The Egyptian Journal of Hospital Medicine (July 2022) Vol. 88, Page 3839-3843

Evaluation of Serum Interferon Gamma in Patients with Vitiligo versus Control Group

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ABSTRACT

Background: Vitiligo is an autoimmune illness that causes uneven depigmentation by gradually destroying melanocytes in the skin. This disfiguring condition commonly affects the face and other visible regions of the body, causing psychological distress. Vitiligo usually appears in young people and progresses throughout their lives, resulting in a high disease burden and a poor quality of life.

Objective: The aim of this study was better clarification the possible participation of interferon gamma (IFN-γ) in the pathogenesis of vitiligo.

Patients and Methods: This case control study included 28 Patients with Vitiligo, and 28 healthy controls. Serum level of IFN-γ was measured by ELISA.

Results: Age showed a non-significant correlation with serum IFN-γ in vitiligo and control group. Serum IFN-γ showed a significant strong positive correlation with the degree of severity in vitiligo group. In vitiligo patients' number of areas affected showed a significant positive correlation with serum IFN-γ. Serum IFN-γ also showed a significant positive very strong correlation with Psoriasis Area Severity Index (PASI) and Vitiligo Area Severity Index (VASI) score. Duration of the disease showed a significant correlation with serum IFN-γ only in vitiligo patients.

Conclusion: It could be concluded that the augmented IFN-γ serum level is found in patients with vitiligo, marking the systemic inflammatory nature of these diseases. The study proved that IFN-γ serum level significantly increases with Vitiligo diseases activity.

Keywords: Serum Interferon Gamma, Vitiligo, Evaluation.

INTRODUCTION

Vitiligo is a depigmentation condition of the skin, hair, and nails caused by a lack of functional melanocytes in the epidermis. Vitiligo pathogenesis is a complex pathogenic mechanism involving cellular and humoral immunity that is initiated by an imbalance in cytokine expression in the skin, serum, and blood of vitiligo patients [(1)].

The lymphocytes, natural killer cells, and CD8+ cytotoxic T cells release interferon gamma, which is a proinflammatory cytokine involved in the aetiolog y of vitiligo [(2)].

Interferon gamma (IFN-γ) influences melanin production, melanosome maturation, anti-melanocyte antibody creation, and cytotoxic T cell activation, all of which contribute to melanocyte apoptosis [(3)].

The existence of new lesions, the growth of old lesions, grafting tests, and koebnerphenomena are all indicators of progressive/active vitiligo. These parameters are subjective and semi- subjective [(4)].

Many studies have found that the amounts of cytokines generated by keratinocytes and inflammatory leukocytes play a role in the initiation and maintenance of the inflammatory process. Changes in cytokine production, both locally and systemically, are thought to be important in monitoring disease activity. Interferon gamma (IFN-γ) is a major proinflammatory cytokine involved in a variety of biological processes [(5, 6, 7)].

The aim of this study was better clarification the possible participation of interferon gamma in the pathogenesis of vitiligo.

PATIENTS AND METHODS

This case-control study included a total of 28 patients with vitiligo, and 28 healthy controls, attending at Outpatient Clinic of Dermatology, Venereology, and Andrology Department, Zagazig University Hospitals. This study was carried out in the period between December 2021 and June 2022.

Participants were divided into 2 groups; Cases group: 28 patients with Vitiligo, 10 males and 18 females and **Control group:** 28 apparently healthy individuals, 15 males, and 13 females.

Inclusion criteria: Subjects of both sexes, all ages, patients with vitiligo, and control group should have no history of vitilgo, or other inflammatory skin disorders.

Exclusion criteria: Patients who are receiving systemic or topical therapy for vitiligo 6 weeks before their visit into our clinic, patients with renal, hepatic or cardiac disease, patients with diabetes mellitus, patients with collagen vascular disorders, and pregnant and lactating female.

Received: 03/04/2022
Accepted: 30/05/2022

All participants were subjected to:

1. **History taking.**
2. **General examination:**
3. **Dermatological examination:**
   a. Dermatological examination of controls was performed
   b. Dermatological examination of patients with vitiligo including wood's lamp as well as examination of scalp, nails, and mucosal surfaces.
   c. 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.

- 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.VASI = ∑ All Body Sites [Hand Units] × [Residual Depigmentation] [8].

4. **Laboratory investigations:**
   a. **Sample collection and measurement of Serum IFN-** serum was separated and kept in Eppendorf tubes at – 80°C till time of measurement. Then assessment of serum IFN-γ was done by enzyme-linked immunosorbent assay (ELISA) method.
   b. **Test principle:** The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of interferon gamma (IFN-γ) in samples.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Written informed consent of all the subjects was obtained. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The Statistical Package for the Social Sciences software (IBM Corporation, v. 20.0, Armonk, NY) was used for data analysis. Qualitative data were expressed as numbers and percentage and quantitative data were expressed as arithmetic mean ±Standard deviation (SD). The differences in Serum IFN-γ among control and vitiligo groups, between males and females, and among the degrees of severity were evaluated by one-way analysis of variance (ANOVA). When differences among them were found to be statistically significant ($P<0.05$), each group was compared with every other group using Tukey's post hoc test. Influence of variables on serum IFN- γ was analyzed using Pearson's correlation coefficient (r). Diagnostic performance of serum IFN- γ in determining vitiligo was assessed using receiver operating characteristic (ROC) curves. The optimal cut-off values for the prediction of the control group were chosen to maximize the sum of sensitivity and specificity. P value < 0.05 was considered significant.

RESULTS

Age varied significantly among the 2 groups. Vitiligo patients (24.32±14.01) tend to be significantly younger than Control group (23.29±7.61) (p=0.000) **(Table 1)**. While, the mean value of serum IFN-γ (pg./ml) in Vitiligo patients (309.74±231.03) was significantly higher than that in Control group (309.74±231.03), and that in control subjects (50.91±46.20) **(Table 2)**. There was also a significant difference between the diseases regarding the recorded duration of the disease and number of areas affected (p=0.003) and (p=0.048) respectively **(Table 1)**.

Table (1): Demographic and clinical data of participants:

	Vitiligo N=28	**Control N=28**	**P***
Age (years)	24.32±14.01	23.29±7.61	**0.000**
Gender	Male=10 (35.71%) Female=18(64.29%)	Male=15(53.57%) Female=13(46.43%)	
Duration (years)	4.46±2.76	NA	**0.003**
	VASI		
Severity	Mild=12(38.71%) Moderate=12(38.71%) Severe=6(19.35%)	NA	
Score	26.53±24.88	NA	
Areas	1-6 (3.18±1.36)	NA	**0.048**

Table (2): Comparison between serum IFN - γ in vitiligo group and control group:

	Vitiligo	**Control**	**P**
Serum IFN (ng/l)	309.74±231.03	50.91±6.20	0.001

Age showed a non-significant correlation with serum IFN- γ in two groups. However, Serum IFN- γ showed a significant strong positive correlation with the degree of severity in vitiligo groups (r=0.92; P=0.000) **(Table 3)**.

Table (3): Correlation of Serum IFN- γ with other variables in vitiligo, and control groups:

	Vitiligo		**Control**	
	r	**p**	**r**	**p**
Age	0.33	**0.085**	0.08	**0.676**
Severity	0.92	**0.001**		
Areas	0.78	**0.001**		
Duration	0.80	**0.001**		

Correlation between serum IFN and variables is analyzed using Pearson's correlation coefficients. P is significant if <0.05.

Serum IFN- γ also showed a significant positive very strong correlation with VASI score (r=0.98; P=0.000) **(Tables 4)**. While duration of the disease showed a significant correlation with serum IFN- γ only in vitiligo patients (r=0.80; P=0.000) **(Table 5).**

Table (4): Correlation between Serum IFN- γ and VASI score in vitiligo patients:

	Vitiligo	
	r	**p**
VASI score	0.98	**0.001**

Correlation between serum IFN- γ and variables is analyzed using Pearson's correlation coefficients. P is significant if <0.05

Table (5): Correlation between Serum IFN- γ and Duration of the disease in vitiligo patients:

	Vitiligo	
	r	**p**
Duration (years)	0.80	**0.001**

Correlation between serum IFN- γ and variables is analyzed using Pearson's correlation coefficients. P is significant if <0.05

IFN- γ was significantly higher in females than in males in vitiligo patients (p=0.012) **(Table 6).**

Table (6): Comparison of serum IFN-γ according to Gender in three groups:

	SERUM IFN-γ		p
	Male	Female	
Vitiligo	**230.38±119.04**	**452.60±313.19**	**0.012**
Control	**49.29± 58.30**	**52.78± 28.81**	**0.846**

There was a significant difference in serum IFN- γ among the three degrees of disease severity in vitiligo patients (p=<0.0001), to be the highest level in severe cases **(Table 7).**

Table (7): Comparison of serum IFN-γ according to disease severity in psoriatic and vitiligo groups:

	Mild	Moderate	Severe	P
Vitiligo serum IFN-γ)	**199.42± 73.45**	**374.23± 54.49**	**748.06±161.80**	**<0.0001**

One-Way ANOVA is used to analyze the difference between the groups.

Receiver operating characteristic curve analyses indicated that the area under the ROC curve was 0.98 (P=0.000, CI 0.94; 01.00). The cutoff value for predicting vitiligo was **101.176** pg./ml with a sensitivity of 100% and specificity of 96%. **(Table 8)**.

Table (8): ROC curve analysis of Serum IFN- γ for detecting the presence of vitiligo:

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.981	0.019	0.000	0.943	1.000
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

DISCUSSION

Vitiligo patients in this study were18 females (64.29%) and 10 males (35.71 %), which was 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 **(9).**

We found that there were statistically significant differences between vitiligo patients and normal healthy controls as regards the serum IFN- γ level (309.74±231.03ng/l versus 50.91± 46.20ng/l; p < 0.005), and this came in agreement with Ala *et al.* **(10)** 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).

Our study founded that serum IFN- γ level is positively correlated with the severity of Vitiligo disease VASI score (r 0.98 p0.000), and these results were similar to **Ezzedine *et al.*** **(11)** (r 0.63 p <0.001), and **Dwivedi *et al.*** **(12)** who observed increased serum level of IFN- γ with increase of the severity of the disease and reported a positive correlation between the concentration of this IFN- γ and the severity of the disease.

Concerning duration, we have found that serum IFN- γ level is positively correlated with the duration of the Disease r 0.80 p 0.000, and these results were similar to **Ala *et al.*** **(10)** r 0.5 p 0.001, and **Dwivedi *et al.*** **(12)** who observed increased serum level of IFN- γ with increase of the duration Of the disease and reported a positive correlation between the concentration of this IFN- γ and the duration of the disease**.**

Concerning gender, IFN- γ was significantly higher in females than in males in vitiligo patients female 452.60±313.19 versus male 230.38± 119.04 (p=0.012), and this not came in agreement With **Ala *et al.*** **(10)** who showed no statistically significant differences between male and female 14.7±5.6 versus 14.5±6.1 (p=0.9). This may be due to exposure to stress in females may affect IFN- γ Level and maybe due to increase autoimmune disease in female patients.

CONCLUSION

It could be concluded that the augmented IFN-γ serum level is found in patients with vitiligo, marking the systemic inflammatory nature of these diseases. The study proved that IFN-γ serum level significantly increases with Vitiligo diseases activity. On the other hand, IFN- γ serum level was around normal values in the control group. These mentioned findings were supported by the statistical evaluations on the relationship between serum IFN- γ level,

severity scores of Vitiligo (VASI scores) and clinical observations. Serum IFN- γ level was affected somehow by the duration and gender of the disease in Vitiligo group. Measurement of serum IFN- γ level could be used as both, diagnostic and prognostic marker for Vitiligo.

Conflict of interest: The authors declare no conflict of interest.

Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution: Authors contributed equally in the study.

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