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The Diagnostic and Prognostic Value of Serum Trypsin Inhibitory Capacity in Infants with Idiopathic Respiratory Distress Syndrome

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Summary

Omene JA, Ihongbe JC, Imoedemhe D and Glew RH. The Diagnostic and Prognostic Value of Serum Trypsin Inhibitory Capacity in Infants with Idiopathic Respiratory Distress Syndrome. *Nigerian Journal of Paediatrics* **1984; 11:1.** Trypsin inhibitory capacity (TIC), a measure of alpha-1-antitrypsin, was determined in the umbilical cord sera of 36 preterm infants. Twenty one of the infants had Idiopathic Respiratory Distress Syndrome (IRDS), while 15 without IRDS served as controls. The mean TIC levels in cord serum were significantly reduced ($p<0.001$) in infants with IRDS. Mortality was 80% among infants with IRDS who weighed 2000g or less and had TIC values of less than 0.65mg per ml. Furthermore, there was a consistent rise in serial TIC levels in 4 infants with IRDS who survived. Thus, the determination of serum TIC levels may have diagnostic and predictive value in the management of newborn babies with IRDS.

Introduction

EVIDENCE for decreased concentration of serum trypsin inhibitors and alpha-1-antitrypsin in preterm infants with idiopathic respiratory distress syndrome (IRDS) was first documented by Evans, Levi and Mandl.[1] Their finding was later confirmed by Kotas, Franzen and Moore.[2] The present prospective study was undertaken in an effort to demonstrate the diagnostic and prognostic value of trypsin inhibitory capacity (TIC) as a simple biochemical method for evaluating neonates with IRDS.

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Materials and Methods

Trypsin inhibitory capacity in cord sera was determined in two groups of neonates. The first group included 21 preterm babies with IRDS. In four infants who survived, serial blood samples were obtained on the third day of life for TIC levels when clinical, biochemical and radiological evidence indicated signs of recovery. The diagnosis of IRDS was based on the presence of

retraction of the chest wall, expiratory grunting and cyanosis in room air. Chest x-ray taken within 6 hours of admission, showed typical reticulogranular pattern and air bronchogram that are diagnostic of IRDS. The second group of babies without IRDS, served as control. This group consisted of 15 preterm babies that were matched in respect of gestational age and sex. In three of these infants, serial TIC values were also determined on the third day of life.

Eight of the 21 infants with IRDS required an ambient oxygen of more than 70%. These infants required continuous positive airway pressure (CPAP) at pressure of 2 to 5 cm of water by nasal prongs. Only three babies in the control group required intermittent use of ambu bag resuscitator for apnoeic attacks. The gestational age was assessed by means of physical characteristics[3] as well as by neurological evaluation.[4] The condition of the infants at birth was assessed with the Apgar scoring system. Infants with evidence of infection or whose mothers had prolonged rupture of membrane were excluded from the study.

Alpha-1-antitrypsin was quantitated by assaying trypsin inhibitory capacity (milligrams of trypsin inhibited by one millilitre of serum). Cord blood and peripheral blood samples were allowed to stand at 22^0C for 45 minutes, after which time the specimen was centrifuged at 1000 x g for 7 minutes with the aid of a bench-top clinical centrifuge. The resulting serum sample was transferred to a clean test tube and stored at -20^0C until the time of analysis. No deterioration of protease inhibitor activity occurred during storage for weeks. Trypsin inhibitory capacity was determined according to the method of Fagerhol and Laurel,[5] using benzoylarginyl paranitro-anilide as the trypsin substrate.

The Student's t-test and chi-square test were used to evaluate the significance of the results.

Results

The Table presents the clinical data and the serum TIC levels in 21 infants with IRDS and 15 preterm babies without IRDS. Six preterm babies with IRDS had Apgar scores ranging between 5 and 6 at 1 min, while 4 babies without IRDS were moderately asphyxiated (Apgar score, 5-6 at one minute). There were no significant differences observed between the mean for gestational age for the IRDS group of infants and the control group. However, the mean birthweight for preterm babies with IRDS differed significantly from that of infants without ($p < 0.001$).

TABLE

Serum Trypsin Inhibitory Capacity in 21 Preterm Babies with IRDS and 15 Control Subjects

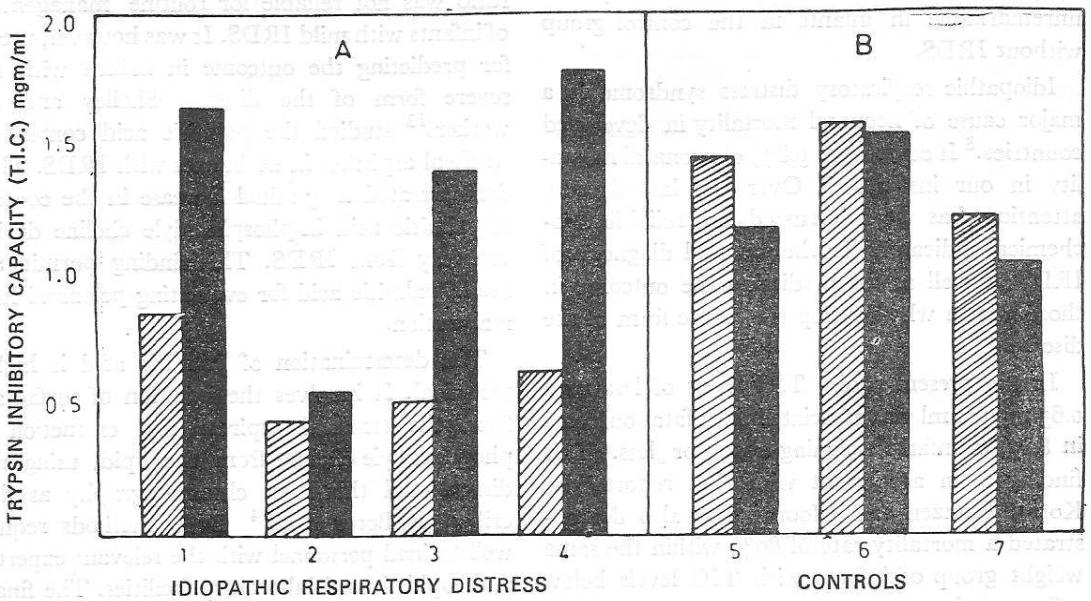
Subject	*No. of Subjects*	*Gestational Age (Weeks)* Mean ± SD	Range	*Birthweight (gm)* Mean ± SD	Range	*TIC (mg Trypsin inhibited/ml serum)* Mean ± SD	Range
Preterm babies with IRDS	21	31.76 ± 3.1	28 – 36	1488 ± 58	750 – 2600	0.62 ± 0.16	0.4 – 0.80
Preterm babies without IRDS	15	31.53 ± 4.7	24 – 36	1720 ± 78	700 – 2700	0.96 ± 0.38	0.44 – 1.57
p		>0.1		<0.001		<0.001	

IRDS = Idiopathic Respiratory Distress Syndrome

TIC = Trypsin Inhibitory Capacity

The mean TIC value (0.62 mg/ml±0.16) was significantly reduced in the preterm babies with IRDS when compared with that (0.96 mg/ml± 0.38) obtained for the control group ($p<0.001$). The relationship between TIC levels and outcome of the disease was examined in infants within a specific weight group. Seventeen infants with IRDS weighed 2000g or less at birth. Eight (80%) out of 10 infants in this weight group died and their TIC levels were less than 0.65 mg per ml. In contrast, only one of the remaining seven (14.3%) infants with TIC levels above 0.65 mg per ml, died. The difference in the survival rate when TIC levels were less than 0.65mg per ml was highly significant ($p<0.001$). In the control group, 9 infants weighed 2000g or less, at birth. One of the three infants with TIC levels below 0.65 mg per ml, died. TIC levels in the control group were not useful in predicting those infants who would survive ($p>0.1$) in this specific weight group.

Serial TIC levels were determined on four infants with IRDS. The Figure was constructed from the results obtained for the individual concentration of this protease inhibitor. The cord serum TIC values for the IRDS infants were 0.85, 0.44, 0.51 and 0.62 whereas the control infants had cord TIC levels of 1.45, 1.57 and 1.22. On the third day of life, after improvement was documented clinically and biochemically, blood sera were again assayed for TIC. As shown in the Figure, there was a consistent rise in TIC levels during recovery from IRDS. The mean value for the protease inhibitor concentration of these infants was 1.35 mg/ml (±0.55). This value was higher than 2 standard deviations of the mean level (0.62 ± 0.16) obtained for the four infants during the acute phase of the disease. By contrast, the TIC levels for the three infants in the control group either remained unremarkable or showed slight decreases.



Figure; Serial TIC levels in subjects and controls during the acute phase of the illness and after recovery.

Discussion

The important findings in the present study were: (*a*) the significant decrease of serum TIC levels in preterm infants with IRDS. It may be argued however, that the observed decrease in TIC levels may be a reflection of differences in birthweight between preterm babies with IRDS and those without, since Kotas, Franzen and Moore[2] found a correlation between TIC levels and specific birthweights. However, a careful study conducted by Evans, Keller and Mandl[6] failed to demonstrate any correlation between the levels of protease inhibitors and birthweight. Furthermore, our previous study demonstrated significant correlation between TIC levels and gestational age only.[7] In the present study, no significant difference was observed between the mean gestational age for the preterm IRDS infants and the control group. It is therefore, reasonable to conclude that the low TIC levels found in IRDS infants are distinct biochemical features of IRDS (*b*) serum TIC levels were appreciably elevated in infants with IRDS who survived, while they were unremarkable in infants in the control group without IRDS.

Idiopathic respiratory distress syndrome is a major cause of neonatal mortality in developed countries.[8] It constitutes 9.8% of neonatal mortality in our institution. Over the last decade, attention has been focussed on reliable biochemical indicators for the prenatal diagnosis of IRDS as well as for predicting the outcome in those infants who develop the severe form of the disease.

In the present study, TIC levels of less than 0.65 mg per ml was associated with fatal outcome in 80% of infants weighing 2000g or less. This finding is in agreement with that reported by Kotas, Franzen and Moore[2] who also demonstrated a mortality rate of 80% within the same weight group of infants with TIC levels below 0.65 mg/ml.

Evans, Keller and Mandl[6] have highlighted the prognostic value of TIC determinations in infants with IRDS. These authors showed that total serum TIC levels were elevated in subsequent serum samples of survivors of infants with IRDS. In contrast, persistently low levels were associated with poor prognosis. Four surviving infants in our series, on whom serial TIC levels were determined, exhibited increase of this protease inhibitor. Sequential serum TIC values that were obtained in the control group did not show such increase, thus eliminating the possibility that the observed increase in TIC levels in survivors may be due to the acute phase reactant properties of alpha-1-antitrypsin which is usually elevated in hospital population.[2] It has been shown that the depressed TIC levels found in IRDS are due to the sequestration of alpha-1-antitrypsin in the hyaline membrane.[9]

Other methods with predictive value for assessing postnatal lung maturation are the lecithin to sphingomyelin ratio (L/S) obtained from tracheal aspirates[10-12] and the determination of palmitic acid content in tracheal aspirates.[13] Kanto *et al*[12] have cautioned that the mean L/S ratio was not reliable for routine management of infants with mild IRDS. It was however, useful for predicting the outcome in infants with the severe form of the disease. Shelley and coworkers[13] studied the palmitic acid content of tracheal aspirates in 20 infants with IRDS. They demonstrated a gradual increase in the content of palmitic acid in phosphatidyle choline during recovery from IRDS. This finding permits the use of palmitic acid for evaluating postnatal lung maturation.

The determination of palmitic acid is highly technical. It involves the isolation of surfactant from the tracheal aspirate and extraction of phosphatidyle choline from the lipid, using two dimensional thin-layer chromatography as described by Borer *et al*.[14] Both methods require well trained personnel with the relevant expertise and sophisticated laboratory facilities. The financial investment for setting up such laboratories is prohibitive for most developing countries. However, the TIC assay method[5] requires simple

and inexpensive colorimeters and readily available substrates that should be within the means of and quite suitable for clinical laboratories in most developing countries. The assay time is approximately one hour. This should encourage clinicians in these countries to consider the determination of TIC levels for monitoring high risk infants with IRDS, whenever feasible. However, adverse perinatal factors, such as intra-uterine infection and prolonged rupture of membranes[2] that are associated with elevated TIC levels must be excluded when this method is employed.

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