Introduction
I. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the most prevalent clinical disorder. It is generally considered to be due to an imbalance between proteolytic enzymes and their inhibitors (Rosenbloom et al., 1991). A specific trypsin inhibitory protein was isolated along with the alpha-1-globulin of human serum. It was named as alpha-1-antitrypsin (α1-AT), as most of the serum trypsin inhibitory activity was found to be associated with the alpha-1 globulin fraction (Schultze et al., 1962). Alpha-1-antitrypsin drew clinical interest when Laurell and Eriksson (1963) described the absence of plasma α1-AT in patients with degenerative lung disease leading to death in middle age.

Genetic deficiencies resulting in the reduced levels of α1-AT in human plasma are particularly prevalent in individuals of north European descent (Hutchison, 1994; Crystal, 1996). The population screening studies in Sweden and United States suggest that α1-AT deficiency occurs among 1 in 2000-7000 of the white European population and 3% are found to be carriers (Crystal, 1990; Hutchison, 1994; Blanco et al., 2001).

Deficiency of α1-AT is a recognized risk factor for COPD and is characterized by the progressive obstruction of airways, which is not fully reversible (Brantly et al., 1988; Crystal, 1996). It is believed to be due to the uninhibited neutrophil elastase released into the lung tissue during inflammation (WHO, 1997; Boschetto et al., 2003). A marked reduction of α1-AT levels in the blood and throughout the body especially in the lungs
leaves the fragile alveolar walls vulnerable to the proteolytic destruction by neutrophil elastase activity slowly destroys alveoli, a process accelerated in cigarette smokers. By the age of 30-40 years the lung destruction becomes clinically apparent with progressive loss of lung function and a 10-15 year reduction in the life span compared with the general population (Crystal 1990; Mason and Crystal, 1998).

Eriksson (1965) showed that the condition was hereditary and was likely to occur in individuals homozygous for mutated or deleted alpha-1-antitrypsin (aat) gene. Heterozygosity was found to exhibit half the normal proteolytic activity of α1-AT (Fagerhol, 1969).

The etiopathogenesis of the α1-AT deficiency related COPD is due to the genetic mutations in the two parental aat genes that cause reduced secretion of α1-AT by the liver (Brantly, 1996). The three most important aat variants are PiM, PiS and PiZ. Their prevalence is reported in most surveys as aat gene frequencies. The PiM allele is the normal aat allele and codes for normal α1-AT serum levels. The PiS allele accounts for 2-3% of the aat alleles and is associated with reduced levels of α1-AT. The PiZ allele accounts for only 1% of aat alleles but is associated with severely reduced α1-AT levels. The PiZ and PiS are referred to as the “at risk” genotypes (Blanco et al., 2001).

Severe α1-AT deficiency is caused by homozygous inheritance of the aat deficient variant, PiZ. Here, a misfolded but functionally active mutant
Hypothesis of the Role of Antitrypsin

A. Antitrypsin present in sufficient amount
   - Normal protease balance
   - Normal pulmonary function

B. Antitrypsin deficient
   - Excessive protease activity
   - Pulmonary emphysema

Fig. 1. Action of alpha-1-antitrypsin on pulmonary structures (Netter, 1980)
α1-AT molecule is retained in the endoplasmic reticulum of liver cells rather than being secreted into the blood and body fluids. Liver injury is thought to be caused by the hepatotoxic effects of the retained mutant α1-AT protein (Perlmutter, 1989; Lomas, 2000; Parfrey et al., 2003).

COPD in PiZ individuals is an excellent example of a condition where genetic predisposition and environmental factors interact resulting in severe lung disease (Davis and Novotny, 1989). Cigarette smoking is the most important environmental risk factor and accounts for 80% of mortality attributable to α1-AT deficiency related COPD (Piitulainen and Eriksson, 1999).

Most northern Europeans are homozygous for the normal PiM allele (Parmar et al., 2002). However approximately 4% of the Caucasian populations are found to carry the homozygous PiZ allele and approximately 10% are heterozygous for either the PiZ or PiS alleles (Janus et al., 1985; ATS/ERS, 2003).

Absence or low prevalence of aat deficiency alleles (PiZ and PiS) from south east Asian and other Asian countries have been reported in the literature (Cox, 1989; Hutchison, 1990; Seyama et al., 1995; Yusa et al., 2001). A few studies reported the presence of deficient alleles in non-Caucasian populations (Mitsuyasu and Oshima, 1981; Awotedu and Adelaja, 1990). A high frequency for PiZ was reported in Afghanistan population (Rahimi et al., 1977). Desa et al (1995) observed the presence of PiZ and PiS variants in
Malaysian population. Very low frequencies of the aat deficient variants were reported from Pakistan (Shahid et al., 2000).

Blanco et al (2001) demonstrated that α1-AT deficiency affects major racial groups worldwide. The prevalence of the "at risk" genotype groups in other countries are much greater than originally thought. In another genetic epidemiological survey on the general population in countries worldwide, de Serres (2002) reported the presence of 116 million carriers of PiMS (heterozygous combination of normal M allele and deficient allele S) and PiMZ (heterozygous combination of normal M allele and deficient allele Z) and 3.4 million deficiency allele combinations – PiZZ (homozygous for the deficient Z allele) and PiSS (homozygous for the deficient S allele).

Recent report by de Serres et al (2005) also indicates the presence of PiS and PiZ in the general populations of Nigeria, Republic of south Africa, Somalia, sub Saharan countries and other non-Caucasian populations suggesting that α1-AT deficiency is not an uncommon scenario in the black and coloured populations.

Alpha-1-antitrypsin deficiency is widely under-diagnosed in other populations with majority of the individuals remaining undetected due to the delay in the onset and variability of respiratory symptoms (Cambell, 2000). In Sweden and Denmark, approximately 15-20% of deficient individuals have been detected. Approximately 80,000 α1-AT deficient individuals have been estimated to be in USA but only 4,000-5,000 (5-6%) has been diagnosed
(Cambell, 2000). However, in the rest of the world the detection of α1-AT deficiency is reported to be very low (de Serres et al., 2003).

WHO (1997) stated that 2-3% of all alpha-1-antitrypsin deficient individuals were homozygous for PiZ and recommended screening for α1-AT deficiency in individuals with COPD, all adults and adolescents with asthma as well as neonates, children and adults with unexplained liver disease.

Present study

Alpha-1-AT deficiency is the only proven genetic risk factor among COPD patients and the “at risk” genotypes PiZ and PiS are mainly associated with the development of α1-AT deficiency related COPD (Blanco et al., 2001). The present study was aimed at screening the “at risk” genotypes of α1-AT deficiency among randomly selected COPD patients from south India using PCR-SSCP and PCR-RFLP methods, so as to identify the genetic susceptibility in these patients.

Objectives

➢ Screening of the *aat* gene for the deficient variants PiZ and PiS among COPD patients and normal controls

➢ To analyze the risk factors such as smoking, occupation, socio-economic status and its association with COPD

➢ To compare the prevalence of α1-AT deficiency in south Indian population with other Asian populations.
The determination of the gene frequencies of the \textit{aat} deficient variants is important to estimate the number of subjects "at risk" of suffering from severe $\alpha$1-AT deficiency related diseases in any given population (Vidal \textit{et al.}, 1996). In India, majority of the COPD is treated as acute manifestations of airways obstructions. The fact that COPD is inherited and that the life span can be increased by altering the lifestyles, if diagnosed, makes the early diagnosis of $\alpha$1-AT deficiency significant. Less literature is available on the prevalence of \textit{aat} genotypes from south Indian populations. Hence the present study was designed.