

Evaluation of Serum Interferon-gamma Level in Vitiligo Patients

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Abstract:

Background: Vitiligo is an acquired depigmentation disorder of the skin, resulting from a loss of functioning epidermal melanocytes. Multiple pathogenetic factors have been proposed, including the neural theory, genetic predisposition and impaired anti-oxidative defense. Cytokines are small immune-regulatory molecules that can generate an inappropriate immune response when imbalanced. IFN- γ is a pleiotropic cytokine that is a key regulator of the immune system. In addition to host defense, IFN- γ also contributes to autoimmune pathology by inducing autoantibodies, activating autologous cytotoxic T cells and inducing target cell apoptosis. It plays an important role to induce depigmentation in vitiligo.

Aim: In this study, we aimed to determine whether vitiligo is associated with alterations in serum level of IFN- γ or not and to find out its association with disease course, severity, and duration.

Methods: This case control study included 40 patients presented with stable (N=22) and active (N=18) vitiligo (non-segmental and segmental), diagnosed on the basis of typical clinical features, were selected as patient group. Forty age and sex matched apparently healthy individuals were also included representing the control group. Also patients with previous skin cancer or premalignant skin lesions, or taking immunosuppressive drugs as methotrexate and patients with hepatitis viral infection and those on INF therapy were excluded. They were 25 females (62.5%) and 15 males (37.5%), their ages ranged from 18 to 45 years old (mean of 33.53). The controls were 20 females (50%) and 20 males (50%), their ages ranged from 18 to 46 years old (mean of 32.85).

Results: The distribution of vitiligo in patients, 60% was vitiligo vulgaris, 25% were segmental vitiligo and 15% were acro-fascial vitiligo. The results showed no statistical significant difference (p-value > 0.05) between patients and control as regard age, sex and family history and showed statistical significant difference (p-value < 0.001) between patients and control as regard serum concentration of IFN- γ . Results also showed statistical significant (p-value < 0.001) positive (r = 0.63) correlation between IFN- γ levels and VASI score in patients group and statistical significant difference (p-value < 0.001) between IFN- γ levels and duration, clinical types and activity of the disease in patients group.

Conclusion: This study proved high serum level of IFN- γ may be risk factor for vitiligo progression suggesting that it could be used as a marker for assessing vitiligo activity and may open the way for further therapeutic approaches for vitiligo. Serum IFN- γ is positively correlated with disease duration and severity, although it does not seem to be influenced by age, sex and family history of the patient.

Keywords: Vitiligo, Serum IFN- γ

Introduction:

Vitiligo is an inflammatory autoimmune skin disorder characterized by the progressive appearance of depigmented skin lesions due to loss of melanocytes at the cutaneous level ⁽¹⁾.

Etiology of vitiligo is still unclear but a growing number of observations have led the researchers to consider altered cellular immunity as a key factor of melanocyte loss ⁽²⁾, a shift in the immune response, characterized by the prevalence of Th1/Th17-related cytokines (pro-inflammatory) instead of a Tregs/Th2-related one (anti-inflammatory), that is killer (NK) cells and CD8+ cytotoxic T lymphocytes (CTLs) ⁽⁶⁾.

responsible for the increase of pro-inflammatory cytokines primarily observed on the border of lesional and perilesional vitiliginous patches ⁽³⁾.

IFN- γ is a pleiotropic cytokine that is a key regulator of the immune system ⁽⁴⁾. In addition to host defence, IFN- γ also contributes to autoimmune pathology by inducing autoantibodies, activating autologous cytotoxic T cells and inducing target cell apoptosis ⁽⁵⁾.

The major IFN- γ -secreting cells in humans are T helper 1 (Th1) cells, natural

To date, since the first description of type II IFN activity more than 4 decades ago, a

considerable amount has been learned about the biological effects of IFN- γ as one of the most important endogenous mediators of immunity and inflammation. IFN- γ plays a key role in macrophage activation, inflammation, host defence against intracellular pathogens, Th1 cell responses, tumour surveillance and immunoediting (7).

In humans, IFN- γ is implicated in the pathology of several autoimmune diseases, including systemic lupus erythematosus, multiple sclerosis(8), allergic encephalomyelitis, rheumatoid arthritis, type-1 diabetes and vitiligo (9).

Aim of the Work:

The aim of the present study is to evaluate the serum level of INF- γ in vitiligo patients to evaluate its role in the pathogenesis of the disease.

Patients and methods:

This case control study included 40 patients presented with stable and active vitiligo (non-segmental and segmental), diagnosed on the basis of typical clinical features, and were selected as patient group. Forty (age and sex matched) apparently healthy individuals were also included representing the control group. All patients were collected from the Outpatient Clinics of Dermatology and Venereology of Al-Azhar University Hospitals (Cairo) during the period from February 2018 till May 2018. An informed written consent was obtained from participant or their guardians before their participation in this study. Approval was obtained from Research Ethics Committee of the Faculty of Medicine, Al-Azhar University.

Patients:

○ **Inclusion criteria:**

1. Age from 18 to 45 years old.
2. Both sexes were included.
3. Patients with stable and active vitiligo.
4. None of the patients was on topical therapy for vitiligo for at least 2 weeks and /or systemic therapy for at least 4-6 weeks before being enrolled in this the study.

○ **Exclusion criteria:**

1. The patients had immune-mediated comorbidities such as Graves' disease, insulin-dependent diabetes, atopic dermatitis or psoriasis, or any other dermatological disease causing depigmentation.

2. Patients with previous skin cancer or premalignant skin lesions, or taking immunosuppressive drugs as methotrexate.
3. Patients with hepatitis viral infection and those on INF therapy.
4. Pregnancy or lactation.

○ **Controls:**

Healthy individuals with no symptoms or signs of vitiligo were selected as control group.

Methods:

All patients in this study were subjected to:

1- History:

- Personal history including; name, age, sex, marital status, occupation, residence and special habits of medical importance.
- History of present illness including onset, course, duration of the disease, precipitating factors, previous treatment, and data of stoppage of the last treatment modality.
- Past history of other skin or systemic diseases.
- Family history of similar condition.

2- General examination:

Careful general examination for clinical manifestations suggestive of any systemic disease was performed for both patients and controls.

3- Dermatological examination:

- Dermatological examination of controls was performed
- Dermatological examination of patients with vitiligo including wood's lamp as well as examination of scalp, nails, and mucosal surfaces.
- Calculation of VASI scores to evaluate the extent of vitiligo lesions.

○ **Assessment of Extent of the disease using vitiligo area scoring index (VASI):-**

The total body VASI is calculated using a formula that includes contributions from all body regions (possible range, 0–100). One hand unit, which encompasses the palm plus the volar surface of all the digits, is approximately 1% of the total body surface area and is used as a guide to estimate the baseline percentage of vitiligo involvement in each body region (10).

The body is divided into five separate and mutually exclusive regions: hands, upper extremities (excluding hands), trunk, lower extremities (excluding feet), and feet. The

axillary region is included with the upper extremities while the buttocks and inguinal areas are included with the lower extremities⁽¹⁰⁾.

The extent of depigmentation is expressed by the following percentages: 0, 10%, 25%, 50%, 75%, 90%, or 100%.

- 100% depigmentation, no pigment is present.
- 90%, specks of pigment are present.
- 75%, the depigmented area exceeds the pigmented area.
- 50%, the depigmented and pigmented areas are equal.
- 25%, the pigmented area exceeds the depigmented area.
- 10%, only specks of depigmentation are present.

The VASI score is then derived by multiplying the values assessed for the vitiligo involvement by the percentage of affected skin for each body site and summing the surface of the lesions of all body sites together.

$$\text{VASI} = \sum \text{All Body Sites [Hand Units]} \times [\text{Residual Depigmentation}]^{(10)}$$

4- Laboratory investigations:

- **Sample collection and measurement of serum INF- γ :**

5 ml venous blood was taken from each patient, let to be clotted, put in plain test tube and centrifuged at 3000 r.p.m. for 5 minutes. Serum from each sample was put after centrifugation in 1.5 ml Eppendorf and was saved at -20°C till time of measurement. Then assessment of serum INF- γ was done by enzyme-linked immunosorbent assay (ELISA) method.

Test principle:

The microtiter plate provided in this kit was pre-coated with an antibody specific to IFN-gamma. Standards or samples were then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IFN- γ and avidin conjugated to horseradish peroxidase (HRP)

was added to each microplate well and incubated. Then a TMB substrate solution was added to each well. Only those wells that contained IFN- γ , biotin-conjugated antibody and enzyme-conjugated avidin would exhibit a change in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of $450 \text{ nm} \pm 2 \text{ nm}$. The concentration of IFN- γ in the samples was then determined by comparing the O.D. of the samples to the standard curve. Calculation of results was done automatically using standard curve which was constructed by instrument computer software.

Statistical analysis:

Data were analyzed using Statistical Program for Social Science (SPSS) version 15.0. Quantitative data were expressed as mean \pm standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done: Independent-samples t-test of significance: was used when comparing between two means. Chi-square test: was used when comparing between non-parametric data. A one-way analysis of variance (ANOVA): when comparing between more than two means. Pearson's correlation coefficient (r): test was used for correlating data. Probability (P-value) (P-value < 0.05 was considered significant, P-value < 0.001 was considered as highly significant and P-value > 0.05 was considered insignificant).

Results:

Demography of the studied groups: This case control study included 40 patients with vitiligo. They were 25 females (62.5%) and 15 males (37.5 %), their ages ranged from 18 to 45 years old (mean of 33.53). In addition, 40 healthy sex and age matched volunteers served as controls were included in this study. They were 20 females (50%) and 20 males (50%), their ages ranged from 18 to 46 years old (mean of 32.85).

Table (1): Demographic data of studied cases.

Variables		Patients (N = 40)	Control (N = 40)
Age (years)	Mean	33.53	32.85
	±SD	7.81	7.98
Gender	Male	15 (37.5%)	20 (50%)
	Female	25 (62.5%)	20 (50%)

Table (2): Comparison between patients and control as regard γ -IFN.

Variables		Patients (N = 40)	Control (N = 40)	P-value
γ -IFN (pg/ml)	Mean	14.55	8.17	< 0.001*
	±SD	5.81	1.96	
	Range	(6.9 – 24.6)	(4.7 – 11.5)	

This table shows statistical significant difference between patients and control as regard γ -IFN.

Table (3): Correlation study between γ -IFN and VASI score in patients group.

Variables	(r)	p-value
γ -IFN vs VASI score	0.63	< 0.001*

This table shows statistical significant positive correlation between γ -IFN and VASI score in patients group.

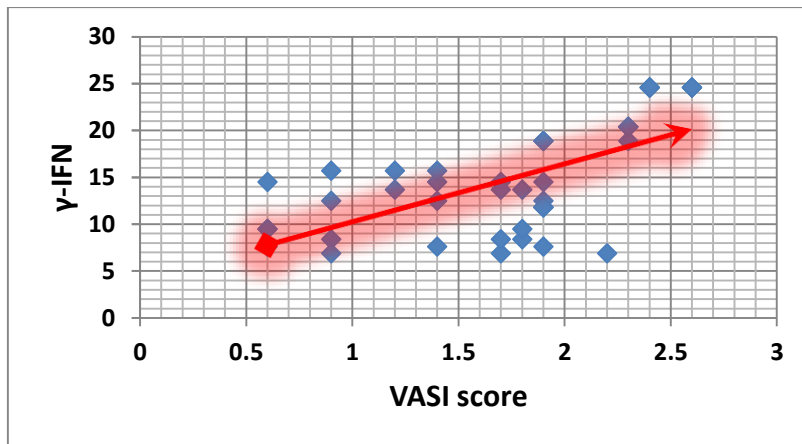


Figure (1): Positive correlation between γ -IFN and VASI score in patients group.

Table (4): Correlation study between γ -IFN and duration of disease in patients group.

Variables	(r)	p-value
γ -IFN vs Duration of disease	0.5	0.001*

This table shows statistically significant positive correlation between γ -IFN and duration of disease in patients group.

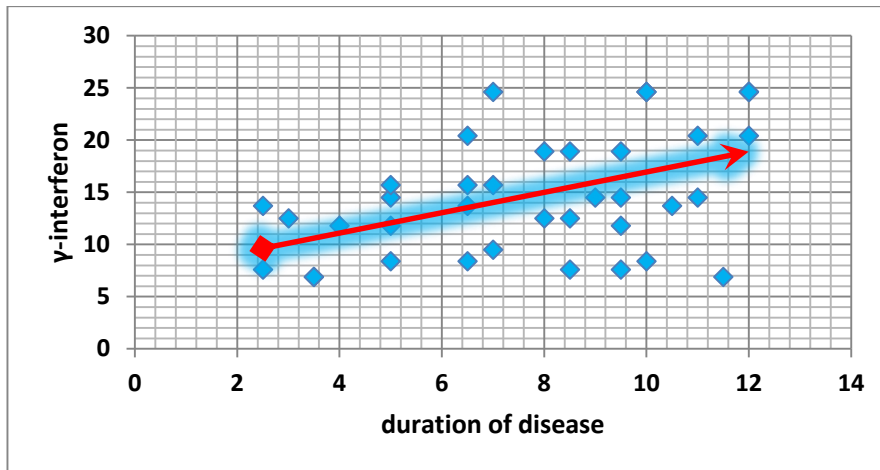


Figure (2): Positive correlation between γ -IFN and duration of disease in patients group.
 Table (5): Correlation study between γ -IFN and age in patients group.

Variables	(r)	p-value
γ -IFN vs Age	0.3	0.07

(r): Pearson correlation coefficient.

This table shows no statistical significant positive correlation between γ -IFN and age in patients group.

Table (6): Comparison between males and females in patients group as regard γ -IFN.

Variables		Males (N = 15)	Females (N = 25)	P-value
γ -IFN (pg/ml)	Mean	14.7	14.5	0.9
	\pm SD	5.6	6.1	

This table shows no statistical significant difference between males and females in patients group as regard γ -IFN.

Table (7): Comparison of γ -IFN as regard course of the disease in patients group.

Variables		Stable (N = 22)	Active (N = 18)	P-value
γ -IFN (pg/ml)	Mean	10.7	19.2	< 0.001*
	\pm SD	3.4	4.6	
	Range	(6.9 – 18.9)	(12.5 – 24.6)	

*: p-value < 0.001 is considered highly significant.

This table shows statistical significant difference between γ -IFN levels as regard course of the disease (stable vs active) in patients group.

Table (8): Comparison of γ -IFN as regard clinical type of the disease in patients group.

Variables		Classic (N = 24)	Segmental (N = 10)	Acro-fascial (N = 6)	P-value
γ -IFN (pg/ml)	Mean	17.4	10.5	9.7	< 0.001*
	\pm SD	5.5	3.2	2.6	
	Range	(7.6 – 24.6)	(6.9 – 15.7)	(6.9 – 14.5)	

This table shows statistical significant difference between γ -IFN levels as regard clinical type of the disease (classic, segmental and acro-fascial) in patients group.

Table (9): Comparison between γ -IFN levels as regard family history

Variables		Positive FH (N = 9)	Negative FH (N = 31)	P-value
γ -IFN (pg/ml)	Mean	13.7	12.9	0.7
	\pm SD	4.6	5.1	

This table shows no statistical significant difference between γ -IFN levels as regard family history.

Discussion:

Vitiligo is an acquired disease with a variable course. It is characterized clinically by well-defined depigmented macules or patches thought to occur secondary to melanocyte dysfunction and loss ⁽¹¹⁾.

Multiple mechanisms might contribute to this loss, including metabolic abnormalities, oxidative stress, generation of inflammatory mediators, cell detachment and autoimmune responses ⁽¹¹⁾.

The clinical course of vitiligo, particularly generalized vitiligo is not easy to be predicted. However, it is generally slowly progressive and hard to be controlled with therapy⁽¹²⁾. Clinical, histological, and biological approach to assess vitiligo progression has been done and each has limitations. In previous studies about vitiligo progression assessment by measuring the pro-inflammatory cytokines levels in vitiligo patient serum, it was reported that there were changes in cytokine expression ⁽¹³⁾.

IFN- γ is a pleiotropic cytokine that is a key regulator of the immune system ⁽⁴⁾. In addition to host defence, IFN- γ also contributes to autoimmune pathology by inducing autoantibodies, activating autologous cytotoxic T cells and inducing target cell apoptosis ⁽⁵⁾.

IFN- γ plays a key role in macrophage activation, inflammation, host defence against intracellular pathogens, Th1 cell responses, tumour surveillance and immunoediting ⁽⁷⁾. In humans, IFN- γ is implicated in the pathology of several autoimmune diseases, including systemic lupus erythematosus, multiple sclerosis⁽⁸⁾, allergic encephalomyelitis, rheumatoid arthritis, and type-1 diabetes and vitiligo ⁽⁹⁾.

This case control study included 40 patients with vitiligo, and 40 age and sex matched healthy control. Serum level of IFN- γ was measured by ELISA, to evaluate its possible role in the pathogenesis of vitiligo.

Patients in this study were 25 females (62.5%) and 15 males (37.5 %), which is in line with other studies that showed a

predominance of female patients, but this observation is most likely because of the greater likelihood of female to seek medical attention for cosmetic problems ⁽¹⁴⁾.

In this study, we aimed to determine whether vitiligo is associated with alterations in serum level of IFN- γ or not and to find out its association with disease course, duration and severity.

We found that there were statistically significant differences between patients and normal healthy controls as regards the serum IFN- γ level and this comes in agreement with **Ala *et al.*** ⁽¹⁵⁾ who found significant elevation in vitiligo patients compared to healthy controls (12.4 ± 3.2 pg/mL versus 9.9 ± 4.4 pg/mL; $p < 0.05$).

As regarding family history we found no significant difference between serum INF level and family history of the patient and this result is similar to **Ala *et al.*** ⁽¹⁵⁾ who found that IFN levels were nearly similar in patients with and without family history of vitiligo; but family history was positively correlated with IFN ; IL 10 ratio but not with their individual levels .

Concerning age and gender there were no statistically significant differences between cases and controls, and this comes in agreement with **Ala *et al.*** ⁽¹⁵⁾.

We also founded that serum IFN level is positively correlated with the duration of the disease, clinical types and severity, and these results are similar to **Ala *et al.*** ⁽¹⁵⁾ and **Dwivedi *et al.*** ⁽¹⁶⁾ who observed increased serum level of IFN with increase of the duration and severity of the disease and reported a positive correlation between the concentration of this IFN and the duration and severity of the disease.

A total of 130 vitiligo patients with a mean age of 27.6 ± 6.9 years and 150 healthy controls with a mean age of 26.9 ± 5.6 years were enrolled in our study. Approximately, equal numbers of males and females were observed in both of the study groups. The

mean age at onset of the disease was 23.3 ± 7.0 years (23.2 ± 7.8 years in females and 23.4 ± 6.5 years in males) and the duration of the disease ranged from 1 to 14 years with a mean of 4.2 ± 3.1 years (4.6 ± 3.5 years in females and 3.8 ± 2.8 years in males). IFN- γ levels were noted to be significantly elevated in vitiligo patients compared to healthy controls (12.4 ± 3.2 pg/mL versus 9.9 ± 4.4 pg/mL). Analysis of variance showed a significant difference with respect to serum IFN- γ levels among the clinical variants of vitiligo (nondermatomal, acrofacial, mucosal, and focal) ($p < 0.05$) and these results are similar to results of our study .

Our study included 18 patients with active vitiligo and 22 patients with stable vitiligo and the results showed statistically significant difference between γ -IFN levels as regard course of the disease (stable vs active) in patients group and that the serum γ -IFN levels were more increased in active vitiligo patients than stable ones.

In accordance with these results, two studies have demonstrated elevated serum levels of IFN- γ in active versus stable cases ^(15, 16).

These findings are most consistent with **Praharsini et al.** ⁽¹⁷⁾ who found that high levels of TNF- α and IFN- γ serum is a risk factor for vitiligo progression, and there were low levels of pro-inflammatory cytokines and low numbers of cytotoxic T cells in stable vitiligo patients. The expression of cytokines TNF- α and IFN- γ were associated with melanocyte destruction in the active phase of vitiligo lesions.

Studies have demonstrated the presence of skin-homing melanocyte- specific cytotoxic T lymphocytes (CD8+ T cells) in the peripheral blood of patients with vitiligo. These T cells are mainly melanocyte reactive CD8+ T cells and can destroy skin melanocytes with subsequent depigmentation in vitiligo. The frequency of these lymphocytes correlates with both the extent and activity of the disease ⁽¹⁸⁾.

In contrast to our finding **Singh et al.** ⁽¹⁹⁾; demonstrated higher serum levels of IL-6 in vitiligo patients versus controls, with no difference between active and stable disease. In addition, they found significantly lower serum IFN- γ in their patients, it was proposed that IFN- γ is increased in bursts for a short period during vitiligo activity, thus explaining discrepancy among different previous studies and why it is not elevated in stable cases.

This discrepancy between our results and the results of previous study could be attributed to the difference in inclusion and exclusion criteria, the difference in number of patients involved, the differences of age of the patients, and the differences of duration of the disease, also exposure to stress which may affect IFN- γ level.

The use of IFN- γ inhibitors as vitiligo treatment has demonstrated positive therapeutic responses ⁽²⁰⁾.

Finally, our study highlights the role of IFN- γ in vitiligo pathogenesis and its association with disease severity, extent, duration and progression.

Conclusion:

Cytokines play a role in regulating the immune response and depigmentation process in vitiligo. There is an imbalance of cytokine levels in patients with vitiligo. The IFN- γ expression play a role in the autoimmune process of vitiligo. The expression of cytokine IFN- γ is associated with melanocyte destruction in the active phase of vitiligo lesions.

This study proved high serum level of IFN- γ may be risk factor for vitiligo progression suggesting that it could be used as a marker for assessing vitiligo activity and may open the way for further therapeutic approaches for vitiligo. Serum IFN- γ is positively correlated with disease duration and severity, although it does not seem to be influenced by age, sex and family history of the patient.

Recommendations:

Further studies about IFN- γ level before and after treatment in vitiligo patients with large samples are needed to confirm this study results. More studies about the role of IFN- γ inhibitor for vitiligo patient are needed as the treatment option in vitiligo.

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