# ALPHA-1-ANTITRYPSIN IN SAUDI POPULATION

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*SUMMARY: Alpha-I-antitrypsin ($\alpha_1$AT) is the major protease inhibitor in the blood plasma and plays a protective role by inhibiting several endogenous proteases. It occurs in the form of several genetically determined variants that differ from the normal $\alpha_1$AT in their physical properties and protease inhibiting capacity. Some variants are completely devoid of all activity while others have very little protease inhibitory capacity and have been implicated as a predisposing factor to emphysema and liver diseases. We investigated the level of $\alpha_1$AT in plasma samples using a spectrophotometric method and expressed the results as Trypsin Inhibitory Units (TIU) and as Trypsin Inhibitory Capacity (TIC) of the serum. The 'reference range' for TIU was 262.7 ± 107.2 TIU/dl and for TIC was 0.881± 0.360 TIC/I. In the Saudi population partial $\alpha_1$AT deficiency was identified at a frequency of 0.0663 and the frequency of complete deficiency was 0.0102.*

*This paper presents the results of a pilot study on Saudis and shows that $\alpha_1$AT deficiency exists in the Saudi population.*

*Key Words: Alpha-1-antitrypsin, $\alpha_1$AT deficiency, emphysema.*

## INTRODUCTION

Alpha-1-trypsin ($\alpha_1$AT), one of the six to eight protease inhibitors (Pi) in blood plasma, is a glycoprotein with a molecular weight of about 54,000. It is the major plasma proteases inhibitor with a broad specificity and can inactivate several protease including trypsin, chymotrypsin and elastase (9). It plays a protective role whereby it protects different tissues, such as the lungs, from the destructive action of endogenous proteases particularly elastase (10, 11).

$\alpha_1$AT exists in the form of several phenotypes that differ from them normal $\alpha_1$AT in their isoelectric pH and inhibitory capacity towards the proteolytic enzymes. Some of these phenotypes e.g. PiZ and PiS have significantly reduced activity and result in a condition referred to as "$\alpha_1$AT deficiency". Genetically determined deficiency of $\alpha_1$AT has been implicated as a factor predisposing to emphysema and benign liver cirrhosis in adults and liver disorders in children (1,5,17). An increase in the level of $\alpha_1$AT occurs in acute inflammatory conditions and thus $\alpha_1$AT is regarded as one of the acute phase reactant proteins (12, 13).

A number of studies have investigated the gene frequency of $\alpha_1$AT phenotypes and have shown considerable differences existing in different populations (2, 3, 4, 7, 14,15). One study from Saudi Arabia established the normal range for $\alpha_1$AT using rate nephelometry (16) and showed the presence of several cases with reduced plasma level of $\alpha_1$AT (Warsy and Sedrani, submitted).

We initiated studies on Saudis to determine the normal reference level of $\alpha_1$AT in healthy Saudi individuals using a spectrophotometric method and to investigate whether or not $\alpha_1$AT deficiency exists in this population. The procedure determined the Trypsin inhibitory Capacity of the serum and was called as the "TIC method". This pilot study reports our results and suggests that further detailed investigations are essential on Saudi population to determine the phenotypes existing in Saudi population.

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## MATERIALS AND METHODS

Blood samples (1-2 ml) were collected in plain tubes from 196 outpatients attending the outpatiens clinics in King Khalid University Hospital, Riyadh. The serum was seprated from the cells and stored frozen until required for analysis. The $\alpha_1$-antitrypsin activity in the serum was measured spectrophotometrically using an enzyme assay method (5,6) which is based on the inhibition of trypsin activity by serum $\alpha_1$ 1-antitrypsin and estimation of the residual trypsin activity using a synthetic substrate, Benzoyl-DL-Arginine-P-Nitroanilide Hydrochloride (BAPA). The level of the product (P-nitroaniline) formed was estimated by measuring the absorbance at 410 nm against a blank.

0.5 ml of trypsin (10 mg/dl) was mixed with 0.02 ml of serum and 0.48 ml tris buffer (0.05 M, pH 8.2) containing 0.02 M $CaCl_2$, for 5 minutes at 37°C. The residual trypsin activity was estimated by reacting with 3.5 ml Benzoyl-DL-arginine-P-Nitroanilide (BAPA) (30 mg dissolved in 1 ml dimethylsulfoxide and diluted at 100 ml with tris buffer, 0.05 M and pH 8.2) at 37°C for 10 minutes. The reaction was stopped by addition of 0.5 ml of 30% acetic acid and the OD 410 nm was measured against the appropriate blank. The control reaction was carried out in the same way without the addition of serum in the trypsin solution. The difference in the OD 410 nm of the control and test reaction was used to calculate the trypsin inhibitory capacity (TIC) i.e. the amount (in grams) of trypsin inhibited/litre of serum and trypsin inhibitor units (TIU) / dl i.e. the number of trypsin units (TU) inhibited by one decilitre of serum. One trypsin unit (TU) is defined arbitrarily as the increase of 0.01 absorbance units at OD 410 nm under the condition of the experiment.

The separation of $\alpha_1$AT phenotypes was also carried out in 60 samples by isoelectric focussing on ready made 0.5 mm thin, flat bed polyacrylamide gels (LKB ampholine PAG plates -LKB Cat. No, 1804-111) containing carrier ampholytes (pH 4-5) purchased from LKB Productor. Samples of (5 µl) serum were applied on filter paper pieces placed 1 cm from the cathodal strip. The anode solution was IM phosphoric acid and the cathode solution was 1M glycine in an LKB 2117 Multiphor Electrophoresis Unit for 2 hours at 10°C. The isoelectric focussing was carried out by applying 1400 V, 50 mA and 30 W. Following isoelectric focussing the gel was placed in a fixing solution containing 10 g sulfosalicyclic acid, 20 ml methanol and 180 ml distilled water for protein fixation. The protein bands were stained using Commassie Brilliant Blue R-250 and destained using ethanol/acetic acid/ water mixture (50:16:134).

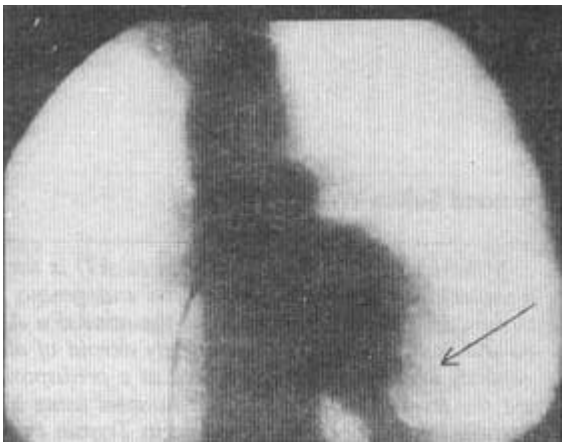

Figure 1: The frequency distribution histogram for TIC/l in the Saudi population.

## RESULTS

The number of trypsin inhibitory units (TIU)/dl and the trypsin inhibitory capacity (TIC)/litre of blood was calculated for each sample. The results obtained were fed on the computer at King Saud University Computer Centre and using the statistical analysis system (SAS), the mean, median, mode, standard deviation and percentile range (2.5 th-97.5 th) was obtained. The normal reference range for TIU and TIC was also calculated using mean ± 2 SD compared with the percentile range. The results are presented in Table 1. The frequency distribution histogram was obtained from the computer and are presented as Figures 1 and 2 for TIC/l and TIU/dl, respectively.

Table 1: The mean, median, mode, standard deviation, and normal reference range for TIU and TIC.

| Parameter | No.* | Mean | Median | Mode | SD | Reference Ranges | |
|---|---|---|---|---|---|---|---|
| | | | | | | Mean ± 2SD | 2.5th-97.5th Percentile |
| TIU/dl | 181 | 262.68 | 273.75 | 252.50 | 53.62 | 262.7 ± 107.2 (155.5 - 369.9) | 130.0 - 343.0 |
| TIC/dl | 181 | 0.881 | 0.942 | 0.960 | 0.180 | 0.881 ± 0.360 (0.521 - 1.241) | 0.430 - 1.086 |

* After exclusion of samples showing partial or complete $\alpha_1$AT deficiency.

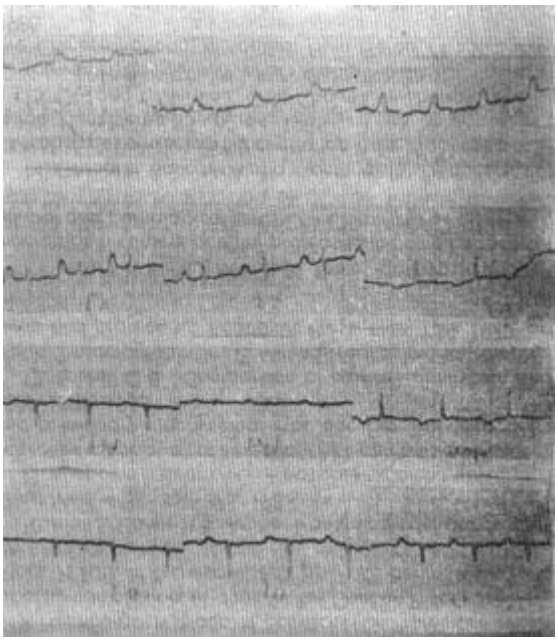

Figure 2: The frequency distribution histogram for TIC/dl in the Saudi population.

On isoelectric focussing $\alpha_1$AT was separated into 3 major bands and two minor bands. The bands were identified as those of $\alpha_1$AT by reacting the gel with anti $\alpha_1$AT obtained from Dako Patts. Figure 3 presents the isoelectric focussing pattern obtained. Two cases have less than 10% of normal $\alpha_1$AT activity and on isoelectric focussing no sharp $\alpha_1$AT bands were observed. In addition, 13 cases were identified with partial $\alpha_1$AT activity. The appeared to be heterozygotes with Pi MZ and Pi MS phenotypes.

## DISCUSSION

This stury was carried out as a pilot study to investigate the normal 'reference' level of Trypsin Inhibitory Capacity (TIC) and Trypsin Inhibitory Units (TIU) in serum using the TIC methods. The procedure was found to be accurate, reproducible and required only a spectrophotometer. The procedure could identify cases with partial and complete $\alpha_1$AT deficiency. Two parameters namely TIU/dl and TIC/1 were calculated and the normal 'reference' level of $\alpha_1$AT were established using 2.5th-97.5th percentile as 130-343 TIU/dl and 0.430-1.086 TIC/1. The range established using parametric method i.e. mean 2SD was not recommended as the 'reference' level since $\alpha_1$AT distribution showed a significant negative skewness (Figures 1 and 2).

Table 2: The frequency of partial and complete $\alpha_1$AT deficiency in Saudi population.

| No. investigated | No. with partial deficiency | Frequency | No. with complete deficiency | Frequency |
|---|---|---|---|---|
| 196 | 13 | 0.0663 | 2 | 0.0102 |

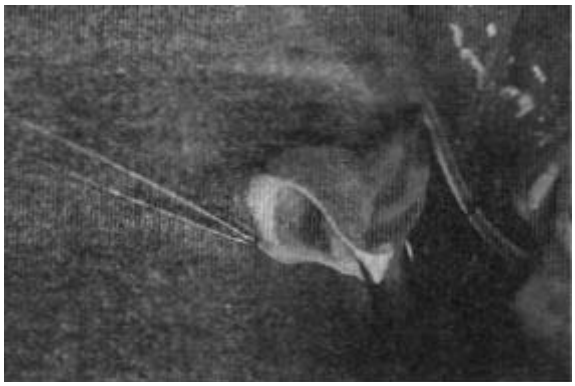

Figure 3: Iso-electric tocussing of $\alpha_1$AT phenotypes on ampholine PAG plates (LKB; pH 4-5.0) Sample 1: PIZZ, Samples 2,4-c: PIMM Sample 3: PISZ.

The estimation of $\alpha_1$AT is often carried out in routine hospital laboratories in order to diagnose cases with genetically determined partial or complete $\alpha_1$AT deficiency or cases with elevated $\alpha_1$AT level. The procedures utilized in different laboratories include immuno-diffusion technique or rate nephelometry. These procedures are accurate in that they are based on the formation of a precipitate upon reaction of the serum $\alpha_1$AT i.e. the antigen, with a specific antibody obtained commercially. In addition, electrophoresis and isoelectric focussing (IEF) are reported as ideal techniques since they can differentiate between the various $\alpha_1$AT variants, though the exact level of $\alpha_1$AT cannot be determined by these procedures. In one study comparing the IEF, the rate nephelometry and TIC method, it was reported that all three methods were comparable and accurately identified the severely deficient phenotypes such as PiZ, PiS, and PiSZ. A few of the heterozygotes cases with mild $\alpha_1$AT deficency i.e. Pi MZ and Pi MS were missed out by the rate nephelometry and TIC method (8).

For screening studies a procedure that would detect all patients with the abnormality including mild or subcilinical (i.e. one with a low specificity and high sensitivity) is better. Though a more rigerous criteria are required for a confirmatory test. The result of our study and those of others (8) showed that the TIC method could be a useful screening procedure for $\alpha_1$AT deficency as it has a high sensitivity. Furthermore, due to its simplicity it can be standardized in

any routine laboratory in hospitals and outpatient clinies or dispensaries for investigation of $\alpha_1$AT status and for screening of $\alpha_1$AT deficiency.

An important aspect of such screening studies in the identification of individuals with partial or complete $\alpha_1$AT deficiency or elevated $\alpha_1$AT level. The individuals with an $\alpha_1$AT deficiency have a higher chance of developing lung and liver diseases particularly if they are also smokers (10). The possibility of developing lung disease is considerably more in people who smoke, and thus early diagnosis and sufficient warning from the clinician to stop smoking can decrease associated morbidity and mortality in these individuals.

In conclusion, the result of our investigation have shown that TIC method for the estimation $\alpha_1$AT activity is an accurate, reproducible and simple method for estimation of $\alpha_1$AT level. Since $\alpha_1$AT has a negatively skewed distribution, the non-parametric methods are more accurate for establishment of the normal reference range. This study has also shown that partial or complete $\alpha_1$AT deficiency exists in Saudis at a frequency of 0.0663 and 0.0102, respectively. It is suggested that further indepth investigations are required to investigate the $\alpha_1$AT phenotype frequency in Saudis and to determine the clinically abnormal states linked to the deficient variants in this population.

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