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Radial Immunodiffusion versus Serum Protein Electrophoresis as a Tool for Diagnosis of Alpha-1-antitrypsin Deficiency

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Alpha-1-antitrypsin (AAT) derived its name from the identification of an alpha-1-globulin on serum protein electrophoresis whose activity was measured by inhibition of trypsin action. Since AAT is actually capable of interfering with the action of a variety of proteolytic enzymes, it is also known as alpha-1-protease inhibitor. In 1963 Laurell and Eriksson first reported a genetically determined deficiency of a major serum protease inhibitor which has strong association with early onset of severe emphysema(1). In 1968 Sharp noted a serum electrophoretic pattern without an a_1-globulin peak and found it belonged, not to an adult with emphysema, but to a child with cirrhosis(2). Studies have shown that in those affected with AAT-deficiency, conjugated hyperbilirubinemia usually occurs within first 3-4 months of life(3-5). Elevated hepatocellular enzyme levels which remain so even after the jaundice has cleared and bile-duct hypoplasia have been reported(6,7). Recently, we encountered AAT-deficiency in a 2-month-old female Libyan patient, which we believe, is the first case of AAT-deficiency reported from Libya.

Case Report

The reference child was born to a non-consanguinous parents and was admitted with diarrhea of 10 days and jaundice of 2 days duration and lethargy. The child was born 3rd in order at 35-36 weeks of gestation and weighed 2200 g at birth. There was no family history of jaundice or any history suggestive of liver disorder. The child was breast fed for 5 days and thereafter received formula feeding.

On general examination, the child was sick looking, moderately jaundiced,

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afebrile and dehydrated with oral thrush. There was no pallor, edema or lymphadenopathy. The pulse rate was 130/min and blood pressure was 80/50 mm Hg. The child weighed 2500 g (<3rd centile) and length and head circumference were also below 3rd centile of measurements. Systemic examination revealed a distended tympanitic abdomen and palpable liver 3.5 cm below right costal margin, with smooth surface, and firm consistency. Roentgenogram of chest abdomen showed no abnormality. A clinical diagnosis of gastroenteritis with moderate dehydration with septicemia was made.

The routine laboratory investigations (hematological, biochemical and microbiological) revealed the following results: hemoglobin 9.0 g/dl, hematocrit 27.2%, mean corpuscular volume 93 μm^3; mean corpuscular of hemoglobin 30.9 pg; Platelet count 487 $\times$ 10^9/L; total leucocyte count 12100 mm^3; with neutrophil 36%; band cells 7%; lymphocyte 51%; monocyte 4%; eosinophil 2%; Prothrombin time 12-13 sec; Partial thromboplastin time of 31-32 sec, Fibrin degradation product 10 μg/L; Sickling test negative; blood group: O Rh^+; Serum Na 131 mmol/L; Serum K 4.8 mmol/L; Serum bicarnbonate 31 mmol/L; Serum Ca 9.4 mg/dl; Serum phosphorus 3 mg/dl; Serum creatinine 0.4 mg/dl; Blood sugar 66 mg/dl; Serum total protein 6.7 g/dl; Serum albumin 3.4 g/dl; Serum globulin 3.3 g/dl; Serum total bilirubin 7.4 mg/dl; Serum bilirubin 5.4 mg/dl direct; Serum bilirubin 2.0 mg/dl indirect; Serum alkaline phosphatase 67 KKU/dl; Serum GOT 301 IU/L; Serum GPT 320 IU/L and Serum HBsAG: negative. The routine microbiological cultures of blood, urine, stool and cerebrospinal fluid for bacterial and fungal growth were negative. The results of special laboratory investigations, serum protein electrophoresis and radial immunodiffusion assay are shown in the *Table.* The patient had hyper-γ-globulinemia with no detectable AAT, although alpha-1-globulin fraction was normal on electrophoresis. Also, the results of liver function tests, serum protein electrophoresis and radial immunodiffusion assays in other two sibls and parents did not reveal any abnormality. In the light of above investigations, the diagnosis was revised as AAT-deficiency with gastroenteritis and septicemia.

The infant was treated conservatively with intravenous fluids, nasogastric aspiration, systemic parenteral antibiotics (ampicillin and gentamycin), blood transfusion and other supportive measures. The infants's general condition improved gradually, was accepting oral feeds and discharged well after 2 weeks stay in the hospital. On discharge, she was still jaundiced (serum bilirubin: 6.8 mg/dl) and there was 3 cm hepatomegaly.

Discussion

AAT deficiency (also known as alpha-1-protease inhibitor deficiency is a rare genetic disorder. The single gene coding for AAT is contained within a 10-kb segment of DNA composed of five axons on chromosome 14(8,9). The laboratory development that has contributed most to an understanding of AAT variants has been that of electrophoresis on starch gel electrophoresis in discontinuous acid buffer(9). A system (Pi) of labelling of the variants based on letters of alphabets has been adopted; the electrophoretically slowest being denominated Z, the usual M, the faster F and S, the second most common type, falls between M and Z.

The diagnosis of the Pi null-null phenotype is usually made by evaluating serum samples of the patient and his family.

TABLE –*Serum Protein Electrophoresis and Radial Immunodiffusion Assays.*

Parameters	Patient values	Normal values
*Serum protein electrophoresis (%)**		
Albumin	48.2	40-50
Alpha-1-globulin	5.1	2-4
Alpha-2-globulin	11.1	5-8
Beta-globulin	9.3	5-8
Gamma-globulin	26.3	3-12
*Radial immunodiffusion assays (mg/dl)***		
Immunoglobulin G	1319.0	270-780
Immunoglobulin A	211.0	6-58
Immunoglobulin M	140.0	12-87
Immunoglobulin D	1.0	0.0-0.6
Immunoglobulin E	38.2	2-17
Complement C3	115.0	56-125
Complement C4	38.0	16-40
Alpha-1-antitrypsin	Not detected	105-200

*Serum protein electrophoresis was carried out on cellulose acetate paper using barbital buffer (0.01 M, pH 8.6) in Beckman Model C-120 and scanning in Beckman Model CDS-200.

**Serum immunoglobulins (G, A, M, D), complements (C3, C4) and alpha-1-antitrypsin were estimated by radial immunodiffusion technique using LC-partigen plates (Behringwerks, West Germany); Serum immunoglobulin E was estimated by enzyme immunoassay (EIA) using IgE-EIA kit of bioMeriux, France. Immunoglobulin E is depicted as IU/ml.

Various immunochemical techniques used include radial immunodiffusion, immunoelectrophoresis, serum protein electrophoresis, isoelectric focussing, enzyme immunoassay, and nephalometry. Serum protein electrophoresis is, however, not helpful as alpha-1-globulin fraction may be shown as normal, at a time when reduced or no AAT in serum can be detected by radial immunodiffusion method as seen in our case reported here. Generally, the results of the determination of inhibitory function correlate well with the measurements of concentration, so that there appears to be no need to perform both types of tests routinely. Radial immunodiffusion technique is simple, specific, accurate, much cheaper and no special instrumentation is required, compared to other immunochemical methods. Therefore, it may be the method of choice for evaluation of AAT deficiency particularly in laboratories where other expensive and sophisticated methods are not available.

From clinical view point, whenever jaundice is encountered in newborn and infants, AAT deficiency should be ruled out as a possible cause. In view of the limited prevalence of AAT deficiency (PiZZ, PiZS) and rare occurrence of Pi null-null phenotype, it is doubtful whether a programme to screen general population for this defect would be worthwhile. What may well be cost effective is the screening for AAT deficiency of all cases of infantile jaundice or obstructive lung disease. This will help to identify affected families so that genetic counselling may be provided. Secondly, the most exciting new development in this field is that replacement therapy is likely to be available soon(10), whereas liver transplantation(11) and gene therapy remain for the near future.

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