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Demonstration of Alpha$_1$-Antitrypsin in Paraffin Sections of Hepatoma and Cirrhosis

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Summary. Alpha$_1$-antitrypsin has been examined in formalin-fixed, paraffin-embedded liver specimens from Greek patients with cirrhosis (35 cases) and hepatoma (55 cases) by peroxidase-antiperoxidase (PAP) method. Ring-like AAT globules were found in the non-neoplastic cells in 12% of the cases of hepatoma and in 11% of the cases of cirrhosis. Atypical globules were seen in neoplastic cells in 5.4% of the cases of hepatoma and in 17% of the cases of liver cirrhosis. A diffuse fine granular pattern of AAT distribution was present in 31% of the cases of hepatoma in the neoplastic cells and in 27% of those in the non-neoplastic cells. The relatively high incidence of ring-like AAT-globules, and of atypical globules in cases of hepatoma and cirrhosis is not in agreement with the extremely low gene frequency of Z allele in a Greek population of patients with cirrhosis and hepatoma. Thus, there is some doubt whether AAT-globules in the liver represent a histopathologic marker of genetically determined AAT deficiency. A relationship between AAT deposits and the degree of differentiation of hepatoma was noted in this series. AAT-positive cells were found in 55% of moderately differentiated, in 29% of highly differentiated and in 20% of poorly differentiated hepatomas.

Key words: Alpha$_1$-antitrypsin – Liver cirrhosis – Hepatoma

Introduction

The association of alpha$_1$-antitrypsin (AAT) deficiency with pulmonary emphysema and with neonatal hepatitis and cirrhosis in the childhood is now well established. Pi^Z allele was found to be responsible for low serum AAT levels, and a number of adults having phenotype ZZ may present hepatic cirrhosis with or without pulmonary disease after middle age, although the severity and the type of histological changes are not related to the

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degree of the AAT deficiency (Berg and Eriksson 1972; Eriksson 1964; Eriksson and Hägerstrand 1974). The mechanism by which this occurs remains obscure (Kelly et al. 1979). There are also numerous reports of hepatocellular carcinoma (HCC) developing in such cases (Aagenaes et al. 1974; Berg and Eriksson 1972; Eriksson 1974; Ganrot et al. 1967; Kelly et al. 1979; Lieberman 1974; Lieberman et al. 1975; Palmer et al. 1980; Rawlings et al. 1974; Triger et al. 1976; Williams and Fajardo 1974). AAT globules have been found in paraffin sections of HCC in about 10% of the cases in a number of studies (Berg and Eriksson 1972; Blenkisopp and Haffenden 1977; Cohen 1976; Dekker and Krause 1973; Norken and Campagna-Pinto 1968) and in embryonic type malignant tumors (yolk sac tumors) (Reintoft 1978; Palmer et al. 1976; Palmer and Wolfe 1976). AAT globules have also been seen in either non-neoplastic liver cells of hepatoma patients (Eriksson and Hägerstrand 1974; Kelly et al. 1979; Lieberman 1974; Reintoft and Hägerstrand 1979), in neoplastic liver cells (Palmer et al. 1977; Palmer and Wolfe 1977), or simultaneously in both neoplastic and non-neoplastic liver cells (Lieberman et al. 1975; Palmer and Wolfe 1976). Although these AAT deposits have been used as a tissue marker of inherited AAT deficiency (usually at least one Z allele) (Palmer et al. 1980) cases of normal serum AAT phenotype associated with AAT deposits have also been reported (Bradfield and Blenkinsopp 1977; Palmer et al. 1977; Palmer et al. 1980; Palmer and Wolfe 1977). These observations suggest that AAT may be a histopathological marker of broader significance than in relation to genotypic factors alone (Palmer et al. 1980). Therefore, we question whether the existence of AAT globules in the liver cells expresses a real, genetically determined AAT deficiency or a histopathological marker of increased synthesis of AAT, during the so-called acute phase reaction.

The aim of this study was to determine the incidence and the distribution of AAT material in the liver of Greek patients with HCC and cirrhosis, using a sensitive and specific peroxidase-antiperoxidase complex method on paraffin sections of large liver specimens obtained after laparotomy or autopsy. We compare our findings to those on other populations.

Material and Methods

AAT was sought in formalin-fixed, paraffin-embedded liver specimens from adult Greek patients with cirrhosis (35 cases) and hepatoma (55 cases). Specimens were taken after laparotomy in 22 and 40 cases and after autopsy in 13 and 15 cases respectively. In 33 cases of HCC non-neoplastic tissue was also present in the specimen.

The hepatocellular carcinomas were typed and graded according to the criteria of Edmondson (1958). The grade of differentiation was as follows: grade I–II (well-differentiated) 27 cases, grade III (moderately differentiated) 18 cases, and grade IV (poorly differentiated) 10 cases.

Sections were deparaffinized with xylene, rehydrated in graded dilutions of ethanol and stained with haematoxylin-eosin, Gomori, and Masson trichrome.

The peroxidase-antiperoxidase (PAP– (Sternberger 1970) method was used for localization of AAT. The sections were deparaffinized and rehydrated. Briefly, the tissue sections were incubated sequentially with appropriate dilutions of: 1) rabbit antihuman AAT serum (Dako-immunoglobulins) in dilution 1:20 in a moist chamber at 37° C for 30′ min, 2) rabbit anti-swine IgG (Dako-immunoglobulins) in dilution 1:40 for 30′ min, 3) horseradish-peroxidase-rabbit antiperoxidase (Dako-immunoglobulins) in dilution 1:60 for 30′ min, 4) 3,3′-diaminobenzidine (Serva Feinbiochemic: Heidelberg, FRG). The peroxidase-stained sections were also counter-

stained with Mayer's haematoxylin in order to permit better morphological evaluation of the immunocytochemical reaction.

The control sections were incubated in all steps except step I.AAT staining was always interpreted by comparing the reaction of the test to the adjacent control section.

Results

The results of this study are summarized in Tables 1 and 2. Typical AAT globules were found in the non-neoplastic cells in 4 (12%) out of the 33 cases of HCC and in 4 (11%) out of the 35 cases of liver cirrhosis (Fig. 1). These globules were located mainly around portal zones and stained strongly at the periphery by the immunoperoxidase technique. Typical globules were not found in neoplastic cells.

In three cases (5.4%) of the 55 cases of HCC, atypical AAT globules were found in neoplastic cells (Fig. 2) as well as in 6 (17%) of the 35 cases of cirrhosis (Fig. 3). These globules were of various sizes in a homogenous smooth pattern.

In 17 cases (31%) of HCC a diffuse fine granular pattern of AAT distribution was present throughout the whole of the cytoplasm of neoplastic cells. Similar findings were observed in 9 cases (27%) of HCC in the cytoplasm of non-neoplastic cells (Fig. 4).

A total of 20 (36%) cases of HCC in the neoplastic part of the liver and of 10 (28%) cases of cirrhosis were stained positively using the immunoperoxidase technique (Table 1).

A relationship between AAT deposits and the degree of differentiation of HCC was noted in this series (Table 2). In the 27 cases of grade I and II carcinoma, AAT cytoplasmic deposits were found in 8 (29%). Among the 18 cases of grade III hepatoma, 10 (55%) were found to be AAT-positive and only 2 among the 10 cases of grade IV liver carcinoma were stained positively using the immunoperoxidase technique.

Table 1. Cytoplasmic distribution of AAT in paraffin sections of hepatoma and cirrhosis

	No. of cases	Ring-like AAT globules	Atypical AAT globules	Granular pattern	Total of cases AAT-positive
Hepatoma					
Neoplastic cells	55	–	3 (5.4%)	17 (31%)	20 (36%)
Non-neoplastic cells	33	4 (12%)	–	9 (27%)	13 (39%)
Cirrhosis	35	4 (11%)	6 (17%)	–	10 (28%)

Table 2. The relationship of AAT deposits to the degree of differentiation of hepatoma

Degree of differentiation	No. of cases	No. of cases AAT-positive
I, II	27	8 (29%)
III	18	10 (55%)
IV	10	2 (20%)

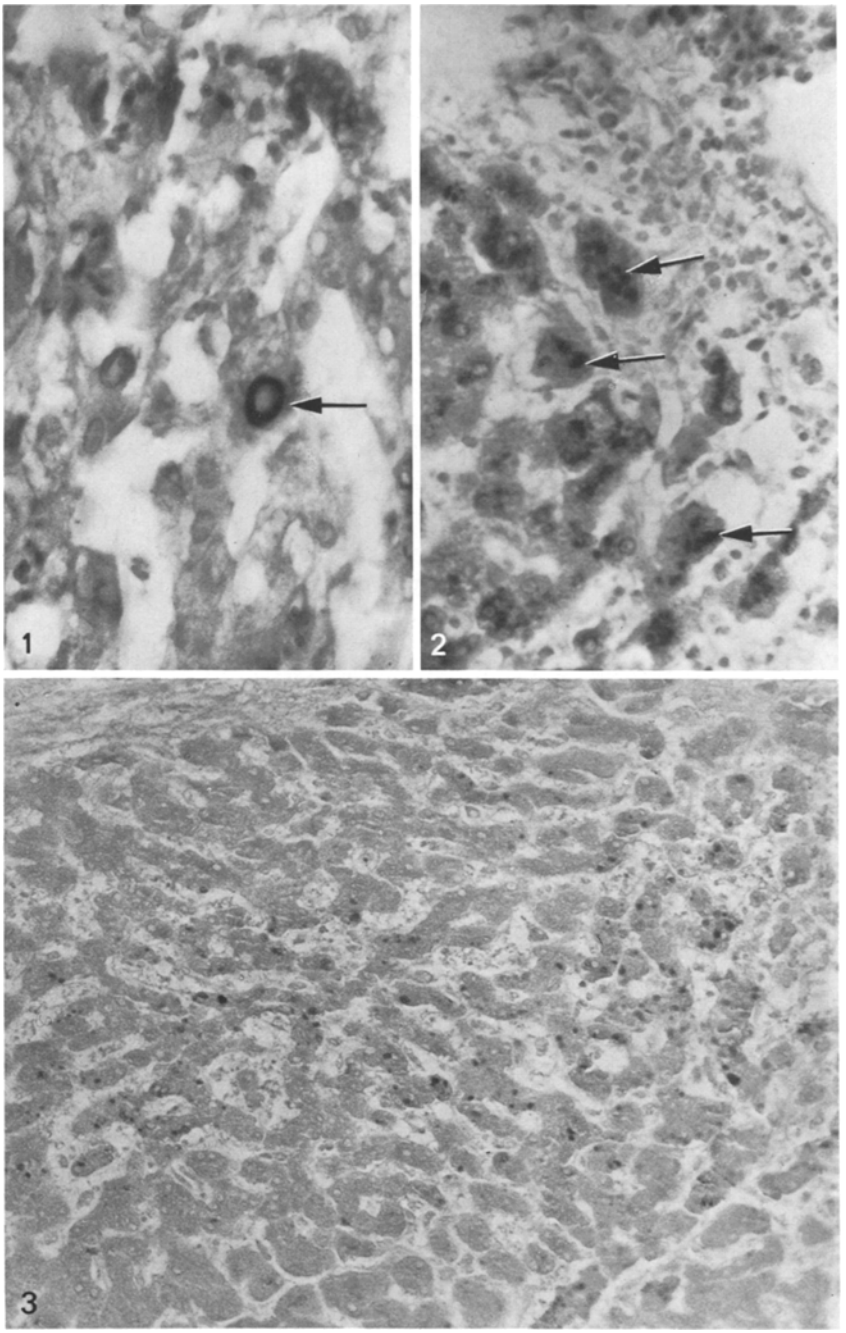

Fig. 1. AAT positve ring-like globule (*arrow*) in a case of liver cirrhosis. PAP × 500

Fig. 2. AAT positive atypical globules (*arrows*) in neoplastic cells in a case of hepatoma. PAP × 300

Fig. 3. AAT positive atypical globules of various sizes in a case of cirrhosis. PAP × 120

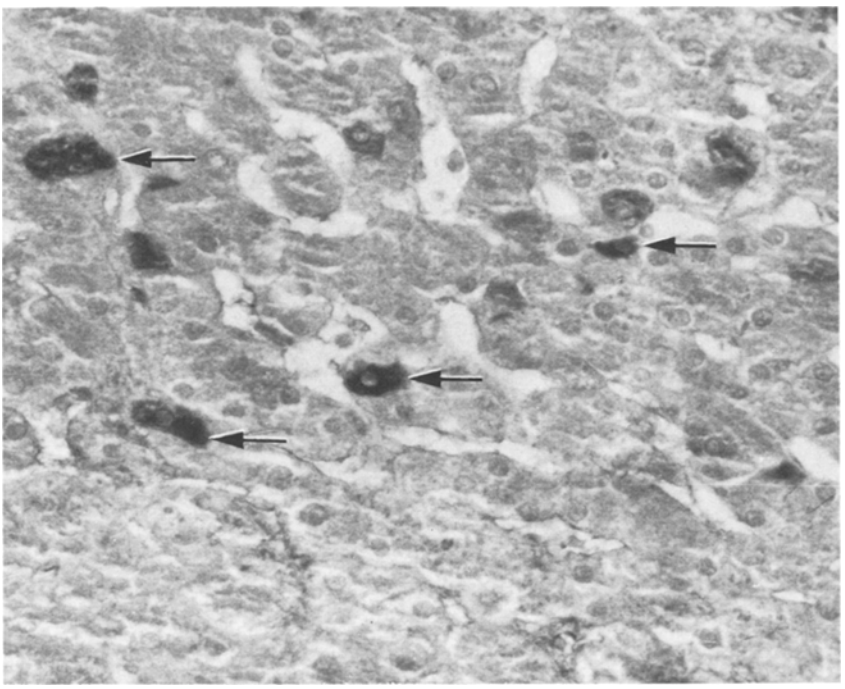

Fig. 4. Diffuse granular pattern of AAT deposition in the whole cytoplasm of some hepatic cells (*arrows*). PAP × 400

Discussion

Immunocytochemical and histological studies using intracytoplasmic liver AAT globules as markers have suggested that there is an increased incidence of the Z allele in cases of HCC (Blenkinsopp and Haffenden 1977; Palmer and Wolfe 1976) or cirrhosis (Blenkinsopp and Haffenden 1977; Eriksson et al. 1975), although phenotypic studies have shown no statistically significant increase of Z allele in cases of HCC and cirrhosis (Peters 1976; Theodoropoulos et al. 1976).

More recently, Kelly et al. (1979), in a series of 42 cases of HCC and 98 controls, described typical AAT globules stained most strongly at the globule periphery in the non-neoplastic liver tissue of two (5%) of HCC. In three cases in the non-neoplastic liver tissue and in one case of HCC atypical PAS-positive, diastase resistant globules were found at the edges of zones of centrilobular congestion and necrosis, resembling those produced in AAT deficiency. With immunocytochemical staining most of them were negative but a few were weakly stained. Finally, AAT was present in nine cases of HCC and in 28 controls as a diffuse and very fine granular pattern throughout the whole cytoplasm of liver cells. Reintoft and Hägerstrand (1979) studied 69 primary liver carcinomas by immunohistochemical methods and found AAT-positive globules or granules in non-tumor liver cells

in 12, while in 10 cases a fine granular pattern was seen. These investigators concluded that these globular AAT deposits may be genetically determined when associated with the Z-gene. In their series of 37 cases of HCC, Thung et al. (1979) detected AAT by the PAP technique in 73% of the patients as fine granules in the cytoplasm of tumor cells.

In a 1980 study of several protein markers, Palmer et al. (1980) described two different AAT-containing structures in one case of cirrhosis (phenotype SZ) and in one case of HCC (phenotype MM). The AAT globules in inherited AAT deficiency were round, regular and showed specific immunoperoxidase positivity as a dark brown granular reaction product distributed in a ring-like peripheral pattern around the globules, mainly within the cytoplasm of hepatocytes. The AAT deposits in HCC were found extracellularly and intracytoplasmically as round, often distorted globules, generally larger and less uniform in shape. These investigators (Palmer et al. 1980) concluded that two separate mechanisms of AAT-deposition are involved in HCC. The first is genetically determined and related to the presence of Z allele. The second is non-genetic in nature, confined to tumor tissue, and probably represents primary protein synthesis in tumor cells.

In this series of 55 cases of HCC and 35 cases of cirrhosis three different patterns of AAT distribution were seen. AAT deposits were seen as:

a) Round, typical globules strongly stained at the periphery (ring-like globules) in the non-neoplastic part of the liver in 12% of cases of HCC and in 11% of cases of cirrhosis. These globules were similar to those described by Kelly et al. (1979) and Palmer et al. (1980) as typical or characteristic of AAT deficiency. They were not seen in tumor cells.
b) Atypical globules of various sizes in neoplastic cells in 5.4% of cases of HCC and in 17% of cases of cirrhosis, resembling those described by Kelly et al. (1979) as negative or weakly stained by immunocytochemical staining and by Palmer et al. (1980) in one case of HCC.
c) A diffuse fine granular pattern throughout the cytoplasm of 31% of the cases of HCC in the neoplastic cells and in 27% of those in the non-neoplastic cells. This pattern was identical to that described by Kelly et al. (1979) in cases of HCC, as well as in controls.

These results agree with those of Kelly et al. (1979) concerning the location of typical ring-like globules in the non-neoplastic part of the liver in cases of HCC, although they were found more frequently (12%) in Greek patients than in British (5%). In Greek cirrhotics ring-like globules were found in about the same proportion (11%).

Although a phenotypic study could not be performed since sera were not available from these patients, the relatively high incidence of typical and atypical globules in cases of HCC and cirrhosis contrasts with the results of a previous study (Theodoropoulos et al. 1976) which found an extremely low gene frequency of Z allele in a Greek population of patients with cirrhosis and HCC. Thus, there is some doubt whether AAT-globules in the liver represent a histopathological marker of genetically determined AAT deficiency or a marker of broader significance, similar to that described in endodermal sinus tumors with normal serum AAT phenotype (Palmer et al. 1976). The simultaneous detection of different protein markers seen in early embryogenesis, such as AAT and $alpha_1$-fetoprotein in individual tumor cells in HCC support this conclusion (Thung et al. 1979, Palmer et al. 1980).

Our results concerning the granular pattern of AAT distribution agree with those of Kelly et al. (1979) which reported that one-quarter of HCCs contained cells showed a diffuse and very finely granular staining for AAT throughout the whole cytoplasm.

Although it is not known whether the presence of AAT in the majority of cases of HCC is due to increased production or faulty secretion of AAT by tumor cells (Thung et al. 1979), the diffuse fine granular pattern of AAT distribution found in the neoplastic and non-neoplastic cells in cases of HCC seems to be an expression of increased synthesis of AAT during the so-called acute phase reaction, as has already been suggested by Reintoft and Hägerstrand (1979). This suggestion is in agreement with the study of Theodoropoulos et al. (1979) who found that the levels of serum AAT, as well as the serum trypsin inhibitory capacity, are relatively high in patients with HCC.

In this series, AAT-positive cells were found in HCC with moderate and high differentiation at a ratio of 55 and 29% respectively, as opposed to the findings of Reintoft and Hägerstrand (1979), who did not note any correlation between the degree of differentiation of HCC and the number of AAT-positive cases.

References

Aagenaes O, Fagerhol M, Elgo K, Munthe E, Hovig T (1974) Pathology and pathogenesis of liver disease in alpha-1-antitrypsin deficient individuals. Postgrad, Med J 50:365–375

Berg NO, Eriksson S (1972) Liver disease in adults with alpha$_1$-antitrypsin deficiency. New Engl J Med 287:1264–1267

Blenkinsopp WK, Haffenden GP (1977) Alpha-1-antitrypsin bodies in the liver. J Clin Pathol 30:132–137

Bradfield JW, Blenkinsopp WK (1977) Alpha-1-antitrypsin globules in the liver and PiM phenotype. J Clin Pathol 30:464–466

Cohen C (1976) Intracytoplasmic hyaline globules in hepatocellular carcinoma. Cancer 37:1754–1758

Dekker A, Krause JR (1973) Hyaline globules in human neoplasma. A report of 3 autopsy cases. Arch Pathol 95:178–181

Edmondson HA (1958) Tumors of the liver and intrahepatic bile ducts, in Atlas of Tumor Pathology. Washington, DC, Armed Forces Institute of Pathology, section 7, p 32

Eriksson S (1964) Pulmonary emphysema and a$_1$-antitrypsin deficiency. Acta Med Scand 175:197–205

Eriksson S, Hägerstrand I (1974) Cirrhosis and malignant hepatoma in a$_1$-antitrypsin deficiency. Acta Med Scand 195:451–458

Eriksson S, Moestrup I, Hägerstrand I (1975) Liver, lung and malignant disease in heterozygous (PiMZ) alpha-1-antitrypsin deficiency. Acta Med Scand 198:243–247

Ganrot PO, Laurell CB, Eriksson S (1967) Obstructive lung disease and trypsin inhibitors in alpha-1-antitrypsin deficiency. Scand J Clin Invest 19:205–208

Kelly JK, Davies JS, Jones AW (1979) Alpha-1-antitrypsin deficiency and hepatocellular carcinoma. J Clin Pathol 32:373–376

Lieberman J (1974) Emphysema, cirrhosis and hepatoma with alpha-1-antitrypsin deficiency. Ann Intern Med 81:850–852

Lieberman J, Silton RM, Agliozzo CM, McMahon J (1975) Hepatocellular carcinoma and intermediate alpha-1-antitrypsin deficiency. Am J Clin Pathol 64:304–310

Norken SA, Campagna-Pinto D (1968) Cytoplasmic hyaline inclusious in hepatoma. Arch Pathol 86:25–32

Palmer PE, Wolfe HJ (1976) a$_1$-antitrypsin deposition in primary hepatic carcinoma. Arch Pathol Lab Med 100:232–236

Palmer PE, Wolfe HJ (1977) Alpha$_1$-antitrypsin and hepatocellular carcinoma. IRCS Med Sci Aliment Syst 5:109

Palmer PE, Christopherson WM, Wolfe HJ (1977) Alpha-1-antitrypsin, protein marker in oral contraceptive-associated hepatic tumors. Am J Clin Pathol 68:736–739

Palmer PE, Safaii H, Wolfe HJ (1976) Alpha-1-antitrypsin and alpha-fetoprotein: Protein markers in endodermal sinus (yolk sac) tumors. Am J Clin Pathol 65:575–582

Palmer PE, Ucci AA, Wolfe HJ (1980) Expression of protein markers in malignant hepatoma. Cancer 45:1424–1431

Peters RL (1976) Pathology of hepatocellular carcinoma. In: Okuda K, Peters RL (eds) Hepatocellular carcinoma, John Wiley, New York and Chichester, pp 107–168

Rawlings WJ, Moss J, Cooper HS, Hamilton SR (1974) Hepatocellular carcinoma and partial deficiency of alpha-1-antitrypsin (MZ). Ann Intern Med 81:771–773

Reintoft I (1978) Periodic acid Schiff-positive nonglycogenic gobules in hepatocytes. Differential diagnostic aspects in screening for a_1-antitrypsin globules in autopsy material. Acta Pathol Microbiol Scand 86:325–329

Reintoft I, Hägerstrand I (1979a) Demonstration of a_1-antitrypsin in hepatomas. Arch Pathol Lab Med 103:495–498

Reintoft I, Hägerstrand I (1979) Does the Z-gene variant of a_1-antitrypsin predispose to hepatic carcinoma? Hum Pathol 10:419–423

Sternberger LA, Hardy PH Jr, Cuculis JJ, Meyer HG (1970) The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-anti-horseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315–333

Theodoropoulos G, Fertakis A, Archimandritis A, Kapordelis C, Angelopoulos B (1976) Alpha-1-antitrypsin phenotype in cirrhosis and hepatoma. Acta Hepato-Gastroenterol 23:114–117

Theodoropoulos G, Archimandritis A, Tsomi A, Chloros G, Rigatos G, Angelopoulos B (1979) Serum trypsin inhibitory capacity and alpha-1-antitrypsin levels in liver cirrhosis and hepatoma. Acta Hepato-Gastroenterol 26:195–197

Thung SN, Gerber MA, Sarno E, Popper H (1979) Distribution of five antigens in hepatocellular carcinoma. Lab Invest 41:101–105

Triger DR, Millward-Sadler GE, Czaykowski AA, Trowell J, Wright R (1976) Alpha-1-antitrypsin deficiency and liver disease in adults. QJ Med 45:351–372

Williams WD, Fajardo LF (1974) Alpha-1-antitrypsin deficiency: A hereditary enigma. Am J Clin Pathol 61:311–320

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