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ORIGINAL ARTICLE

Circulatory Level of Interferon-gamma and its Relation to Disease Severity and Activity Among Vitiligo Patients

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Abstract

Background: **A skin depigmentation illness known as vitiligo is brought on by malfunctioning epidermal melanocytes. Cytokines are tiny immune-regulatory chemicals that may cause an unwarranted immunological response. An important immune system regulator is the pleiotropic cytokine interferon-gamma (IFN-γ).**

IFN-γ induces autoantibodies, activates autologous cytotoxic T cells, and causes target cells to undergo apoptosis, all of which support host defense in addition to contributing to autoimmune disease. It is crucial to vitiligo depigmentation induction.

Aim: **To measure the IFN-γ concentration in the serum of vitiligo patients and to explain how this level relates to the disease degree and activity.**

Methods: **This research was cross-sectional and analytical, carried out at dermatology the outpatient clinic of the Suez Canal University Hospitals, Ismailia on 60 participants divided into 2 groups: Patient group: comprised 30 patients who had segmental and nonsegmental vitiligo that was stable and active, as determined by the conventional clinical characteristics, and a control group: included 30 healthy people without vitiligo symptoms or indicators were chosen ages and sexes matched to the patients.**

Results: **There is statistically significant statistically relation between IFN-γ concentration and each of vitiligo area score index (VASI) and vitiligo area score index (VIDA) score activity and the duration of disease.**

Conclusion: **In vitiligo, cytokines control the immune response and depigmentation process. Patients with vitiligo have unbalanced amounts of cytokines. The autoimmune cause of vitiligo involves the expression IFN-γ. In the active stage of vitiligo lesions, the production of the cytokine IFN-γ is connected to the destruction of melanocytes.**

Keywords: Cytokines, Enzyme-linked immunosorbent assay, Interferon-gamma, Immune response, Vitiligo

1. Introduction

With vitiligo, melanocyte loss occurs at the epidermal level, leading to the formation of chronic depigmented skin lesions on the skin's surface.[1]

Interferon-gamma (IFN-γ) is a pro-inflammatory cytokine that is mostly produced by NK cells, $CD8^+$ cytotoxic cells, and H1 lymphocytes. This cytokine is important for both the innate and adaptive immunological response. It may also have an antagonistic or synergistic effect on how other cytokines behave.[2]

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IFN-γ may work in a variety of ways and its specific role in the pathogenesis of vitiligo is unknown. IFN-γ, for example, may help $CD8^+$ lymphocytes homing to the skin by generating chemokines locally and driving endothelial cells to produce adhesion molecules.[2]

Although the cause of vitiligo is still unknown, a rising number of studies have led scientists to believe that altered cellular immunity is a major contributor to melanocyte loss.[3]

NK cells and $CD8^+$ cytotoxic T lymphocytes (CTL) are the immune cells responsible for the change in immune reaction, which is marked by the predominance of Th1/Th17-related (pro-Inflammatory) cytokines rather than Tregs/Th2-related (anti-Inflammatory) ones. This change in immune reaction is primarily seen on the border of lesional and peri lesional vitiliginous patches.[4]

IFN-γ is linked to the pathophysiology of a number of autoimmune illnesses in humans, such as type 1 diabetes, vitiligo, multiple sclerosis,[5] allergic encephalomyelitis, and systemic lupus erythematosus.[6]

Singh and colleagues,[7] showed elevated serum IL-6 levels in vitiligo patients versus controls without a difference between the disease's active and stable states. Additionally, they discovered significantly low levels of serum IFN-γ in their patients. This was contrary to the theory that IFN-γ declines in bodies for a short time during vitiligo activity, explaining the discrepancy between various previous studies and the reason why it is not raised in stable cases.[7]

It has been thought that one of the main effector mechanisms of autoreactive, cytotoxic T lymphocytes is the activation of apoptosis (CTLs). As a consequence, recruited $CD8^+$ T lymphocytes may selectively trigger autologous melanocyte apoptosis, which would cause melanocytes to vanish. On the other hand, efficient activation of antigen-specific $CD8^+$ T cell effector responses necessitates $CD4^+$ Th1 cells, one of the major sources of IFN-production. Considered to be the primary effector cell in Th1 response are $CD8^+$ T cells.[8]

Therefore, this research will examine the serum IFN-γ level in vitiligo patients and how it relates to the severity and activity of the condition.

2. Patients and methods

A total of 60 individuals were included in this cross-sectional analytical research. They had been chosen from the Ismailia Suez Canal University Hospitals' dermatological outpatient clinic.

2.1. Study population

2.1.1. Patient group

Based on the usual clinical criteria, 30 people with stable and active vitiligo (nonsegmental and segmental) were identified.

2.1.2. Control group

30 healthy people without vitiligo symptoms or indicators were chosen (patients' ages and sexes were matched). All vitiligo patients visiting the dermatological outpatient clinic at the Suez Canal University Hospitals, Ismailia, were conveniently sampled. The individuals' enrolled age ranged from 18 to 45 years old, both sexes were included, and stable and active vitiligo patients were included.

Any patients who had immune-mediated diseases such as Graves illness, insulin-dependent diabetes, atopic dermatitis or psoriasis, or any other depigmenting dermatological condition are excluded. Also, patients with a history of skin cancer or premalignant skin lesions, those taking immunosuppressive medications such as methotrexate, those with hepatitis viral Infection and those receiving IFN therapy, patients who were pregnant or nursing, and those who had been receiving topical treatment for vitiligo for at least 2 weeks and/or systemic treatment for at least 4–6 weeks prior to enrollment in this study were excluded from participation.

2.2. Every patient was subjected to the following

2.2.1. Informed consent

Before participating in this trial, every patient provided written Informed permission.

2.2.2. History taking

Personal Information such as name, age, sex, marital status, occupation, place of residence, and any special habits that are medically significant. Information about the current illness, such as its onset, course, duration, precipitating factors, prior treatments, and information on the last form of treatment was discontinued.

2.2.3. General examination

Vital indicators (temperature, pulse rate, blood pressure, and breathing rates), symptoms of disease, and signs of (Pallor, jaundice, cyanosis, and enlarged lymph nodes).

2.2.4. Skin examination

To identify the skin type, the quant location, size, and color of vitiligo, as well as the distribution and clinical variation of the illness. Each patient had a thorough clinical examination to determine the number, location, and extent of vitiligo. Check the patient out. Where are the lesions located on the body? There are how many lesions? If there are many lesions, do they have a pattern or do they all appear in one place? What are the lesions? What is the lesion's biggest diameter? Is color present? Are there any secondary alterations (such as lichenification, crusting, excoriation, ulceration, erosion, fissure, hypertrophy, or granuloma)? Is the boundary well delineated? Is it typical? Touch the lesion. Feel the warmth, surface, consistency, movement, and mobility (use gloves to prevent Infection). Make sure the patient is healthy overall. Examine the mucous membranes, hair, scalp, and nails. Exists lymphadenopathy, if so? Does the patient's temperature exist?

Patients with vitiligo have a dermatological examination that includes a wood lamp examination as well as checks of their scalps, nails, and mucosal surfaces.

2.2.5. VASI scores were calculated to determine the severity of vitiligo lesions. Applying the vitiligo area score index (VASI), the disease's severity is determined

A method that accounts for contributions from all body areas is used to get the whole body vitiligo area score index (VASI) (possible range: 0–100).

2.2.6. Assessment of vitiligo activity: vitiligo disease activity score (VIDA)

The vitiligo area score index (VIDA) is a six-point scale for evaluating the severity of vitiligo activity based on the patient's perception of the current illness, whether it be via the growth of already-existing lesions or the emergence of new lesions.

2.2.7. Laboratory investigations

Samples were collected to measure serum IFN: from each patient, three ml of venous blood was drawn, allowed to clot, placed in a plain test tube, and centrifuged for 10 min at 3000 rpm. After centrifugation, the serum from each sample was placed in 1.5 ml of Eppendorf and kept there at −20 °C until the measuring time. Sunlong Biotech Company kits were used to perform a sandwich enzyme-linked immunosorbent assay (ELISA) approach to evaluate the serum IFN-γ level.

2.3. Data management and statistical analysis

Statistical software for social sciences (IBM-SPSS), version 24 (May 2016); IBM-Chicago, USA; was utilized for data input, processing, and statistical analysis. The significant tests of the Kruskal-Wallis, Wilcoxon, χ^2, logistic regression analysis, and Spearman's correlation were utilized. According to the kind of data (parametric and non-parametric) collected for each variable, data were presented, and an appropriate analysis was carried out. *P* values of 0.05 or below (5%) were regarded as statistically substantial.

3. Results

Our results showed that in the patient's group 33.3% are males and 66.7% are females and in the control group 53.3% are males and 46.7% are females and regarding each age and sex, there was no statistically substantial variation between the groups (Table 1). According to VASI score in the patient group mean was 35.79 with SD of 24 (Table 2). Regarding VIDA score activity in the patient's group, 10 (33.3%) patients were +4, 6 (20.0%) patients were +3, 5 (16.7%) patients were +1, 5 (16.7%) patients were +2 and 4 (13.7%) patients were 0 score activity (Table 3). As shown in (Table 4) most patients (73.3%) were of the generalized type, then generalized acrofacial (16.7), All rest types (acral and segmental and generalized acral) included one (3.3%) patient. 83.3% of patients had vitiligo from less than or equal to 5 years and 16.7% had vitiligo from greater than 5 years and the mean disease duration was 3.23 ± 2.89 years (Table 5). The mean

Table 1. Comparison of the two study groups based on demographic Information.

	Patients ($n = 30$) No. (%)	Control ($n = 30$) No. (%)	Test of Significance	*P*
Sex				
Males	10 (33.3)	16 (53.3)	$\chi^2 = 2.443$	0.118
Females	20 (66.7)	14 (46.7)		
Age (years)				
Mean ± SD.	29.87 ± 10.27	32.37 ± 9.14	$t = 57.238$	0.323

χ^2, Chi-square test; SD, Standard deviation; t, Student *t*-test.
p: *P*-value for analyzing the groups under study.

Table 2. Analysis descriptive of the analyzed cases based on VASI score in the patient group (n = 30).

	Mean ± SD.
VASI score	35.79 ± 24.0

SD, Standard deviation.

Table 3. Distribution of analyzed cases based on VIDA score activity in the patient population (n = 30).

VIDA score activity	No. (%)
0	4 (13.3)
+1	5 (16.7)
+2	5 (16.7)
+3	6 (20.0)
+4	10 (33.3)

Table 4. Distribution of examined cases by the type of lesion (n = 30).

Type	No. (%)
Segmental	1 (3.3)
Generalized	22 (73.3)
Acral	1 (3.3)
Generalized acral	1 (3.3)
Generalized acrofacial	5 (16.7)

Table 5. Distribution of the examined patients according to disease duration (n = 30).

Duration of disease (years)	No. (%)
≤5	25 (83.3)
>5	5 (16.7)
Mean ± SD.	3.23 ± 2.89

SD, Standard deviation.

IFN-γ concentration (pg/ml) in the patient's group was 15.74 with an SD of 1.86. However, in the control group mean concentration was 7.93 with an SD of 2.42 (Table 6, Fig. 1). There was a substantial variation between the two groups. IFN-γ concentration (pg/ml) was of significant positive weak or moderate correlation with VASI score, VIDA scores activity and duration of disease (years) (Table 7).

4. Discussion

The course of the acquired illness vitiligo varies. Clinically, it is indicated by clearly defined depigmented macules or patches that are assumed to result from melanocyte malfunction and loss Ezzedine and colleagues.[1]

It is difficult to anticipate the clinical course of vitiligo, especially widespread vitiligo.

However, it usually progresses gradually and is difficult to manage with treatment Searle and colleagues.[9]

Multiple factors, such as aberrant metabolic processes, oxidative stress, the production of inflammatory mediators, cell detachment, and immunological reactions, might be to blame for this loss Harris and colleagues.[10]

An important immune system regulator is the pleiotropic cytokine IFN-γ. Through the induction of autoantibodies, the activation of autologous cytotoxic T lymphocytes, and the induction of target cell death, IFN-γ also contributes to autoimmune disease in addition to host defense Ezzedine and colleagues.[11]

Thirty patients with vitiligo were included in this case-control research, and the blood level of IFN-γ was assessed using an ELISA to assess its potential contribution to the etiology of vitiligo.

There have been attempts to evaluate the development of vitiligo using clinical, histological, and biological methods, but each has drawbacks. There were observed alterations in cytokine expression in earlier research concerning evaluating the pro-inflammatory cytokine levels in vitiligo patient blood to determine the course of the condition.

They had a gender split of 20 (66.7%) females and 10 (33.3%) men, which is consistent with past research that found a majority of female patients. However, this discovery is most likely due to the fact that ladies are more prone than males to seek medical assistance for aesthetic issues.

Praharsini[12] found that Low concentrations of pro-Inflammatory cytokines and low numbers of cytotoxic T cells were found in individuals with stable vitiligo, but high serum levels of TNF and IFN-γ are risk factors for vitiligo development. In the active stage of vitiligo lesions, the production of the cytokines TNF and IFN-γ was connected to the destruction of melanocytes.

In contrast, Singh and colleagues[7] revealed that there was no variation between active and stable disease and that vitiligo patients had greater blood

Table 6. Comparison of IFN-γ concentration between the two examined groups.

IFN-γ concentration (pg/ml)	Patients (n = 30)	Control (n = 30)	T	P-value
Mean ± SD.	15.74 ± 1.86	7.93 ± 2.42	54.377*	<0.001*

SD, Standard deviation; t, Student *t*-test.
*Statistically significant at *P* less than or equal to 0.05.

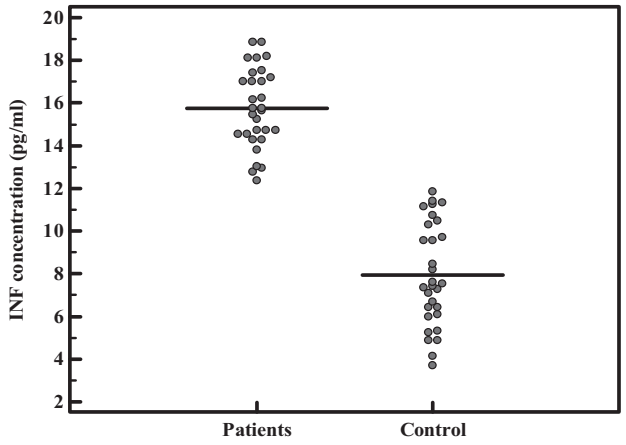

Fig. 1. Comparison of IFN-γ concentration between the two examined groups.

levels of IL-6 than controls. Additionally, they discovered much lower blood IFN-γ levels in their patients; it was hypothesized that IFN-γ is raised briefly during vitiligo activity in order to explain discrepancies between several earlier research and why it is not enhanced in stable instances.

We found that patients had statistically significantly higher levels of serum IFN-γ (15.74 ± 1.86) than controls (7.93 ± 2.42) ($P < 0.001$).

This is in accordance with Ezzedine[13] presented a study in which statistically significant difference between patients and control as regard IFN-γ (14.55 vs. 8.17, $P < 0.001$).

Ala[14] revealed that considerably higher levels were seen in vitiligo patients (12.4 ± 3.2 pg/ml vs. 9.9 ± 4.4 pg/ml; $P < 0.05$) than in healthy controls.

As regards family history, we found no significant difference between serum IFN-γ level and the family history of the patient and this result is similar to Ala[14] who found that Individuals with and without a family history of vitiligo had IFN-γ levels that were almost identical, although family history was favorably connected with IFN-γ/IL10 ratio but not with patients' individual levels.

Table 7. Correlation between IFN-γ concentration and various patient parameters (n = 30).

	IFN-γ concentration (pg/ml)	
	r	*P*
VASI score	0.439	0.015*
VIDA score activity	0.403	0.027*
Duration of disease (years)	0.482	0.007*

r, Pearson coefficient.
*Statistically substantial at *P* less than or equal to 0.05.

Tomaszewska[15] revealed both AA and NSV patients' sera had greater concentrations of IFN-γ than healthy controls. This may suggest that these cytokines have a function in the biology and development of diseases.

In the current study, the mean VASI score of the patients (35.79 ± 24.0) showed a significant direct moderate correlation with serum IFN-γ ($r = 0.439$, $P = 0.015$).

Similarly, Ezzedine[13] mean VASI score of the patients (35.79 ± 24.0) showed a significant direct moderate correlation with serum IFN-γ ($r = 0.630$, $P < 0.001$).

Also, this study found that the VIDA score of the patients showed a significant direct moderate correlation with serum IFN-γ ($r = 0.403$, $P = 0.027$).

In agreement with Ezzedine[13] in which VIDA score of the patients showed significant direct moderate correlation with serum IFN-γ ($r = 0.501$, $P < 0.001$).

Tomaszewska[15] study has showed the relationships between elevated IFN-γ serum levels and NSV patients' vitiligo severity index (VASI) and disease duration. The serum level of IFN-γ was strongly linked with disease severity (VIDA).

These findings are most consistent with Praharsini[12] who found that Low rates of pro-inflammatory cytokines and low numbers of cytotoxic T cells were found in individuals with stable vitiligo, but high serum levels of TNF and IFN-γ are risk factors for vitiligo development. In the active stage of vitiligo lesions, the production of the cytokines TNF and IFN-γ was linked to the degeneration of melanocytes.

In this study, patients had mean duration of disease of (3.23 ± 2.89) years that showed significant direct moderate correlation with serum IFN-γ ($r = 0.482$, $P = 0.007$).

This is come in agreement with Ezzedine[13] in which mean duration of disease had significant direct moderate correlation with serum IFN-γ ($r = 0.500$, $P = 0.001$).

These outcomes resemble Ala[14] and Dwivedi[16] revealed that Increased blood levels of IFN-γ revealed a correlation between the concentration of this IFN-γ and the duration and severity of the sickness. Increased serum levels of IFN- γ were also correlated with an increase in the disease's duration and severity.

In contrast to our finding Singh and colleagues[7] demonstrated that IFN-γ is raised in bursts for a brief duration during vitiligo activity, explaining the contradiction between several prior research and why it is not enhanced in stable instances after they discovered considerably lower serum IFN-γ in their patients.

This disparity between our findings and those of the prior research may be due to changes in the

inclusion and exclusion criteria, the number of patients participating, the ages of the patients, the lengths of the patients' illnesses, as well as stress exposure, which may have an impact on IFN-γ level. IFN-γ inhibitors have shown effective therapeutic results when used to cure vitiligo Tomaszewska.[15]

Finally, this research sheds insight on IFN-γ function in the etiology of vitiligo and its correlation with the severity, extent, duration, and development of the condition.

4. 1. Conclusion

This research shows that a high blood level of IFN-γ may be a risk factor for the advancement of vitiligo, indicating that it may be utilized as a marker to measure the disease's activity and pave the way for other treatment strategies for vitiligo. Although serum IFN-γ seems to be unaffected by the patient's age, sex, or family history, it is positively connected with the length and severity of the illness.

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Conflicts of interest

The authors declared that there were no conflicts of Interest.

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