate cancer patients (100%) were found to be of PiMM phenotype. Phenotypes of breast cancer patients were 94% PiMM, 3% PiFM and 3% PiMS.

Conclusion: These findings demonstrated that there were no significant differences in the distribution of α1-AT phenotypes among Jordanian patients with lung, prostate and breast cancer and they matched those reported for healthy individuals. Thus, we cannot recommend a given α_1-AT phenotype for early diagnosis of the above mentioned types of cancer.

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Human Alpha$_1$-antitrypsin (α_1-AT) is the major component of α_1-globulin electrophoresis band of the plasma protein. It is known as α_1 protease inhibitor (α_1-Pi). It inhibits serine proteinases and acts as an acute phase glycoprotein.[1] Alpha$_1$-AT is the principle inhibitor of leukocyte elastase, trypsin, chymotrypsin, cathepsin G, plasmin, thrombin, tissue kallikrein, factor Xa, plasminogen and proteinase III.[2,3] The molecule of α_1-AT is relatively small and polar; it is comprised of single polypeptide chain of 394 amino acids with 3 N-asparaginyl-linked complex type carbohydrate side chains giving it. A total molecular weight of 52 kDa.[1] Electrofocusing of α_1-AT showed multiple banding that demonstrated the variations in its carbohydrate structure.[4] Alpha$_1$-AT variants are classified alphabetically by the Pi nomenclature, in order of their migration on acid starch electrophoresis or isoelectric focusing at pH 4.2-4.9.[1,5] The majority of the population has the M allele.[4,6] The variants, which are associated with deficiency, are the Z and S mutants. Deficient phenotypes of α1-AT have been associated with a number of diseases including renal disease, arthritis, malignancies, and the strongest association is with lung and liver diseases.[1,8] Homozygous and heterozygous α_1-AT deficient phenotypes (PiZZ, PiSS, PiMZ, PiSZ) are known to be associated with increased risk of liver cirrhosis, primary liver carcinoma (PLC), chronic obstructive pulmonary

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disease (COPD) and emphysema, hepatocellular carcinoma (HCC), lung cancer specifically squamous cell or bronchoalveolar carcinoma and other diseases.[1,4,7,9-12] A significant increase in the serum PiZ allele was shown in bladder cancer patients.[13] MZ phenotype was found in liver carcinoma, hepatocellular carcinoma, and liver cirrhosis.[14-16] FM phenotype was found to have a positive correlation with PLC.[14] However, the results of several studies which were carried out on patients with different types of malignant diseases such as breast cancer, hepatocellular carcinoma and gastric carcinoma have shown phenotype distribution and allele frequencies similar to those of normal subjects.[17-19] In this study, we tested the α_1-AT phenotypes in lung, prostate and breast cancers. The aims of this study were to determine α_1-AT phenotypes in Jordanian patients with lung, prostate and breast cancer and to find if there is a correlation between the phenotypes and the above stated types of cancer to be used in the early diagnosis of these tumors.

Methods. Pharmalyte pH 4.2-4.9, acrylamide, N,N-methylenebisacrylamide, ammonium persulfate, triton X-100, was purchased from SIGMA Chemical Co. (Saint Louise, Missouri, United States of America, [USA]). N,N,N,N-tetramethylethylenenediamine, glycine, sulfosalicylic acid, coomassie Blue (R250), were obtained from Fluka Chemie AG) The following chemicals were obtained from sources that indicated for each: Na2EDTA (Promega Madison, USA), L-cysteine HCl (Scharlan), phosphoric acid (850g/L analytical grade), trichloroacetic acid (Riedel-de Haen), methanol, glacial acetic acid, glycerol, α1-AT antiserum (IgG-fraction, DAKO, Denmark), cellulose acetate membrane (Sartorius GmbH, West Germany). Flatbed electrophoresis apparatus (Multiphor 2117, FBE-3000- Pharmacia) was used for isoelectric focusing. Blood samples were obtained from 83 patients with 3 different types of cancer, 33 with breast cancer, 25 with lung cancer, and 25 with prostate cancer at the Radiotherapy and Chemotherapy Treatment Department in Albashir Hospital. Venous blood was drawn into plain tubes. Serum was separated and stored in small aliquots at -20˚C. To reduce the risk of bacterial contamination, serum was treated with sodium azide (0.2g per liter final concentration). Before isoelectric focusing was commenced, serum specimens were treated with cysteine by mixing 90µl of serum with 10µl of cysteine-HCl solution and incubating overnight at 4˚C. This treatment of samples with cysteine reduces and blocks the active thiol groups in α_1-AT that increases the stability of α_1-AT and increases the sharpness of the bands. Isoelectric focusing to determine the α_1-AT phenotypes was performed using polyacrylamide gel electrophoresis as described by John et al.[20] Statistical analysis was performed using Chi-square test.

Results. Phenotyping of α_1-AT variants in cancer patients was performed by isoelectric focusing pH 4.2-4.9 on polyacrylamide gels and was followed by immunofixation on cellulose acetate membrane. Isoelectric focusing results demonstrated that 96% of lung cancer patients were of PiMM phenotype and 4% of PiFM phenotype. All prostate cancer patients (100%) were found to be of PiMM phenotype. Phenotype of breast cancer patients were 94% PiMM, 3% PiFM and 3% PiMS, **Table 1**.

Statistical analysis was carried out to test the difference in the distribution of α_1-AT phenotypes among the 3 types of cancer. It was found that there is no significant difference between the 3 groups. The phenotype homozygotes (MM) is the prevalent one in all 3 groups. The incidence of the MM phenotype among the 83 cancer patients was 96.4%. Other phenotypes have shown low frequency distribution 3.6%.

Discussion. Proteolytic enzymes play an important role in cancer physiology, but the role of the body's natural inhibitors of these enzymes in this process is not very well studied. Alpha$_1$-AT is the major serine protease inhibitor in plasma. Various studies were performed on the behavior of α_1-AT in different types of human cancer such as lung, breast, liver, prostate, pancreas, cervix, and colorectal cancer.[21-25] It has been suggested that α1-AT deficiency may favor the invasion of healthy tissue by neoplastic cells, since some tumors are capable of synthesizing and excreting protease to facilitate invasion during neoplasia. The α_1-AT would protect the organism either in the area next to the tumor by opposing its growth or by inhibiting the circulating proteases. Consequently, individuals having α_1-AT deficient alleles (null, S and Z) may be at greater risk of neoplastic invasion due to their lower capacity to protect the organism from the proteolytic enzymes. Therefore, in the last 30 years the increase interest to study the various phenotypes of α_1-AT, especially the deficient types, and their association with cancer has been noticed.[13-15,21,26] The majority of these reports were on hepatocellular carcinoma and primary liver cancer. These studies demonstrated that individuals with either homozygous or heterozygous deficiency of α1-AT might be at risk of having HCC and PLC.[13,27-29] Bladder cancer patients were studied as well and it was found that they have an increase in PiZ allele.[13] Yang et al[12] have studied 260 lung patients and found that 32 patients carried deficient α1-AT, S and Z, alleles. Therefore, they suggested that deficient α_1-AT

Table 1 - Alpha$_1$ antitrypsin phenotypes in Jordanian patients with lung, prostate and breast cancer.

α_1-AT phenotype	Lung cancer patients n=25 %	Prostate cancer patients n=25 %	Breast cancer patients n=33 %
MM	96	100	94
FM	4	-	3
MS	-	-	3

α_1-AT - Alpha$_1$ antitrypsin, MM - homozygotes, FM - heterozygotes, MS - the appearance of M and S (heterozygotes) in the gel.

carriers might have an increased risk of developing lung cancer, especially squamous cell or bronchoalveolar carcinoma.[12] The main goal of our work was to determine the prevalent α_1-AT phenotype among Jordanian patients with lung, prostate and breast cancer. Our results demonstrated that the phenotype distribution frequency among the 83 cancer patients studied is in agreement with that reported in Europe on healthy population. It has been reported that PiMM phenotype distribution is 86-99%. This range of distribution was found to be dependent on which population was studied.[4]

Our findings confirm the results of Garcia-Orad et al[17] who worked on breast cancer patients of Basque population and Lu et al[19] who worked on gastric cancer patients in Shanghai area. Both investigators found that there were no significant differences in phenotype frequencies between the patients and the controls. Also, Hitzetoth et al[30] have demonstrated that in 176 white south Africans breast cancer patients that were studied, the Pi phenotype and gene frequency distributions were found to be similar to those of healthy controls. Labadie et al[31] have studied 66 French patients with hepatocellular carcinoma and were compared with 1030 healthy controls. Their results have shown no significant differences between the 2 groups for the α_1-AT phenotype. Govindarajan et al,[32] results had shown that the prevalence of PiMZ phenotype in hepatocellular carcinoma patients was very close to that of normal control population (4% in HCC and 3% in controls).[32]

In conclusion, α_1-AT phenotypes distribution among patients with lung, prostate and breast cancer in Jordan is in agreement with what have been reported in other regions of the world and is similar to that reported for healthy population. Thus, we cannot recommend α_1-AT phenotypes as diagnostic tumor marker in the above stated tumors.

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