Journal of Population Therapeutics & Clinical Pharmacology

A Prospective Cohort Study to Assess Soluble FAS Ligand as a Marker of Fetal Programming and an Early Predictor of Feto-Maternal Sensitization in Relation to In Vivo IgE Assessment and cord Blood Mononuclear Cells

Doha Magdy Emam,[*], Magda Yehia El Seify[+], Terez Boshra Kamel[+], Dalia Helmy El-Ghoneimy[+] ,Enas Samir Nabih[ψ] , Randa Ibrahim Taha[*] , Eman AboElmaaty Mohamed[*].

* Paediatric and Neonatology department, El Galaa teaching hospital.

+ Paediatric department , Faculty of Medicine, Ain Shams university.

ψ Medical Biochemistery and Molecular Biology department, faculty of medicine, Ain Shams university.

Abstract

Background: Allergy is a state of sensitization of the immune system, Programming of disease in fetal and early postnatal life has been hypothesized to be an important mechanism for atopic diseases, peripheral blood mononuclear cell responses to allergen can be detected from as early as 22 weeks gestation, Many immune cells, including neutrophil, monocytes, macrophages and lymphocytes, express Fas (CD95) on their surface.

Objectives: We assessed soluble Fas Ligand as a marker of fetal programming and early predictor of feto-maternal aeroallergen sensitization in relation to in-vivo IgE assessment and peripheral blood mononuclear cells.

Methods: a total of 60 subjects were included, divided into 2 groups, group I included 30 newborn infants born to mothers with positive history of allergic diseases and /or positive SPTs to aeroallergens, group II included 30 newborn infants born to mothers with negative history of allergic diseases and negative SPTs to aeroallergens. Cord blood s.FAS ligand and CBC with differential leucocytic cell count was measured, Skin prick test was done to all mothers within the first 2 weeks after delivery, and to all newborns after 1 year.

Results : 20 infants from group I had positive skin prick tests, 4 from group II had positive results (P=0.000), The level of s.Fas ligand was significantly higher in the cord blood of the non allergic group (Median=389.3 range =112.70 – 949.8) when compared to the study group**(Median= 261.1 Range =(149.2 – 406.7) (P=0.016),** and in group I its level was higher in children with negative SPT results **(P=0.068),** percentage of MNCs in the allergic group was significantly higher than in the control group. (P= 0.025), the number of CBMNCs and their percentage were significantly higher in infants with positive SPT results in group I **(P= 0.036 and 0.007 respectively).**

Conclusion: Cord blood MNCs percentage was more specific (90.32 %) than MNCs number (74.19%) and cord blood sFas ligand as an early predictor of allergic diseases, S.Fas ligand is the least sensitive (75%) and specific (67.74 %) marker

Introduction

Allergy is a state of sensitization of the immune system, a disease mechanism, rather than a specific disease entity [1] , The importance of studying allergic diseases is attributed to; they are increasing in prevalence in many countries, they are the commonest chronic diseases of childhood and the commonest cause of school absence and acute hospital admission and they cause significant morbidity and can be fatal[2]

Few studies evaluated asthma prevalence in Egypt; In 2009 ***Zedan et al*** [3] studied the prevalence of questionnaire-diagnosed asthma and revealed that the overall prevalence of childhood asthma was 7.7 % in the Nile Delta region of Egypt. This rate was different from previously estimated in Cairo, 2006, of 9.4% [4].

Programming of disease in fetal and early postnatal life has been hypothesized to be an important mechanism for atopic diseases [5] ,Peripheral blood mononuclear cell responses to allergen can be detected from as early as 22 weeks gestation [6]**.** Studies have utilized that cord blood MNCs (CBMCs) proliferation assays in response to specific antigen is a surrogate measure for sensitization.

This suggests that fetal life is a critical period for development of atopic diseases and may be an important "window of opportunity" for prevention of disease [7].

Many immune cells, including neutrophil, monocytes, macrophages and lymphocytes, express Fas (CD95) on their surface[8]**,** Fas Ligand (FasL or CD95L) is a type II transmembrane protein that belongs to the tumor necrosis factor (TNF) family. Its binding to its receptor produces apoptosis (programmed cell death). Fas ligand /receptor interactions play an important role in the regulation of the immune system [9].

Epicutaneous skin prick test (SPTs) are widely used to demonstrate an immediate IgE-mediated allergic reaction [10,11,12]**.** They are used to confirm clinical sensitivity induced by aeroallergen, foods, some drugs and a few chemicals [13] .In conjunction with clinical history, SPT is highly specific and sensitive, 70-95% and 80-97%, respectively, to diagnose inhalant allergies [14]**.**

sFas Ligand has been correlated to allergic diseases in several studies as atopic dermatitis [9] , asthmatic and allergic rhinitis in children [15,16] , however, whether sFasL can serve as a predictor for allergy still under trials, This study was designed to to assess soluble Fas Ligand as a marker of fetal programming and early predictor of feto-maternal allergens sensitization in relation to in-vivo IgE assessment and peripheral blood mononuclear cells.

Study subjects

This is a prospective cohort study that was conducted in the obstetric Outpatient Clinic, Al Galaa Teaching Hospital over a period of two years ,For follow up of the study subjects, further assessment of the participants was conducted at the Pediatric Allergy, Immunology and Rheumatology Unit and Pulmonology Unit, Children's Hospital, Ain Shams University.

The study included 60 full term newborns and their mothers. They were divided into two groups according to maternal history of allergic diseases or current allergic diseases, **Group I (study group):** Newborns of allergic mothers**,** 30 newborn infants

born to mothers with positive history of allergic diseases and /or positive SPTs to aeroallergens and **Group II (control group):** Newborns of non- allergic mothers, 30 newborn infants born to mothers with negative history of allergic diseases and negative SPTs to aeroallergens were taken as the control group. All studied mothers were delivered by normal vaginal delivery. Patients with multiple gestations (e.g., twins and triplets), delivery by caesarian section, associated diseases of the mother (e.g. DM, hypertension, autoimmune diseases), Apgar score <8 were excluded from the study. 5 cases dropped during follow up (Four in group I and one in group II).

Study Methods

At enrollment, history taking from the mothers to determine presence of allergies and type of allergic disease was taken, newborns weight and Apgar score were taken at birth to ensure normal parameters. At 2 weeks of age, newborns were further examined, weight and length were assessed, Follow up visits were assigned every 2 months during which, history taking was taken with special stress on type of feeding of the infants, recurrent infections, exposure to smoke or pets, early weaning and type of weaning food. Clinical examination was done to determine the presence of allergic diseases or concurrent infections.

Laboratory investigations

Five milliliters of fresh venous cord blood were withdrawn immediately after birth from all newborns included in the study. Every sample was divided into two parts; The first was left to clot, then centrifuged, plasma was collected and stored at -80C for soluble FAS ligand measurement using a commercially available enzyme-linked immune-sorbent assay kit. The second part was taken on K3EDTA for complete blood count "CBC" with differential leucocytes count.

Skin prick test

Skin prick test was done to all mothers over the arm for the following aeroallergens (dermatophagoides Farinae, dermatophagoides pteronyssinus, Aspergillus fumigates, cockroach and rye grasses) within the first 2 weeks after delivery,Skin prick test was done to all newborns after 1 year on the back, it was done for the same aeroallergens done to mothers, as well as milk and egg white, Histamine solution (10mg/ml) and glycerinated saline were used as positive and negative controls .SPT was considered positive if there was a wheal of 3 mm in diameter or larger [(17)].

Skin was cleaned with alcohol prior to skin prick testing. Positions for skin pricks were marked by given numbers to each allergen used on the skin,Skin prick test was done at least 2 cm apart to avoid overlapping reactions and false-positive results,Overall, we red the histamine result at 10-15 minutes after the skin prick and the allergens at 15-20 minutes.

Measurement of wheal and flare: The standard and accepted method for quantifying the skin prick reaction is to measure the mean diameter of the wheal, using a ruler marked in mm (a transparent ruler is often most convenient; if the result is a circular wheal, one measurement of the diameter (in mm) is sufficient, if ovoid or irregular, it should be measured on the longest and shortest perpendicular axis and the numbers are added and divided by 2 (mean diameter).

Results

This study included 60 newborn infants divided into 2 groups, Group I: 30 infants born to mothers with history of allergy and/ or positive skin prick tests. Group II: 30

infants born to mothers with no history of allergy and with negative skin prick tests,5 cases dropped during follow up (Four in group I and one in group II).

There was a statistically significant difference in the maternal age between the two studied groups being higher in group I (27.77 +5.01 years vs 25.3 + 4.19 years, P= 0.043), no statistically significant difference in the sex, weight of infants at the time of birth between both groups, There was no significant difference in the type of feeding, mean time of weaning between both groups, and paternal smoking among the studied groups.

Table (1): Baseline characteristics of the study groups

		Group I	Group II	Chi-square test	
		No. = 30	No. = 30	X^2/t*	P-value
Mother's age (years)	Mean±SD Range	27.77 ± 5.01 21 – 42	25.30 ± 4.19 17 – 33	2.067	**0.043**
Sex of the infant	Female Male	15 (50.0%) 15 (50.0%)	19 (63.3%) 11 (36.7%)	1.086	0.297
Weight at birth (kg)	Mean±SD Range	2.97 ± 0.25 2.3 – 3.5	2.95 ± 0.30 2.5 – 3.7	0.233	0.817
Feeding	BF Bottle Both	10 (33.3%) 9 (30.0%) 11 (36.7%)	13 (43.3%) 7 (23.3%) 10 (33.3%)	0.689	0.709
Mean age of weaning (months)	Mean±SD Range	5.48 ± 1.12 3 – 7.5	5.52 ± 1.17 3 – 8	0.124*	0.902
Weaning before 6 months	Positive	16 (53.3%)	16 (53.3%)	0.000	1.000
Paternal smoking	Negative Positive	13 (43.3%) 17 (56.7%)	14 (46.7%) 16 (53.3%)	0.067	0.795

BF = exclusive breast feeding, Bottle = bottle fed, both = breast and bottle fed

Table (2): Distribution of allergy among mothers of group I

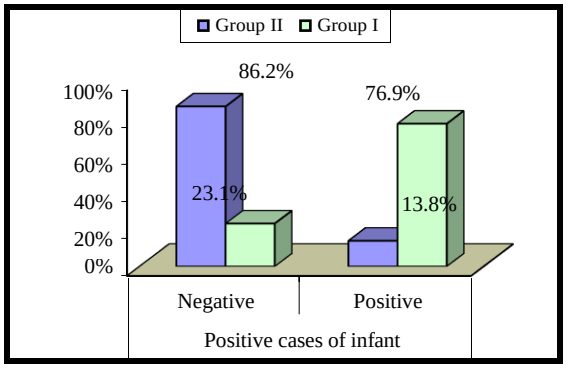
Type of atopy among Mothers	No.	%
Asthma	22	73.3%
Allergic rhinitis	3	10.0%
Atopic dermatitis	3	10.0%
Anaphylaxis	1	3.3%
Allergic conjunctivitis	1	3.3%

Bronchial asthma was most prevalent type of allergy among mothers of group I (73 %), and the least prevalent was allergic conjunctivitis and anaphylaxis (A single case for each).

Results of Skin Prick tests (Table 3)

		Group I	Group II	Mann-Whitney test	
		N=26	N=29	Z/X^{2*}	P-value
Positive cases of Mother	Negative Positive	2 (7.7%) 24 (92.3%)	29 (100.0%) 0 (0.0%)	47.494	0.000
Positive cases of infant	Negative Positive	6 (23.1%) 20 (76.9%)	25 (86.2%) 4 (13.8%)	22.214	0.000

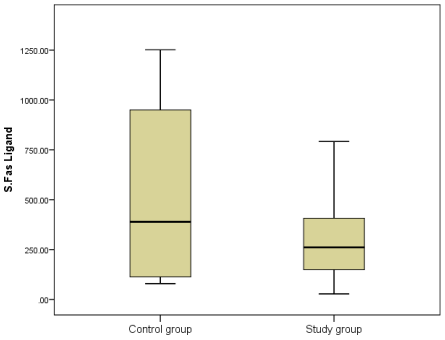
As shown above 24 cases from the group I had positive SPT results and only 2 cases had negative SPT results. No mothers from the group II had a positive SPT result.The number of positive cases of SPT in the infants results were significantly higher in the group I (76.9 %) than in group II (13.8 %) with a P value =0.000.



Results of cord blood analysis (Table 4)

		Group I	Group II group	Independent t-test	
				t/z*	p-value
MNCs No	Median (IQR) Range	1646.5 (1022 – 2379) 762 – 4070	996.50 (880 – 1950) 603 – 3642	-1.839*	0.071
% of MNCs	Median (IQR) Range	13.9 (10.2 – 21.5) 8.5 – 37	12.0 (10.2 – 14.8) 7.4 – 26	-2.296*	0.025
S.Fas Ligand (Pg/ml)	Median (IQR) Range	261.1 (149.2 – 406.7) 27.7 – 810.3	389.35 (112.70 – 949.8) 79.1 – 1251.6	2.477*	0.016

There was no significant difference in MNCs count between both groups, however the percentage of MNCs in group I was significantly higher than in the group II (P= 0.025), the level of sFas ligand was significantly higher in the cord blood of the group II when compared to the group I (P=0.016)



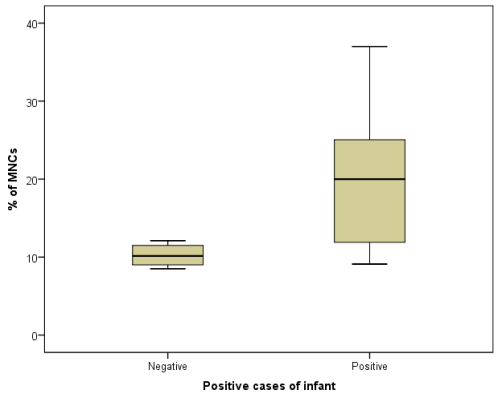
Relation between results of skin prick tests in the infants of group I and other parameters (Table 5)

		SPT of infant		Chi-square test	
		Negative	Positive	X^2/t*/z♦	P-value
Feeding	BF Both Bottle	5 (83.3%) 1 (16.7%) 0 (0.0%)	4 (20.0%) 7 (35.0%) 9 (45.0%)	8.552	0.014
Weaning before 6 months	Negative Positive	2 (33.3%) 4 (66.7%)	9 (45.0%) 11 (55.0%)	0.257	0.612
Mean time of Weaning (Months)	Mean±SD Range	5.17 ± 1.03 4 – 6.5	5.53 ± 1.16 3 – 7.5	0.677	0.505
sex of the infant	Females Males	3 (50.0%) 3 (50.0%)	10 (50.0%) 10 (50.0%)	0.000	1.000
Passive smoking	Negative Positive	4 (66.7%) 2 (33.3%)	7 (35.0%) 13 (65.0%)	1.896	0.169
MNCs No	Median (IQR) Range	1043.5 (889 – 1944) 762 – 2101	2142 (1119 – 2917) 809 – 4070	-2.100	0.036
% of MNCs	Median (IQR)	10.1 (9 – 11.5)	20 (11.9 – 25.1)	-2.679	0.007

	Range	8.5 – 12.1	9.1 – 37		
S. Fas Ligand	Median (IQR) Range	392.9 (287.1 – 686.7) 192.3 – 791.8	229.75 (138 – 389.8) 55.3 – 810.3	-1.826	0.068

The number of positive cases of SPT in the infants of the group I was statistically higher in those who were fed by bottle feeding and the least in those who were exclusively breast fed. No significant relation was found between results of Skin prick test and with time of weaning, sex of the infants as well as passive smoking.

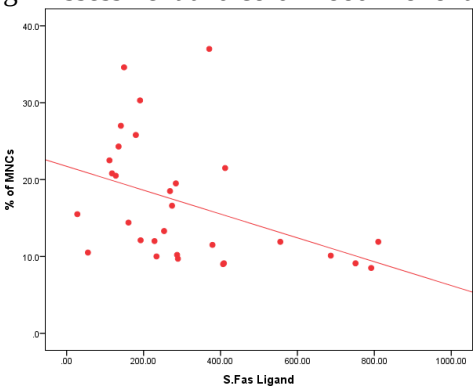
The number of CBMNCs (cord blood MNCs) and their percentage were significantly higher in infants with positive SPT results (P= 0.036 and 0.007 respectively ,the level of s.Fas ligand in the cord blood of infants with negative SPT was higher than its level in those with a positive test however this didn't reach statistical significance (P= 0.068).



Correlation between sFas ligand and cord blood MNCs number and percentage (Table 6)

There was no statistically significant direct correlation between sFas ligand level in the cord blood and MNC number .However there was a significant inverse correlation between sFas ligand level in the cord blood and percentage of CBMNCs (r= -0.553, P=0.002).

	S.Fas Ligand	
	R	**p-value**
MNCs No	-0.357	0.053
% of MNCs	-0.553**	0.002



Determination of the cut off values of MNCs number and percentage and sFas L levels as a predictor for positive SPT results

	Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
MNCs number /mm^3	>1116	0.858	83.33	74.19	71.4	85.2
MNCs percentage %	>14.8	0.813	66.67	90.32	84.2	77.8
sFas ligand level (Pg/ml)	≤289.3	0.685	75.00	67.74	64.3	77.8

After calculation of the area under the curve (ROC curve), the cut off value of MNCs number above which there is increased risk for development of atopy is 1116 /mm3 with a sensitivity of 83.33 % and specificity 74.19 %, and a positive predictive value of 71.4 % and negative predictive value 85.2%, The cut off value of MNCs percentage above which there is increased risk for development of atopy is 14.8 % with a sensitivity of 66.67 % and specificity 90.32 %, and a positive predictive value of 84.2 % and negative predictive value 77.8%,And The cut off value of sFasL levels below which there is increased risk for development of atopy is 289 Pg/ml with a sensitivity of 75 % and specificity 67.3 %, and a positive predictive value of 64.3 % and negative predictive value 77.8%

Discussion

The development of allergic disease is complex and not fully understood, with both environmental and genetic components influencing not only the development of IgE-mediated sensitivity but also the subsequent development of clinical symptoms in a range of tissues, including skin, nose, and lung tissue [18] .

In order to institute preventive treatment appropriately, it is important to identify infants at risk and to identify early markers in young children before the allergic symptoms have occurred.

For many years there has been a search for a reliable marker for allergy prediction. Although elevated cord blood IgE (CB-IgE) is associated with the development of atopy and asthma [19,20,21] , after several years of research it is widely accepted that cord blood IgE is of less value than a positive family history in predicting allergy [22], and is not of value as a screening test [23] .

Hence the identification of a prognostic markers pointing to increased risk of allergy development is of importance. Cord blood represents a suitable source of cells for searching for such prognostic markers

Primary mononuclear cells (MNCs) are derived from the umbilical cord blood (CB) include hematopoietic lineage cells such as lymphocytes, monocytes, stem and progenitor cells as well as mesenchymal stromal cells, these cells are critical components of the immune system which are involved in both humoral and cell mediated immunity.

FasL is a 40-kD type II transmembrane protein; Fas ligand (FasL) plays an important immunoregulatory role in limiting the host immune response. Immunotolerance is achieved by binding of FasL to its receptor (Fas) on activated immune cells, which results in cell apoptosis [24]**,** The Fas-FasL pathway of apoptosis is abnormally activated in diseases associated with impaired immune-tolerance or chronic inflammation, keratinocyte apoptosis is a key pathogenetic mechanism in atopic dermatitis, the concentration of soluble FasL in blood and bronchial lavage fluid has been noted to increase in asthmatics and allergic patients, especially during allergy season [25 -26] .Soluble FasL in cord blood has been associated with atopic dermatitis in children, [27] however the relationship between early life soluble FasL and allergy still is not clear [28]**.**

The heritability of allergy in our study was 76.9% in the study group of 30 sensitized infants with a maternal family history of sensitization to common environmental allergens, compared with only (13.8%) of the control group of 30 non-sensitized infants .

Upon performing SPT to allergic mothers 22 Mothers (84 %) had positive reaction to house dust mites (***Dermatophagoides pteronyssinus*** and ***D. farina)*** allergen, 12 (46.15 %) had positive reaction to cockroach allergen, 4 to Aspergillus antigen (15.3 %) and only 3 cases(11.5 %) had positive results to rye grass allergen, and in their offsprings the most prevalent allergen sensitization was also house dust mites (18 infants ,69.2%), 12 (46.1 %) infants were sensitized to cockroach allergen, 7 (26.9%) were sensitized to Aspergillus antigen and only 3 (11.5%) cases had positive results to rye grass allergen.

Moreover the number of MNCs and their percentage were significantly higher in infants with positive SPT results (P=0.036 & 0.007 respectively).

This was in accordance with ***Miller et al in 2001*** [30] *who assayed* after delivery, newborn cord and maternal blood for IgE and mononuclear cell proliferation in response to antigen. They found that increased mononuclear cell proliferation occurred in 54% of newborns in response to cockroach, 65 % to house dust mites (25% in response to *Dermatophagoides pteronyssinus*, 40% in response to *D.farinae)*.

Devereux et al in 2002 [31] supported our results in his study that tested the proliferative response of CBMCs from a sample of 223 neonates; he found that the magnitude of CBMNCs proliferative responses to allergens increased significantly in association with a family history of atopic diseases.

However ***Chan-Yeung et al*** [32] found that there is no difference in the CBMCs and immediate SPT reactivity, this was discordant with our study that showed that there is significantly higher number and percentage of CBMNcs with positive SPT results ***(P=0.036 and 0.007 respectively).***

The current study showed that sFas ligand levels was significantly higher in the cord blood of non-allergic group when compared to allergic group, when assessing its level in the study group, infants with positive SPT results showed more

lower levels of sFasL levels when compared to those with a negative SPT results however this didn't reach statistical significance, this was in concordance with the results of the study done by ***Kato et al***, in 1999 [33], who measured sFas levels in serum of 36 patients with allergic rhinitis and 22 patients with bronchial asthma and 32 healthy patients as the control group during both the stable and attack disease phases and found that Fas levels were significantly lower in allergic rhinitis patients during attack when compared to healthy individuals, while in asthmatic patients the levels were significantly higher than those in healthy one.

This came in discordance with ***Su et al ,*** [28,29]who measured s.Fas L levels in the cord blood and detected that s.FasL were significantly elevated in AD patients than in control group ,This difference could be due to their study was only performed on AD patients which either IgE mediated or not associated with IgE mediated sensitization, atopic dermatitis is a disease that mainly results from a defective skin barrier, reduced skin innate immune responses and apoptosis [34] furthermore, The follow up period for the patients after 2 years by ISAAC questionnaire for allergy , while in our study we included all types of allergy , and we tested the patients after 1 year by a skin prick test, also

Another valued inverse correlation was found in the current study between sFasL levels and MNCs percentage (P=0.002) whilst it was a with a trend inverse correlation with the number of CBMCs.

The type of infant feeding may have a great influence on the risk of allergy development; numerous studies have investigated the potential of early breastfeeding as a protective influence against the development of allergy and asthma. Meta-analyses of prospective studies of exclusive breastfeeding for 4 or more months from birth have been associated with less AD and asthma (summary odds ratios of 0.68 and 0.70, respectively) [35,36].

The present study confirmed these results by showing that the number of positive cases of SPT in the infants of the study group was statistically higher in those who were on bottle feeding and the least in those who are only breast fed (P=0.014).

Conclusions

The primary sensitization to allergy can occur prenatally, CBMNCs percentage was more specific (90.32 %) than CBMNCs number (74.19%) and cord blood sFas ligand as an early predictor of allergic diseases. S.Fas ligand is the least sensitive (75%) and specific (67.74 %) marker, type of infant feeding plays a role in the development of allergic diseases and this harmonizes with the epigenetic hypothesis.

References

1. Krishnan K ,Dolen *DK*. Allergy and the Allergic Diseases. Textbookof clinical paediatrics, 2nd edition. Springer-Verlag Berlin Heidelberg 2012:1351.
2. Boyle RJ. Paediatric allergy. Illustrated textbook of paediatrics. 4th edition.elsevier.2012; 271-276.

3. Zedan M, Settin A, Farag M. Prevelance of bronchial asthma among Egyptian school children. *Egyptian Journal of Bronchology*. 2009; 3:124-130.
4. Georgy V, Fahim HI, El-Gaafary M. Prevalence and socioeconomic associations of asthma and allergic rhinitis in northern Africa. *Eur Respir J*. 2006;28:756-62.
5. Jones CA, Holloway JA, Warner JO. Does atopic disease start in foetal life? *Allergy* 2000; 55(1):2-10
6. Jones AC, Miles EA, Warner JO, et al. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimulate during gestation. *Pediatr Allergy Immunol* 1996;7:109–16.
7. Tang MLK, Kemp AS, Thorburn J, et al. Reduced IFN-c and subsequent atopy. *Lancet* 1994; 344:983–985.
8. Stuart P.M., Griffith T.S., Usui N., et al: CD95 LIGAND (FasL)-induced apoptosis is necessary for corneal allograph survival. J Clin Invest 1997; 99:396-402.
9. Trautmann A, Akdis M, Kleemann D et al. T cell-mediated Fas-induced keratinocyte apoptosis plays a key pathogenetic role in eczematous dermatitis. *J Clin Invest* 2000; 106:25–35, 2000.
10. Bousquet J, Khaltaev N, Crruz AA, Denburg J,Fokkens WJ, Togias A et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the world health organization, GA (2) LEN and AllerGen. *Allergy* 2008; 63(Suppl 86):8-160.
11. Demoly P, Bousquet J, Romano A. In Vivo methods for the study of allergy.In:Adkinson NF Jr, Bochner BS,Buss WW, Holgate ST,Lemanske RF Jr, Simons FER, editors. Middleton's Allergy, principles and practice, Seventh edition. Philadelphia, PA: Mosby Elsevier Inc, 2009:1267-1280.
12. Cox L, Williams B, Sicherer S, Oppenheimer J, Sher L, Hamilton R et al. Pearls and pitfalls of allergy diagnostic testing: report from the American college of allergy, asthma and Immunology / American academy of Allergy, Asthma and immunology specific IgE Test task Force. *Ann Allergy Asthma Immunol* 2008; 101:580-592.
13. Bernstein L, Li J, Bernstein DI et al. Allergy Diagnostic Testing: An Updated Practice Parameter. *Annals of allergy, Asthma & Immunology*. 2008; 100:15-28.
14. Demoly P, Bousquet J, Romano A: In vivo methods for the study ofcallergy. In Middleton's Allergy - Principles and Practice. 6th edition. Edited by Adkinson NJ, Yunginger J, Busse W, Bochner B, Holgate S, Simons F. Philadelphia: Mosby; 2003:430–439.
15. Mezei G et al. Seasonal changes of proapoptotic soluble fas Ligand level in allergic rhinitis combined with asthma. *Pediatr Allergy Immunol* 2006;17:444-449.
16. Zakrzewska et al evaluation of CD25, CD152, Fas Ligand expression in the adenoids of allergic and non allergic children: a pilot study. *Int J Pediatr Otorhinolaryngy*.2003; 67:205-208.
17. Konstantinou GN, Bousquet PJ, Zuberbier T et al: The longest wheal diameter is the optimal measurement for the evaluation of skin prick tests. Int Arch Allergy Immunol 2010, 151(4):343–345.
18. Vercelli D. Discovering susceptibility genes for asthma and allergy. Nat Rev Immunol 2008;8:169-82.

19. Croner S, Kjellman NIM. Development of atopic disease in relation to family history and cord blood IgE levels. Eleven year follow–up in 1654 children. Pediatr Allergy Immunol 1990; 1:14–20.
20. Kjellman N, –I, Croner S. Cord blood IgE determination for allergic prediction – a follow–up to seven years of age in 1651 children. Ann Allergy 1984; 53:167–71.
21. Hansen LG, Halken S, Host A et al. Prediction of allergy from family history and cord blood IgE levels. Pediatr Allergy Immunol 1993; 4:34–40.
22. Magnusson C. Maternal smoking influences cord serum IgE and IgD levels and increases the risk for subsequent infant allergy. J Allergy Clin Immunol 1986; 78:898–904.
23. Kjellman NIM. IgE in neonates is not suitable for general allergy risk screening. Pediatr Allergy Immunol 1994; 5:1–4.
24. Kauma S, Huff TF, Hayes N et al. Placental Fas Ligand Expression Is a Mechanism for Maternal Immune Tolerance to the Fetus. The Journal of Clinical Endocrinology & Metabolism.1999;84:2188-2194
25. S.Daneshmandi ,A.A Poufathallah,Z.Ppourpak et al. Soluble formof FasL in adult asthma. Iran Red Crescent Med J.2009 ;11: 301-305.
26. G.Menzi M.Levay , Z Sepler et al. Seasonal changes of proapoptotic soluble fas ligand level in allergic rhinitis combined with asthma . Pediatri Allergy Immunol .2006;17 :444-449.
27. K.W .Su ,P.C Chen, J.Wang. Cord blood soluble fas ligand and pediatric atopic dermatitis . Allergy Asthma Proc .201;32 :366 – 371.
28. Su KW, Chen AC, Wang IJ. Cord blood soluble Fas ligand and pediatric atopic dermatitis. Allergy Asthma Proc.2011;32:366-371.
29. *Su KW, Chiu C-Y , Tsai MH et al. Cord blood soluble fas ligand linked to allergic rhinitis and lung function in seven year old children. Journal of microbiology, Immunology and Infection . 2022;55 : 300-306.*
30. Miller RL, Chew GL, Bell AC et al, Prenatal Exposure, Maternal Sensitization, and Sensitization *In Utero* To Indoor Allergens in an Inner-City Cohort. Am J Respir Crit Care Med 2001:164;995–1001.
31. Devereux G, Barker RN and Seaton A. *Antenatal determinants of neonatal immune reosponse to allergens. Clin Exp All.2002;32:43-50*
32. Chan-Yeung M, Ferguson A, Chan H et al,Umbilical cord blood mononuclear cell proliferative response to house dust mite does not predict the development of allergic rhinitis and asthma .J Allergy Clin Immunol August 1999:317-321.
33. Kato M, Nozaki Y, Yoshimoto T. Different serum soluble Fas levels in patients with allergic rhinitis and bronchial asthma. Allergy .1999; 54: 1299-1302.
34. *Leung D, Sicherer* S. Atopic Dermatitis (Atopic Eczema**).** *Nelson textbook of pediatrics. 20th edition. Published by Elsevier, 2016. P1116-1121.*
35. Gdalevich M, Mimouni D, David M et al. Breast-feeding and the onset of atopic dermatitis in childhood: a systematic review and meta-analysis of prospective studies. J Am Acad Dermatol 2001;45:520–7.
36. Gdalevich M, Mimouni D, Mimouni M. Breast-feeding and the risk of bronchial asthma in childhood: a systematic review with meta-analysis of prospective studies. J Pediatr 2001;139:261.