Original Article

Vitiligo: Correlation with Cytokine Profiles and its Role in Novel Therapeutic Strategies: A Case–Control Study

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Abstract

Background: The inflammatory and immune factors play a key role in the pathogenesis of vitiligo, and there are very few studies that have investigated the levels of major cytokines produced by T helper (Th) 1, Th2, and Th17 cells. This can enable better understanding of the, pathogenesis, and severity of vitiligo. **Objectives:** To evaluate the serum levels of interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1β, interferon (IFN)-γ, and IL-10 in patients with vitiligo and to correlate them with the disease severity and activity and to compare them with normal healthy controls. **Materials and Methods:** A case–control study was conducted with 100 study participants: 50 cases clinically diagnosed as vitiligo and 50 controls. All patients underwent complete evaluation with detailed demographic parameters, history, and physical examination. The severity of the disease was assessed clinically by Vitiligo Area Scoring Index (VASI) and Vitiligo Disease Activity Score (VIDA). Blood investigations performed were IL-6, TNF-α, IL-1β, IFN-γ, and IL-10. **Results:** We observed significantly higher levels of serum IFN-γ levels in the patient group when compared with those of the normal controls ($P = 0.002$) and showed a positive correlation with the activity and severity of the disease with a significant VASI ($P = 0.05$) and VIDA score ($P = < 0.001$). The mean serum IL-10 ($p < 0.001$) in patients with vitiligo was significantly lower than that in the control group. There was no significant difference in the serum level of TNF-α level ($P = 0.347$), IL-6 ($P = 0.365$), and IL-1β ($P = 0.362$) between vitiligo and healthy controls. **Conclusion:** This study proved that high serum level of IFN-γ may be a risk factor for vitiligo progression and significantly low levels of IL-10, which has an anti-inflammatory role, suggesting that they could be used as a marker for assessing vitiligo activity and may open the way for further therapeutic approaches for vitiligo.

Keywords: *Interferon gamma, interleukin, vitiligo*

Introduction

Vitiligo is a primary acquired disorder characterized by the presence of well-circumscribed, milky white, or chalk-white macules on the skin and mucous membranes due to the result of loss of functioning melanocytes from the involved areas.[1] Vitiligo affects 1–4% of the world's population with an incidence of 0.5–2.5% in India.[2]

Recent data suggest that there is skewing of immune responses toward T helper (Th) 1 and Th17 cells, and this may be responsible for the development and progression of the disease.[3]

The pathogenesis of vitiligo includes both intrinsic defects within melanocytes that activate cellular stress response and the autoimmune mechanisms targeting the melanocytes involving both humoral and cell-mediated immunity.[2,4,5] Infiltration of cytotoxic T cells in perilesional lesions is the characteristic hallmark of vitiligo.[6] There is growing evidence that cytokines are important in the depigmentation process and show a cytokine imbalance in the skin of vitiligo patients suggesting their prominent role in autoimmune pathogenesis.[7,8] Systemic biological therapies used for treating psoriasis and other autoimmune diseases by targeting cytokines indicate that a similar approach might be effective for vitiligo.[4]

However, there are very few studies that have investigated the levels of major cytokines produced by Th1, Th2, and Th17 cells and correlated their levels

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with the activity, duration, and extent of the disease. The major cytokines produced by Th1 are interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Th2 cells produce interleukin (IL)-10, and Th17 cells produce IL-6, IL-1β, and TNF-α as well.

Since inflammatory and immune factors play a key role in the pathogenesis of vitiligo, we aimed to assess the relationship between serum levels of IL-6, TNF-α, IL-1β, IFN-γ, and IL-10. This can enable better understanding, pathogenesis, and severity of vitiligo.

Materials and Methods

Subjects and sampling

A retrospective case–control study was conducted in the department of dermatology, venereology, and leprosy at a tertiary care hospital from december 2020 to may 2022 after clearance from the institutional ethics committee (IEC/183/2020-2021). We calculated the sample size considering the number of vitiligo patients attending outpatient department (OPD) of dermatology, Rajarajeswari Medical College and Hospital, which was approximately 3 to 4 per week on an average.

According to the Yamane formula, where N = population size, e = margin of error (95% confidence level, margin of error = 0.05), a sample size of 100 with 50 cases clinically diagnosed as vitiligo (any type) and 50 controls were enrolled. Before enrollment, bilingual and well-informed consent was taken from every patient. Patient details (age, gender, family history of vitiligo, duration of disease, associated diseases, etc.) were recorded in a preset pro-forma designed for the study. Our inclusion considered patients between the age group of 18–60 years, patients with any type of non-segmental vitiligo, and those not on treatment for vitiligo in the past 3 months. Patients with segmental vitiligo, any active infections, and autoimmune diseases (autoimmune thyroiditis, pernicious anemia, diabetes mellitus, and Addison's disease) were excluded from the study. Clinical examination was carried out to determine the body surface area (BSA) involved and to assess the activity of the disease. Age and sex matched consenting healthy volunteers belonging to the same geographic region were enrolled as controls. The baseline venous blood samples (10 ml) were collected from patients and control groups under sterile conditions. Sera were separated and stored at 80°C until the time of cytokine estimation.

Analysis of TNF-α, IL-6, IL-1β, IFN-γ, and IL-10

Serum levels of TNF-α, IL-6, IL-1β, IFN-γ, and IL-10 were detected by enzyme-linked immunosorbent assay (ELISA). The calibration curves were plotted on semi-log papers, and values of the optical density of samples were calculated from the standard curve for the above mentioned five assays.

Principle of sandwich ELISA

- Analyte capture: The microplate provided in the kit is pre-coated with capture antibody (Ab). Any analyte in the standard or samples added to the well is captured. The unbound molecules are washed away.
- Horseradish peroxidase (HRP) detection antibody binding to captured analyte: A second antibody conjugated with HRP is added, which binds to the captured analyte, and the free detection antibody is washed away.
- HRP/substrate color display: HRP substrate tetramethylbenzidine (TMB) is added to the wells, and the blue color develops proportionally to the amount of analyte in the sample. The blue color development is stopped, and yellow color develops when stop solution is added. OD450 is measured to make a standard curve and calculate analyte concentration.

Statistical analysis

Continuous variables were presented as mean and standard deviation (SD). The results were analyzed statistically using a paired Student's t-test, Chi-square test, Mann–Whitney U-tests, and Spearman's correlation method ($P \leq 0.05$ was assumed significant in statistical tests). All calculations were performed using Statistical Package for the Social Sciences (SPSS) version 20 (SPSS Inc., Chicago).

Results

Demographic characteristics

A total of 50 cases of vitiligo and 50 control patients with vitiligo were interviewed during the study period. Age and gender were comparable between cases and controls in our study. The male-to-female ratio was 0.8:1. Among the 100 study subjects, 44% were males and 56% were females [Table 1]. The mean age of study subjects in our study is 41.1 years with a SD of 10.9. No statistical difference was observed between vitiligo patients and control subjects for gender and age ($P > 0.05$).

The majority of our vitiligo cases had a duration of vitiligo between 1 and 3 years (56%) followed by those diagnosed recently, that is, less than 1 year (28%). The majority of

Table 1: Comparison of demographic characteristics among cases and controls

Variables	Cases	Controls	*P*
Gender			
Male	22 (44%)	22 (44%)	1
Female	28 (56%)	28 (56%)	
Mean age	41.4±10.5 years	40.8±11.3 years	0.784
Age			
<30 years	12 (24%)	15 (30%)	0.798
31−50 years	29 (58%)	26 (52%)	
>50 years	9 (18%)	9 (18%)	

our vitiligo cases were progressive in nature (72%). We had 28% of vitiligo cases, which were stable [Table 2].

Serum levels of cytokines in patients and controls

The mean serum IFN-γ levels in the patient group were significantly higher in cases with vitiligo when compared with those of the normal controls (P = 0.002) [Table 3]. The mean serum IL-10 (P < 0.001) in patients with vitiligo was significantly lower than that in the control group. There was no significant difference in the serum level of TNF-α level (P = 0.347), IL-6 (P = 0.365), and IL-1β (P = 0.689) between vitiligo and healthy controls [Figure 1].

Assessment of serum levels of cytokines in relation to disease characteristics

The Vitiligo Area Scoring Index (VASI) score was ≤15 in 44% of patients and >15 in 56% of patients. According to these results, there were significantly higher values of IFN-γ in VASI >15 than in VASI <15 group (p < 0.05) [Table 4]. There was a significant correlation between the cytokine levels of IFN-γ and IL-10 with their VASI score [Table 5].

Table 6 data show correlations between Vitiligo Disease Activity Score (VIDA) score and cytokine serum levels among cases of vitiligo. There were statistically significant correlations between VIDA and IFN-γ, IL-10, IL-6, and IL-1β cytokine serum levels except for TNF-α.

Discussion

Vitiligo is a multifactorial polygenic disorder with complex pathogenesis.[9] The etiology is still unknown, but the loss of melanocytes has been so far explained as either caused by an autoimmune mechanism or by an autocytotoxic/metabolic mechanism or by a neural dysfunction.[10] Close relationships important for melanocyte survival and differentiation likely exist between these two cell types, which are mainly because of keratinocyte-derived cytokines acting on melanocytes via specific receptors.[11,12]

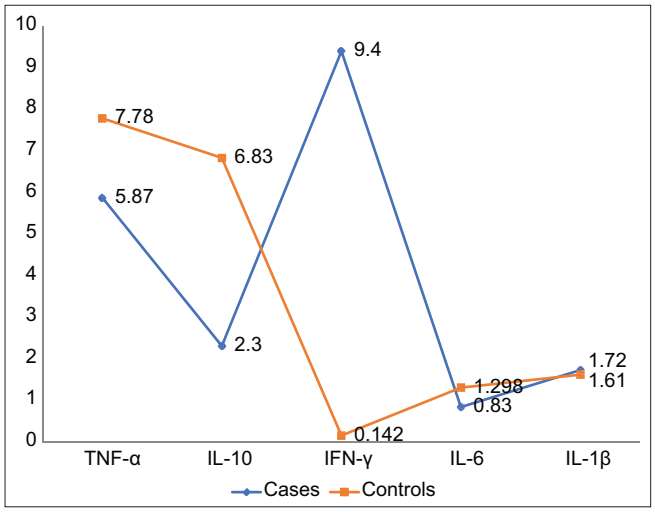

Figure 1: Line graph showing mean cytokine in vitiligo patients and controls

Therefore, it is possible to hypothesize that an impairment in keratinocyte secretory activity may be involved in melanocyte disappearance in vitiligo; hence, the study of cytokines can help us understand the pathogenesis. In our study, the majority of the vitiligo cases (72%) were progressing, that is, lesions increasing in size and

Table 2: Distribution of duration and stability of vitiligo in cases

	Frequency (%)
Duration	
<1 year	14 (28%)
1 to 3 years	28 (56%)
3 to 5 years	6 (12%)
>5 years	2 (4%)
Stability of vitiligo	
Stable	14 (28%)
Progressive	36 (72%)

Table 3: Serum levels of cytokines in vitiligo patients and controls

Cytokines (mean±SD)	Cases (n=50)	Controls (n=50)	P
TNF-α	5.87±1.64	7.78±14.21	0.347
IL-10	2.3±1.89	6.83±0.31	<0.001
IFN-γ	9.4±5.38	0.142±0.733	0.002
IL-6	0.83±1.86	1.298±0.01	0.365
IL-1β	1.72±0.24	1.61±0.89	0.689

Table 4: Serum levels of cytokines and VASI scores in vitiligo cases and controls

Cytokines (mean±SD)	Cases		P
	VASI >15 (n=28)	VASI <15 (n=22)	
TNF-α	0.31±1.62	0.41±1.32	0.816
IL-10	0.16±0.031	0.15±0.048	0.3769
IFN-γ	11.49±3.02	1.38±0.66	<0.001
IL-6	3.92±1.35	2.21±0.27	0.121
IL-1β	1.54±0.89	0.561±0.91	0.384

Table 5: Correlation between VASI and cytokines levels evaluated by the Spearman rank correlation

Correlations	Statistics	TNF-α	IL-10	IFN-γ	IL-6
VASI score	Spearman's Rho	-0.054	-0.425	0.659	0.596
	P	0.708	0.015	0.05	0.091

Table 6: Serum levels of cytokines and VIDA scores in vitiligo cases and controls

Cytokines (mean±SD)	VIDA			P
	0 (n=23)	+1 (n=16)	+2 (n=11)	
TNF-α	0.12±0.4	0.26±0.92	0.38±1.1	0.64
IL-10	0.2±0.1	0.08±0.03	0.11±0.048	<0.001
IFN-γ	1.1±0.67	1.31±0.66	9.49±2.98	<0.001
IL-6	1.3±0.32	1.89±0.27	3.91±1.31	<0.001
IL-1β	0.37±0.18	0.561±0.91	1.51±0.83	<0.001

number in the past year. The mean serum IFN-γ levels were significantly higher in cases with vitiligo when compared with those of the normal controls, and on further evaluation, there was a significant ($P = < 0.01$) positive correlation between the severity of the disease and the BSA involvement (VASI >15). Supporting our observation, Th1 predominance has been reported in association with vitiligo in previous studies. Ala *et al*.[13] found a significant elevation in vitiligo patients compared with healthy controls (12.4 ± 3.2 pg/mL versus 9.9 ± 4.4 pg/mL; $p < 0.05$). Dwivedi *et al*.[14] observed increased serum level of IFN-γ with an increase in the duration and severity of the disease and reported a positive correlation between the concentration of this IFN-γ and the duration and severity of the disease. Rashigi *et al*.[4] reported that both vitiligo patients and mouse model of vitiligo reflect a uniquely IFN-γ-specific Th1 cytokine signature in the skin that includes IFN-γ-dependent chemokines such as chemokine C-X-C motif ligand (CXCL) 9, CXCL10, and CXCL11, which induce T-cell homing into peripheral tissues. IFN-γ indirectly increases the expression of intercellular adhesion molecule-1 (ICAM-1) on melanocytes and enhances T-cell melanocyte attachment in the skin and thus establishes a link between cytokine and T-cell-mediated destruction of melanocytes in vitiligo.[15,16]

Decreased serum concentrations of the anti-inflammatory cytokine IL-10 were observed in our patients compared with controls suggesting a low Th2 cytokine profile in the pathogenesis of vitiligo. Shi and Erf suggested that the physiological inducer of T regulatory cell (Treg) function and proliferation, IL-10 cytokine, was found to be decreased in active vitiligo lesions.[17] Teher *et al*. and Tembhreet *et al*. reported increased levels of this immunosuppressive cytokine in vitiligo patients who showed the repigmentation process upon treatment with tacrolimus and narrowband ultraviolet B (NB-UVB), respectively.[18,19] This indicates that the upregulation of IL-10 may be responsible for the drug response, which indirectly supports the decreased levels of IL-10 in vitiligo pathogenesis observed in our study. The results vary widely, with some studies finding higher concentrations of IL-10 or hypermethylation of IL-10 loci, and others showing a reduction in IL-10 or inverse correlation between hypermethylation of IL-10 loci and its levels in the blood. These contradictory results make it clear that future studies are needed to investigate the role of IL-10 in the pathogenesis of vitiligo.

In our study, we reported no significant difference in TNF-α between cases and controls. Studies such as Mitra *et al*.,[20] Singh *et al*.,[21] Yu *et al*.,[22] and Sushama *et al*.[3] have reported an increase in TNF-α levels in patients with vitiligo compared with the control group; also, in our study, we reported no significant difference in IL-6 between cases and controls. Sushma *et al*.[3] and Yang *et al*.[23] have reported an increase in serum levels of IL-6 in patients with vitiligo. Moreover, another study by Moretti *et al*.[8] has reported an increase in the expression of both IL-6 and TNF-α in keratinocytes. Serum levels of pro-inflammatory cytokines (TNF-α and IL-6) in patients with generalized vitiligo with new lesions emerging in the previous three months were significantly higher than in the control group. These pro-inflammatory cytokines may have other effects apart from disease activity. Although this is controversial, testosterone is thought to reduce pro-inflammatory cytokines and increase anti-inflammatory cytokines.[24] The decrease in TNF-α production by mononuclear cells in active vitiligo may partially explain the reduced inflammatory reaction and cell infiltration in lesions.[22]

In our study, we reported no significant difference in IL-1β between cases and controls. For the first time, Laddha *et al*.[25] reported an association between IL-1β promoter polymorphism and vitiligo along with higher transcript levels of IL-1β in vitiligo patients as compared to controls. Neuropeptide Y (NPY) synthesis and release are also governed by IL-1β. Taken together, altered IL-1β transcript levels due to genetic variability in IL-1β might be associated with elevated NPY levels in patients with vitiligo. A study by Bharadwaj *et al*.[26] showed increased expression of IL-1β in active non-segmental vitiligo. However, no significant increase in stable vitiligo is found. The possible role of IL-1β in the pathogenesis of vitiligo remains a problem to be further studied.

Newer therapies have been developed to target key mediators in the vitiligo inflammatory cascade. Our study has shown that IFN-γ levels were increased in patients with vitiligo and suppressors of cytokine signaling (SOCS1) have been shown to inhibit IFN-γ signaling, presumably by preventing Janus kinase (JAK) activation.[27] These data warrant further research studies for newer therapeutics in the treatment of vitiligo. As the scope of understanding of vitiligo pathogenesis improves, various modes achieving each of these targets combined together would give the best results.

Conclusion

Patients with vitiligo have unbalanced amounts of various cytokines. In the autoimmune process that leads to vitiligo, IFN-γ expression is important. This study shows that a high serum level of IFN-γ may be a risk factor for the advancement of vitiligo. IL-10 has an anti-inflammatory role, and these levels were significantly decreased in our study. These findings can give us a clue to measure the disease's activity and pave the way for other therapeutic strategies in the treatment of vitiligo. Newer strategies, such as combination of IFN-γ blockade with inhibition of other signaling pathways, may further improve IFN-γ targeted immunotherapy of human diseases.

Limitations

Though our study involved a large number of cases and controls pertaining to a certain demographic area, the

inclusion of different strata across various demographics can give us a better understanding of the cytokine profile of the disease.

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Conflicts of interest

There are no conflicts of interest.

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