Original article

The value of cord serum interferon-gamma estimation in the prediction of first year allergies.

Background: *It was previously assumed that interferon-gamma (IFN-γ) underexpression in newly born infants could be a risk factor for atopic diseases.*

Objective: *We sought to investigate the value of cord serum IFN-γ in the prediction of infantile allergy and its possible correlations with other relevant markers.*

Methods: *Eighty mother-newborn pairs were enrolled consecutively at delivery. The family history of allergy was inquired about and then cord blood was tested for eosinophil and basophil counts and serum total IgE, IgD, and IFN-γ. The infants were followed up for one year for subsequent development of allergic disorders.*

Results: *Twenty-eight infants (35%) developed first year allergies, of whom 19 (68%) had a positive family history of atopy. Atopic dermatitis constituted 57% of the forms of allergy detected. Cord serum IFN-γ concentration at birth was significantly lower in infants who developed allergies during the first year of life (2.8±2 pg/ml) as compared to those who did not (13.6±6.1 pg/ml, p<0.05). Only 11 cord serum samples (14%) contained detectable levels of total IgE. However, 64% of neonates with measurable cord serum IgE developed allergy subsequently. Cord serum IgD concentrations were below the detection limit (5 mg/L) of the method employed. Cord blood basophil, eosinophil and total leucocytic counts were negatively correlated to cord serum IFN-γ levels.*

Conclusions: *Our findings imply that the family history of atopy is still the most important predictor of allergy. Estimation of cord serum IFN-γ in genetically predisposed babies might raise the predictive value.*

Key words: IFN-γ; cord blood; prediction; allergy; Ig-E; Ig-D; eosinophils; basophils; infants.

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INTRODUCTION

Early abnormalities in immune development of allergic individuals may help defining disease pathogenesis and may direct future prevention strategies[1]. Atopy has been linked to skewing of immune responses away from a T_H1 toward a T_H2 profile with subsequent imbalance between T_H1 cytokines such as interferon-γ (IFN-γ) and T_H2 cytokines such as IL-4, IL-5 and IL-13[2,3].

The influence of IFN-γ on atopy and allergic inflammation seems to be pleiotropic. IFN-γ has got an inhibitory effect on mast cells and together with IL-12 it inhibits IL-5 production by T cells and activates T_H1 responses[4,5]. It may also inhibit IL-4 and IL-13 induced IgE production[6]. On the other hand, IFN-γ has been found to maintain chronic allergic inflammation particularly in asthmatic airways[7] and this may explain the elevated IFN-γ levels in chronic asthmatics in addition to the expected high level of T_H2 cytokines[8,9].

It was assumed that IFN-γ deficiency in newly born infants could be a risk factor for the development of atopic diseases and deficient production of this cytokine by cord blood mononuclear cells (CBMC) was demonstrated in infants with family history of atopy and those who developed allergic diseases during the first two years of life[3,6,10]. Nevertheless, Row and associates[7] identified elevated IFN-γ production by CD8+ T cells from cord blood samples and this was strongly associated with atopy development in high risk children at 2 years. It seems that the role of IFN-γ in progression from high risk status to active disease is less clear.

The increasing prevalence of allergy has focused attention on primary prevention and the great need for early life prediction of allergy so that

"at-risk" children can be accurately defined and preventive measures promptly instituted[11]. Up till now, no test for allergy holds high predictivity and this was the stimulus for the study. It is aimed to evaluate the role of cord serum IFN-γ expression in the prediction of allergy within the first year of life and its possible relations to other relevant markers of allergic disease. The alterations in this cytokine may influence the future strategies for prevention and treatment in allergy-prone neonates.

METHODS

Study population:

The study was conducted on 80 consecutive term deliveries that took place in the Maternity Hospital of Ain Shams University, Cairo. Preterm and post-term deliveries were excluded to avoid interference

Mothers:

The mothers were interrogated prior to labor for the duration of pregnancy, active or passive smoking, prenatal maternal illness and personal or family history of atopy. Atopic predisposition of the offspring or positive family history of atopy was defined as at least one parent, older sibling or second degree relative who has an atopic disease[12].

Neonates:

The neonates were classified according to the family history of allergy (FHA) into two groups. The first group comprised 44 neonates (55%) with a negative FHA. These were 21 males and 23 females whose gestational ages ranged between 37 and 40 weeks (mean 38.8 ± 1.2 weeks). Forty one neonates (93%) were born by normal vaginal delivery (NVD) and 3 (7%) were born by cesarean section (CS). Thirty six neonates (45%) with a positive FHA constituted the second group and they comprised 22 males and 14 females. Their gestational ages ranged between 38 and 40 weeks (mean 38.0 ± 1.2 weeks). Thirty one babies (86%) were born by NVD and five (14%) were born by CS.

The newly born babies underwent clinical evaluation at birth for the presence of congenital anomalies. Apgar scoring was estimated at 5 minutes and scores ≥ 8 were considered normal [13]. Birth weight, crown heel length, and skull circumference measurements were recorded in relation to normal percentiles for gestational age[14].

The infants were followed up for a period of 1 year and the following was recorded:

- Type of milk feeding whether breast, formula or mixed feeding.
- Age of weaning in months.
- Exposure to passive smoking.

- Presence of symptoms and/or signs of atopic diseases such as:
  - Atopic eczema (atopic dermatitis).
  - Wheezing attacks relieved by β_2-stimulants.
  - Urticaria and angioneurotic edema.
  - Allergic rhinitis, allergic conjunctivitis or both (allergic rhinoconjunctivitis).
  - Anaphylaxis.
  - Symptoms and signs suggestive of food allergy including gastrointestinal disorders.

Study measurements:

Umbilical cord whole blood samples (5 ml) were subjected to complete blood counting especially for eosinophils, basophils and platelets using coulter electronic automated system (Sysmex, K-model-1000, Japan). Aliquots of serum were separated from another 5 ml sample by centrifugation at 1500 rpm at room temperature. IFN-γ estimation in cord serum was performed by the ELISA technique (Boehringer Mannheim GmbH, Sandhofer Str. 116, D-68305 Mannheim). It is a photometric enzyme linked assay for the quantitative determination of human IFN-γ in streptavidin-coated microtiter plates. Serum total IgE was carried out by enzymatic immunoassay (Eurogenetics, Seppim, Zone industrielle, 61500 SEES, France). Serum total IgD was assayed by radio-immunodiffusion (BINDARID, The Binding Site LTD, Birmingham, B29 6AT, England). The method involves antigen diffusion radialy from a cylindrical well through an agarose gel containing an appropriate mono-specific antibody. Antigen-antibody complexes then form a precipitin ring. A calibration curve was constructed by measuring the ring diameters produced by a number of samples of known concentration. The IgD concentration was determined by measuring the ring diameter and plotting it against the calibration curve.

Statistical Analysis:

Data were analyzed using a statistical Software package V.5 (StatSoft, Tulsa OK, USA). All numeric data were expressed as mean ± standard deviation (SD). Data were analyzed using the student "t" test to compare mean values. Non-parametric variables were analyzed using the Man Whitney or Wilcoxon rank signed tests "z". Pearson "r" correlation coefficient was used to determine the relationship between different numeric variables. Chi-square (χ^2) test was used to compare the frequency of qualitative variables among the different groups. For all tests, a probability (p) of less than 0.05 was considered significant.

RESULTS

Eighty babies were enrolled in this study and followed up for one year to look for symptoms and signs of allergic disorders. Their birth weight percentiles (mean 51.4 ± 25.5), crown heel length (mean 56.8 ± 22.5) and occipito-frontal skull circumference (mean 76.2 ± 18.9) percentile values ranged between the 10th and 95th for age. The Apgar scores at 5 minutes ranged from 7 up to 10 with a mean score of 8.7 ± 0.9. Seventy four mothers (92%) were healthy with no history of prenatal illness, while six (8%) had a positive history of prenatal maternal disease. Of these, two had gestational diabetes mellitus (DM), two had hypertension and two mothers gave a history of threatened abortion in the first trimester.

Family history of allergy (FHA) was evident in 36 infants (45%) while 44 infants (55%) did not have any history suggestive of atopy. Fifty three mothers were considered smokers by self-report, of whom two were active smokers and 51 were passive smokers. On the other hand, 27 mothers (34%) were not exposed to tobacco smoke by history. Follow up revealed that 36 infants (45%) were breast fed (BF), 33 (41%) were formula fed (AF) and 11 infants (14%) were both breast and formula fed in a mixed way (MF). The infants' weaning age ranged from 2 to 6 months with a mean age of 3.4 ± 0.8 months.

Cord serum immunoglobulin E (IgE) was below the detection limit in 69 neonates (86%) and was detectable in 11 (14%). In the latter group, the IgE levels ranged from 0.5 up to 15 IU/ml with a mean value of 7.6 ± 4 IU/ml. Cord serum interferon-gamma (IFN-γ) was undetectable in 64 neonates (80%) while its level was measurable in 16 neonates (20%). Their IFN-γ concentrations ranged from 1 up to 18 pg/ml with a mean value of 9.27 ± 6.34 pg/ml and a median value of 10 pg/ml. The immunoglobulin D (IgD) in cord blood of the enrolled neonates was below the detection limit of the method employed.

Follow up of the studied sample for one year revealed that 28 infants (35%) developed allergic disorders of whom 16 (57%) showed manifestations of atopic dermatitis, 8 developed recurrent wheezing attacks (28.6%), 3 had other forms of skin allergy including papular urticaria and/or hives and a single infant developed manifestations of allergic rhino-conjunctivitis.

Of infants who developed allergic disorders, 19 had a positive family history of atopy (68%). On the other hand, only 9 out of 28 infants (32%) with a negative family history of allergy developed a subsequent allergic disorder during the one year follow up period. Maternal allergy was associated with a higher frequency of allergy in the offspring; 14 infants (74%) of the allergic group belonged to allergic mothers whether they had another allergic person in the family (in 4 infants) or not, while 5 allergic infants (26%) had a history of allergy in the father ± another family member.

The presence of a positive family history of atopy did not influence the cord serum IgE and IFN-γ levels or total leukocytic, eosinophil or platelet counts. However, the mean cord blood basophil count (CBBC) was higher in infants with a positive family history of allergy (mean 54.9 ± 70.4/mm^3) as compared to infants with no such history (mean 20.3 ± 45.1/mm^3).

Infants who did show frank allergic manifestations had significantly lower IFN-γ concentrations in their cord serum at birth as compared to infants who did not develop allergies by their first birthday (Fig. 1). Babies with measurable levels of IFN-γ in cord serum were comparable to the rest of the series in terms of cord blood basophil and eosinophil counts. Although, the mean value of serum IgE seemed higher in infants with absent IFN-γ in cord blood samples, the difference did not reach statistical significance (Table 1). The cord serum IFN-γ concentrations correlated negatively with the total leucocytic, basophil and eosinophil (Fig. 2) counts. Moreover, a positive correlation could be elicited between the cord basophil and eosinophil counts of infants enrolled in the study (Fig. 3).

Seven out of 11 infants (64%) with measurable IgE in their cord blood developed allergy subsequently. The IgE mean concentrations did not vary significantly between infants who developed allergies and those who did not but the number of neonates with measurable IgE in their cord sera was significantly higher among the allergic group. Also, both groups were comparable as far as their eosinophil and basophil counts were concerned (Table 2).

Other parameters such as the gestational age, body weight, crown heel length, skull circumference at birth, Apgar score at 5 minutes and weaning age in months could not be significantly related to the subsequent development of allergy (Table 3). Also, the occurrence of allergic disorders during the first year was not influenced by the mode of delivery, prenatal maternal illness, congenital anomalies of babies, or history of maternal and/or paternal smoking in our series. Breast fed infants were comparable to those who

were formula fed or on mixed feeding as far as the subsequent development of allergy is concerned. However, the number of allergic infants on mixed feeding were significantly less than those on formula (Table 4).

Infants who developed respiratory allergies such as allergic rhinitis and bronchial asthma did not differ from those who had various dermal allergies such as atopic dermatitis, papular urticaria or hives in terms of basophil and eosinophil counts and cord serum IgE and IFN-γ expression.

Gender did not influence any of the laboratory parameters of the enrolled neonates. Male and female babies were quite comparable in terms of hemoglobin concentration, TLC, CBBC, CBEC, platelet count, and cord serum IgE and IFN-γ at birth.
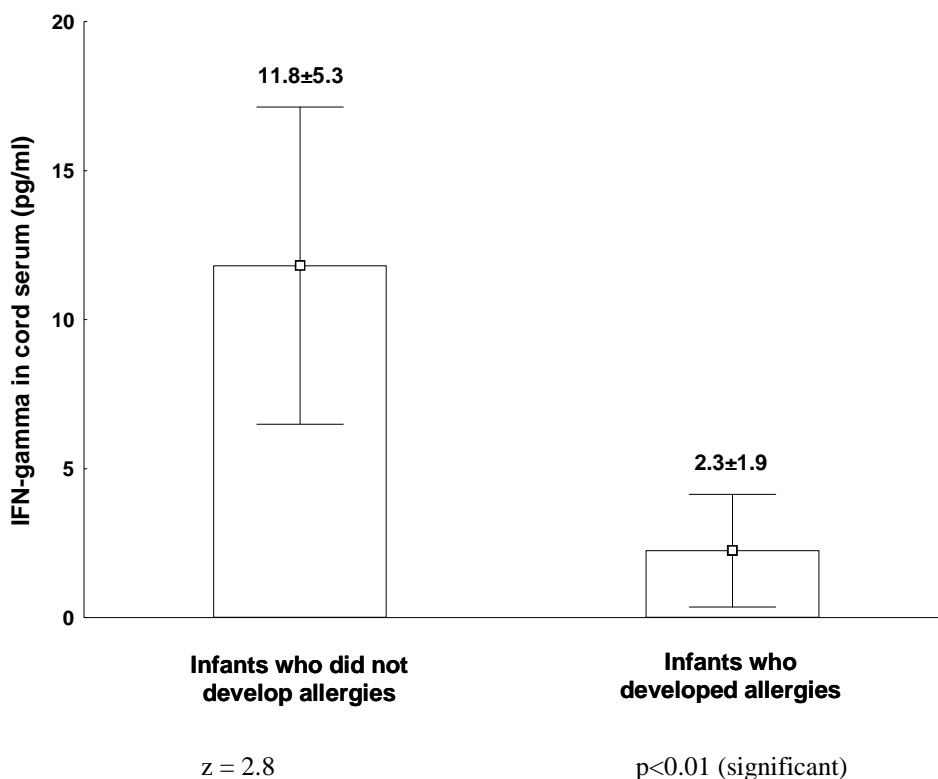

z = 2.8   p<0.01 (significant)

Figure 1. Variation of cord serum IFN-γ concentrations with the subsequent development of allergic disorders.

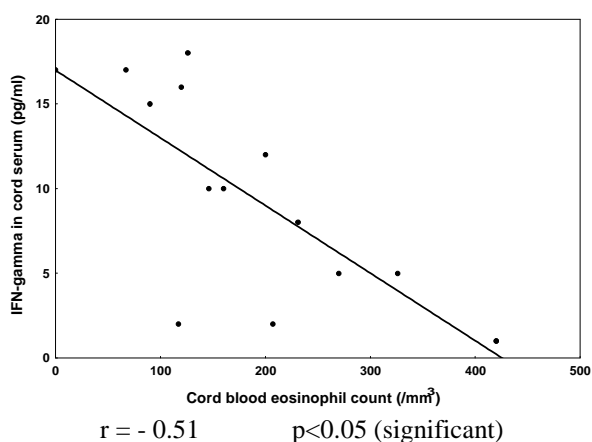r = - 0.51   p<0.05 (significant)

Figure 2. Negative correlation between the eosinophil count and serum IFN-γ in cord blood.

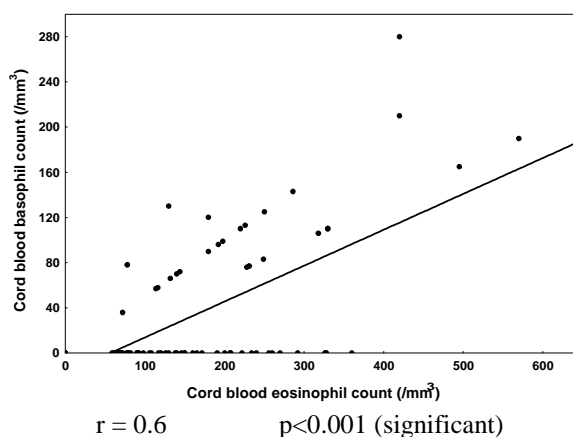r = 0.6   p<0.001 (significant)

Figure 3. Positive correlation between the basophil and eosinophil counts in cord blood.

Table 1. Relation of serum IFN-γ detectability to basophil and eosinophil counts and serum IgE levels in cord blood.

Variable in cord blood	IFN-γ absent		IFN-γ detected		Statistical analysis
	n	Mean± SD	n	Mean± SD	
CBEC ($/mm^3$)	64	165.5±114.5	16	186.1±122.1	z = 0.62 p = 0.54
CBBC ($/mm^3$)	64	36±52.9	16	35.4±85	z = 1.0 p = 0.32
Serum IgE (IU/ml)	8	7.9±3.5	2	3.8±4.6	z = 1.2 p = 0.24

CBBC: cord blood basophil count; CBEC: cord blood eosinophil count; IgE: immunoglobulin E; SD: standard deviation.

Table 2. Variation of cord blood basophil and eosinophil counts and cord serum IgE with the subsequent development of allergy.

Variable in cord blood	Non-allergic infants		Infants with allergy		Statistical analysis
	n	Mean± SD	n	Mean± SD	
CBEC ($/mm^3$)	52	160.1±101.2	28	187.3±138.2	z = 0.56 p = 0.57
CBBC ($/mm^3$)	52	27.3±47.1	28	51.9±77.1	z = 1.27 p = 0.21
Serum IgE (IU/ml)	4	7.3±2.1	6	7.0±4.9	z = 0.32 p = 0.75

CBBC: cord blood basophil count; CBEC: cord blood eosinophil count; IgE: immunoglobulin E; SD: standard deviation.

Table 3. Variation of some quantitative clinical variables with the subsequent development of allergy.

Data	Non-allergic infants (n = 52)	Infants with allergy (n = 28)	Statistical analysis
	Mean± SD	Mean± SD	
Gestational age (weeks)	38.9±0.8	38.7±1.1	t = 0.3 p = 0.58
Body weight (percentile)	50±26.5	54.1±23.7	z = 0.7 p = 0.48
Crown-heel length (percentile)	58.4±22.6	53.8±22.3	z = 0.89 p = 0.37
Skull circumference (percentile)	74.7±19.7	78.9±17.2	z = 0.79 p = 0.43
Apgar score at 5 minutes	8.5±0.9	8.6±0.8	z = 0.76 p = 0.45
Weaning age (months)	3.5±0.8	3.4±0.7	t = 0.6 p = 0.59

SD: standard deviation.

Table 4. Variation of some qualitative variables with the subsequent development of allergy.

Variable		Non-allergic infants (n = 52)	Infants with allergy (n = 28)	Statistical analysis
MOD	CS	6	2	$\chi^2 = 0.39$ p = 0.53
	NVD	46	26	
Perinatal maternal disease	Negative	49	25	$\chi^2 = 0.64$ p = 0.42
	Positive	3	3	
Congenital anomalies	Negative	50	28	$\chi^2 = 1.1$ p = 0.29
	Positive	2	0	
Type of milk feeding	FF	17	16	$\chi^2 = 2.3$ p = 0.13
	BF	25	11	
	FF	17	16	$\chi^2 = 5.4$ p = 0.02*
	MF	10	1	
	BF	25	11	$\chi^2 = 2.0$ p = 0.15
	MF	10	1	
Maternal or paternal smoking	Negative	16	11	$\chi^2 = 0.6$ p = 0.44
	Positive	36	17	

* Significant

BF: breast fed; CS: Cesarean section; FF: formula fed; MF: mixed feeding; MOD: mode of delivery; NVD: normal vaginal delivery.

DISCUSSION

Twenty eight out of 80 neonates (35%) enrolled in this study developed allergic manifestations during the first year of life and this may reflect the rise in the prevalence of allergic symptoms in Egyptian infants. Of those who developed various allergies, 19 (68%) had a positive family history of allergy (FHA). In other words, 19 (53%) out of 36 infants with a positive FHA showed symptoms and signs of allergy during the first year of life in comparison to 9 (20%) out of 44 infants with a negative history. These findings, reinforce the concept that a positive FHA remains the dominant predictive factor for subsequent development of allergy sensitization and disease[1,15]. In a population based prospective study conducted on 1111 newborns who were followed up for 1 year, the family history of atopy was by far the most sensitive factor in detecting infants at risk of atopy and little was added by knowledge of cord blood IgE[16].

The most common allergic manifestation observed in our series was atopic dermatitis (57%) and this conforms with previous reports which also linked the development of atopic eczema to the positive parental or family history of atopy[17]. Maternal allergy conferred a higher allergy risk for infants in our series suggesting that maternal factors can directly influence the immune development of the offspring. It is established that the induction of allergen specific T-cell memory is initiated in utero and it was previously reported that maternal history of allergy is inversely related to the perinantal IFN-γ production capacity[18]. Prescott and associates[19] recently demonstrated that allergic disease at 6 years of age was associated with significantly higher maternal responses to fetal alloantigens which suggests that events at the materno-fetal interface have an important influence on early immune development. The cytokine profiles of deciduas from allergic women were found different from those of deciduas from non-allergic women and this appeared to be related to cytokine production by cord blood mononuclear cells (CBMC) further enforcing the influence of maternal allergy on the fetal immune profile[20].

Among our series, infants who did show frank allergic manifestations during their first year of life had significantly lower IFN-γ concentrations in their cord serum at birth as compared to infants who grew up without developing allergies (Fig. 1). This observation suggests a relation between cord serum IFN-γ expression and the subsequent development of allergic disorders.

Liao and associates[21] compared the difference in cytokine production by CBMC between newborns with high-risk for allergy [family allergy score (FAS) ≥ 3] and those with low risk [FAS = 0]. They found that increased production of IL-6 and decreased production of IFN-γ appeared to be the hallmark of newborns with high risk. Several investigators made the same conclusion from studies on cord or peripheral blood mononuclear cells[3,6,22-24]. The reduced cord blood secretion of IFN-γ and IL-10 were associated with subsequent sensitization to egg at 12 months in a follow up study[6]. The combination of food allergy and atopic dermatitis at 12 months in a high risk cohort was

the strongest risk factor for the development of asthma at 24 months of age[25].

An obstacle to the routine use of serum or plasma level of IFN-γ in research is the very low level of IFN-γ produced spontaneously by cord blood cells. Several investigators proved that CBMC, T cells and NK cells produce much lower levels of IFN-γ compared with adult mononuclear cells[25,27]. The low profile of IFN-γ in neonates was assumed to reflect the dominance of type 2 immunity during the entire neonatal period whereas type 1 immunity dominates during childhood leading to a lower plasma IFN-γ concentration in neonates from non-atopic parents than in healthy children[28].

The predictive value of neonatal IFN-γ for allergy was questioned in some studies. Although infants at genetic risk (family history) of allergy definitely had weaker IFN-γ responses in one study, those who developed allergic disease at 6 years of age had almost comparable neonatal responses (p = 0.05) compared to those with no symptoms[1]. The same conclusion was made by Rowe and coworkers[7] who identified, by logistic regression analysis, that although high genetic risk is associated with attenuated neonatal IFN-γ responses, its production by CD8+ T cells may synergize with T_H2 cytokines in driving atopy development in children with high risk. This may explain the reported elevation in IL-4, IL-5 and IFN-γ production by sputum CD4+ and CD8+ cells in adult asthmatics[29].

The cord serum IFN-γ predictivity in our series seemed higher than that of IgE and no significant correlation could link both of them. Cord serum IgE was detectable in 7 (25%) out of the 28 neonates who developed subsequent allergies while it was detectable in 4 neonates (8%) out of 52 who did not show allergic manifestations. IgE does not cross the placenta, but fetuses are capable of synthesizing it. The human fetus has B cells that are primed to undergo IgE class switching from the earliest stages of ontogeny and can produce endogenous IgE by 20 weeks' gestation. However, IgE producing cells were found rare until 9 months after birth. This may explain the low detectability of IgE in cord blood at birth[30].

Cord blood IgE was claimed to be a significant predictor for urticaria due to food allergy at 12 months but not for other allergic disorders[31], and that the positive correlation between cord blood IgE expression and serum total IgE levels in the first 2 years of life[31] is of lower significance during childhood[16]. However, there seems to be a general agreement that a high neonatal IgE concentration is connected with later allergic disease but still with a low positive predictive value[33].

The cord blood eosinophil count correlated negatively with the cord serum IFN-γ in our series meaning that the lower the IFN-γ was the higher got the eosinophil count in cord blood (Fig. 2). This may be due to lack of the inhibitory effects of IFN-γ on IL-5, the major cytokine in eosinophil differentiation, proliferation, and survival. IFN-γ producing CD4+ T cells were reported to be inversely correlated with peripheral blood eosinophils and had a significant correlation with airway hyperresponsivieness in atopic and non atopic asthmatic children[2]. Again, the cord blood basophils were inversely related to the cord serum IFN-γ in our series probably reflecting the influence of IFN-γ on T_H2 cytokines which may enhance basophil differentiation and release. A positive correlation was found to link the eosinophil and basophil counts in the cord blood samples (Fig. 3) reflecting the common developmental lineage of both cells and the hemopoietic influence of IL-5 on their proliferation[34].

The total serum IgD was reported to be elevated in atopic than non-atopic subjects[35,36]. We therefore sought to investigate its expression in cord serum as well as any possible predictive value. IgD levels in our series were below the detection limit of the method employed (5mg/L). Haraldsson and colleagues[37] noted that there was no IgD in cord serum. They detected very low levels of IgD in older infants and young children that gradually increased until the age of 10 then decreased with age. It was stated that few nanograms of IgD are actually present in cord serum and are thus not easily detectable[38] and that serum IgD is positively correlated to age throughout childhood.

The mode of delivery in our series did not influence the cord serum IFN-γ results or the subsequent development of allergy. Brown and coworkers[40] demonstrated a higher level of IFN-γ and IL-12 production from stimulated CBMC of vaginally delivered infants as compared to infants born by unlabored cesarean section. The fact that we measured spontaneously produced free serum IFN-γ rather than its production from CBMC may count for the difference. In the current study, measuring IFN-γ in the cord serum was more simple and less tedious than tracing its release from CBMC but it was obviously less expressed.

When we compared the allergic to non-allergic infants in terms of all variables studied (Tables 1-4), the only significant difference observed was the

higher frequency of first year allergy among formula fed infants in comparison to those on mixed feeding (Table 4). This might imply some protective effect for breast feeding although infants on pure breast feeding did not show lower affection rates in our series. The findings are indeed limited by the sample size and the short duration of follow up.

In conclusion, cord serum IFN-γ at birth was found significantly low in infants who subsequently developed skin and/or respiratory allergies during the first year of life. Cord serum IFN-γ detectability at birth seemed higher than that of total IgE. Family history of atopy is still the most important predictor of allergy and can determine neonates at high risk. Indeed, combining more than one factor would improve the predictability such as the presence of a positive family history with the reduced IFN-γ and/or elevated IgE in cord serum. Wider scale studies are needed to be able to accurately define the predictive value of each marker in terms of sensitivity, specificity and overall performance. Experimental trials on the effect of recombinant IFN-γ or IFN-γ inducing cytokines (IL-12, IL-15 and IL-18) on the expression of allergic manifestations could be worthwhile.

REFERENCES

1. **Prescott SL, King B, Strong TL, Holt PG.** The value of perinatal immune responses in predicting allergic diseases at 6 years of age. Allergy 2003; 58(11):1187-94.

2. **Kim JH, Kim BS, Lee SY, Seo JH, Shim JY, Hong TJ, et al.** Different IL-5 and IFN-gamma production from peripheral blood T-cell subsets in atopic and non atopic asthmatic children. J Asthma 2004; 41(8):869-76.

3. **Contreras JP, Ly NP, Gold DR, He H, Wand M, Weiss ST, et al.** Allergen-induced cytokine production, atopic disease, IgE, and wheeze in children. J Allergy Clin Immunol 2003; 112(6):1072-7.

4. **Ngoc LP, Gold DR, Tzianabos AO, Weiss ST, Celedon JC.** Cytokines, allergy and asthma. Curr Opin Allergy Clin Immunol 2005; 5(2):161-6.

5. **Varga EM, Wachholz P, Nouri-Aria KT, Verhoef A, Corrigan CJ, Till SJ, et al.** T cells from human allergen-induced late asthmatic responses express IL-12 receptor beta 2 subunit m-RNA and respond to IL-12 in vitro. J Immunol 2000; 165(5):2877-85.

6. **Neaville WA, Tisler C, Bhattacharya A, Anklam K, Gilbertson-White S, Hamilton R, et al.** Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. J Allergy Clin Immunol 2003; 112(4): 740-6.

7. **Rowe J, Heaton T, Kusel M, Suriyaarachchi D, Serralha M, Holt BJ, et al.** High IFN-gamma production by CD8+ T cells and early sensitization among infants at high risk of atopy. J Allergy Clin Immunol 2004; 113(4):710-6.

8. **Ten Hacken NH, Oosterhoff Y, Kauffman HF, Guevarra L, Satoh T, Tollerud DJ, et al.** Elevated serum interferon-gamma in atopic asthma correlates with increased airways responsiveness and circadian peak expiratory flow variation. Eur Resp J 1998; 11(2): 312-6.

9. **Loza MJ, Peters SP, Penn RB.** Atopy, asthma and experimental approaches based on the linear model of T cell maturation. Clin Exp Allergy 2005; 35(1):8-7.

10. **Rinas U, Horneff G, Wahn V.** Interferon-gamma production by cord blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE levels. Pediatr Allergy Immunol 1993; 4(2):60-4.

11. **Tariq SM, Arshad SH, Matthews SM, Hakim EA.** Elevated cord serum IgE increases the risk of aeroallergen sensitization without increasing respiratory allergic symptoms in early childhood. Clin Exp Allergy 1999; 29(8):1042-8

12. **Johnson CC, Ownby DR, Peterson EL.** Parental history of atopic disease and concentration of cord blood IgE. Clin Exp Allergy 1996; 26(6):624-9.

13. **Golden SM, Peters DY.** Delivery room care-Apgar score. In: Menenstein GB, Gardner SL, editors. Handbook of neonatal intensive care. St. Louis: Mosby Co; 1993. p. 59-64.

14. **Needlman DR.** Growth and development. In: Behrman RE, Kliegman RM, Jenson HB, editors. Nelson textbook of pediatrics 17th ed. Philadelphia: WB Saunders; 2004. p. 23-66.

15. **Jacobsen HP, Herskind AM, Nielsen BW, Husby S.** IgE in unselected like-sexed monozygotic and dizygotic twins at birth and at 6 to 9 years of age: high but dissimilar genetic influence on IgE levels. J Allergy Clin Immunol 2001; 107(4):659-63.

16. **Hide DW, Arshad SH, Twiselton R, Stevens M.** Cord serum IgE: an insensitive method for prediction of atopy. Clin Exp Allergy 1991; 21(6):739-43.

17. **Beyer K, Wahn U.** Is atopic dermatitis predictable? Pediatr Allergy Immunol 1999; 10(12 Suppl):7-10.

18. **Prescott SL, Holt PG, Jenmalm M, Bjorksten B.** Effects of maternal allergen-specific IgG in cord blood on early postnatal development of allergen-specific T-cell immunity. Allergy 2000; 55(5):470-5.

19. **Prescott SL, Taylor A, Roper J, Wahdan A, Noakes P, Thornton C, et al.** Maternal reactivity to fetal alloantigens is related to newborn immune

responses and subsequent allergic disease. Clin Exp Allergy 2005; 35(4):417-25.

20. Brown M, Gustafson M, Saldana S, Baradaran A, Miller H, Halonen M. Correlation of human decidual and cord blood mononuclear cell cytokine production. Hum Immunol 2004; 65(11):1336-43.

21. Liao SY, Liao TN, Chiang BL, Huang MS, Chen CC, Chou CC, et al. Decreased production of IFN-gamma and increased production of IL-6 by cord blood mononuclear cells of newborns with a high risk of allergy. Clin Exp Allergy 1996; 26(4):397-405.

22. Kondo N, Kobayashi Y, Shinoda S, Takenaka R, Teramoto T, Kaneko H, et al. Reduced interferon-gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders: 6 year-follow-up study. Clin Exp Allergy 1998; 28(11):1340-4.

23. Teramoto T, Fukao T, Tashita H, Inoue R, Kaneko H, Takemura M, et al. Serum IgE level is negatively correlated with the ability of peripheral mononuclear cells to produce interferon-gamma (IFN-gamma): evidence of reduced expression of IFN-gamma mRNA in atopic patients. Clin Exp Allergy 1998; 28(1):74-82.

24. Hagendorens MM, Van Bever HP, Schuerwegh AJ, DeClerok LS, Bridts CT, Stevens WJ. Determination of T-cell subpopulations and intracellular cytokine production (interleukin-2, interleukin-4, and interferon-gamma) by cord blood T-lymphocytes of neonates from atopic and non-atopic parents. Pediatr Allergy Immunol 2000; 11(1):12-9.

25. Laan MP, Baert MR, Bijl AM, Vredendaal AE, De Waard-van der Spek FB, Oranje AP, et al. Markers for early sensitization and inflammation in relation to clinical manifestations of atopic disease up to 2 years of age in 133 high-risk children. Clin Exp Allergy 2000; 30(7):944-53.

26. Scott ME, Kubin M, Kohl S. High level IL-12 production, but diminished interferon-gamma production, by cord blood mononuclear cells. Pediatr Res 1997; 41(4 Pt 1):547-53.

27. Hodge S, Hodge G, Flower R, Han P. Cord blood leucocyte expression of functionally significant molecules involved in the regulation of cellular immunity. Scand J Immunol 2001; 53(1):72-8.

28. Kanakoudi-Tsakalidou F, Drossou-Agakidou V, Noutsia C, Tzimouli V, Taparkou A, Mavridis P, et al. Intracellular and plasma cytokine profile in neonates born to non-atopic parents: the impact of breast feeding. Eur J Pediatr 2004; 163(7):395-401.

29. Cho SH, Stanciu LA, Holgate ST, Johnston SL. Increased interleukin-4, interleukin-5, and interferon-gamma in airway CD4+ and CD8+ T cells in atopic asthma. Am J Respir Crit Care Med 2005; 171(3):224-30.

30. Lima JO, Zhang L, Atkinson TP, Philips J, Dasanayake AP, Schroeder HW Jr. Early expression of iepsilon, CD23 (Fcepsilon RII), IL-4Ralpha, and IgE in the human fetus. J Allergy Clin Immunol 2000; 106(5):911-7.

31. Kaan A, Dimich-Ward H, Manfreda J, Becker A, Watson W, Ferguson A, et al. Cord blood IgE: its determinants and prediction of development of asthma and other allergic disorders at 12 months. Ann Allergy Asthma Immunol 2000; 84(1):37-42.

32. Wetzig H, Schulz R, Diez U, Herbarth O, Viehweg B, Borte M. Associations between duration of breast-feeding, sensitization to hens' eggs and eczema infantum in one and two year old children at high risk of atopy. Int J Hyg Environ Health 2000; 203(1):17-21.

33. Edenharter G, Bergmann RL, Bergmann KE, Wahn V, Forster J, Zepp F, et al. Cord blood IgE as risk factor and predictor for atopic diseases. Clin Exp Allergy 1998; 28(6):671-8.

34. Ochi H, De Jesus NH, Hsieh F, Austen KF, Boyce JA. Il-4 and -5 prime human mast cells for different profiles of IgE-dependent cytokine production. Proc Natl Acad Sci USA 2000; 97(19):10509-13.

35. Peng Z, Fisher R, Adkinson NF Jr. Total serum IgD is increased in atopic subjects. Allergy 1991; 46(6):436-44.

36. El-Gamal Y, Awad Z, Zaghloul M, Fouad A. Total serum IgD in atopic infants and children. Egypt J Pediatr 1995; 12(1-2):65-7.

37. Haraldsson A, Weemaes CM, Jonasdottir S, Olafsson O, Van de Wiel G, Goertz J, et al. Serum immunoglobulin D in infants and children. Scand J Immunol 2000; 51(4):415-8.

38. Buckley RH. The T-, B-, and NK- cell systems-B-cell development and differentiation. In: Behrman RE, Kliegman RM, Jenson HB, editors. Nelson textbook of pediatrics, 17th ed. Philadelphia: WB Saunders; 2004. p. 686-7.

39. Litzman J, Ward AM, Wild G, Znojil V, Morgan G. Serum IgD levels in children under investigation for and with defined immunodeficiency. Int Arch Allergy Immunol 1997; 114(1):54-8.

40. Brown MA, Rad PY, Halonen MJ. Method of birth alters interferon-gamma and interleukin-12 production by cord blood mononuclear cells. Pediatr Allergy Immunol 2003; 14(2):106-11.