International Journal of Health Sciences
Available online at http://sciencescholar.us/journal/index.php/ijhs
Vol. 2 No. 2, August 2018, pages: 1~8
e-ISSN: 2550-696X, p-ISSN: 2550-6978
http://dx.doi.org/10.29332/ijhs.v2n2.109

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

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Article history: Received 9 August 2017, Accepted in revised form 1 March 2018, Approved 17 April 2018, Available online 23 April 2018

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Keywords

IFN-γ;
TNF-α;
VASI;
VIDA;
Vitiligo Progressivity;

Abstract

Vitiligo is an autoimmune disease that causes melanocyte of dysfunction. Cytokines played an important role in the pathogenesis of vitiligo. *Interferon-gamma* and TNF-∝ 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-∝ 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-∝ 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-∝ and IFN-γ to predict the therapeutic response in vitiligo.

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Contents

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-∝ 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-γproduced 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-∝ and IFN-γ 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-α and IFN-γ 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	*Leukotrichia*	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 6 1 (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-∝ was higher (2.07 ± 1.03) than stable vitiligo (1.06 ± 0.20).

Table 2
Multivariate correlation between TNF-∝ 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-κB). It plays a major role in regulating cell survival and *repair genes*. An increased NF-κB 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 t*yrosinase-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 CXC-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-αlevel induces an excessive production of ROS (NO, H_2O_2) 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 bounds with IFN-γ receptor via JAK I and JAK 2, leading to phosphorylation initiation of transducer signal, STAT 1+ transcription activation and translocation to the further nucleus induced IFN-γgene transcription, including gene chemokine ligan 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.

Funding: This research was financially supported by Unit Penelitian dan Pengembangan (LITBANG), Faculty of Medicine, Udayana University

Conflict of interest: The authors have nothing to disclose

Acknowledgments
The authors thank the staff Department Dermatology and Venereology of Sanglah Hospital for supporting and participating in this project. We would also thank to I Wayan Suryasa (Scopus ID: 57200211897) as an advisor and editor of IJCU, IJMRA, SKIREC, Euro Asia as well as ScienceScholar who has reviewed and approved this study to be published.

References

1. Aydıngöz, I. E., Kanmaz-Özer, M., Gedikbaşi, A., Vural, P., Doğru-Abbasoğlu, S., & Uysal, M. (2015). The combination of tumour necrosis factor-α− 308A and interleukin-10− 1082G gene polymorphisms and increased serum levels of related cytokines: susceptibility to vitiligo. *Clinical and experimental dermatology*, *40*(1), 71-77.
View in (Google Scholar)

2. Lotti, T., Hercogova, J., & Fabrizi, G. (2015). Advances in the treatment options for vitiligo: activated low-dose cytokines-based therapy. *Expert opinion on pharmacotherapy*, *16*(16), 2485-2496.
View in (Google Scholar)

3. Dwivedi, M., Laddha, N. C., & Begum, R. (2013). Correlation of increased MYG1 expression and its promoter polymorphism with disease progression and higher susceptibility in vitiligo patients. *Journal of dermatological science*, *71*(3), 195-202.
View in (Google Scholar)

4. Jadali, Z. (2015). IFN-γ Promotes Apoptosis of Melanocytes in Vitiligo. *Journal of Skin and Stem Cell*, *2*(4).
View in (Google Scholar)

5. Dwivedi, M., Laddha, N. C., Shah, K., Shah, B. J., & Begum, R. (2013). Involvement of interferon-gamma genetic variants and intercellular adhesion molecule-1 in onset and progression of generalized vitiligo. *Journal of Interferon & Cytokine Research*, *33*(11), 646-659.
View in (Google Scholar)

6. Abreu, A. C. G., Duarte, G. G., Miranda, J. Y. P., Ramos, D. G., Ramos, C. G., & Ramos, M. G. (2015). Immunological parameters associated with vitiligo treatments: a literature review based on clinical studies. *Autoimmune Diseases*, *2015*.
View in (Google Scholar)

7. Sahni, K., & Parsad, D. (2013). Stability in vitiligo: is there a perfect way to predict it?. *Journal of cutaneous and aesthetic surgery*, *6*(2), 75.
View in (Google Scholar)

8. Alghamdi, K. M., Kumar, A., Taïeb, A., & Ezzedine, K. (2012). Assessment methods for the evaluation of vitiligo. *Journal of the European Academy of Dermatology and Venereology*, *26*(12), 1463-1471.
View in (Google Scholar)

9. Ezzedine, K., & Silverberg, N. (2016). A practical approach to the diagnosis and treatment of vitiligo in children. *Pediatrics*, *138*(1), e20154126.
View in (Google Scholar)

10. Lee, H., Lee, M. H., Lee, D. Y., Kang, H. Y., Kim, K. H., Choi, G. S., ... & Lee, A. Y. (2015). Prevalence of vitiligo and associated comorbidities in Korea. *Yonsei medical journal*, *56*(3), 719-725.
View in (Google Scholar)

11. Webb, K. C., Tung, R., Winterfield, L. S., Gottlieb, A. B., Eby, J. M., Henning, S. W., & Le Poole, I. C. (2015). Tumour necrosis factor-α inhibition can stabilize disease in progressive vitiligo. *British Journal of Dermatology*, *173*(3), 641-650.
View in (Google Scholar)

12. Glassman, S. J. (2011). Vitiligo, reactive oxygen species and T-cells. *Clinical Science*, *120*(3), 99-120.
View in (Google Scholar)

13. Lopes, C., Trevisani, V. F. M., & Melnik, T. (2016). Efficacy and safety of 308-nm monochromatic excimer lamp versus other phototherapy devices for vitiligo: a systematic review with meta-analysis. *American journal of clinical dermatology*, *17*(1), 23-32.
View in (Google Scholar)

14. Yang, L., Wei, Y., Sun, Y., Shi, W., Yang, J., Zhu, L., & Li, M. (2015). Interferon-gamma inhibits melanogenesis and induces apoptosis in melanocytes: a pivotal role of CD8+ cytotoxic T lymphocytes in vitiligo. *Acta dermato-venereologica*, *95*(6), 664-671.
View in (Google Scholar)

15. Son, J., Kim, M., Jou, I., Park, K. C., & Kang, H. Y. (2014). IFN-γ inhibits basal and α-MSH-induced melanogenesis. *Pigment cell & melanoma research*, *27*(2), 201-208.
View in (Google Scholar)

16. Frisoli, M. L., & Harris, J. E. (2017). Vitiligo: Mechanistic insights lead to novel treatments. *Journal of Allergy and Clinical Immunology*, *140*(3), 654-662.
View in (Google Scholar)

17. Rashighi, M., & Harris, J. E. (2015). Interfering with the IFN-γ/CXCL10 pathway to develop new targeted treatments for vitiligo. *Annals of translational medicine*, *3*(21).
View in (Google Scholar)

18. Liu, L. Y., Strassner, J. P., Refat, M. A., Harris, J. E., & King, B. A. (2017). Repigmentation in vitiligo using the Janus kinase inhibitor tofacitinib may require concomitant light exposure. *Journal of the American Academy of Dermatology*, *77*(4), 675-682.
View in (Google Scholar)

19. Ala, Y., Pasha, M. K., Rao, R. N., Komaravalli, P. L., & Jahan, P. (2015). Association of IFN-γ: IL-10 cytokine ratio with Nonsegmental Vitiligo pathogenesis. *Autoimmune Diseases*, *2015*.
View in (Google Scholar)

20. Malaiya, S., Shrivastava, A., Prasad, G., & Jain, P. (2017). Impact of Medical Education Trend in Community Development. *International Journal of Health Sciences (IJHS)*, *1*(1), 23-27.
View in (Google Scholar)

21. Agarwal, R., Jain, P., Ghosh, M. S., & Parihar, K. S. (2017). Importance of Primary Health Care in the Society. *International Journal of Health Sciences (IJHS)*, *1*(1), 6-11.
View in (Google Scholar)

22. Wirawan, I. G. B. (2018). Surya Namaskara Benefits for Physical Health. *International Journal of Social Sciences and Humanities (IJSSH)*, *2*(1), 43-55.
View in (Google Scholar)

Biography of Author

IG A A Praharsini has been a General practice doctor in 1983-1990 at the Faculty of Medicine, Udayana University, Bali-Indonesia. She is interested in Dermatology-Venereologist in 1997-2001 at Faculty of Medicine, Diponegoro University, Central of Java-Indonesia. She was a Doctor in 2014 at the Faculty of Medicine, Udayana University, Bali-Indonesia. Her previous publication included **(1)** Expression of Stem Cell Factor and Tyrosinase Positively Correlate with Expression of Ki-67 in Seborrheic Keratosis (Indonesia Journal of Biomedical Science. 2015:9 (1). **(2)** Correlation between Dermatology Quality of Life and Melasma Area Score Index (Intisari Sains Medis. 2017: 8 (3). **(3)** The mechanism of Repigmentation and the Role of Melanocyte Stem Cell Hair Follicle in Vitiligo (Intisari Sains Medis .2018: 9(1). **(4)** Hair Colour of Disorders (publication in the book everything about hair, ISBN 978-979-469-818-5). **(5)** Systemic Photoprotection (publication in book Skin Pigmentation ISBN 978-979-469-863-5). **(6)** Terapi laser excimer 308 pada penyakit kulit alergi (publication in book Psychoneuroimmunology in Dermatology, ISBN 978-602-294-117-0). Her unpublished publication included **(a)** Peeling kimiawi (PIAAM,2014). **(b)** Profile Melasma In RSUP Sanglah (2007). **(c)** Profile hypopigmentation disorders in RSUP Sanglah (2012**). (d)** Management acne vulgaris (2010). **(e)** Profile Occupational Dermatosis on Garment Workers in Denpasar (2008). She currently works in Dermatology- Venereologist at Sanglah Hospital-Bali Indonesia.

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