

# Interferon Gamma Gene Polymorphism as a Biochemical Marker in Egyptian Atopic Patients

YM Hussein,<sup>1</sup> AS Ahmad,<sup>2</sup> MM Ibrahim,<sup>1</sup> SA El Tarhouny,<sup>1</sup> SM Shalaby,<sup>1</sup> AS Elshal,<sup>1</sup> M El Said<sup>1</sup>

<sup>1</sup> Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Egypt

<sup>2</sup> Chest Department, Faculty of Medicine, Zagazig University, Egypt

## ■ Abstract

*Objectives:* The aim of this study was to clarify the role of interferon (IFN)  $\gamma$  in the diagnosis and follow-up of atopic patients. We genotyped the IFN- $\gamma$  polymorphism at position +874 to examine the relationship between serum levels of IFN- $\gamma$  and disease severity and the role of IFN- $\gamma$  as a biochemical and immunologic marker.

*Methods:* The study population comprised 75 patients suffering from atopic asthma, atopic dermatitis, and allergic rhinitis (25 each), and 25 control participants. Total immunoglobulin (Ig) E and serum IFN- $\gamma$  were measured by enzyme-linked immunosorbent assay, the IFN- $\gamma$  polymorphism at position +874 was determined by amplification refractory mutation system-polymerase chain reaction, and eosinophil counts were recorded.

*Results:* There was a significant association between genotype and the frequency of the A allele of the +874T/A polymorphism in atopic patients when compared with controls ( $P < .001$ ). In all 3 groups, there was a significant increase in total IgE levels and eosinophil counts, and a decrease in serum IFN- $\gamma$  levels towards the presence of homozygous AA compared with homozygous TT.

*Conclusions:* The IFN- $\gamma$  gene polymorphism at position +874 contributes to susceptibility to atopic diseases by decreasing the amount of IFN- $\gamma$ . Identification of variants of IFN- $\gamma$  gene signalling and its role in the development of atopic diseases provides a focus for the development of novel diagnostic and therapeutic strategies for these diseases.

**Key words:** Interferon  $\gamma$ . Polymorphism. Atopic patients.

## ■ Resumen

*Objetivos:* El objetivo de este estudio ha sido aclarar el papel del interferón (IFN)  $\gamma$  en el diagnóstico y seguimiento de los pacientes atópicos. Genotipamos el polimorfismo de IFN- $\gamma$  en la posición +874 para examinar la relación entre los niveles séricos de IFN- $\gamma$  y la gravedad de la enfermedad y su papel como marcador bioquímico e inmunológico.

*Métodos:* La población de estudio comprendía 75 pacientes afectados de asma atópica, dermatitis atópica, y rinitis alérgica (25 en cada grupo), y 25 participantes control. Se midió la inmunoglobulina (Ig) E total e IFN- $\gamma$  sérico mediante ensayo inmunoanálisis enzimático adsorbente, se determinó el polimorfismo del IFN- $\gamma$  en la posición +874 mediante reacción en cadena de la polimerasa-amplificación refractaria de sistema de mutaciones, y se recogieron el número de eosinófilos.

*Resultados:* Había una asociación significativa entre el genotipo y la frecuencia del alelo del polimorfismo +874T/A en los pacientes atópicos cuando se compararon con los controles ( $P < 0,001$ ). En los 4 grupos, había un aumento significativo de los niveles de IgE total y el recuento de eosinófilos, y un descenso en los niveles séricos de IFN- $\gamma$  hacia la presencia de homocigosis AA comparado con la homocigosis TT.

*Conclusiones:* El polimorfismo del gen IFN- $\gamma$  en la posición +874 contribuye a la susceptibilidad de las enfermedades atópicas mediante el descenso de la cantidad de IFN- $\gamma$ . La identificación de la señalización de las variantes del gen de IFN- $\gamma$  y su papel en el desarrollo de las enfermedades atópicas ofrece un enfoque para el desarrollo de nuevas estrategias diagnósticas y terapéuticas para estas patologías.

**Palabras clave:** Sensibilización a aeroalérgenos. Comorbilidad entre rinitis y asma. Pruebas cutáneas.

## Introduction

An allergy is any clinical event caused by immune mechanisms in response to exposure to allergens. This event may or may not harm the host. These mechanisms are activated by immunoglobulin (Ig) E, which leads to the release of mast cells. The most common familial IgE-related diseases include allergic rhinitis, atopic dermatitis, allergic asthma, and allergic conjunctivitis. These are known as atopic diseases [1].

Beltrani et al [2] described atopy as the expression of polygenic and phenotypic immunologic aberrations, with a systemic expansion of the activity of helper T cells of the type 2 subset ( $T_H2$ ) leading to the release of interleukin (IL) 5, IL-4, IL-13, and IL-3, which cause eosinophilia, increase IgE levels, and boost the growth and development of mast cells.

Immune responses may result from immune suppression of T regulatory cells (Tregs) as well as T helper cells of the type 1 subset ( $T_H1$ ) [3].

$T_H1$  cytokines such as interferon (IFN)  $\gamma$ , IL-12, and IL-2 are thought to have an inhibitory effect on  $T_H2$  cells and decrease the amount of IL-4, IL-5, IL-13 and IgE production [4]. IFN- $\gamma$ -producing  $T_H1$  cells have been reported to protect against allergic responses by attenuating the activity of  $T_H2$  effector cells [5].

The development of atopy depends on several genes, and disease expression is influenced by exposure to environmental factors. Several genome-wide searches have linked the development of atopy to different autosomal chromosomal regions, many of which directly or indirectly regulate IgE production and affect the activation and proliferation of cells involved in inflammatory processes associated with atopy. These regions include chromosome 5q31-q33, which contains a  $T_H2$  cytokine gene cluster (IL-5, IL-4, IL-13, and IL-9) [6], and 12q14, which contains an IFN- $\gamma$  gene consisting of 4 exons and 3 introns [7].

More than 20% of the world's population is thought to have IgE-mediated allergic diseases. This prevalence is rising dramatically, with its consequent effects on health care and costs [8,9].

Various explanations for the increase in the prevalence of allergic diseases have been put forward, the most attractive being the hygiene hypothesis, which states that reduced exposure to infection during early childhood can lead to subsequent susceptibility to allergic diseases through a  $T_H2$  bias [10].

We analyzed the allelic distribution of the +874T/A polymorphism of the IFN- $\gamma$  gene in atopic diseases, its relationship with atopic markers, and the relationship between the allele and disease severity.

## Patients and Methods

### Study Groups

The study population comprised 100 participants: 75 atopic patients from the outpatient allergy unit of our institution (Zagazig University Hospital) and 25 age-matched healthy and unrelated volunteers as controls. None of the participants

received antihistamines or systemic or topical corticosteroids during the 3 weeks before clinical evaluation, and they all underwent skin prick testing. Atopy was diagnosed on the basis of a positive skin prick test result and clinical signs and symptoms. Participants were further classified into the following groups:

*Group 1:* A control group comprising 25 healthy, nonatopic, age-matched, and unrelated volunteers with a negative skin test result and no history of allergic conditions or smoking.

*Group 2 (atopic asthma):* This group included 25 nonsmokers, diagnosed with extrinsic atopic asthma in accordance with the criteria of the American Thoracic Society [11]. A plain posteroanterior and lateral chest radiograph was taken to exclude any associated radiological abnormality. These patients were further classified into subgroups according to previously published guidelines [12].

*Group 3 (atopic dermatitis):* This group comprised 25 patients who met the diagnostic criteria for atopic dermatitis with no other atopic conditions. The severity of atopic dermatitis was measured using the Nottingham Eczema Severity Score [13].

*Group 4 (allergic rhinitis):* This group included 25 patients with allergic rhinitis. Signs and symptoms were identified and scored [14].

All participants gave their written consent before blood sample extraction, and they all underwent a full clinical examination. A complete history was taken, and stool and urine were analyzed to rule out any factors that could affect study determinations.

### Blood Sample Collection

A 6-mL blood sample was taken under aseptic conditions and divided into 2 portions: 1.5 mL of whole blood was collected in sterile EDTA-containing tubes for DNA extraction and eosinophil counts, and the remainder was left for 30 to 60 minutes for spontaneous clotting at room temperature before being centrifuged at 3000 rpm for 10 minutes. Serum samples were kept frozen at  $-20^{\circ}\text{C}$  for determination of total IgE and IFN- $\gamma$  levels.

Serum IFN- $\gamma$  was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) (RayBio Human IFN- $\gamma$  kit, Norcross, Georgia, USA). Total IgE level was measured by ELISA using AccuBind IgE Quantitative Kits, (Lake Forest, California, USA). Eosinophil counts were determined according to Burrows et al [15].

### Genotyping

Genomic DNA was isolated using the Wizard Genomic DNA Purification Kit purchased from Promega (Madison, Wisconsin, USA).

Genotyping for the polymorphisms in IFN- $\gamma$  was performed according to Pravica et al [16], and genes were typed using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). To assess the success of PCR amplification in both reactions, an internal control was amplified using a pair of primers designed from the nucleotide sequence of the human growth hormone (HGH).

The primer sequences used for amplification were as follows: allele-specific sense primer T 5'-TTC TTA CAA CAC AAA ATC AAA TCT-3'; allele-specific sense primer A 5'-TTC TTA CAA CAC AAA ATC AAA TCA-3'; antisense common primer of IFN- $\gamma$  5'-TCA ACA AAG CTG ATA CTC CA-3'; control primer (HGH) (sense), 5'-CCTTCCAAC CATTC CTTA-3'; control primer (HGH) (antisense), 5'-TCA CGG ATTCT GTT GTG TTTC-3'. The reaction mix of the total volume of 15.1  $\mu$ L included 5  $\mu$ L of genomic DNA, 5  $\mu$ L Dd H<sub>2</sub>O, 1.5  $\mu$ L 10X PCR buffer 0.3  $\mu$ L 8 mM dNTPs (SIGMA Chemical Co, St. Louis, Missouri, USA), 0.3  $\mu$ L of 10  $\mu$ M allele-specific and common primers (Biosynthesis), 0.3  $\mu$ L of 2  $\mu$ M control primers (HGH), 1.5  $\mu$ L Mg<sup>2+</sup> and 0.6  $\mu$ L 5U/ $\mu$ L Taq Gold Polymerase (Life Technologies Inc, Gaithersburg, Maryland, USA).

Amplification was performed using a PTC-100 thermal cycler (MJ Research, Inc., Watertown, Massachusetts, USA) according to the following protocol; 95°C for 1 minute followed by 10 cycles of 95°C for 15 seconds, 62°C for 50 seconds, and 72°C for 40 seconds, then 20 cycles of 95°C for 20 seconds, 56°C for 50 seconds and 72°C for 50 seconds.

The amplified products were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. The gel was visualized under a UV transilluminator with a 100-base pair (bp) ladder (Bioron, Ludwigshafen am Rhein, Germany) and photographed.

Two sample products were available for each participant (1 for each specific T or A allele of the IFN- $\gamma$  alleles).

### Statistical Analysis

Data were analyzed using SPSS software version 11 (SPSS Inc, Chicago, Illinois, USA)

## Results

### Skin Prick Test

The allergens with the highest number of positive skin

prick test results in atopic patients were mixed pollens (48%), followed by hay dust (32%), smoke (24%), house dust mite and mixed fungi (17.3%), cotton and wool (8%), mixed feather (6.6%), and animal dander (5.3%). Most patients were sensitive to more than 1 type of allergen.

### IFN- $\gamma$ Gene Polymorphism at Position +874T/A

ARMS-PCR for the polymorphisms in IFN- $\gamma$  (+874T/A) (Figure) revealed a 500-bp product for HGH as a control gene, and a 300-bp product for T874 (homozygous for allele T; TT), or A874 (homozygous for allele A; AA), or both alleles T and A (heterozygous; TA).

Our results revealed a significant association between genotype and the allele frequencies of the +874T/A gene polymorphism in patients with atopic asthma, atopic dermatitis, and allergic rhinitis, compared with controls (odds ratio for the A874 allele: 7.67, 5.92, and 7.67, respectively;  $P < .05$  for each group). The  $\chi^2$  statistic for the atopic asthma group was 10.5 ( $P < .005$ ), for atopic dermatitis it was 9.4 ( $P < .001$ ), and in allergic rhinitis it was 11.5 ( $P < .001$ ) (Table 1).

We analyzed the relationship between allele and genotype frequency and disease severity and found that there was a nonsignificant association between genotype and allele frequency in atopic patients with mild disease and those with severe disease in each group studied ( $P > .05$  for each group).

We also studied the association between the parameters measured and the allelic variants of +874T/A (Table 2) and we concluded that the mean values for serum IFN- $\gamma$ , eosinophil count, and total IgE were significantly different according to the allelic variants in the atopic groups (Table 2). Furthermore, there was a highly significant decrease in IFN- $\gamma$  levels in homozygous AA patients compared with homozygous TT patients ( $P < .001$ ), whereas there was a significant increase in both the eosinophil count and serum IgE levels in homozygous AA patients compared with homozygous TT patients in the atopic groups ( $P < .001$ ).

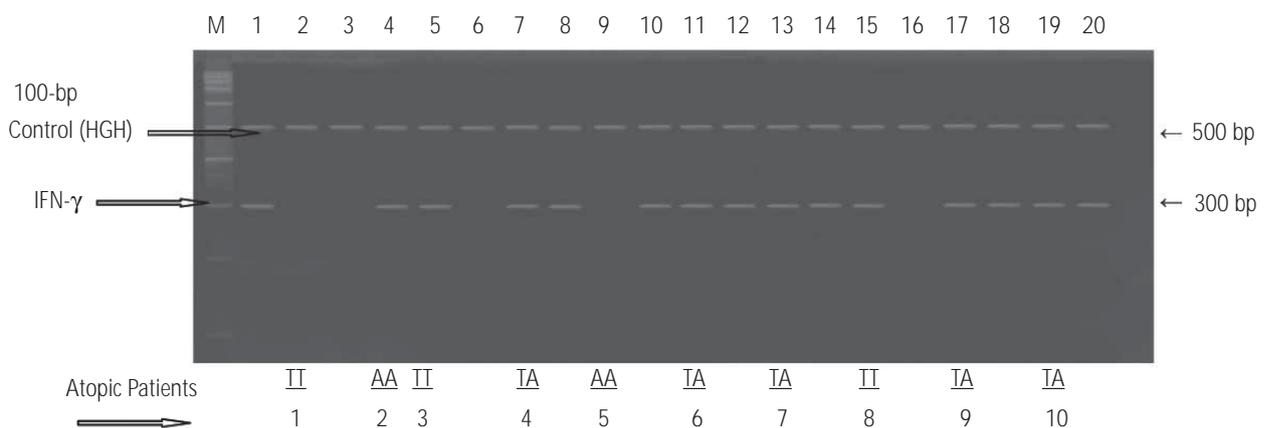


Figure. Amplification refractory mutation system-polymerase chain reaction Representative agarose gel electrophoresis, findings of T874A polymorphism of IFN- $\gamma$  gene. Marker (100 bp) containing every participant was presented by 2 lanes for T and A alleles, respectively.

Table 1. Frequency of the Genotypes of the T874A Polymorphism of the IFN- $\gamma$  Gene in All the Groups Studied

	Control Group, Number %	Atopic Asthma, Number %	Atopic Dermatitis, Number %	Allergic Rhinitis, Number %
AA	0 (0%)	6 (24%)	3 (12%)	5 (20%)
TA	4 (16%)	8 (32%)	11 (44%)	10 (40%)
TT	21 (84%)	11 (44%)	11 (44%)	10 (40%)
$\chi^2$		10.5	9.4	11.5
$P^a$		.005	.001	.001

<sup>a</sup>  $P < .05$  when compared with controls

Table 2. Genotypes in Atopic Groups<sup>a</sup>

Parameters	AA (n=6)	TA (n=8)	TT (n=11)	$P$ Value <sup>b</sup>
Serum IFN- $\gamma$ , pg/mL	55.8 (3.3)	63.9 (2.5)	73.4 (4.5)	<.001
Eosinophils/ mm <sup>3</sup>	756.7 (76.8)	618.7 (26.9)	470 (51.76)	<.001
Serum IgE, IU/mL	266.8 (49.5)	218.9 (14.3)	149.6 (28.8)	<.001
Parameters	AA (n=3)	TA (n=11)	TT (n=11)	$P$ Value
Serum IFN- $\gamma$ , pg/mL	51.3 (8)	61 (3.2)	72.1 (2)	<.001
Eosinophils/ mm <sup>3</sup>	822 (52)	660.9 (62)	486.3 (61)	<.001
Serum IgE, IU/mL	327.2 (16.6)	228.9 (24.1)	148.7 (24.6)	<.001
Parameters	AA (n=5)	TA (n=10)	TT (n=10)	$P$ Value
Serum IFN- $\gamma$ , pg/mL	50.3 (5.7)	60.7 (1.7)	71.6 (4.2)	<.001
Eosinophils/ mm <sup>3</sup>	782.6 (50.6)	577.2 (42.7)	430.2 (56.9)	<.001
Serum IgE, IU/mL	285.4 (45.8)	214.9 (20.4)	137.5 (22.9)	<.001

Abbreviations: IFN, interferon; Ig, immunoglobulin

<sup>a</sup> Values are expressed as the mean (SD)

<sup>b</sup> Compared with the control group

### Serum IFN- $\gamma$

The present study revealed a significant decrease in serum IFN- $\gamma$  levels in atopic groups compared with the control group ( $P < .001$ ) (Table 3). There was significant association between serum IFN- $\gamma$  levels and disease severity in each group (Table 4).

Table 3. Serum IFN- $\gamma$ , Eosinophil Count, and Serum IgE Levels<sup>a</sup>

Measure parameters	Control Group (n=25)	Atopic Asthma (n=25)	Atopic Dermatitis (n=25)	Allergic Rhinitis (n=25)
Serum IFN- $\gamma$ , pg/mL	128.8 (13.6)	66.1 (8.1)	64.75 (8.1)	62.9 (8.9)
Eosinophils/ mm <sup>3</sup>	180 (73.8)	586.4 (127.9)	603.4 (130.9)	595.4 (140.7)
IgE, IU/mL	73.25 (20.8)	199.9 (57.5)	205 (63.9)	198.1 (62.7)
$P^b$	–	<.001	<.001	<.001

Abbreviations: IFN, interferon; Ig, immunoglobulin

<sup>a</sup> Values expressed as mean (SD)

<sup>b</sup> Compared with the control group

Table 4. Relationship Between Parameters and Disease Severity<sup>a</sup>

Parameters	Mild n=9	Moderate n=7	Severe n=9	$P$ Value <sup>b</sup>
Serum IFN- $\gamma$ , pg/mL	77.5 (3.1)	68.4 (2.6)	58.22 (4.1)	<.001
Eosinophils/ mm <sup>3</sup>	424 (33.3)	544 (49.5)	710 (84)	<.001
Serum IgE, IU/mL	123.7 (19.5)	186.1 (22.5)	251.7 (41.8)	<.001
Parameters	n=9	n=11	n=5	$P$ Value
Serum IFN- $\gamma$ , pg/mL	72.5 (1.6)	66.3 (1.76)	57.9 (5.7)	<.001
Eosinophils/ mm <sup>3</sup>	479 (58.9)	576.7 (20.8)	713.8 (83.2)	<.001
Serum IgE, IU/mL	144.1 (20.3)	195.9 (24.7)	258.1 (45.8)	<.001
Parameters	n=8	n=7	n=10	$P$ Value
Serum IFN- $\gamma$ , pg/mL	71.6 (4.1)	57.2 (3.8)	52.14 (2.6)	<.001
Eosinophils /mm <sup>3</sup>	430 (56.9)	558 (90.56)	675 (156.37)	<.001
Serum IgE, IU/mL	137.5 (22.9)	238.4 (45.3)	263.4 (43.69)	<.001

Abbreviations: IFN, interferon; Ig, immunoglobulin

<sup>a</sup> Values expressed as mean (SD)

<sup>b</sup> By analysis of variance

### Serum Total IgE Levels and Eosinophil Count

There was a significant increase in total IgE levels and eosinophil count in the atopic groups compared with the control group ( $P < .001$ ) (Table 3), whereas there was no significant change in the atopic subgroups ( $P > .05$ ). We also detected a

significant association between total IgE levels and eosinophil count and disease severity in the atopic groups (Table 4).

In the present study, there was a significant negative correlation between serum IFN- $\gamma$  levels and both eosinophil count and total IgE levels in the atopic groups ( $P < .001$ ).

## Discussion

We found that, in atopic patients, mixed pollens had the highest number of positive skin prick test results (48%), and that most patients were sensitive to more than 1 type of allergen. These results are comparable with those of Bener et al [17] and Hussein et al [18].

The current findings demonstrate that the frequency of allele A874 was greater in atopic patients than in control patients, and that this correlates with markers of atopy (increased IgE levels and eosinophil count and decreased IFN- $\gamma$  titers).

Pravica et al [16] reported that the single nucleotide polymorphism, T—A, at the 5' end of the CA repeat of the human IFN- $\gamma$  gene (+874T/A) directly affects the level of IFN- $\gamma$  production and correlates with the presence of the A874 allele and low production of IFN- $\gamma$ . The authors proposed that this polymorphism coincided with a putative nuclear factor  $\kappa$ B (NF- $\kappa$ B) binding site that could have functional consequences for transcription of the human IFN- $\gamma$  gene, with the result that the polymorphism could directly influence the level of IFN- $\gamma$  production. However, Ohly [19] revealed that there was no significant association between the +874T/A polymorphism and IgE level in atopic German newborns. This discrepancy could be due to differences in population and age groups; in other words, each analysis may identify the allele or haplotype responsible for the phenotype in that specific population.

In the present study, the serum INF- $\gamma$  level was significantly lower in the atopic asthma group than in the control group, and the significant correlation between serum INF- $\gamma$  levels and severity of asthma is consistent with the results of Nurse et al [20] in black South African patients.

IFN- $\gamma$  plays an important anti-inflammatory role in asthma, as it suppresses tumor necrosis factor (TNF)  $\alpha$  signalling in atopic patients [21], and expression of IL-6, IL-8, and eotaxin induced by exposure to TNF- $\alpha$  [22]. It also induces inflammatory genes such as vascular endothelial growth factor, and expression of IL-17 receptors [23].

Increased acetylation of the NF- $\kappa$ B p65 subunit as a result of TNF- $\alpha$  signalling was considerably reduced by IFN- $\gamma$ . These findings suggest that IFN- $\gamma$  suppresses the expression of some, but not all, proinflammatory genes induced by TNF- $\alpha$  by interfering with the transcriptional activity of NF- $\kappa$ B, possibly through changes in acetylation levels of the key regulatory proteins [24].

The present study revealed that the IFN- $\gamma$  level was significantly lower in patients with atopic dermatitis than in the control group, and that it correlated with the Nottingham Eczema Severity Score.

These results are consistent with those of Lafaille [25], who suggested that T<sub>H</sub>1 and T<sub>H</sub>2 cells can antagonize each other either by blocking the generation of the other cell type

or by opposing effector functions. IFN- $\gamma$  secreted by T<sub>H</sub>1 cells blocks the proliferation of T<sub>H</sub>2 cells.

T<sub>H</sub>2 cytokines (IL-4) rather than T<sub>H</sub>1 cytokines (IL-2, IFN- $\gamma$ ) showed statistically significant higher serum levels in patients with atopic dermatitis than in the control patients [26].

However, Grewe et al [27] stated that IFN- $\gamma$ , but not IL-4, is correlated with the clinical severity of atopic dermatitis. This may be related to the capacity of IFN- $\gamma$  to enhance eosinophil viability and activate vascular endothelial molecules, which in turn increases infiltration by eosinophils and induces atopic dermatitis. We found that the peripheral blood eosinophil count was significantly higher in patients with atopic asthma than in the control group, and that there was a highly significant negative correlation between peripheral blood eosinophil count and forced expiratory volume in the first second (FEV<sub>1</sub>).

The results of studies [18] on patients living in the same area (Sharquia, Egypt) and with almost the same criteria as the patients in the present study are consistent with the results for eosinophil count and atopic asthma of other etiologies (eg, parasitic infestation). Studies on asthmatic patients in different areas have revealed similar results [28].

There was a significant increase in eosinophil count in the atopic dermatitis group compared with the control group, and there was a strong positive correlation between the peripheral blood eosinophil count and the Nottingham Eczema Severity Score. Other authors have found similar results [29].

In the allergic rhinitis group, there was a highly significant increase in eosinophil count compared with the control group, and there was a strong positive correlation between peripheral blood eosinophil count and the Rhinitis Severity Scoring System [14]. These results conflict with those of Winther et al [30], who found no statistically significant differences between the atopic asthma, dermatitis, or rhinitis groups as regards peripheral blood eosinophil count.

We also found that total IgE was higher in atopic asthmatic patients than in control patients, and that there was a significantly negative correlation between increased serum total IgE levels and FEV<sub>1</sub>.

Johansson et al [31] found that mean total serum IgE in atopic asthmatic children was higher than that of the control patients. Staikuniene et al [32] showed that a more than 2-fold elevated serum IgE level in acute and symptom-free periods of pollinosis was considered a significant risk factor for pollinosis with asthma.

However, other studies question the role of total IgE as a useful indicator of atopic asthma. Kaliner [33] found that 40% of allergic asthmatics had normal total IgE levels. Akpinarli et al [34] found that both atopic and nonatopic asthmatics had high total IgE levels.

Kawai et al [35] found a positive correlation between total IgE and severity of atopic dermatitis, and Hussein et al [36] reported a significant correlation between the severity of dermatitis and total IgE levels. In allergic eczema non-IgE-mediated inflammatory mechanisms may play a significant role, and even low IgE concentrations are capable of playing a key role in the pathogenesis of the disease. This overlap in IgE levels made it suggestive but not diagnostic of atopic diseases [37].

The present study revealed a significant negative correlation

between serum IFN- $\gamma$  and peripheral blood eosinophil count, and total IgE levels in all the groups studied. These may simply reflect the fact that IFN- $\gamma$  expression in this situation is a surrogate marker of T<sub>H</sub>1 cell activation and reflects its downregulation in blood eosinophilia and serum IgE levels. These data support the hypothesis that a normal level of IFN- $\gamma$  synthesis regulates disease severity in atopic diseases, as activated B-cell clones could remain active (perhaps for years) and produce IgE. Nevertheless, IgE-mediated local release of mast cells in atopic areas could lead to acute exacerbations of atopic manifestations after acute allergen exposure, although this does not imply an obligatory role for IFN- $\gamma$ /IgE in the pathogenesis of chronic atopic diseases.

These results agreed with those of Tang et al [38], who observed IFN- $\gamma$  to be a major inhibitor of IgE synthesis *in vitro*. They found less intense production of IFN- $\gamma$  in children and adults with atopic dermatitis and elevated serum IgE levels than in nonatopic controls, thus suggesting a similar role for this cytokine *in vivo*.

In conclusion, the IFN- $\gamma$  gene polymorphism at position +874 increases susceptibility to atopic diseases by decreasing the amount of IFN- $\gamma$  and elevation of total IgE levels in the sera of atopic patients. The negative correlation between these parameters supports the effect of IFN- $\gamma$  on the inhibition of IgE, either directly or indirectly, and its role in the pathogenesis of atopic diseases. Finally, the identification of variants of the IFN gene and their role in the development of atopic diseases provides a focus for the development of novel diagnostic and therapeutic strategies.

## Acknowledgments

This work was funded by support of academic research in Zagazig University Projects, Zagazig University Post Graduate & Research Affairs

## References

1. Sicherer SH and Leung DY. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. *J Allergy Clin Immunol*. 2007 Jun;119(6):1462-9.
2. Beltrani VS, Boguniewicz M. Atopic Dermatitis. *Dermatol Online J*. 2003;9(2):1.
3. Ngoc PL, Gold DR, Tzianabos AO, Weiss ST, Celedon JC. Cytokines, allergy, and asthma. *Curr Opin Allergy Clin Immunol*. 2005;2:161-6.
4. Shirai T, Suzuki K, Inui N, Suda T, Chida K, Nakamura H. Th1/Th2 profile in peripheral blood in atopic cough and atopic asthma. *Clin Exp Allergy*. 2003;33(1):84-9.
5. Renaud JC. New insights into the role of cytokines in asthma. *J Clin Pathol*. 2001;54 (8):577-89.
6. Marsh DG, Nely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science*. 1994;264(5162):1152-6.
7. Ahmadi KR, Lanchbury JS, Reed P, Chiano M, Thompson D, Galley M, Line A, Lank E, Wong HJ, Strachan D, Spector TD. Novel association suggests multiple independent QTLs within chromosome 5q21-33 region control variation in total humans IgE levels. *Genes Immun*. 2003;4(4):289-97.
8. Sengler C, Lau S, Wahn U, Nickel R. Interactions between genes and environmental factors in asthma and atopy: new developments. *Respir Res*. 2002;3:7.
9. Asher I, Dagli E, Johansson SG, Karen H, Tari H. Environmental Influences on Asthma and Allergy Prevention of Allergy and Allergic Asthma. World Allergy Organization Project Report and Guidelines. Chem Immunol Allergy. Basel, Karger, 2004;36-101.
10. Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol*. 2004;113(3):395-400.
11. American Thoracic Society: Standardization of spirometry. *Am J Respir Crit Care Med*. 1995;152(3):1107-36.
12. Sheffer AL, Taggart VS. The National Asthma Education Program. Expert Panel Report Guidelines for Diagnosis and Management of Asthma. National Heart, Lung and Blood Institute/ Med Care. 1993;31(3):MS20-8.
13. Emerson RM, Charman CR, Williams HC. The Nottingham Eczema Severity Score: preliminary refinement of Rajka and Langeland grading. *Br J Dermatol*. 2000;142(2):288-97.
14. Meltzer EO. Evaluating rhinitis: Clinical, rhinomanometric, and cytologic assessments. *J Allergy Clin Immunol*. 1988;82:900-8.
15. Burrows B, Hasan FM, Barbee RA, Halonen M, Lebowitz MD. Epidemiologic observations on eosinophilia and its relation to respiratory disorders. *Am Rev Respir Dis*. 1980;122(5):709-19.
16. Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. *Hum Immunol*. 2000;61(9):863-6.
17. Bener A, Safa W, Abdulhalik S, Lestringant GG. An analysis of skin prick test reactions in asthmatics in a hot climate and desert environment. *Allergy Immunol*. 2002;34(8):281-6.
18. Hussein YM, Abou El, Magd Y, Mahmoud YA, Mahmed HM, Rasha LA. Some Biochemical Markers in Atopic Patients and PCR-Based Assay for Detection of R576 IL-4 Receptors Allele Gene. *Zagazig University Medical Journal*. 2006;12(4):4307-21.
19. Ohly A. The common variant / common disease hypothesis on the example of the allergy [doctoral thesis]. Giessen: Justus-Liebig University; 2006.
20. Nurse B, Haus M, Puterman AS, Weinberg EG, Potter PC. Reduced interferon-gamma but normal IL-4 and IL-5 release by peripheral blood mononuclear cells from Xhosa children with atopic asthma. *J Allergy Clin Immunol*. 1997;100:662-8.
21. Wen FQ, Liu X, Manda W, Terasaki Y, Kobayashi T, Abe S, Fang Q, Ertl R, Manouilova L, Rennard SI. TH2 Cytokine-enhanced and TGF-beta-enhanced vascular endothelial growth factor production by cultured human airway smooth muscle cells is attenuated by IFN-gamma and corticosteroids. *J Allergy Clin Immunol*. 2003;111:1307-18.
22. Keslacy S, Tliba O, Baidouri H, Amrani Y. Inhibition of tumor necrosis factor-alpha-inducible inflammatory genes by interferon-gamma is associated with altered nuclear factor-kappaB transactivation and enhanced histone deacetylase activity. *Mol Pharmacol*. 2007;71:609-18.
23. Lajoie-Kadoch S, Joubert P, Letuve S, Halayko AJ, Martin JG, Soussi-Gounni A, Hamid Q. TNF-alpha and IFN-gamma inversely

- modulate expression of the IL-17E receptor in airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol*. 2006; 290: 1238–1246.
24. Tliba O, Amrani Y. Airway smooth muscle cell as an inflammatory cell: lessons learned from interferon signaling pathways. *American Thoracic Society*. 2008;5:106-112.
  25. Lafaille JJ. The role of helper T cell subsets in autoimmune diseases. *Cytokine Growth Factor Rev*. 1998;9(2):139-51.
  26. Nomura I, Goleva E, Howell MD, Hamid QA, Ong PY, Hall CF, Darst MA, Gao B, Boguniewicz M, Travers JB, Leung DY. Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. *Immunology*. 2003;171(6):3262-9.
  27. Grewe MC, Buijnzeel-Koomen CA, Schopf BE, Thepen T, Langveld-Wildschut AG, Ruzicka T, Krutmann J. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today*. 1998;19:359-61.
  28. Wosinska-Becler K, Plewako H, Hakansson L, Rak S. Cytokine production in peripheral blood cells during and outside the pollen season in birch-allergic patients and non-allergic controls. *Clin Exp Allergy*. 2004;34(1):123-30.
  29. Bettiol J, Bartsch P, Louis R, De Groote D, Gevaerts Y, Louis E, Malaise M. Cytokine production from peripheral whole blood in atopic and nonatopic asthmatics: relationship with blood and sputum eosinophilia and serum IgE levels. *Allergy*. 2000;55:1134-41.
  30. Winther L, Moseholm L, Reimert CM, Stahl Skov P, Kaergaard Poulsen L. Basophil histamine release. IgE, eosinophil counts, ECP, and EPX are related to the severity of symptoms in seasonal allergic rhinitis. *Allergy*. 1999; 54:436.
  31. Johansson SG, Lundahl J. Asthma, atopy, and IgE: what is the link? *Curr Allergy Asthma Rep*. 2001;1:89-90.
  32. Staikuniene J, Sakalauskas R. The immunological parameters and risk factors for pollen-induced allergic rhinitis and asthma. *Medicina (Kaunas)*. 2003;39(3):244-53.
  33. Kaliner MA. Pathogenesis of asthma. In: Rich RR, Fleisher TA, Shearer WT, Schwartz BD, Strober W, editors. *Clinical Immunology: Principles and Practice*. St. Louis: Mosby; 1996:909-23.
  34. Akpınarlı A, Güc D, Kalaycı O, Yigitbas E, Ozon. Increased interleukin-4 and decreased interferon gamma production in children with asthma: function of atopy or asthma? *Asthma*. 2002;39(2):159-65.
  35. Kawai K, Kamei N and Kishimoto S. Levels of serum IgE, serum soluble-Fc epsilon RII, and Fc epsilon RII [+/-] peripheral blood lymphocytes in atopic dermatitis. *J Dermatol*. 1992;19(5):285-92.
  36. Hussein YM, Abd Allah SH, Mahmoud S, Ahmed AS. Impact of IL-13 gene mutations in atopic diseases. *EJBMB*. 2006;24:123-7.
  37. Wuthrich B, Schmid-Grendelmeier P. The atopic eczema/dermatitis syndrome. Epidemiology, natural course, and immunology of the IgE-associated ["extrinsic"] and the nonallergic ["intrinsic"] AEDS. *J Investig Allergol Clin Immunol*. 2003;13(1):1-5.
  38. Tang ML, Coleman J, Kemp AS. Interleukin-4 and interferon-gamma production in atopic and non-atopic children with asthma. *Clin Exp Allergy*. 1995;25(6):515-21.

■ *Manuscript received November 4, 2008; accepted for publication January 21, 2009.*

■ **Prof Dr YM Hussein**

Medical Biochemistry Department  
Faculty of medicine  
Zagazig University  
Zagazig  
Egypt  
E-mail: yousrhussein@hotmail.com