



High Level of Tumor Necrosis Alpha and Serum Interferon Gamma as Risk Factors for Progression of Vitiligo Disease



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Abstract



Keywords

IFN- γ ;
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Vitiligo Progressivity;

Vitiligo is an autoimmune disease that causes melanocyte of dysfunction. Cytokines played an important role in the pathogenesis of vitiligo. *Interferon-gamma* and TNF- α were cytokines that induce apoptosis of melanocyte cell. The increase of cytokine levels affects the clinical course of vitiligo. The stable and progressive phase of vitiligo clinically is not easy to predict. Assessment of vitiligo stability could be used to determine treatment options, duration of therapy and prognosis. This study was a cross-sectional observational study which intended to prove high levels of TNF- α and IFN- γ serum is a risk factor for vitiligo progression. The demographic, clinical, and laboratory data in active vitiligo subjects (n=30) were compared with stable vitiligo subjects (n=40). The relationship was analyzed with multivariate. Median of the subject age with active vitiligo was 44 years (8~60) and on the subject with stable vitiligo was 45 years (15~66). The most subjects were male (58.5%) and the most common type of vitiligo was non-segmental vitiligo (87.1%). Multivariate analysis showed a high level of TNF- α serum increased the risk of vitiligo progressivity (Adjusted PR 390.89; CI 95 % 27.98-5460.12 ; p<0,001) and high level of IFN- γ serum increased the risk of vitiligo progressivity (Adjusted PR 341.06; CI 95% 33.40-3482.26 ; p<0,001). The high level of TNF- α and IFN- γ serum as a risk factor for progression of vitiligo could be used to assess the activity of vitiligo disease. The further research about the association between TNF- α and IFN- γ to predict the therapeutic response in vitiligo.

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1. Introduction

Vitiligo is a skin depigmentation disorder of the skin, hair, and mucosa. Which is characterized by functional melanocyte defect in the epidermis. Pathogenesis of vitiligo is a complex pathological mechanism involving cellular and humoral immunity, induced by the cytokine expression imbalance in the skin, serum, and blood in vitiligo patients [1], [2]. *Tumor necrosis alpha* is a multifunctional proinflammatory cytokine that plays an important role in apoptosis through the activation of *receptor-mediated apoptosis* in various cells. Keratinocytes interact closely with melanocytes, secrete $TNF-\alpha$ which acts as an inhibitor in melanocyte proliferation and melanogenesis. It causes the initiation of melanocyte apoptosis and inhibits melanocyte *stem cell* differentiation [3]. Other pro-inflammatory cytokines that are involved in the pathogenesis of vitiligo are $IFN-\gamma$ produced by Th1 lymphocytes, NK cells, and CD8+ cytotoxic T cells. Interferon-gamma affects melanin production, melanosomes maturation induces the formation of anti-melanocyte antibodies and activation of cytotoxic T cells which further leads to melanocyte apoptosis [4], [5].

The clinical course of vitiligo is difficult to predict as it involves stable phase and active phase or spontaneously regression. The previous studies have shown that in stable vitiligo, there are low levels of pro-inflammatory cytokines and low numbers of cytotoxic T cells [6]. Progressive/active vitiligo can be assessed by the presence of new lesions, the proliferation of old lesions, grafting tests and Koebner phenomena, VASI and VIDA scores. These parameters are subjective and semi- subjective [7], [8]. The higher level of $TNF-\alpha$ and $IFN-\gamma$ is more objective in predicting the progression of vitiligo. In vitiligo, the treatment choices, treatment duration, and prognosis could be determined by assessing the course of vitiligo disease [9].

2. Research Method

The study used a cross-sectional observational design. The research subject is vitiligo patients who visited the Dermatology and Venereology polyclinics at Sanglah Hospital, Denpasar Bali. The number of research samples was 70 vitiligo patient. They were chosen by *consecutive sampling* based on inclusion and exclusion criteria. The VASI score was used as vitiligo severity assessment. Vitiligo activity degree was assessed using VIDA score. Demographic and clinical data were obtained using a questionnaire. The serum level of $TNF-\alpha$ and $IFN-\gamma$ were assessed using ELISA technique by drawing venous blood. The samples were divided into two groups, active vitiligo, and stable vitiligo. Active vitiligo defined by new lesions or expanding of old lesions within the last one year with the VIDA score ≥ 1 . Stable vitiligo defined as a nonexpanding old lesion and/or no new lesion within the last one year with VIDA score of 0.

The data were analyzed using IBM SPSS Statistics ver. 20. Descriptive analysis was used to describe characteristics of subjects with vitiligo, such as age, gender, and clinical manifestation. Data normality was assessed using Shapiro Wilk test. Bivariate analysis using chi-square were applied to observe associations between qualitative variables. Multivariate analysis using logistic regression was performed to observe adjusted prevalence ratios (PR) with 95% confidence intervals. A p-value of $<0,05$ was considered statistically significant.

3. Results and Analysis

Research results

The data of the research subject characteristics that include gender, age, type of vitiligo, and *leukotrichia* is presented in Table 1.

Table 1
Characteristics of research subjects

No	Characteristics	Active vitiligo (n = 40)	Stable vitiligo (n = 30)
1	Gender		
	Male	24 (60)	17 (56.7)
	Female	16 (40)	13 (43.3)
2	Age (years)	44 (8~60)	45 (15~66)
3	Vitiligo pattern		
	Segmental	7 (17.5)	2 (6.6)
	Non-segmental	33 (82.5)	28 (93.3)
4	<i>Leukotrichia</i>	6 (15)	2 (6.7)
5	VASI score	4.1 ± 3.3	1.5 ± 0.9
6	IFN- γ level	7.16 ± 11.52	0.85 ± 0.38
7	TNF- α level	2.07 ± 1.03	1.06 ± 0.20

*The value is presented in median form (range), n = number (%), mean ± standard deviation

The total samples were 70 cases of vitiligo, the median age of active vitiligo was 44 (8~60) and stable vitiligo was 45 (15~66). The most subjects were 41 (58.5%) males compared to 29 (41.5%) females. The most common type of vitiligo was non-segmental 61 (87.1%) followed by segmental 9 (12.9%). *Leukotrichia* was more prevalent in active vitiligo than stable vitiligo. The mean of VASI score was higher in active vitiligo (4.1 ± 3.3) compared to stable vitiligo (1.5 ± 0.9). Mean serum levels of IFN- γ in active vitiligo was higher (7.16 ± 11.52) than stable vitiligo (0.85 ± 0.38). Mean serum levels of TNF- α was higher (2.07 ± 1.03) than stable vitiligo (1.06 ± 0.20).

Table 2
Multivariate correlation between TNF- α and IFN- γ levels with vitiligo progressivity towards age and gender

Parameter	Adjusted PR	Confidence interval	p-value
TNF- α	390.89	27.98-5460.12	0,001
IFN- γ	341.06	33.04-3482.26	0.001

95% confidence interval, PR: *Prevalence Ratio*, p-value <0.05 significant.

The adjusted PR value towards serum levels of TNF- α was 390.89 (CI 95% 27.98-5460.12; p <0,001). It defined that high serum levels of TNF- α significantly increase the risk of vitiligo progression. The adjusted PR value towards serum levels of IFN- γ was 341.06 (CI 95% 33.40-3482.26; p <0,001). It defined that high serum levels of IFN- γ significantly increase the risk of vitiligo progression.

Analysis

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 [10]. 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. [1], [2]. The result showed that high serum levels of TNF- α and IFN- γ as a risk factor for vitiligo progression.

Leukotrichia is usually found on the surface of the depigmented skin or in vitiligo patches due to loss of melanocytes in the hair bulb after the destruction of the epidermal melanocytes. Due to the immunologic process attested to the discovery of lymphocyte infiltration in dermis or perifollicular.

This phenomenon is found during the active vitiligo phase [11]. In this study, *leukotrichia* was found to be in higher proportion in active vitiligo.

Inactive vitiligo, there is a change in the number and function of T cells, that is increased number of CD 8 + T-cell and decreased of T reg cells that correlate with the vitiligo severity [11]. In vitiligo lesions, it is found that Th1 is the predominant cells that release various cytokines, one of them is TNF- α . It is proven by a research conducted by Laddha et al, were reported serum levels of TNF- α increased in vitiligo patients compared with healthy control [3].

Tumor necrosis factor- α plays a role in the immunopathogenesis of vitiligo. This cytokine induces mortality and melanocyte cell dysfunction through various mechanisms. An infiltration and induction of Th1 cell response will increase TNF- α level resulting in increased expression of *nuclear factor kappa-B* (NF- κ B). It plays a major role in regulating cell survival and *repair genes*. An increased NF- κ B expression will cause apoptosis of melanocyte cells. The high TNF- α level is also caused expression of adhesion molecules such as *intercellular adhesion molecule-1* (ICAM-1) in melanocytes that will be marked by T cells as a target to be destroyed through immunologic cytotoxic processes [12].

TNF- α cytokine inhibits melanogenesis through decreasing level and activity of tyrosinase enzyme and *tyrosinase-related protein-1*, decreasing MITF expression which is a transcription factor that plays a role in proliferation, survival, the death of melanocyte and regulates the melanogenesis process. TNF- α also inhibits the melanocytes proliferation by increasing CXCL-chemokine receptor II expression. It has also been shown that high levels of TNF- α decreases *pigment-associated antigen* HMB-45 and K.1.2 expression in melanocyte culture. Another mechanism that causes melanogenesis changes in vitiligo is TNF- α decreases α -*Melanocyte-Stimulating Hormone* (α -MSH) activity and *Melanocortin-1 Receptor* (MCI-R) expression that plays a role in the pigmentation and melanocyte survival [12]. Redox hemostasis changes in vitiligo pathogenesis were reported by Glassman. High TNF- α level induces an excessive production of ROS (NO, H₂O₂) which leads to an increase in melanocyte apoptosis in vitiligo [12], [13], [20].

IFN- γ is a pleiotropic cytokine that plays a role to regulate the immune system produced by *T helper cells*, *natural cell killer*, and keratinocytes. It plays an important role to induce depigmentation in vitiligo. The cytokines cause the formation of anti-melanocyte antibodies or activate cytotoxic T cells. Yang et al reported many CD8+ cytotoxic T lymphocyte cell infiltration and elevation of IFN- γ levels in progressive vitiligo [14]. The activation of CD8+ cytotoxic T lymphocyte is a source of IFN- γ levels elevations.

An *invitro* research (*primary culture human melanocyte*) showed IFN- γ inhibited melanogenesis by changing the expression of mRNA of the melanogenic enzyme and the more important was IFN- γ induced direct apoptosis of melanocytes [14]. Son et al reported that IFN- γ is also inhibited α -MSH hormone by decreasing MITF expression [15], [21].

IFN- γ signal binds with IFN- γ receptor via JAK 1 and JAK 2, leading to phosphorylation initiation of transducer signal, STAT 1+ transcription activation and translocation to the further nucleus induced IFN- γ gene transcription, including gene chemokine ligand 9 (CXCL9) and chemokine ligand 10 (CXCL10). Those chemokines (CXCL9 and CXCL10) recruits an autoreactive CD 8 + T cells to the skin cause melanocyte apoptosis. CXCL 10 is directly contributes to T cells migration [15], [16], [17]. CXCL10 receptor in CD 8+ autoreactive T cells, CXCR3 is highly expressed on the skin of vitiligo lesions. Based on those study findings, current vitiligo therapy research focuses on IFN- γ -CXCL10-CXCR3 axis pathway, a potential therapeutic target in vitiligo [16], [18].

Interferon-gamma indirectly increases ICAM-1 expression in melanocytes and increases melanocyte attachment with T cells in the skin, resulting in melanocyte defective. ICAM-1 expression also induces B cells activity that increases the autoantibodies production leading to melanocyte cell defective [19], [22].

4. 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 cytokines of TNF- α and IFN- γ expression play a role in the autoimmune process of vitiligo. The expression of cytokines TNF- α and IFN- γ are associated with melanocyte destruction in the active phase of vitiligo lesions. This study proved high serum levels of TNF- α and IFN- γ were the risk factors for vitiligo progression. Further studies about TNF- α levels and IFN- γ levels before and after treatment in vitiligo patients with large samples are

needed to confirm this study results. More studies about the role of TNF- α and IFN- γ inhibitor for vitiligo patient are needed as the treatment option in vitiligo.

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