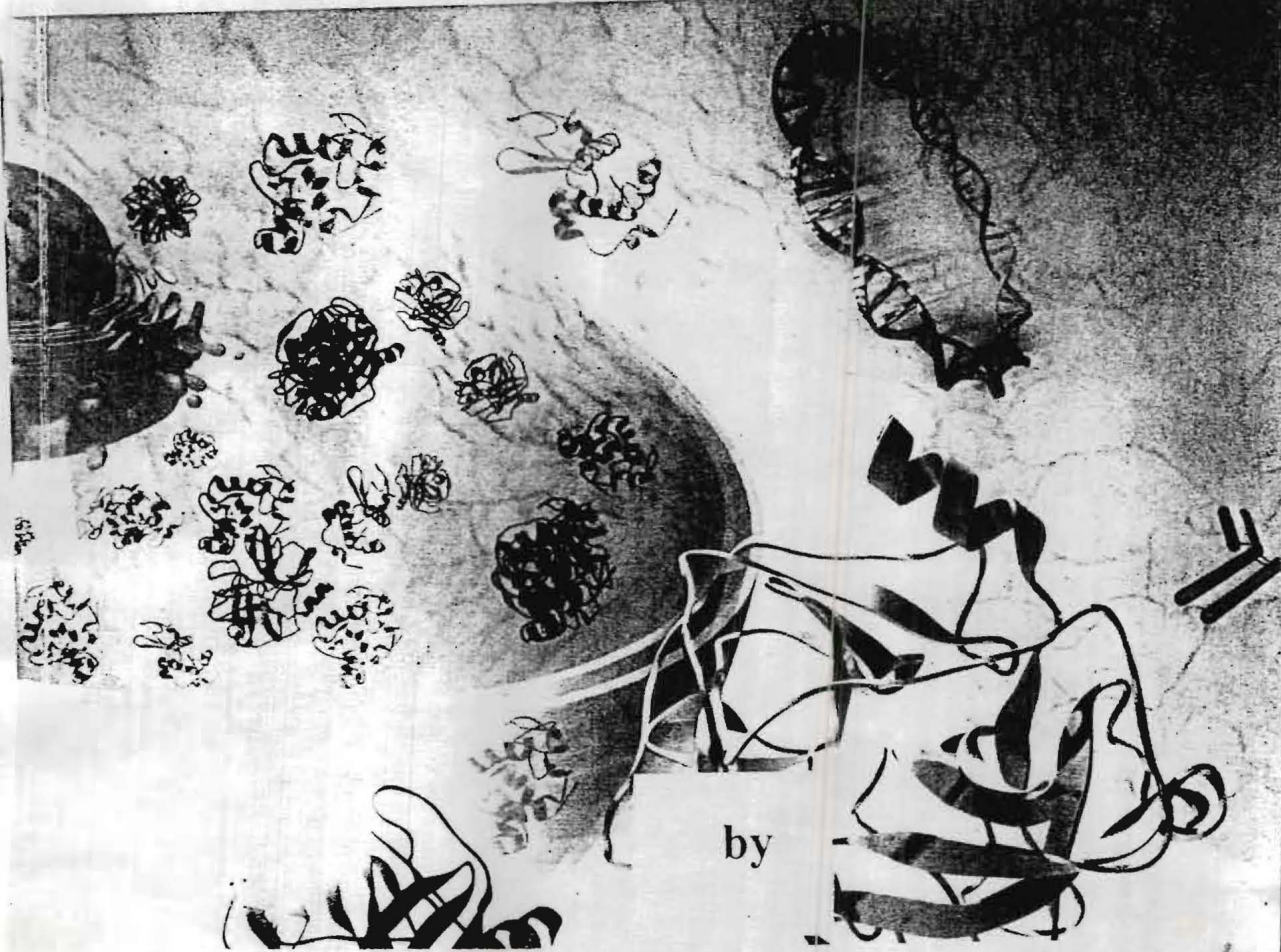
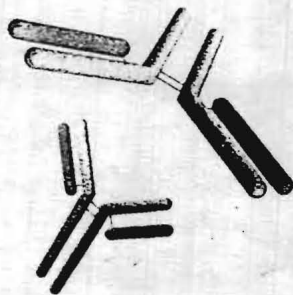


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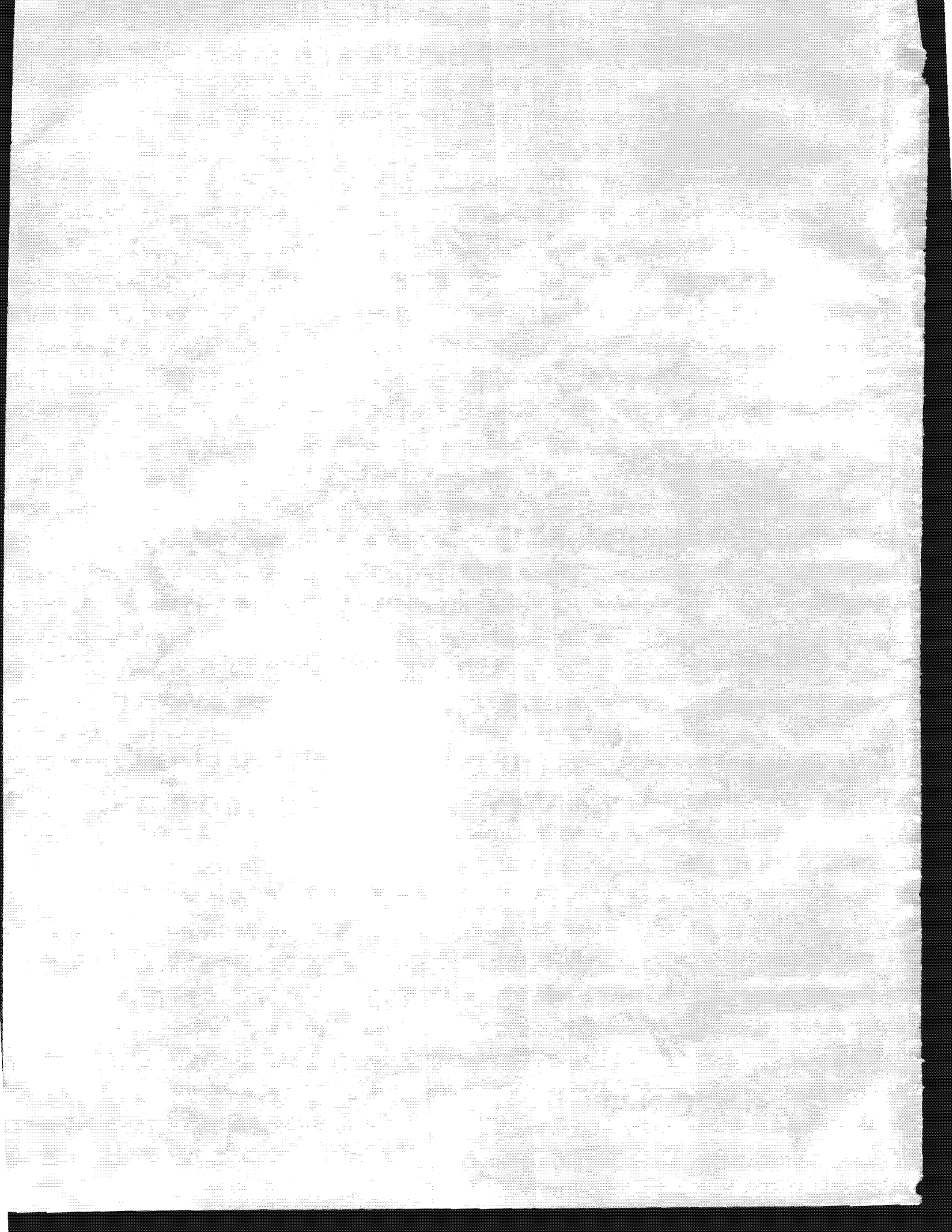
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PERIPHERAL BLOOD Th₁ AND Th₂ CYTOKINES PROFILE IN CHILDREN WITH BRONCHIAL ASTHMA

By

Safeya Deyab^{*}, Somaia Elwan^{**} and Magda A. Gamil^{***}

Department of Clinical Pathology^{*} and Department of Pediatrics^{**}, Benha Faculty of Medicine,
Department of Microbiology, Faculty of Medicine (girls), Al-Azhar University.

Abstract

The aim of this study was done to examine the changes in cytokines production profiles secreted by Th₁ and Th₂ of asthmatic children at acute attacks and after the treatment by glucocorticoid therapy. Also we investigated whether oral prednisone modulate the balance of peripheral blood Th₁ and Th₂ cytokines. The study was conducted on twenty patients with acute bronchial asthma. Twelve were males and eight were females, with mean age (10.2 ± 2.44) years. Ten apparently normal children of age and sex matched to the patients were control. Six were males and four were females with mean age (9.55 ± 2.58) years.

To investigate the difference between Th₁ and Th₂ cytokines secretion, pattern of peripheral blood mononuclear cells (PBMCs) in such patients and control were cultured. Subsequently the immunoreactive interferon gamma (IFN-γ) (Th₁ cytokines) and interleukin (IL-4) (Th₂ cytokines) levels were measured in the supernatant by immunoabsorbance (ELISA) assay to evaluate the role of these cytokines in bronchial asthma and the effect of corticosteroid therapy on their level.

The results showed that there were a significantly higher IL-4, IL-4/IFN-γ ratio and significantly lower IFN-γ production detected from activated PBMCs of asthmatic patients than control children (133.65 ± 9.98 pg/dl versus 97.1 ± 8.59 pg/dl, p<0.001), (1.36 ± 0.2 versus 0.71 ± 0.11, p<0.001) and (99.8 ± 12.28 pg/dl versus 139 ± 14.12 pg/dl, p<0.001) respectively.

After treatment there were a significantly high IFN-γ and significantly low IL-4 levels and IL-4/IFN-γ ratio (99.81 ± 12.28 pg/dl versus 124.65 ± 16.24 pg/dl, P<0.001), (133.65 ± 95.98 versus 95.35 ± 14.38 pg/dl, P<0.001) and (1.36 ± 0.2 versus 0.78 ± 0.16, P<0.001) respectively.

Before treatment there was a significant positive correlation between IL-4 and the absolute eosinophil count (A.E.C.), (P < 0.05) and the ratio of IL-4/IFN-γ and inversely correlated to IFN-γ, but not significant (P > 0.05). After treatment there was a significantly positive correlation between IL-4 and the ratio of IL-4 - IFN-γ (P < 0.05).

Conclusion: 1-Defective Th₁ and enhanced Th₂ cytokine responses have been implicated in the development of bronchial asthma and enhanced airway inflammation and obstruction after allergen exposure. 2- Short term oral corticosteroid may be modulate the balance of Th₁ and Th₂ cytokines in patients with asthma. 3-This immune deviation could be suggested as the future therapy of atopic bronchial asthma in children.

Introduction

Asthma is an inflammatory disorder of the airways. It is the most common chronic disease of childhood and the most frequent reason for pediatric hospital admission (*Adam and Marano, 1994*), and its incidence is in rise (*Marino et al, 1998*). Chronic inflammation of the airways in asthma is a complicated process in which many cytokines and different type of immune cells take part (*Fraenkel and Holgate, 1996*). Among these cells corresponding T- lymphocytes clone that are activated by allergen (*Mori et al, 1996*). CD₄ T- cells have been divided at the clonal level into two broad functional subsets according to their profile of cytokine secretion. The first Th₁ cell secrete predominantly interferon gamma and are particularly implicated in all mediated immune responses against invading intercellular pathogen such as virus (*Mossman and Sad, 1996*). IFN- γ is thought to have adown regulatory effect on Th₂ immune response. The second Th₂ cells on the other hand secrete predominantly IL- 4, IL-5 which support antibody synthesis. Such as IL-4 and IL-5 are generally believed to play an important role in the pathogenesis of allergic asthma (*Barbara et al,2000*). CD₄ T-cells (Th₁ and Th₂) and their cytokines play an essential role in asthma, both as part of sensitization phase, providing help for B-cells for IgE synthesis and as part of the inflammatory cascade. These through the release of the Th₂ type cytokines which are critical for IgE synthesis and accumulation of eosinophil in inflamed airway (*Sophie et al, 2000*). IL- 4 is secreted by Th₂ cells and mast cells and along with IL- 13, it is the major cytokine responsible for B- cell class switching from IgM to IgE. Furthermore, IL- 4 acts on Th₀ phenotype and promotes their progression to Th₂ phenotype, ultimately, leading to the secretion of more IL- 4 and other Th₂ derived cytokines (*Turnoy et al,2002*).

Because of the regulatory role of the T- lymphocytes especially T- helper cell (Th) cytokine in the pathogenesis of atopic asthma (*Kay 2003*), it is interesting to investigate the profiles of the Th₁ and Th₂ cytokines and IL- 4 and IFN- γ in the peripheral mononuclear cells of asthmatic patients and after short- term

corticosteroid therapy by in vitro culture study, to assess the release of two cytokines namely IL- 4 and IFN- γ .

The aim of this study was to measure the role of T- helper cytokines in atopic asthmatic children and to predict the response of corticosteroid therapy, also immune intervention based on Th₁-Th₂ cytokines manipulation may ultimately lead to an effective control and altering natural course of the disease.

Subject and method:

This study included twenty patients with acute bronchial asthma, their ages ranged from 2- 13 years. They were 12 males (60%) and 8 females (40%) with a mean \pm SD 10.2 \pm 2.44, admitted to pediatric department of the Benha University Hospital. All of them were suffering from acute, asthma attack diagnosed both clinically and by using peak flow meter. The patients received treatment as nabulized B agonist, plus short course of perdnisone therapy. Later, after a variable period of time patients were brought back to the hospital for reexamination and to confirm that they were being in remission. Ten apparently healthy children of age and sex matched to patients were taken as control. All of our cases and control were subjected to the following:-

- A full history taking and full clinical examination
- Plain X-ray for chest.
- Routine laboratory investigations including:
 - Complete blood count by Sysmex KX-21 automated heematology analyzer with thorough examination of leishman-stained peripheral blood smears especially for eosinophil count.
 - Erythrocyte sedimentation rate (E.S.R.) (*Dacie and Lewis, 1991*).
 - Stool analysis.
 - Total serum IgE levels (*Halonon et al, 1982*).
 - Short- term lymphocyte culture for assessment of Interferon-gamma and Interlukin-4 in vitro culture.

Sample collection:

About five ml. of venous blood was aseptically withdrawn for each child. 1ml. was collected on EDTA (for complete hemogram). 3ml. on sterile preservative free heparin (30 I U/ml) for lymphocyte culture and 1ml. on tri-sodium citrate for ESR.

Lymphocyte culture :

Fresh peripheral blood from patients and control subjects was fractionated by Ficoll-Histopaque-1077 (sigma, St Louis) sedimentation (Boyer, 1967). After lymphofiltrate separation, cells were washed twice with phosphate buffer solution (PBS). Cellular viability and cell counts were assessed. Mononuclear were resuspended in RPMI-1640 (GIBCO- BRL, Gaithersburg) supplemented with streptomycin (100ug/ml) penicillin (100 unit/ml) and 10% heat inactivated fetal calf serum (FCS/GIBCO) at concentration of 2×10^6 cell/ml in T-25 tissue culture flask. The culture cells were stimulated using Conavalin A (Con A) (250 mg/ml) which was dissolved in the same culture medium and added to the flask for induction of cytokine release. All the flasks were incubated in CO₂ incubator (5% CO₂) at 37°C

five days. After that the supernatants were collected and stored at -70°C for cytokine determination. IL-4 and IFN- γ were measured by sandwich enzyme immunoassay utilizing the following kits: 1-IL-4 (Quantikine HIS human IL-4. Quantitative colorimetric sandwich EISA R and D system Inc. Catalogue no D5040 USA). 2-IFN- γ Quantikine HIS human IL-4. Quantitative colorimetric sandwich EISA R and D system Inc. Catalogue no DIF00 (USA)

Statistical analysis:

Data were expressed as mean \pm SD. The comparative study was performed using "student t-test". "Paired t" analysis were used to evaluate the result, while correlation study was done employing the Pearson's correlation statistics. A level of (P<0.05) was considered to be statistically significant.

Results

Table (1) Characteristics of children with asthma patients versus Control.

Character	Cases, n=20	Control, n=10	t	P
Age Mean \pm SD	10.22 \pm 2.49	9.55 \pm 2.5	1.82	>0.05
Sex (M/F) ratio	3 : 2	3 : 2		
E.S.R. (mm/hr) mean \pm SD	11.75 \pm 3.87	9.3 \pm 1.77	1.93	>0.05

This table showed that there was nonsignificant difference between patients and control regarding age, sex, and E.S. R (P> 0.05).

Table (2) Hemogram parameters in studied children.

Character	Cases, n=20	Control, n=10	t	P
Hb (g/dL).	11.46 \pm 1.59	12.61 \pm 0.66	2.18	>0.05
A.E.C./Cmm	435.45 \pm 50.185	83.6 \pm 8.92	3.49	<0.01

A.E.C: Absolute eosinophil count

This table showed that there was nonsignificant difference between patients and control regarding haemoglobin (P> 0.05), and highly significant for absolute eosinophil count (P< 0.01).

Table (3) Comparison between control and asthmatic patients regarding other variables.

Variables \ Groups	Cases, n=20	Control, n=10	t	P
IL-4 / pg / dL	133.65 ± 9.98	97.1 ± 8.59	9.87	<0.001
IFN - γ pg / dL	99.8 ± 12.28	139.3 ± 14.12	7.91	<0.001
IL-4 / IFN - γ %	1.36 ± 0.2	0.71 ± 0.11	9.58	<0.001

This table revealed that: The mean value ± SD of IL-4 in acute bronchial asthma was statistically significant higher than that in control. The mean value ± SD of IFN - γ in acute bronchial asthma was statistically significant lower than that in control. The mean value ± SD of IL-4/IFN-γ in acute bronchial asthma was statistically significant higher than that in control.

Table (4) Comparison between asthmatic patients before and after treatment regarding to other variables.

Variables \ Groups	Before Treatment	After Treatment	Paired t	P
IL-4 / pg/dL	133.65 ± 9.98	95.35 ± 14.38	17.9	P <0.001
IFN - γ pg/dL	99.8 ± 12.28	124.65 ± 16.24	11.77	P <0.001
IL-4 / IFN - γ %	1.36 ± 0.2	0.78 ± 0.163	24.48	P <0.001

This table revealed that:

- Level of IL-4 was significantly higher in acute asthmatic patients before than after treatment (P <0.001).
- Level of IFN- γ was significantly lower in acute asthma before than after treatment (P <0.001).
- Level of IL-4 / IFN- γ ratio was significantly higher in acute asthmatic patients before treatment than after (P <0.001).

Table (5) Correlation between IL-4 level and other laboratory variables among patients before treatment.

Variables \ IL-4	r	p
Δ E C	0.3853	0.05
IFN - γ	- 0.0531	0.05
IL-4 / IFN - γ %	0.4963	0.001

This table illustrated that there was positive correlation between IL-4 and AEC (p < 0.05), also between IL-4 and IL-4 / IFN - γ ratio (p < 0.001) after treatment.

Table (6) Correlation between IL-4 level and other laboratory variables among patients after treatment.

Variables \ IL-4	r	p
IFN - γ	-0.1306	> 0.05
IL-4 / IFN - γ %	0.7803	<0.001

This table revealed that there was a negative correlation between IL-4 and IFN - γ after treatment ($p > 0.05$). On the other hand there was highly positive correlation between IL-4 and IL-4 / IFN - γ ratio ($P < 0.001$).

Chart () correlation coefficient between IL4 and IL4/INF- gamma ratio after treatment

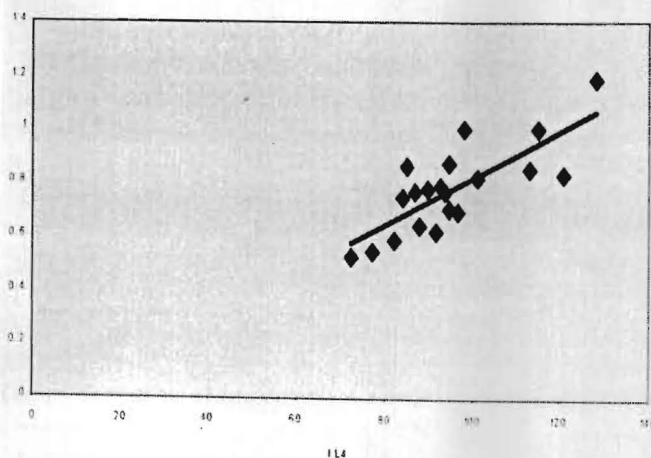
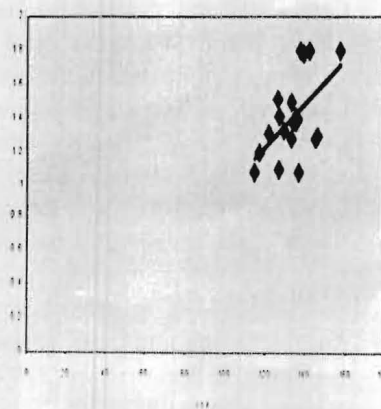


Chart () correlation coefficient between IL4 and INF-gamma after treatment



Discussion

Asthma was a chronic inflammatory disorder of airways mediated by a multitude of cell types and inflammatory mediator (*Sundeeb et al, 2001*). Mast cells and eosinophil were initially believed to play central role in driving the airway inflammation associated with asthma. However the emphasis has now shifted to T-lymphocyte, in particular helper T-type2 (Th_2) cells and Th_1 . These two subclasses of helper T-cell differ in their production of cytokines (*Mossman et al, 1986*). The Th_2 lymphocytes produce interleukin (IL-4), IL-5, IL-9 and IL-13 which activate the mechanisms important in defense against parasites and allergic inflammation. Such mechanism include Ig-E production, mast cell differentiation and eosinophil growth, migration and activation. Th_1 lymphocyte

produce interferon gamma (IFN- γ) and IL-2 which activate

mechanisms important in defense virus and bacteria (*Mossman and Coffman, 1989*).

The result of the present study showed a statistically significant increase in activated PBMCs cytokines IL-4 and a statistically significant decrease of the IFN- γ when compared with those of healthy control ($P < 0.001$). This clearly indicated higher activity of blood Th_2 over Th_1 cells in children with bronchial asthma (*Milgrom, 2003*). The airway inflammation in allergic asthma is orchestrated by CD_4^+ Th_2 cells that secrete the cytokines 4, 5 IL- 13 while IFN- γ secreted by Th_1 cells (*Ray and Cohn, 2000*) (*Prescott, 2003*) suggested that atopic disease including asthma are characterized by T-helper cell (Th_2) cytokine pathology. Contradictory results

were reported by (Antoine et al, 2000) who showed that increase of IFN- γ in adults with atopic asthma. The cell culture conditions can explain such discrepancies because they cultured cells in the whole blood, while we used separated PBMCs. Our study was applied only on children and not on adults.

The result of the present work also revealed a statistically significant increase in the IL-4/ IFN- γ ratio when compared with control group ($P < 0.001$). The increased ratio would lead to aberrant IgE production. This comes in agreement with previous studies, which suggest that bronchial asthma was the result of an impaired IFN- γ production leading to IL-4 synthesis and IgE dependent sensitization allergy (Tang et al, 1995). The high level of IL-4 (Th₂ cytokine) in patients with bronchial asthma may be a marker of an ongoing inflammatory process and can cause mean patho-physiological abnormalities characteristic of the disease (Shiria et al, 2003). On the other hand there was a high significant difference in A.E.C. in patients when compared to the control children which indicate the role of IL-5 in the development of chronic phase of bronchial inflammation (Till et al, 1997) and (Ohasi et al, 1998).

After treatment with prednisone therapy IL-4 cytokine was significantly lower while the IFN- γ was significantly higher than those children before treatment. So glucocorticoid play an important role in the treatment of the allergic disease. The glucocorticoid inhibit IL-4 and IL-5 secretion by Th₂ - cell, and may be required for improvement in the lymphocytic inflammation (Nagano et al, 2002) and (Crocker et al, et al, 1998). (Kazuyoshi et al, 2001) suggest that short-term corticosteroid (modulate the balance of CXCR₃⁺ cells T-helper type1 (Th₁) and CCR₄⁺ (Th₂) cells in patients with asthma. Reduced the percentage of blood CCR₄⁺ expression CD₄ (Th₂) cells resulting in the shift to CXCR₃⁺ expression CD₄ (Th₁) cells. due to specific reduction in mRNA for IL-4 and IL-5 and increase into the nucleus and bind to specific regulatory target gene (Scheinman et al, 1995). While (Miyaura and Iwata, 2002) found that glucocorticoid directly inhibit the Th₁ and enhances Th-

development. Their results suggest that P₃₈ mitogen activated protein kinase (MAPK) inhibitor SB₂₀₃₅₈₀ inhibited Th₁ and enhanced Th₂ development, and glucocorticoid indicated that P₃₈ (MAPK) and extracellular signal regulated kinase pathway, and involved in the Th₁, Th₂ development. Moreover the result of this study revealed a significant positive correlation between IL-4 and AEC before treatment. A correlation could also be detected between IL-4 and IFN- γ but (Lanter et al, 1997) and (ÓByrne and Wood, 1999) nonsignificant. So stimulated Th₂ secrete both IL-4 and IL-5 which is the most specific cytokines to eosinophil and have been suggested to be the key cytokines in the express of allergic disease (Tang et al, 1998) and (Noma et al, 1999).

As observed in the present work a significant positive correlation was found in patients with asthma between IL-4 and AEC, also between IL-4 and IL-4/IFN- γ ratio before treatment. These results confirm the Th₂ hypothesis for asthma which was based on: that IgE and eosinophils play a major and crucial role in asthma pathogenesis (Martin, 2002). There was a negative correlation between IL-4 and IFN- γ after the prednisone therapy and also positive correlation between IL-4 and IL-4/IFN- γ ratio. So glucocorticoid are the most effective drugs used in asthma treatment. This drug has been shown a markedly improve asthma symptoms, quality of life as well as it reduced the underlying airway inflammation associated with asthma (Castro, 2000). this drug should affect Th₁/Th₂ cytokines imbalance and deviate response toward the Th₁ phenotype or away from the Th₂ phenotype (Boushey and Fahy, 2000).

Conclusion and recommendation:

The association between Th₁, Th₂ and their cytokines give the new insights in the pathophysiology of the T-cell response in asthma and provide exciting opportunities for the development of novel immunotherapeutic strategies such as nutritional influences. A group that included methionine, cysteine, arginine, vitamins A, B, C, and E, zinc (Zn) and selenium (Se), are necessary for the synthesis and maintenance of sufficient amounts of nitrogen monoxide (NO), glutathione (GSH)

and metallothionein (MT) which liberate metal ions, such as zinc and copper(Cu). Where their deficiencies shift the Th₁/Th₂ balance toward Th₂ (CAPI,2001). It may be possible someday to treat or prevent these disorders of children, who require unacceptably high doses of oral or inhaled corticosteroid and those who are suffering

from steroid induced side effects by selective manipulation of Th₁ and Th₂ cells, as this may ultimately leads to an effective control of these T-helper subsets, potentially capable of altering the nature course of the disease.

References

- 1- Adams P.F., Marano M.A., (1994): Current estimates from the National health Interview survey: data from the National survey, vital and health statistics Bethesda: National Institutes of Health, National Center for Health Statistics Series 10.
- 2- Antoine O. Magnan, Laurent G., et al (2000): Assessment of Th₁/Th₂ paradigm whole blood in atopy and asthma. *Am. J. Respir. Crit. Care Med.*, V.161, No.6; 1790- 1796.
- 3- Barbara N., Allan S., Mathias H., et al (2000): -PBMCs from both atopic asthma and nonatopic children show a Th₂ cytokine response to house dust mite allergen. *Journal of Allergy and Clinical Immunology*; 106 No1: 1-13.
- 4- Boushey H.A., and Fahy J.V. (2000): Targeting cytokines in asthma therapy. *Lancet*, 356, 2114- 2116.
- 5- Boyem A., (1967): Isolation of lymphocytes, granulocytes and macrophages: *Scand.J.Immunol* 5: 12- 319.
- 6- Canadian Asthma Prevention Institute (CAPI); (2001): The Th₁/Th₂ balance zinc, glutathione, prostaglandin production and the possible relationship to the establishment of asthma: @ asthmaworld.org, (4 pages).
- 7- Castro M., Chaplin D.D., Walter M. J., and Holtzman M. J. (2000): Could asthma be worsened by stimulating the T-helper Type1 immune response? (Comment). *Am. J. Respir. Cell Biol.* 22: 143- 146.
- 8- Crocker L.C., Church M. K., Newton S., and Townley R. G. (1998): Glucocorticoids inhibit proliferation and IL-4 and IL-5 secretion by aeroallergen-specific T-helper type₂ cell lines. *Ann allergy Asthma Immunol.* 80: 509-16.
- 9- Dacie J.V. and Lewis S.M. (1991): Practical hematology, Seventh edition. Edinburgh, London, Melbourne and New York.
- 10- Fraenkel A.J. and Holgate S.T. (1996): Etiology of asthma: Pathology and Mediators. In: *Allergy, Asthma, and Immunology from infancy to adulthood*. 3rd Ed. Bierman C W; Pearlman.D.S., Shapiro, G.G. and Buss, W. W.(eds). Saunders Company, Philadelphia, 443.
- 11- Halonen M., Barbee R. A., Lebowitz M.D. et al (1982): An epidemiological study of the interrelationships of total serum IgE, allergy skin test reactivity and coinophilia. *J. Allergy and Clin Immunol* 69 : 221.
- 12- Kay A.B. (2003) Immunomodulation in asthma: mechanisms and possible pitfalls. *Curr Opin Pharmacol* ; 3 (3) : 220- 6.
- 13- Kazuyoshi K. et al (2001): Effect of oral steroid on blood CXCR₃⁺ and CCR₄⁺ T cells in patients with bronchial asthma. *Am. J. Respir. Crit. Care Med.* 164, No5, 754-758.
- 14- Lantero S., Sacco O., Scala C., Rossi G.A., (1997): Stimulation of blood mononuclear cells of atopic children with relevant allergen induces the release of eosinophil chemotaxins such as IL-3,IL-5 and GM-CSF. *J Asthma*.34:141-52.
- 15- Marino D.M., Homa D.M., Petrowski C.A. et al. (1998) : Surveillance for asthma United States, 1960-1995. *MMWR Morb Mortal Wkly Rep*.47.
- 16- Martin J.T. (2002): Asthma, airway biology, and nasal disorder. *Am. J. Cri. Care Med.*, 165.No.5,598- 618.
- 17- Miyaura H., Iwata M. (2002): Direct and indirect inhibition of Th₁ development by progesterone and glucocorticoids. *J Immunol* 168 (3): 1087- 94.
- 18- Milgrom H. (2003): Is there a role for treatment of asthma with omalizumab. *Archives. of Disease inChildhood*.88:71-74.
- 19- Mori A., Kamiruma O., Suko M. etal (1996): Two distinct pathways of IL-5 synthesis in allergen- specific T- cell clones blood. 89: 2891.
- 20- Mossman T. R., Chexainki H. Bond M.W., Giedlin M. A., Coffman R. L. (1986): Two types of murine helper T-cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol.* 136: 2348-2357
- 21- Mossman T. R., Coffman R. L. (1989): Th₁

- and Th₂ cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7:145-173.
- 22- Mossman T.R., and Sad, S. (1996): The expanding universe cell subsets: TH₁; TH₂ and more *Immunol Today* 17 :138.
- 23- Nagano Y, Kondo M, Tamaoki J, Isono K, Nagai A (2002) Peripheral blood Th₁ and Th₂ profile in patients with moderate asthma: effect of inhaled corticosteroid. *J. Asthma.* 39 (3): 247-53.
- 24- Noma T., Hayashi M., Kawano Y., Yoshizawa I., Sackii T., et al (1999): Functional interleukin-5 activity in peripheral blood mononuclear cells from adolescence with mite antigen asthma in remission. *Clin Exp Allergy;* 29: 780-5.
- 25- ÓByrne P.M. and Wood L., (1999) Interleukin- 5 and allergic inflammation; *Clin Exp Allergy;* 29: 573-5.
- 26- Ohashi Y., Nakai A., Kakinoki T., Kato A., et al(1998): Allergen-induced synthesis of interleukin-5, but not of IgE, is a key mechanism linked to symptomatic episodes of seasonal allergic rhinitis in sensitized individuals. *Scand J Immunol;*47: 596-602.
- 27- Prescott S.L. (2003): New Concept of cytokines in asthma: is the Th₂/Th₁ Paradigm out the window? *J. Pediatr Child Health;* 39 (8): 575-9.
- 28- Ray A., Cohn L. (2000): Altering Th₁/Th₂ balance as a therapeutic strategy in asthma disorder. *Curr. Opin. Investig. Drugs;* 442- 8.
- 29- Scheinman R.I. et al (1995): Role of transcriptional activation of B-alpha in mediation immunosuppression glucocorticoids. *Science;* 270: 282- 286.
- 30- Shirai T, Suzuki K, Inui N, et al (2003): Th₁/Th₂ profile in peripheral blood in atopic cough and atopic asthma. *Clin. Exp. Allergy;* 33 (1) : 84- 9.
- 31- Sophie L., M.D., Stephane P., BSC, Eva-Maria Vagra, M.D. et al (2000): Increased expression of IL-16 immunoreactivity in bronchial asthma after segmental allergen - challenge in patients with asthma. *J Allergy Clin Immunol;* 106 :293.
- 32- Sundeep S., Salvi K., Suresh Babu, and Stephen T. Holgate. (2001): Is asthma really due to polarized T- cell repose toward a helper T- cell type2 phenotype. *Am. J. Respir. Crit. Care Med., V.164, No.8,1343-1346*
- 33- Tang, M.L.K., J. Cleman, and A.S. Kemp. (1995): Interlukin-4 and interferon-γ production in atopic and atopic children with asthma. *Clin. Exp. Allergy;* 25:515-521
- 34- Tang C., Rolland J.M., Ward C., Thien F., Li X., Gollant S., et al (1998): differential regulation of allergen-specific Th₂ but not Th₁ type responses by alveolar macrophages in atopic asthma. *J Allergy Clin. Immunol;* 102: 368-75.
- 35- Till S., Dicason R., Huston D., Humbert M., Robinson D., Larche M., et al (1997): IL-5 secretion by allergen- stimulated CD₄⁺ T cells in primary culture relationship to expression of allergic disease. *J Allergy Clin. Immunol;* 99:563-9.
- 36- Turnoy K.G., Kips J.C., Pauwels R.A. (2002): Is Th₁ the solution for Th₂ in asthma. *Clin Exp Allergy;* 32 (1): 17-29.

