RESEARCH PAPER

Cord Serum Screening Test and the Newborn's Allergic Status

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Objective: To evaluate the predictive value of a cord serum screen test and possible subsequent development of allergic disease in infants.

Design: Cohort study.

Setting: 100 pregnant women were randomly recruited for the study.

Methods: The maternal serum and the cord serum of their matched newborn were analyzed for total serum immunoglobulin E (IgE), gamma interferon (γ IFN), house dust mite- Dermatophagoides pteronyssinus allergen (Der p1) and Blomia tropicalis allergen (Blo t5) using immunoassay methods. All infants were followed up for one year.

Results: Infants who had allergic diseases in the one year follow-up (n=45) had significantly (P<0.001) elevated IgE, Der p1, Blo t5, and significantly low γ IFN levels in cord serum as compared to the same parameters of infants who did not develop allergic disease in the one year follow-up (n=43).

Conclusion: In utero exposure to HDM allergens Der p1 and Blo t5 is prevalent. We have successfully established a cord serum screening test for predicting allergic diseases in infancy with 93% specificity and sensitivity.

Key Words: *Allergy, Cord serum, Infant, IgE, House dust mite, γ interferon.*

Allergic symptoms usually appear early in life implying early priming for allergic disease(1). Evidence that there is a significant *in-utero* component to allergic disorders came from studies of twins by Edfors-Lubs(2). Reports suggest that immune "imprinting" may actually begin *in utero* through the fetal exposure to inhalant allergens(3). It is also well established that the induction of allergen-specific T-cell memory is frequently initiated *in utero*, and maternal factors may exert their influence during this period. Prescott, *et al*.(4) observed an inverse relationship between maternal atopy and perinatal γ IFN (gamma interferon) production. Cord serum (CS) IgE is a significant risk factor for the development of allergy in offspring. However, there is a dilemma about its application, with some studies concluding that it is better than family history for predicting sensitization(5), and others, that it has low predictive value(6). The present research was carried out between 2004-2006, to establish a CS screening test for newborn by correlating the *in-utero* exposure to the ubiquitous perennial house dust mite allergens *Dermatophagoides pteronyssinus* (Der p1) and *Blomia tropicalis* (Blo t5), γIFN and IgE at birth, with the development of allergic symptoms at the age of one year.

METHODS

The study was approved by the Board of Studies in Biochemistry, University of Mumbai, India. We recruited 100 pregnant women for the study using random number table. An informed written consent

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was taken prior to enrolment. Name, age, address, phone number and history of allergy to house dust were noted. The Person's 'doctor's diagnosis of allergy to house dust' was taken as positive history of allergy to house dust. Her negative history of allergy to house dust and no records of visit to doctors for any allergic conditions was taken as negative history of allergy to house dust.

On admission for delivery, 5mL venous blood was collected in the vaccutainer straight from the peripheral vein using the needle holder to avoid any external contamination. After delivery, 5mL cord blood was collected by aspiration from the umbilical vein of the placenta into sterile plain glass tube. Date of delivery, gender and health status of the baby was noted. Tube containing cord blood was kept at room temperature for the retraction of blood clot. Serum was separated and aliquots were made in screw-cap sterile plastic vials. Each aliquot was labeled with the matched mother's name, date and serial number and was stored at –20ºC. CS total IgA was estimated by nephelometery (Beckman, USA)(7). Total serum IgE was estimated by the immunoradiometric assay (DPCt, USA)(8). γ IFN was estimated by ELISA (Immunotech, France)(9). Der p1 and Blo t5 allergen in serum was detected by using the ELISA (Indoor Biotechnologies, UK)(10). For a period of one year, monthly follow up of the infants was carried out. Symptom based postnatal questionnaire was updated after each follow up. Pediatrician's diagnosis was recorded and babies allergic status was defined based on the same. Descriptive statistics, Chi-square test and Fisher exact test were used. As variation was high amongst subjects, throughout the statistical analysis, non-parametric tests were used to evaluate significance. Medians were considered as the central value and compared between the groups.

TABLE I HOUSE DUST MITE ALLERGEN IN CORD SERUM VS. MATERNAL ALLERGIC STATUS

Allergens in cord serum	Mother allergic to house dust	Mother not allergic to house dust
Der p1 and Blo t5	52.3% (23/44)	9.1% (4/44)
Der p1	18.2% (08/44)	2.3% (1/44)
Blo t5	13.6% (06/44)	4.5% (2/44)
Total	84.1% (37/44)	15.9% (7/44)

RESULTS

Twelve cases were excluded from the study (9 were pre-term deliveries, 1 was miscarriage, 1 was stillbirth and 1 aborted). Of the rest, 49 (55.7%) women were having allergy problems. Allergic mothers (*n*=49) had significantly raised IgE (280 vs 40 IU/mL), lower γ IFN (36.5 vs. 139.0 IU/mL), higher Der p1 (1.9 *vs.* 0 ng/mL) and higher Blo t5 (1.9 *vs.* 0 ng/mL) level as compared to non-allergic mothers (*n*=39) (*P*<0.001).

All cord sera (*n*=88) had serum IgA less than <0.06 IU/mL confirming that there was no contamination with maternal blood. HDM allergens were detectable in 50% (44/88) matched maternal and CS samples (***Table* I**). Infants who had Der p1 and/or Blo t5 allergen present in CS were at a significantly higher risk (*P*<0.001) than infants who had Der p1 and/or Blo t5 allergen absent in CS (OR =49.5; 95% CI 13.9-175.6; *P*<0.001) (***Table* II**).

At the end of one year follow up, 51.1% (45/88) infants were doctor-diagnosed to have symptoms of allergy whereas 48.9% (43/88) infants were not. Of these, 17.8% (8/45) infants had allergic rhinitis, 17.8% (8/45) infants had wheezing and 53.3% (24/45) infants had atopic dermatitis in the one year follow-up and had significantly elevated CS IgE (0.7 *vs* 0.4 IU/mL), CS Der p1 (0.9 *vs.* 0 ng/mL), CS Blo t5 (0.8 *vs.* 0 ng/mL), and significantly low CS γIFN (13 *vs.* 37.7 IU/mL) as compared to (*n*=43) infants who did not develop allergic disease in the one year follow up (*P*<0.001).

CS Screening Test (CSST) **(*TABLE* III):** For newborns (n=88), median CS IgE was 0.55 IU/mL and median CS γ IFN was 14.65 IU/mL, so these values were considered as cut off. CS Screening tests were aimed to check sensitization, Th_1 helper function and *in-utero* allergen exposure to HDM allergens Der p1 and Blo t5.

TABLE II HOUSE DUST MITE (HDM) ALLERGEN IN CORD SERUM AND ALLERGY IN INFANTS

Allergy in infants	HDM allergen in cord serum	
	Present	Absent
Allergy	39	6
No allergy	5	38

A test was considered positive if CS IgE>0.55 IU/mL and negative if ≤0.55 IU/mL. *B test* was considered positive if CS γ IFN ≤14.65 IU/mL and negative if >14.65 IU/mL. *C test* was considered positive if CS Der p1 and/or CS Blo t5 allergen were present and negative if CS Der p1 and CS Blo t5 allergen were absent. *D test* was considered positive when CS had IgE greater than 0.55 IU/mL and γIFN was less than or equal to 14.65 IU/mL, and negative when IgE was less than or equal to 0.55 IU/mL and γIFN was greater than 14.65 IU/mL. *E test* was considered positive when CS had IgE greater than 0.55 IU/mL and Der p1 and/or Blo t5 allergen present. It was considered negative when CS had IgE less than or equal to 0.55 IU/mL and Der p1 and Blo t5 allergens were absent. *F test* was considered positive when CS had γ IFN less than or equal to 14.65 IU/mL and Der p1 and/or Blo t5 allergen present, whereas it was considered negative when CS had γ IFN greater than 14.65 IU/mL and CS Der p1 and CS Blo t5 allergen were absent. *G test* was considered positive when CS had IgE greater than 0.55 IU/mL, γ IFN was less than or equal to 14.65 IU/mL and Der p1 and/or Blo t5 allergen present. It was considered negative when CS had IgE less than or equal to 0.55 IU/mL, γ IFN was greater than 14.65 IU/mL and Der p1 and Blo t5 allergen were absent. *H test* is modified G. This test was considered positive if any 2 of the G test criteria were positive and negative if any 2 of the G test criteria were negative.

Comparisons of D, E, F and G Test: (***Table* III**) D could not detect 5.7% (5/88) cases of allergy, E could not discriminate 14.8% (13/88) cases, F could not differentiate 11.4% (10/88) cases and G missed out 15.9% (14/88) cases which indicated that on the basis of any one of the above mentioned screening test – D, E, F or G test there is a possibility of missing out the babies at high risk of allergy. Only H test could discriminate all 88 cases.

DISCUSSION

Three main findings can be emphasized. (*i*) *In utero* exposure to HDM allergens Der p1 and Blo t5 is prevalent; (*ii*) reduced Th_1 helper function imbalance due to in-utero allergen exposure and (*iii*) the newborn may already be on the way to the development of allergic disease. The cord serum screening test has diagnostic and therapeutic value for the first couple of years that are so crucial regarding immunomodulation. It can provide more discriminative information about the allergic status of the newborn thereby assisting for early diagnosis, deter-mination of preventive measures and appropriate therapy.

Cord serun IgE, γIFN and HDM allergens (CS Der p1 and CS Blo t5) individually can not predict the child at-risk of allergies. The lower sensitivity and specificity of C test in comparison with A and B tests, perhaps indicated the possibility of transplacental exposure to some other allergens. Evaluating each factor in the context of the other should provide a better comprehensive picture so that at-risk populations can be accurately defined. Keeping this in mind, different available tests and the permutation combinations of various parameters values were compared to find out the best suitable test "H" with 93% specificity and sensitivity for the detection of probability of occurrence of allergy in the infant (***Table* III**).

In the present study HDM allergens could be detected in 50% (44/88) matched maternal and cord sera. Holloway, *et al*.(11) have also reported detectable Der p1 in matched maternal and cord blood samples. Children who develop a high level of sensitization to house-dust mite allergens are at the greatest risk of having a diagnosis of asthma(12). Exposure to high concentrations of mite allergens in early infancy is a risk factor for developing atopic dermatitis during the first 3 years of life(13). HDM allergen avoidance interventions may have a role in preventing the development of allergic sensitization and airways disease in early childhood(14) and that avoidance of inhalant allergens during late pregnancy may be more important strategy for the reduction of cord blood IgE levels(15). In this study, 7 HDM allergen positive newborns, matched mothers had no house dust allergy. This is in accordance with Rachel, *et al*.(16) that cord blood mononuclear cell proliferation can occur even in absence of maternal sensitization. This clearly suggested that maternal T-cell reactivity or sensitization is not required for the baby to develop an immune reaction.

Due to lack of an established gold standard for

WHAT IS ALREADY KNOWN?

- The development of an atopic immune response may begin during fetal life.

WHAT THIS STUDY ADDS?

- A cord serum screening including IgE, γ IFN and house dust mite allergens Der p1 and Blo t5 carries 93% specificity and sensitivity for predicting occurrence of allergy in Indian infants.

TABLE III SENSITIVITY AND SPECIFICITY OF CORD SERUM SCREENING TESTS

Test	Criteria / Parameter	Allergy diagnosed	Sensitivity %	Specificity %
A	CS IgE>0.55 IU/mL			
	Positive	40/43 (93%)	89	93
	Negative	5/45 (11%)		
B	CSgIFN<=14.65 IU/mL			
	Positive	41/44 (93%)	91	93
	Negative	4/44 (9%)		
C	CS Der p1 and/or CS Blo t5 allergen present			
	Positive	39/44 (89%)	87	88
	Negative	6/44 (14%)		
D	CS IgE> 0.55 IU/mL and γ IFN <= 14.65 IU/mL			
	Positive	38/41 (93%)	95	93
	Negative	2/42 (5%)		
E	CS IgE >0.55 IU/mL and Der p1 and/or Blo t5 allergen present			
	Positive	35/37 (95%)	97	95
	Negative	1/38 (3%)		
F	CS γ IFN ≤14.65 IU/mL and Der p1 and/or Blo t5 allergen present			
	Positive	37/39 (18%)	95	95
	Negative	2/39 (5%)		
G	CS IgE > 0.55 IU/mL, γ IFN ≤14.65 IU/mL and Der p1 and/or Blo t5 allergen present			
	Positive	34/36 (94%)	97	95
	Negative	1/38 (3%)		
H	Any 2 of the G test criteria			
	Positive	42/45 (93%)	93	93
	Negative	3/43 (7%)		

CS= Cord Serum.

allergy in 1 year infants, the test was evaluated only clinically imparting it a subjective bias. Moreover, within the constraints of the facilities, funds, time and inclusion criteria, we could study only 88 newborns and could carry out only 1 year follow-up. Therefore, there is a need for a larger study with a long-term follow-up.

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