JMSCR Vol||05||Issue||02||Pages 17821-17828||February 2017

www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i2.101

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Alpha-1-Antitrypsin Activity in Bronchiectasis

Authors
Dr Humera Khan (MBBS, MD Biochemistry, PhD Biochemistry)[1],
Dr Khushtar A. Salman (PhD Biochemistry)[2]
[1]Associate Professor, Department of Biochemistry, Mayo Institute of Medical Sciences, Faizabad, Road, Gadia, Barabanki- 225001, UP, India
[2]Professor, Department of Biochemistry,J. N. Medical College, Aligarh Muslim University, Aligarh – 202002, UP, India
Email: *khan_humera@hotmail.com, Mobile No. 09997411172*

ABSTRACT
Bronchiectasis is an uncommon disease with the potential to cause devastating illness.[1] Although fewer than 100 cases of bronchiectasis associated with alpha-1-antitrypsin deficiency have been reported there is little doubt of an association between bronchiectasis and alpha-1-antitrypsin deficiency.[2,3,4] Aim of the present study was to determine the level of alpha-1-antitrypsin activity in case of bronchiectasis patients. The method that was adopted in the present study of serum alpha-1-antitrypsin activity was by measuring the inhibition of trypsin by serum. The serum alpha-1-antitrypsin level was found to be 2.46 ± 0.21 mg/ml among the control group, which lies in the normal range. The serum alpha-1-antitrypsin level was found to be 2.40 ± 0.10 mg/ml among thirty one bronchiectasis patients, which also lies in the normal range.
Key Words: *proteinase inhibitor; serpin superfamily; neutrophil elastase; bronchiectasis.*

INTRODUCTION

Alpha-1-antitrypsin (or alpha-1-proteinase inhibitor) is the most abundant circulating proteinase inhibitor and the archetypal member of the serine proteinase inhibitor or serpin superfamily.[5,6] It is synthesised in the liver and protects lung alveolar tissues from destruction by neutrophil elastase.[7,8] Alpha-1-antitrypsin (α-1 antitrypsin) deficiency is a common autosomal recessive condition (1:1600 to 1:1800) in which liver disease results from retention of abnormal polymerised α-1 antitrypsin in the endoplasmic reticulum of hepatocytes, and emphysema results from alveolar wall damage.[9] Control of elastase-mediated degradation of elastic tissue in the lung requires both an optimal concentration and function of anti-elastase. The damage wrought by uninhibited neutrophil elastase in the lung takes many years to manifest itself clinically.[10]

The alpha-1-antitrypsin (AAT) phenotype, described as the protease inhibitor (Pi) system, is determined by the independent expression of two parental alleles. It is defined on the basis of electrophoretic pattern of alpha-1-antitrypsin in serum.[11] Deficiency can be homozygous or heterozygous in character. Homozygotes have alpha-1-antitrypsin below 35% of the normal concentration while the level in heterozygotes is 40-80% of normal.[12] Data suggest that cigarette smokers may lose some of the normal antielastase

protective screen of the lower respiratory tract, making them more vulnerable to destructive lung diseases.[13] Alpha-1-proteinase inhibitor may be inactivated by activated neutrophils by an oxidant-dependent mechanism.[14,15]

Hutchinson (1988) and Eriksson (1989) provided insight into the natural history of severe alpha-1-antitrypsin deficiency. Alpha-1-antitrypsin (AAT) deficiency is one of the most common inherited disorders among whites. Its primary manifestation is early-onset panacinar emphysema. **In the US:** This genetic defect affects 1 per 3000-5000 individuals. AAT is one of the most common lethal genetic diseases among whites. **Internationally:** Similar rates are found among whites worldwide.[18] Bronchiectasis is primarily a disease of the bronchi and bronchioles involving a vicious circle of transmural infection and inflammation with mediator release.[19] There is permanent dilatation of bronchi.[20] Bronchiectasis is an uncommon disease with the potential to cause devastating illness, including repeated respiratory infections requiring antibiotics, disabling productive cough, shortness of breath, and occasional hemoptysis.[1] Retained inflammatory secretions and microbes cause obstruction and damage of the airway and recurrent infection. Cigarette smoking may worsen pulmonary function and accelerate the obstructive impairment.[21]

It was Longstretch *et al.*[22] (1975) who first reported an association between alpha-l-antitrypsin deficiency and bronchiectasis in the absence of emphysema. In a study on fifteen patients with bronchopulmonary infection Kagan *et al.*[23] 1975, found one patient with bronchiectasis who also had alpha-l-antitrypsin deficiency. Although pulmonary complications of alpha 1-antitrypsin deficiency are most commonly manifested by panlobular emphysema, in a 21 year old man with massive haemoptysis and homozygous deficiency of alpha 1-antitrypsin neither panlobular emphysema nor cirrhosis of the liver were present. So bronchiectasis must be considered part of the spectrum of the pulmonary pathology that may be encountered in individuals with alpha 1-antitrypsin deficiency.[24]

In a study of adults with bronchiectasis there was no increase in the prevalence of α-1 antitrypsin deficiency alleles, but more emphysema if both diseases coexisted.[25] If α-1 antitrypsin deficiency is found in a child with lung symptoms it should not therefore be accepted as the underlying cause of the problem, but it might be an exacerbating factor in disease progression. High prevalence of emphysema in patients with bronchiectasis has been reported by Loubeyre *et al.*[26] (1996). Bronchiectasis is also a feature associated with emphysematous changes of the lung in AAT deficiency, affecting 1: 2000 in the United Kingdom.[27] Varpela *et al.*[28] (1978) found that six out of 60 patients with bronchiectasis had alpha-l-antitrypsin deficiency. It has been suggested that patients with diffuse bronchiectasis should be screened for alpha1-antitrypsin deficiency.[29]

A few case reports have suggested a putative association between bronchiectasis and AAT deficiency in the absence of emphysema.[22,24,29,30] Jones *et al.*[31] (1985) described three patients with the clinical and radiographic features of progressive bronchiectasis, and without evidence of emphysema, associated with deficiency of alpha-1-antitrypsin. No elastase inhibition capacity, but free elastase activity, was found in 82% of studied 11 bronchiectasis patients. Bronchiectasis patients show a severe imbalance between neutrophil activity and protective molecules leading to possible lung destruction.[32] PiZ, the mutant responsible for more than 95% of cases of pulmonary and hepatic disease associated with α-1 antititrypsin deficiency, is most frequent in Scandinavia and progressively less common as one travels south in Europe.[9]

MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry and Department of T.B. & Respiratory Diseases at Jawaharlal Nehru Medical College and Hospital, Aligarh Muslim University, Aligarh (India). Twenty five samples

were taken from normal healthy adults which served as controls (Fig: 1). Study group comprised thirty one samples (Fig. 2) from patients attending J. N. Medical College, Hospital, Aligarh, with clinical manifestations pointing to bronchiectasis. Detailed history and clinical examination of patients and investigations such as haemogram, X-Ray chest, PA View and Lateral View (if needed), sputum for AFB, culture sensitivity, routine urine examination and Lung Function Test (if needed). Samples of blood were collected from patients and serum separated from the sample and subjected to measurement of serum trypsin inhibitory activity according to the procedure of Waheed and Salahuddin[33] (1975). Patients with acute infection were excluded. Blood samples were collected separately and were planned for serum alpha-l-antitrypsin activity measurement.

Protein concentration was routinely determined by the method of Lowry *et al.*[34] (1951), using crystalline bovine serum albumin as standard. The serum trypsin inhibitor activity of all samples were determined by measuring the inhibition of trypsin by the sera according to the method of Waheed and Salahuddin (1975). The substrate that has been used for trypsin is N Alpha-Benzoyl–DL-Arginine P-Nitroanilide (BAPNA). Trypsin was allowed to react with BAPNA resulting in the formation of P-nitroanilide which has intense yellow colour. After the reaction of trypsin with BAPNA, the absorbance of the solution was measured colorimetrically at 410 nm.

The intensity of colour was used for checking the activity of trypsin in the presence and absence of serum.

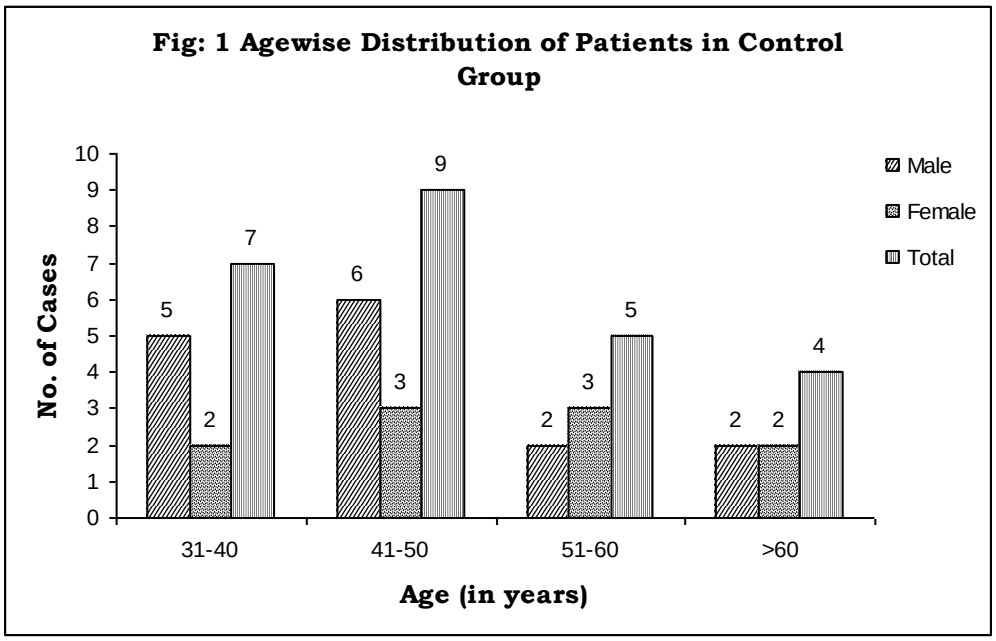
RESULTS AND DISCUSSION

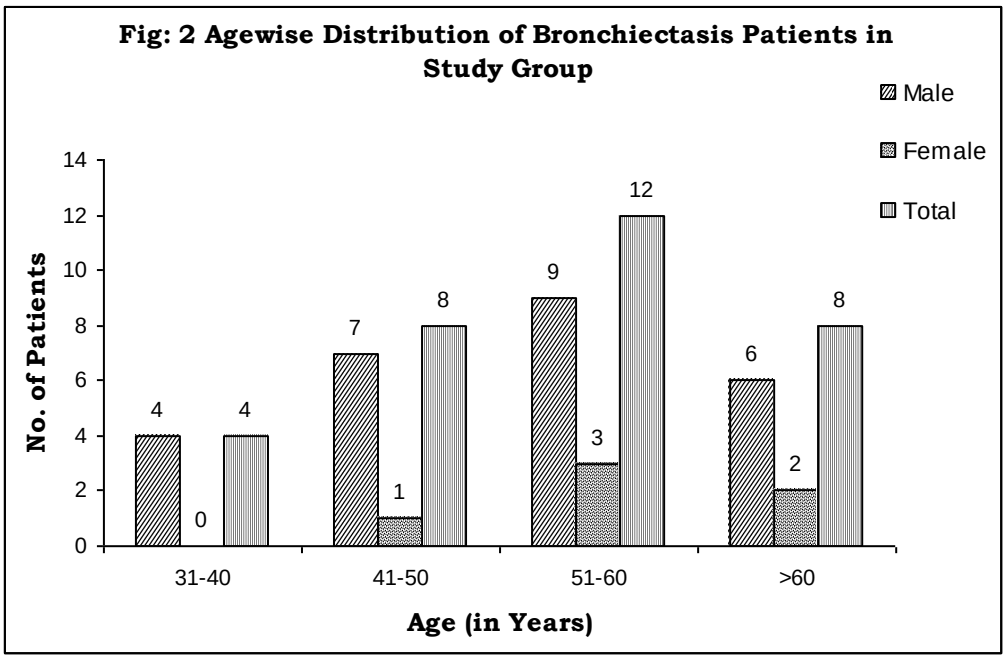
The serum alpha-1-anti-trypsin level was found to be 2.46 ± 0.21 mg/ml among the control group, which lies in the normal range. These findings are in agreement to those of Shahid *et al.*[35] (1995) who in their study on 100 healthy adults (52 males and 48 females) found mean serum alpha-1-antitrypsin concentration to be 2.47 ± 0.08 g/l. In a similar study, Habibullah *et al.*[36] (1980) had not reported any deficiency among 25 healthy controls. The findings of the present study seem to be also in line with those of Fagerhol and Tenfjord[37] (1968) who noted that alpha-1-antitrypsin is rarely found in dark and Asian populations while being relatively common inherited condition in US. Silverman *et al.*[38] (1989) reported the prevalence of alpha-1-antitrypsin in US ranging one in 2850. On the basis of findings from extant and the present study in the Indian context, it can be safely surmised that alpha-1-antitrypsin deficiency is rather uncommon but the same needs to ascertained through studies covering larger populations.

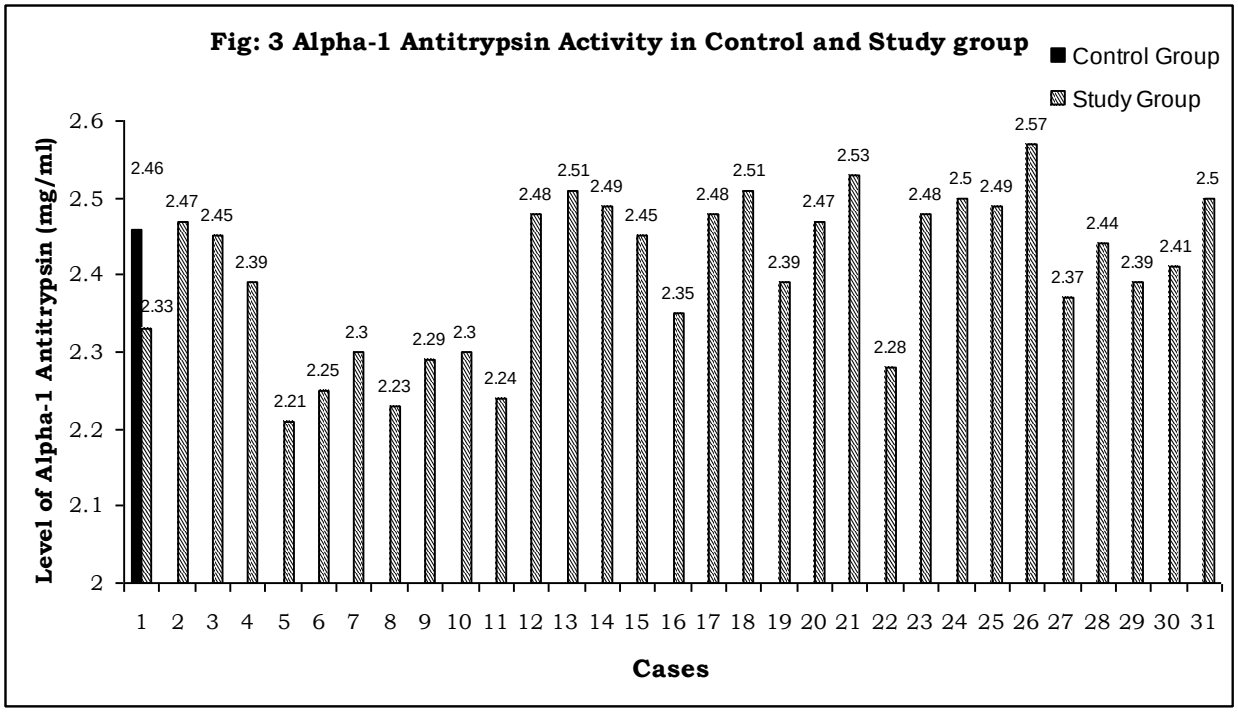
All the studied thirty one cases of bronchiectasis had normal alpha-1-antitrypsin level (Fig: 3). Only seven of them were smokers. Level of alpha-1-antitrypsin level was found to be 2.40 ± 0.10 mg/ml. Level of alpha-1-antitrypsin in females was found to be 2.35 ± 0.10 mg/ml and in males level is 2.41 ± 0.09 mg/ml all within normal range. None of the bronchiectasis patients had low levels of alpha-1-antitrypsin. Similarly the study conducted by Cuvelier *et al.*[25] (2000) did not implicate the alpha-1-antitrypsin genes in the development of bronchiectasis. They suggested that bronchiectasis may be a consequence of emphysema in PI*Z patients rather than a primary effect. In a study in India, Ray[39] (1994) found that out of 20 bronchiectasis cases 2 had intermediate levels of alpha-1-antitrypsin deficiency. A study found significantly higher serum levels of alpha 1-antitrypsin in 35 patients with widespread bronchiectasis compared with healthy controls. However, the validity of the association has been questioned in the literature on the grounds that most of the patients in those reports had other possible etiologies for their bronchiectasis. It was reported that the raised level AAT in our patients represents a non-specific acute phase response to bronchial infection.[3]

Bronchiectasis may appear in AAT deficient patients who are exposed to repeated pulmonary infections, even before the development of emphysema.[4] Therefore, bronchiectasis in PI*Z

patients may be caused by chronic bronchitis that is seen in emphysematous patients. Because approximately 50% of patients with AAT deficiency have symptoms of airway disease, especially chronic sputum production.[40] Emphysema is a confounding factor that may lead to bronchiectasis, but the presence of emphysema in bronchiectatic patients also is associated with an abnormal distribution of AAT alleles. Studies show that bronchiectatic patients without emphysema do not have a different AAT distribution as compared with control subjects in contrast to bronchiectatic patients with emphysema. suggesting that bronchiectasis may be a consequence of emphysema in PI*Z patients rather than a primary cause. This indicates that AAT phenotype distribution and gene frequencies are not different between patients with bronchiectasis and control subjects. Thus this comparative study does not provide any evidence that AAT deficiency may play a role in the pathophysiology of bronchiectasis.[25]







CONCLUSION

Bronchiectasis may be more common in patients with alpha 1-antitrypsin deficiency than has been previously recognized. The diagnosis of alpha 1-antitrypsin deficiency should be considered in patients with emphysema and diffuse cystic bronchiectasis.[29,41,42]

Investigations using CT scans should reveal the true incidence regarding presence of bronchiectasis among patients with AAT deficiency which might not be as low as generally believed.[4,42,45,46,47]

The data from the former USSR, the Eastern Mediterranean Region and India are based on relatively small samples and on quantitation of alpha-1-antitrypsin in serum as well as PI typing. More data are needed from these regions to establish the frequency of specific alleles and the health burdens due to the genetic susceptibilities. There is no firm evidence that any of the alleles with a modest reduction (>35% normal) in the plasma concentration of alpha-1-antitrypsin (I, S, P, etc.) are associated with the disease. The best approach to demonstrate a potential link between AAT deficiency and bronchiectasis is to compare the AAT allelic distribution in an unselected and large population of bronchiectatic patients with that of a control population.[3,28,31]

Our study had several methodological limitations: diagnosis of bronchiectasis was usually assessed by methods other than CT scan (chest radiography or bronchography). Moreover, the patients did not have blood samples analyzed by isoelectric focusing; this is a very critical point because AAT serum concentrations may be influenced by sex or inflammatory conditions and have been found to slightly increase in deficient individuals. In as much as AAT is an acute-phase reactant protein, its serum concentration may increase in patients with stable bronchiectasis as compared with control subjects.[3,43] Therefore, because it is not possible to use only AAT serum concentrations as a screening method for the diagnosis of AAT deficiency.

Alpha 1-antitrypsin deficiency frequently escapes diagnosis despite many medical encounters and most individuals are often unaware of basic details of their disease. Strategies to enhance guideline-based diagnostic testing are warranted.[48] Delay in diagnosing this disease is associated with adverse psychosocial effects.[44,49] Loss of alpha-1 AT impacts immune function both directly and through complex indirect mechanisms in the

damaged lung is a critical element of the disease progression. [50,51] Large scale studies are needed.[52] Hence, further research is to be focused on those alleles which produce a marked deficiency of alpha-1-antitrypsin.

REFERENCES

1. Alan FB - Bronchiectasis. The New England Journal of Medicine 2002; 346:1383-1393.
2. Jones DK, Godden D, and Cavanagh P - Alpha-l-antitrypsin deficiency presenting as bronchiectasis. Br. J. Dis. Chest 1985; 79(3): 301-304.
3. El-Kassimi FA, Warsy AS, Uz-Zaman A, and Pillai DK - Alpha-1-antitrypsin serum levels in widespread bronchiectasis. Respir. Med. 1989; 83:119-121.
4. Shin MS and Ho KJ - Bronchiectasis in patients with alpha 1-antitrypsin deficiency. A rare occurrence? Chest 1993; 104:1384-1386.
5. Huber R and Carrell RW - Implications of the three-dimensional structure of alpha-1-antitrypsin for structure and function of serpins. Biochemistry 1989; 28:8951-8966.
6. Potempa J, Korzus E, and Travis J - The serpin superfamily of proteinase inhibitors: structure, function, and regulation. J. Biol. Chem. 1994; 269:15957-15960.
7. Parmar JS and Lomas DA - Alpha-1-antitrypsin deficiency, the serpinopathies and conformational disease. J. R. Coll. Physicians Lond. 2000; 34:295-300.
8. Carrell RW, Jeppsson JO and Laurell CB - Structure and variation of human alpha-1-antitrypsin. Nature 1982; 298:329-334.
9. Primhak RA and Tanner MS - Alpha-1 antitrypsin deficiency. Arch. Dis. Child. 2001; 85(1): 2 - 5.
10. Brantly ML, Paul LD, Miller BH, Falk RT, Wu M, Crystal RG - Clinical features and history of the destructive lung disease associated with alpha-1-antitrypsin deficiency of adults with pulmonary symptoms. Am. Rev. Respir. Dis. 1988; 138:327-336.
11. Cox DW, Johnson AM and Fagerhol MK - Report of nomenclature meeting for alpha-l-antitrypsin. Hum. Genet. 1980; 53(3): 429.
12. Eriksson S - Studies in alpha-1 antitrypsin deficiency. Acta. Med. Scand. 1965; 177:1-85.
13. Gadek JE, Fells GA and Crystal RG - Cigarette smoking induces functional antiprotease deficiency in the lower respiratory tract of humans. Science 1979; 206(4424): 1315-6.
14. Carp H and Janoff A - Inactivation of bronchial mucous proteinase inhibitor by cigarette smoke and phagocyte-derived oxidants. Exp. Lung Res. 1980; 1: 225-237.
15. Kramps JA, van Twisk C and Dijkman DH - Oxidative inactivation of antileukoprotease is triggered by polymorphonuclear leukocytes. Clin. Sci. (Colch.) 1988; 75: 53-62.
16. Hutchinson DCS - Natural history of alpha-1-protease inhibitor deficiency. Am. J. Med. 1988; 84 (suppl 6A): 3-12.
17. Eriksson S - Alpha-1-antitrypsin deficiency: lessons learned from the bedside to the gene and back again. Chest 1989; 95: 181-189.
18. Fairman P - Alpha1-Antitrypsin Deficiency. Copyright 2004, eMedicine.com, Inc. http://www.emedicine.com/MED/topic108.htm
19. Cole PJ - Inflammation: a two-edged sword -- the model of bronchiectasis. Eur. J. Respir. Dis. Suppl 1986; 147:6-15.
20. Reid LM - Reduction in bronchial subdivision in bronchiectasis. Thorax 1950; 5:233-247.
21. Nicotra MB, Rivera M, Dale AM, Shepherd R and Carter R - Clinical, pathophysiologic, and microbiologic

characterization of bronchiectasis in an aging cohort. Chest 1995; 108:955-961.

22. Longstretch GF, Weitzman MD, Browning RJ and Lieberman J - Bronchiectasis and homozygous alpha-1-antitrypsin deficiency. Chest 1975; 67: 233.
23. Kagan E, Saskolene CL, Zwi S, Hurwitz S, Maier GM, Ipp T, Rabson AR - Immunologic studies in patients with recurrent bronchio pulmonary infection. Am. Rev. Respir. Dis. 1975, 111: 441-51.
24. Rodriguez-Cintron W, Guntupalli K and Fraire AE - Bronchiectasis and homozygous (P1ZZ) alpha 1-antitrypsin deficiency in a young man. Thorax 1995; 50(4): 424-5.
25. Cuvelier A, Muir JF, Hellot MF, Benhamou D, Martin JP, Bénichou J and Sesboüé R - Distribution of alpha(1)-antitrypsin alleles in patients with bronchiectasis. Chest 2000; 117:415-419.
26. Loubeyre P, Paret M, Revel D, Wiesendanger T and Brune J - Thin-section CT detection of emphysema associated with bronchiectasis and correlation with pulmonary function tests. Chest 1996; 109: 360-365.
27. Sansom ME, Ferry BL, Sherrell ZPMC and Chapel HM - A preliminary assessment of alpha-1 antitrypsin S and Z deficiency allele frequencies in common variable immunodeficiency patients with and without bronchiectasis. Clinical & Experimental Immunology 2002; 130(3): 489.
28. Varpela E, Koistinen J and Morhola O - Deficiency of alpha-l-antitrypsin and bronchiectasis. Ann. Clin. Res. 1978; 10: 79.
29. Scott JH, Anderson CL, Shankar P and Stavrides A - Alphal-antitrypsin deficiency with diffuse bronchiectasis and cirrhosis of the liver. Chest 1977; 71:535-538.
30. Falk GA and Smith JP - Chronic bronchitis: a seldom noted manifestation of homozygous alpha-1-antitrypsin deficiency. Chest 1971; 60: 166-169.
31. Jones DK, Godden D and Cavanagh P - Alpha-l-antitrypsin deficiency presenting as bronchiectasis. Br. J. Dis. Chest 1985; 79:301-304.
32. Schaaf B, Wieghorst A, Aries SP, Dalhoff K and Braun J - Neutrophil inflammation and activation in bronchiectasis: comparison with pneumonia and idiopathic pulmonary fibrosis. Respiration (Switzerland) 2000; 67 (1): 52-9.
33. Waheed A and Salahuddin A - Isolation and charaterisation of a variant of ovomucoid. Biochem. J. 1975; 147: 139-144.
34. Lowry O. H., Rosebrough M. J., Farr A. L. and Rardal R. J., J. Biol. Chem. 1951; 193: 265-275.
35. Shahid A, Siddiqui AA, Zuberi SJ and Waqar MA - Genetic variants of serum alpha 1 antitrypsin. J. Pak. Med. Assoc. 1995; 45(9): 245-6.
36. Habibullah CM, Somayajulu GL, Reddy KS and Vasantha VC - Study of alpha-l-antitrypsin activity in liver diseases. Jr. Assoc. Phys. Ind. 1980; 28: 177.
37. Fagerhol MK and Tenfjord OW - Serum Pi types in some European, American, Asian and African populations**.** Acta. Path. Microbiol. Scand. 1968**;** 72: 601-608.
38. Silverman EK, Miletich JP, Pierce JA, Sherman LA, Endicott SK, Broze GJ Jr. and Campbell EJ - Alpha-l-antitrypsin deficiency, high prevalence in the St. Louis area determined by direct population screening. Am. Rev. Respir. Dis. 1989; 140:961-966.
39. Ray D, Kanagasabapathy AS and Jairaj PS - Alpha 1-Antitrypsin deficiency in Indian patients with chronic obstructive airways and other pulmonary diseases. Ceylon Med. J. 1994; 39(2): 77-81.
40. Tobin MJ, Cook PJL and Hutchison DCS - Alpha-1 antitrypsin deficiency: the clinical

and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z; a survey by the British Thoracic Association. Br J Dis Chest 1983; 77: 14-27.

41. Guest PI and Hansell DM - High resolution computed tomography (HRCT) in emphysema associated with alpha-1-antitrypsin deficiency. Clin. Radiol. 1992; 45:260-266.
42. King MA, Stone JA, Diaz PT, Mueller CF, Becker WJ and Gadek JE - Alpha 1-antitrypsin deficiency: evaluation of bronchiectasis with CT. Radiology, (USA) 1996; 199 (1): 137-41.
43. Ip M, Lam WK, Chan JC, Liong E - Systemic effects of inflammation in bronchiectasis. Respir Med. 1991; 85(6): 521-5.
44. Stoller JK, Smith P, Yang P and Spray J - Physical and social impact of alpha 1-antitrypsin deficiency: results of a survey. Cleve. Clin. J. Med. 1994; 61(6): 461-7.
45. AL-Shirawi N, H AL-Jahdali H and Al Shimemeri A - Pathogenesis, etiology and treatment of bronchiectasis. Annals of Thoracic Medicine 2006; 1:41-51
46. Kelly E, Greene CM, Carroll T, McElvaney NG. O'Neill SJ - Alpha-1 antitrypsin deficiency. Respiratory Medicine 2010; 104(6):763-72.
47. Parr DG, Guest PG, Reynolds JH, Dowson LJ, and Stockley RA - Prevalence and Impact of Bronchiectasis in α_1-Antitrypsin Deficiency. American Journal of Respiratory and Critical Care Medicine 2007; 176(12):1215-1221.
48. Jain A, McCarthy K, Xu M and Stoller JK - Impact of a Clinical Decision Support System in an Electronic Health Record to Enhance Detection of α_1-Antitrypsin Deficiency. Chest. 2011;140(1):198-204
49. Stoller JK and Aboussouan LS - A Review of α_1-Antitrypsin Deficiency. American Journal of Respiratory and Critical Care Medicine 2012; 185(3):246-259
50. Stockley RA - The multiple facets of alpha-1-antitrypsin. Ann Transl Med 2015; 3:130
51. Russell DW, Gaggar, A and Solomon GM - Neutrophil Fates in Bronchiectasis and Alpha-1 Antitrypsin Deficiency. Annals of the American Thoracic Society April 2016; Vol. 13, No. Supplement-2 pp S123–S129
52. Araújo D, Sucena M- Association between Alpha 1 Antitrypsin and Bronchiectasis. European Respiratory Journal 2015; 46: PA1248.