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Preliminary Studies on Serum Alpha-1-Antitrypsin in Children with Sickle-cell Anaemia

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Summary

Ezeoke ACJ. Preliminary Studies on Serum Alpha-1-Antitrypsin in Children with Sickle-cell Anaemia. *Nigerian Journal of Paediatrics* **1985; 13: 13.** Serum levels and phenotype distribution of alpha-1-antitrypsin (A–1–AT) were determined in 107 children with sickle-cell anaemia and in 100 healthy controls of similar ages. The mean level of A–1–AT in the sicklers was 4.39 g/l±1.61 g/l (range 1.68 g/l - 7.37 g/l) and this was significantly higher than the mean of 2.62g/l ± 0.53g/l (range 2.05 g/l - 4.4g/l) in the controls ($p<0.001$). It was considered that this difference was most likely the result of a non-specific response to tissue damage in the sicklers. Preliminary phenotype study on starch gel electrophoresis revealed only the common allele Pi^M.

Introduction

REPORTS on serum proteins in sickle-cell disease had been scanty and had in the main, involved American negroes[1–4] until the study involving Nigerians, which was reported by Isichei in 1979.[5] Apart from the small numbers of patients reported in these studies, the results obtained were generally inconsistent. Furthermore, all these reports were mainly on total serum protein, albumin and globulin. It has long been known that patients with sickle-cell disease are prone to infections particularly, bacterial. Various proteolytic enzymes are liberated in human plasma during such bacterial infections.[6] The actions of these proteases are opposed by various protease inhibitors such as serum alpha-1-antitrypsin (A-1-AT) which is produced in the liver.[7] In the present study, we have determined the serum concentrations and the phenotype distribution of A-1-AT in children with sickle cell anaemia.

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Materials and Methods

The subjects were 107 children (43 males: 64 females) aged 9 months—16 years (mean, 8.6 years) with sickle-cell anaemia in steady state. These children were not in crisis, or on steroid therapy, neither did they have acute infections at the time of the study. Diagnosis of sickle-cell anaemia was based on clinical and haemoglobin electrophoretic examinations. Controls consisted of 100 apparently healthy children of similar ages and sexes. They were homozygous for haemoglobin A and had no present, past or family history of liver disease, chronic respiratory and renal disorders.

Five to ten millilitres of venous blood were obtained under aseptic technique, from each subject and control. Sera separated from the blood samples were stored at –20°C until required. Electrophoresis of the serum protein was done on gelatinized cellulose acetate strips. Serum A-1-AT levels were estimated using low concentration (LC) and M-Partigen plates purchased from *Hoechst Nigeria Limited*. For standard, *Hoechst's* protein standard plasma (human) lyophillized was used. Alpha-1-antitrypsin standard was diluted 1:2, 1:5 and 1:10 and used in plotting a dilution curve. The square of the diameter of the ring precipitate was plotted against the concentration of antigen. From the curve, the values of the concentration of A-1-AT was read off. Quantitative serum protein was estimated by the Biuret method. Phenotyping of serum A-1-AT was performed by starch gel electrophoresis.[8]

Results

Table I compares the serum A-1-AT levels in the subjects and controls. The sicklers had significantly higher mean A-1-AT level (4.39± 1.61 g/l) than the controls (2.62±0.53 g/l) ($p<0.001$).

Cellulose acetate electrophoresis did not show any significant deviation from the normal; the alpha-1-band was present in all the materials examined. The deficient Z gene was not detected in the homozygous state in any of the specimens investigated. Those samples which, from the starch gel or assays, appeared to have a low concentration of A-1-AT were classified heterozygous. Phenotype result showed 85% were Pi^M homozygotes.

The means of total serum protein and albumin for the control group were found to be significantly higher than those for sicklers ($p<0.001$ and $p<0.01$ respectively) (Table II). However, the reverse was the case for serum globulin ($p<0.01$).

TABLE I

Serum Alpha-1-antitrypsin Levels in Children with Sickle-cell Anaemia and in Controls

Serum Alpha-1-antitrypsin (g/l)	*Sicklers n = 107*	*Controls n = 100*	*P*
Mean	4.39	2.62	
Standard Deviation	1.61	0.53	<0.001
Range	1.68 - 7.37	2.05 - 4.40	

TABLE II

Total Serum Protein, Albumin and Globulin Levels in Children with Sickle-cell Anaemia and in Controls

Parameters	*Sicklers n = 107*	*Controls n = 100*	*P*
Age (years)			
Mean	8.6	8.4	
SD	5.2	4.9	>0.5
Range	0.75 - 16	1-16	
Total Protein (g/l)			
Mean	63.4	78.3	
SD	13.9	5.6	<0.001
Range	38.4 - 98.7	60.5 - 84.3	
Albumin (g/l)			
Mean	37.6	43.0	
SD	7.3	4.5	<0.01
Range	24.1-52.6	39.6-50.3	
Globulin (g/l)			
Mean	35.0	32.2	
SD	10.2	5.6	<0.01
Range	28.0-52.8	25.5-45.2	

SD = Standard deviation

Discussion

Serum A-1-AT levels are known to vary in various diseases. The association between genetic deficiency of serum A-1-AT and both emphysema[6,9] and cirrhosis of the liver,[10] is well established. Furthermore, the serum level of the enzyme is frequently elevated in malignancy,[11] acute infection,[12] rheumatoid arthirtis,[13] pregnancy,[14] sarcoidosis,[15] leprosy[16] and during corticosteroid therapy[17] and oral contraceptive administration.[18,19] Serum alpha-1-globulin contains about 90–95 per cent A-1-AT. Therefore, the measurement of serum alpha-1-globulin can be used as a screening procedure to detect cases with A-1-AT elevation[20] or deficiency.[21]

In the present study, the serum A-1-AT levels were significantly higher in sicklers than in controls. Although the serum trypsin inhibitory capacity (STIC) was not measured, it has been established that there is a strong positive correlation between STIC and serum A-1-AT levels. This means that either of the two can be used in a preliminary screening for a possible genetically controlled A-1-AT deficiency.[22] The immediate significance of elevated A-1-AT levels in sickle-cell anaemia is not clear. One can only speculate that since the Pi system is one of the three systems involved in immune response function of serum proteins, it would be elevated in repeated infections in the same way as serum immunoglobulins. Besides, since there is probably a higher rate of tissue damage in sickle-cell anaemia than in normal individuals, high levels of A-1-AT may be a non-specific response.

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