Journal of Pakistan Association of Dermatologists.2023;**33(1)**:137-141.

Original Article

Immunoregulatory factors (IRF3 and IRF7) serum levels and its relation to disease activity in vitiligo patients

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Abstract

Background Occurrence of vitiligo was reported in patients treated with IFN (Interferons) for melanoma, hepatitis C, and hepatitis B. This suggests IFN and its immunoregulatory factors may play a role in the pathogenesis of vitiligo. However, the immunopathogenesis remains largely unknown.

Objective Explore the role of immunoregulatory factor 7 (IRF7) and immune-regulatory factor 3 (IRF3) in the serum of vitiligo patients.

Methods We studied (n=94) vitiligo patients and compared them with healthy control subjects. All included subjects were submitted to full history taking, dermatological examination, VASI score calculation. IRF7 and IRF3 levels were assessed by IRF7 and IRF3 Immunoassay ELISA kit.

Results Serum IRF-7 and IRF-3 are not elevated in vitiligo patients, and is associated with the least severity of the disease.

Conclusion Our data suggest that the serum levels of type 1 IFN (IRF7) and (IRF3) are not implicated in vitiligo pathogenesis. However, Age and duration of disease showed a negative correlation with IRF3 serum levels with statistically significant correlation.

Key words
Vitiligo, Interferon gamma, IRF7, IRF3.

Introduction

Vitiligo is an autoimmune de-pigmenting skin disease that afflicts 0.5-1 percent of the world's population.[1] Melanin synthesis occurs in specialized cellular organelles in epidermis called melanosomes.[2] Oxidative stress, immunologic factors, neurogenic disturbance, melanocyte degeneration contribute together to the pathogenesis of vitiligo.[3] CD8+cytotoxic T cells produces IFN-γ in skin lesions and blood of vitiligo patients which is responsible for the destruction of melanocytes by apoptosis.[4] Cytokines and their inflammatory cofactors play an important role in lymphocyte stimulation and skin localization. However, the mechanism of immune response is still under study.[5] Interferon gamma,[6] chemokine (C-C motif) ligand 22 (CCL22)[7] and tumor necrosis factor a (TNFα).[8]

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They are significantly suppressed in vitiligo patients' lesional skin and serum, and they control melanocyte propagation and/or distinctions.[9] IFN-γ is important in the incidence and development of auto - immune vitiligo.[10]

To avoid the transmission of viral infections, the innate immune system identifies pathogen-associated molecular patterns (PAMPs) and releases pro-inflammatory cytokines. PAMPs are double-stranded DNA (dsDNA) and double-stranded RNA (dsRNA) in viruses as well as some bacteria. DExD/ H-box usually contains RNA helicases, such as retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated protein 5, recognize dsRNA in dendritic cell cytoplasm (MDA5).[11]

Both RIG-I and MDA5 attach to the adaptor molecule IPS-1, stimulating apoptosis and the expression of pro-inflammatory cytokine genes or type I interferon genes via a TBK-1 and IRF3-dependent path.[12] TLR3 is a Toll-like receptor (TLR) dsRNA receptor. TLR3 on endosome membranes detects extracellular dsRNA endocytosed by cells.[13] TLR3 activates NFkβ which results in transcription of pro-inflammatory cytokines. TLR3 also activates TBK-1, resulting in the phosphorylation and nuclear translocation of IRF3 and IRF7, as well as the interpretation of IFNβ and TNF-α genes.[14]

In skin lesions of vitiligo patients IFN-γ and IFN-γ-induced genes are expressed.[15] Yang *et al*. correlated the production of IFN-γ in serum from patients with disease activity.[16] The relationship between vitiligo and IFN therapy is rarely reported in studies, and the relationship between its transcription factors IRF3, IRF7, and vitiligo remains unknown. The current research aims to better understand the immune-pathogenesis of vitilgo in order to better understand disease pathogenesis.

Methodology

This case control study carried on in the Dermatology outpatient clinic of Suez Canal University hospital. Ninety four patients with vitiligo were randomly enrolled in the study according to eligibility criteria. Patients with other dermatological disorders, receiving treatments for vitiligo or suffering from systemic disease as (Diabetes, hypertension, renal, hepatic or heart diseases were all excluded from the study. IRF7 and IRF3 Elisa kits (CUSABIO product) were used which employs the quantitative sandwich enzyme assay technique to determine serum IRF7 and IRF3 levels.

Results

94 subjects were enrolled in the study 47 patients with vitiligo assigned as cases and 47 normal subjects assigned as controls. Cases and controls were matched for age and sex. In **Table 1** the sociodemographic characteristics of study groups 29.8% of cases were older than or equal to 45 years old. Both males and females had the same number in cases and control groups. In **Table 2** the distribution of vitiligo patients according to disease characteristics, 42.6% of patients had duration of vitiligo between 1-5 years.

Table 1 Sociodemographic characteristics of study groups (n=94).

Characteristics	*Cases n=47* No. (%)	*Controls n=47* No. (%)	*p value**
Age (years)			
< 15	4 (8.5)	2 (4.3)	
15 - < 30	16 (34)	22 (46.8)	
30 - < 45	13 (27.7)	17 (36.2)	0.14
≥ 45	14 (29.8)	6 (12.8)	
Mean±SD	34.7±16.1	29.7±10.6	
Gender			
Female	26 (55.3)	26 (55.3)	0.08
Male	21 (44.7)	21 (44.7)	1.0

* Statistically significant at $p<0.05$.

Table 2 Distribution of vitiligo patients according to disease characteristics (n=47).

Effects of Pruritus		*Frequency*
Type of vitiligo	Segmental	7 (14.9%)
	Focal	18 (38.3%)
	Universal	7 (14.9%)
	Acral	3 (6.4%)
	Vulgaris	12 (25.5%)
Family history	Negative	38 (80.9%)
	Positive	9 (19.1%)
Course	Progressive	37 (78.7%)
	Stationary	8 (17.0%)
	Regressive	2 (4.3%)
Duration of vitiligo	< 1 year	8 (17.0%)
	1 - < 5 years	20 (42.6%)
	5 - < 10 years	10 (21.3%)
	10 - < 15 years	4 (8.5%)
	15- 20 years	5 (10.6%)
	Mean±SD (years)	6.36 ± 6.35

Table 3 Duration of disease and VASI score results among patients.

Characteristic	*Mean ± SD*	*Range*
Duration of disease in years	4.63±5.13	2 month-20 years
VASI score	7.76±18.26	0.25-86

Interferon regulatory factor 7 (IRF7) was measured in all participants and the VASI score ranged from 0.25-86 **(Table 2)**. IRF7 and IRF3 levels were compared in regard to age groups. The difference between cases and controls showed no statistically significant difference **(Table 4)**. Age and duration of disease showed negative correlation with IRF3 serum levels with statistically significant correlation. However, IRF3 levels showed no significant correlation between different vitiligo types **(Table 5)**.

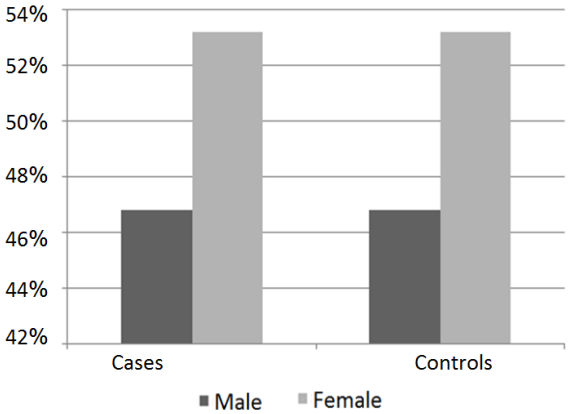

Figure 1 Gender distribution among study groups.

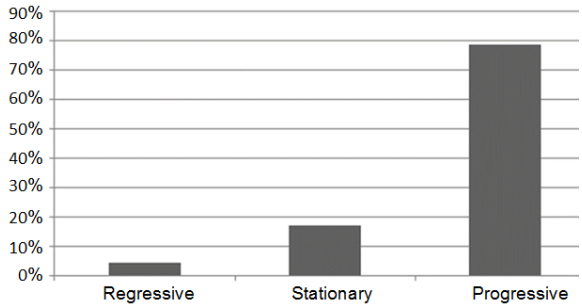

Figure 2 Distribution of patients according to course of disease.

Table 4 IRF3 and IRF7 levels according to gender.

		N	*Control group (n = 47)*	*Vitiligo group (n = 47)*	p-value*
			IRF3 level (ng/ml)		
Sex	Female	26	2.28±0.48	2.12±0.10	0.349
	Male	21	2.17±0.48	2.09±0.08	0.900
			IRF7 level (ng/ml)		
	Female		0.31±0.15	0.44±0.39	0.395
	Male		0.44±0.28	0.36±0.36	0.261

* Significant at p-value < 0.05. Mann-Whitney test was used.

Table 5 Correlation coefficients (r) of disease duration, course and VASI score with IRF7 and IRF3 levels.

Variable	*IRF7(ng/ml)*		*IRF3(ng/ml)*	
	Correlation coefficients (r#)	*p- value**	*Correlation coefficients (r#)*	*p- value**
Age	-0.174	0.241	-0.277	0.007
Duration of disease	-0.045	0.762	-0.316	0.031
Course of disease	-0.034	0.761	-0.018	0.902
VASI	-0.046	0.761	0.090	0.548

* Significant at p value < 0.05. Spearman correlation was used.

Discussion

Many questions still without answers regarding the pathogenesis of vitiligo and how genes interact to promote or prevent vitiligo. In mammals The (IRF) family contains nine members; IRF1, IRF2, IRF3, IRF4/PIP/ LSIRF/ICSAT, IRF5, IRF6, IRF7, IRF8/ICSBP, and IRF9/ISGF3γ.[17] This family of transcription factors function mainly in the immune system, they act in innate immune reaction as well as the formation of immune cells, IRFs were proved to be involved in biological processes, like oncogenesis[18] and metabolism.[19] In the current study IRF3 and IRF7 serum levels didn't have significant statistical relation between men and women, course of disease or VASI score and levels of both factors didn't differ between patients and controls. We suggested that their action have some redundancy with other family members of IRFs.

Basak *et al*. proved that IFN-c level was significantly increased in skin lesions of vitiligo and nearby unaffected skin, and increased when measured in the serum of vitiligo patients.[20] Assessment of cytokines, interferons, and IRFs in the involved skin and their circulating levels and comparing with healthy controls can help to understand the difference between our results and Basak *et al*. results.

Scientists investigated the effect of some IRF family members in the CNS of infected mice by lymphocytic choriomeningitis virus (LCMV). They discovered that neither IRF3 nor IRF7 were completely required for the induction of type I IFN responses in the LCMV-infected CNS, and that removing IRF3 and IRF7 significantly reduced the virus-induced host response. These findings demonstrated that the early type I IFN reaction to LCMV infection with in CNS was governed by a coordinated action of IRF3 and -7.[21]

Comparing of IRF3 and IRF7 in relation to course of vitiligo disease revealed no significant relation. In contrast to our findings, one Indian study discovered higher IFN- serum levels in active vitiligo especially in comparison to stable disease.[22]

Also, Scientists found that the IFN-γ gene expressed in vitiligo skin lesions.[16] Yang *et al*. stated that CD8+ T cells produced IFN-γ, and correlated IFN-γ production in peripheral blood from patients related to activity of the disease.[16]

Vitiligo run in families which revealed a genetic origin. Inheritance was related to several factors including the interaction between several genes and environmental causes. Around 25% to 50% of vitiligo patients had a genetic background.[1] This study included around 20% of the patients with the family history. As a result, there were no reports relating IRFs concentration in patients with vitiligo with a familial history of the disorder. However, large sample size was needed to support our observation.

The only significant finding of this research was a correlation between a rise in IRF3 levels and a decrease in the age and duration of vitiligo disease, indicating that there may be a concomitant increase in IRF3 in young and early diseased patients.

More understandings of the flexible functions IRF family were required. Indeed, in spite of the wide data made on the function of IRFs, questions still remain. What functions did IRFs play in the regulation of innate immunity, and how did the 2 transcription factor family groups, IRF3 and IRF7, interact with one another?

Moreover; large development in understanding the key immune signaling pathways involved in the pathogenesis of vitiligo disorder, specifically an IFN-γ-driven immune response that included

IRFs family, has aided to pursue new management lines for this autoimmune dermatologic disorder. Patients will benefit from novel targeted immunotherapies, which will support the start of larger controlled clinical trials. Nonetheless, more research is needed to gain a better understanding of the actual mechanism of viral infection-caused vitiligo.

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