ORIGINAL ARTICLE

Pre and post treatment assessment of inflammatory markers in active vitiligo patients: A prospective study from a large tertiary center in Northern India

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ABSTRACT

Background: Data is scarce in literature regarding systemic inflammation in vitiligo patients. Alteration in cytokine levels and altered neutrophil lymphocyte ratio (NLR) has been described. However exact details of these alterations are not fully understood. Therefore we did this study to find out alterations in inflammatory markers in active vitiligo patients before and after treatment.

Objectives: To study NLR, PLR (platelet lymphocyte ratio), IL6 and TNF alpha levels in active generalized vitiligo patients before and after treatment with NB-UVB and 0.1% topical tacrolimus.

Material And Methods: In 31 cases of active generalized vitiligo and an equal number of healthy controls, serum levels of IL-6, TNF alpha, NLR & PLR was performed. After 3 months of treatment, inflammatory markers levels were re-analyzed.

Results: We observed significantly raised levels of IL-6 and TNF-alpha in active generalized vitiligo patients at baseline compared to controls (P = 0.001). There was significant reduction in IL-6 mean (P = <0.001) after 3 months of treatment.

Conclusions: In our study we found significant increase in IL-6 and TNF alpha levels at baseline, which suggest that these cytokines may play an important role in the pathogenesis of vitiligo. Also there was reduction in the levels of these cytokines after treatment and this reduction was significant for IL-6 which further substantiate its role in vitiligo pathogenesis and may be an important mechanism for efficacy of combination of Narrowband-UVB and topical tacrolimus in cases of active vitiligo.

KEY WORDS: IL-6, TNF- alpha, active vitiligo

INTRODUCTION

In India, the incidence of vitiligo is 0.5%. The presence of *in-situ* immune infiltration and their interaction with epidermal melanocytes are major features in vitiligo patients. Active vitiligo is defined as new patches or extension of old lesions in the past 3 months and the presence of hypochromic border and/or confetti-like depigmentation under a Wood's lamp examination.[1]

Tumour necrosis factor (TNF) alpha has an important role in the development of cytotoxic T lymphocytes, which are implicated in the disease initiation in vitiligo. T cell mediated autoimmunity in particular plays a prominent role in disease progression, as skin infiltrating cytotoxic T lymphocytes react with melanocytes.[2] IL-6 might play an important role in melanocyte cytotoxicity through the enhancement of effector cell migration and effector target attachment.[3] Neutrophil Lymphocyte Ratio (NLR) and Platelet Lymphocyte Ratio (PLR)

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have emerged as good indicators of subclinical inflammation and prognostic markers in variety of diseases like cancer, cardiovascular and autoimmune inflammatory diseases. To the best our knowledge, data is scarce in the literature regarding systemic inflammation in vitiligo patients.[4] Systemic inflammation has been described previously in vitiligo patients for which meagre literature is available. There is an alteration in the serum cytokine levels after treatment with NB-UVB and tacrolimus in patients with vitiligo. However exact details of this cytokine alterations are yet to be fully understood. Raised NLR has been found in vitiligo patients previously however there is no study showing alteration in NLR after treatment similarly there is no previous study evaluating PLR in vitiligo patients. Therefore, we did this study to find alteration in the inflammatory markers (IL-6, TNF alpha, NLR and PLR) in active vitiligo patients before and after treatment with NB-UVB and topical tacrolimus.

METHODS AND MATERIALS

A hospital based observational study was conducted in the Department of Dermatology, Venereology, and Leprosy Department of Dermatology & STD and Biochemistry, Smt. Sucheta Kriplani Hospital (SSKH) And Kalawati Saran Children's Hospital (KSCH) November 2018 - April 2020. The study was approved by the institutional ethics committee. Thirty six patients of active generalized vitiligo were initially enrolled after applying inclusion & exclusion criteria. Five patients did not follow the study protocol (Patients received less than 18 exposures of Narrowband-UVB were considered as drop out) & were excluded from study. Thirty one patients completed the study as per the protocol. An equal number of age and sex matched healthy controls were enrolled. Inclusion criteria for patients: All clinically diagnosed cases of active generalized vitiligo (active vitiligo is defined as new patches or extension of old lesions in the past 3 months and the presence of hypochromic border and/ or confetti-like depigmentation under a wood's lamp examination) patients and age more than 12 years.

Generalized active vitiligo, involving >5% body surface area or disseminated skin lesions involving more than 5 sites or more than 10 lesions at different sites of size more than 1cm.

Exclusion criteria for patients: Patients with acute or chronic inflammatory diseases, focal, mucosal and segmental vitiligo patients, patients with chronic infections, photosensitivity disorders, pregnancy and lactating mothers, patients who have been on systemic treatment or topical treatment for vitiligo during last 8 weeks, patients with claustrophobia and patients with immunosuppression.

Patient details (age, gender, family history of vitiligo, duration of disease, associated diseases, etc) were recorded in a preset proforma designed for the study. The baseline venous blood samples (6ml) were collected from patients and control groups under sterile conditions. Sera were separated and stored until the time of cytokine estimation.

NLR was calculated by dividing neutrophil count by lymphocyte count and PLR by dividing platelet count by lymphocyte count. Serum levels of IL6 and TNF alpha were measured by ELISA kits following detailed methodology mentioned on the kit.Once daily evening application of topical tacrolimus

0.1% ointment and concomitant narrow band UVB phototherapy was given thrice a week for 3 months. After 3 months again patient's inflammatory marker levels (NLR, PLR, IL-6 and TNF alpha) were analyzed.

RESULTS

Patient's demographic details and clinical characteristics are described in Table 1. Upper & lower limbs were the most common sites of involvement, each being affected in 90.3% (n=30) of the patients. Trunk was the next common site involved in 28 patients (90.3%), followed by face/neck (n=21, 67.7%) and scalp (n=13, 41.9%). IL-6 and TNF alpha positively correlated with body surface area, however this correlation was statistically insignificant (p value 0.811, 0.593 respectively). IL-6 and TNF alpha negatively correlated with duration of the disease, however again, this correlation was statistically insignificant (p value 0.612, 0.709 respectively).

Serum levels of IL-6 and TNF alpha levels were higher in active generalised vitiligo patients as compared to controls. The difference between the mean levels of IL-6 and TNF alpha in cases and controls was statistically significant (p value 0.001, 0.001). The difference between mean NLR and PLR in cases and controls was not statistically significant (p value 0.862, 0.223 respectively). However mean NLR and PLR were definitely higher in cases as compared to controls.

After 3 months of treatmentmean levels of IL-6 and TNF alpha were reduced as compared to the baseline. Also IL-6 levels were significantly (p value 0.004) reduced after treatment with NB-UVB and topical tacrolimus. After treatment, NLR reduced to 2.36±0.73 and PLR reduced to 105.87±28.94. However, both the values were found to be statistically insignificant (p value 0.450 and 0.466 respectively).

Table 1 Demographic and clinical characteristics

Variables	Percentage inactive vitiligo patients (n = number)
Age (mean± SD in years)	10.63 ±35.10
Type of vitiligo 1. Vitiligo vulgaris 2. Acrofacial vitiligo	 83.9% (n=26) 16.1 (n=6)
Duration (mean± SD in years)	8.1±5.96
Leukotrichia	41.9% (n=13)
Koebnerisation	6.5% (n=2)
Premature greying	25.8% (n=8)
Family history	3.2% (n=1)

DISCUSSION

Auto immunityis the most long standing and popular hypothesis for the pathogenesis of vitiligo.

CD8 T cells play an important role in the pathogenesis of vitiligo.[3] Cell mediated immunity is mediated by various subset of T

Table 2 Serum levels (mean level/value) of various inflammatory markers in cases (at baseline) and controls

INFLAM-MATORY MARKERS	Mean value of level/value of inflammatory markers		
	CASES	CONTROLS	p value (as compared to base line)
	MEAN±SD	MEAN±SD	
IL-6 (pg/ml)	7.20±10.23	0.90±0.93	0.001
TNF ALPHA (pg/ml)	8.16±5.30	4.26±2.73	0.001
NLR	2.45±1.01	2.41±0.78	0.862
PLR	108.90±38.20	96.57±40.03	0.223

Table 3 Serum levels/value of various inflammatory markers at various intervals

Inflammatory markers Mean±sd		Value/ levels of inflammatory markers			
		Minimum	Maximum	Paired t test P value	
IL 6(pg/ml)	Before treatment	7.20±10.23	1.12	57.65	0.004
	After treatment	4.86±8.15	.86	45.10	
TNF alpha(pg/ml)	Before treatment	8.16±5.30	2.91	21.87	0.060
	After treatment	6.29±6.54	2.01	31.80	
NLR	Before treatment	2.45±1.01	1.30	5.10	0.450
	After treatment	2.36±0.73	1.20	4.00	
PLR	Before treatment	108.90±38.20	49.00	220.00	0.466
	After treatment	105.87±28.94	65.00	200.00	

Table 4 Correlation of inflammatory markers with bsa and duration

VARIABLES	PEARSON CORRELATION R	p value (as compared to baseline by chi square test)
BSA * IL6 before treatment	0.045	0.811
BSA * TNF alpha before treatment	0.100	0.593
Duration * IL6 before treatment	-0.095	0.612
Duration * TNF alpha before treatment	-0.070	0.709

cells and cytokines secreted by them, that is, Th1 (IL-2, TNF-a), Th2 (IL-6), and Th17 (IL-17, IL-22). Vitiligo was long considered a Th1-mediated disease, and the evidence for the same has been reported earlier. However, skewing of immune responses toward Th1 or Th17 cells has now been shown in cases of vitiligo.[5]

Cytokines like IL-2, IL-6, IL-13, IL-17 and TNF alpha were found to be increased in generalised vitiligo patients.[6,7] IL-6 is produced by mononuclear cells. It activates the polyclonal B cell with subsequent increase in antibody production which further leads to melanocyte damage.

IL-6 significantly upregulates melanocyte intracellular adhesion molecule 1 (ICAM1). ICAM1 is necessary for leukocyte and melanocyte attachment for immunologic cytotoxicity. Moreover, IL-6 is a potent inhibitor of melanocyte growth.[3]

In a study by Tu et al,[8] mean levels of IL-6 were 121.04±29.33 pg/ml in generalized vitiligo and it was found to be statistically significant (p value<0.05). Similar findings were observed in a study by Singh S et al,[9] where IL-6 mean levels were raised in cases (13.21±2.25 pg/ml) as compared to controls and the results were statistically significant (p value 0.000). In a study by Sushama et al,[10] again similar findings were observed (IL-6 level mean levels were 65.636±39.912 pg/ml as compared to controls, statistically significant p value 0.000). Singh S et al,[9] found negative correlation of IL-6 with the duration of the disease of less than 15 years, findings similar to our study. This was in contrast

to the study by Sushama S et al,[10] where no such correlation was found. In our study positive correlation was noted between IL-6 and body surface area however this correlation was found to be statistically insignificant (p value 0.811). This was similar to the results observed in the study by Sushama S et al,[10] In a study by Zaki AM et al,[11] before treatment IL-6 mean levels were 228.43±88.52 pg/ml and after 16 sessions of NB-UVB levels were reduced to 112.10± 45.69 pg/ml and were found to be statistically significant (p value <0.001). However there is no study to estimate IL-6 levels before and after treatment with combination of Narrowband-UVB and topical tacrolimus.

Abdallah et al,[12] in their study mentioned IL-6 as the sensitive marker of activity if its levels are more than 2.5 pg/ml. In our study levels of IL-6 more than 2.5 pg/ml were seen in 21 (67.7%) patients and mean levels of IL-6 in cases was also higher (7.20±10.23 pg/ml) than this.

IL-6 levels were more than 2.5 pg/ml in 21 (67.7%) patients before treatment. After 3 months of treatment level of IL-6 was reduced to, less than 2.5 pg/ml in 10 (47.6%) patients out of these 21 patients. Our findings suggest that IL-6 plays an important role in the pathogenesis of vitiligo and can be considered as a sensitive marker to look for activity in vitiligo patients. Reduction in levels of IL-6 may be an important mechanism for efficacy of this combination (Narrowband-UVB and topical tacrolimus) in cases of active vitiligo.

TNF-alpha induces the expression of ICAM1 on the cell surface of melanocytes. Increased expression of ICAM-1 on the melanocytes enhances the T-cell and melanocytes attachment in the skin resulting in destruction of melanocytes in vitiligo patients.[3] In study by Sushama S et al,[10] TNF alpha mean levels were 185.988±86.792 pg/ml significantly higher than the controls in generalised vitiligo patients (p value 0.000). Sushama et al,[10] found negative correlation of TNF alpha with the duration of disease, findings similar to our study. In our study positive correlation was noted between TNF alpha and body surface area however this correlation was found to be statistically insignificant (p value 0.593). This was similar to the findings in the study by Sushama S et al.[10]

In contrast to above in studies by Singh S et al,[9] and Tu et al[8] mean levels of TNF alpha were not significantly (p value 0.32, >0.05 respectively) raised in vitiligo patients as compared to controls. Before treatment TNF alpha mean levels were 8.16±5.30 (pg/ml). After 3 months of treatment it was reduced to 6.29± 6.54 pg/ml and this was found to be statistically insignificant (p value 0.060). In the literature it has been described previously that serum levels of TNF alpha correlates with levels in the skin lesions.[9] Attawa E et al,[13] immunohistochemically evaluated TNF alpha expression in 20 generalised vitiligo patients and healthy controls after 60 sessions of Narrowband-UVB. In this study after treatment, lesional and perilesional TNF alpha expression was increased as compared to baseline and was statistically significant (P<0.05). This is in contrast to our study where TNF alpha levels were decreased after the treatment. However we have used combination of Narrowband-UVB and topical tacrolimus which may be responsible for the reduction of TNF alpha levels after treatment. Our study is the first to estimate TNF-alpha levels in patients with active generalized vitiligo after treatment with this combination (Narrowband-

UVB and topical tacrolimus).

In a study conducted by Webb KC et al,[14] they concluded TNF alpha inhibitors are effective in disease stabilization in patients with vitiligo and can be used as an adjuvant in active vitiligo patients to achieve stabilization. Reduction in TNF alpha levels seen in our study also suggest that therapeutic agents targeting TNF alpha can be useful in active vitiligo.

Reduced melanogenesis in the adipose tissue may lead to metabolic disturbance in vitiligo patients, since oxidative stress plays a role in the pathogenesis of metabolic syndrome.[15,16] NLR and PLR are novel inflammatory parameters. Patients with generalised vitiligo can have increased systemic inflammation as compared to localised vitiligo. The melanocytes present in the adipose tissue exert an anti-inflammatory action. They have a role in the reduction of ROS.[16] NLR is calculated as the absolute count of neutrophils divided by the absolute count of lymphocytes. Platelets are a rich source of inflammatory cytokines, and play an active role in inflammation while having regulatory effects on immune cells. PLR by calculating the absolute platelet count divided by the absolute lymphocyte count is again suggested as a potential marker to determine inflammation.[17]

Our study is the first to estimate the NLR and PLR after treatment with Narrowband- UVB and topical tacrolimus. After 3 months of treatment NLR reduced to 2.36± 0.73 and PLR reduced to 105.87± 28.94. However both the values were found to be statistically insignificant (p value 0.450 and 0.466 respectively).

CONCLUSION

IL-6 levels were raised significantly at baseline in our patients therefore it can be considered as a marker to look for activity in vitiligo patients which may be helpful in taking the decision regarding treatment in such patients. IL-6 levels were decreased significantly after treatment with Narrowband-UVB and topical tacrolimus. Reduction in levels of IL-6 post treatment suggests its role in the pathogenesis and also could be a possible mechanism for efficacy of above treatment in vitiligo. Larger studies measuring levels of IL-6 pre and post treatment may be considered to explore this aspect further. TNF alpha levels were also increased at baseline in our study therefore it can also be considered as a marker to look for activity in vitiligo patients. Reduction in TNF alpha levels after treatment seen in our study suggests that therapeutic agents targeting TNF alpha can be useful in active vitiligo to stop the progression of the disease. More studies with large sample size, vitiligo of varying duration and varying severity (especially more body surface area) are required to look for systemic inflammation in vitiligo as levels of inflammatory markers including IL-6 and TNF alpha were raised whereas NLR and PLR values were not conclusive.

REFERENCES

1. Nguyen S, Chuah SY, Fontas E, Khemis A, Jhingan A, Thng STG et al. Atorvastatin in combination with narrowband UV-B in adult Patients with active vitiligo a randomized Clinical Trial. JAMA Dermatol. 2018; 154(6):725-26.
2. Moretti S, Spallanzani A, Amato L, Hautmann G, Gallerani I, Fabiani M et al. New insights into the pathogenesis of vitiligo: Imbalance of Epidermal Cytokines at Sites of Lesions. Pigment Cell Res. 2002; 15(2):87-92.
3. Tu CX, Gu JS, Lin XR. Increased interleukin-6 and granulocyte-macrophage colony stimulating factor levels in the sera of patients with non-segmental

vitiligo. J Dermatol Sci. 2003; 31(1):73-78.
4. Solak B, Dikicier BS, Cosansu NC, Erdem T. Neutrophil to lymphocyte ratio in patients with vitiligo. Postepy DermatolAlergo. 2017; 34(5):468-70.
5. Yeo UC, Yang YS, Park KB, Sung HT, Jung SY, Lee ES, et al. Serum concentration of the soluble interleukin-2 receptor in vitiligo patients. J Dermatol Sci. 1999; 19:182-88.
6. Singh RK, Lee KM, Vujkovic-Cvijin I, Ucmak D, Farahnik B, Abrouk M, et al. The role of IL-17 in vitiligo: A review. Autoimmun Rev. 2016; 15:397-404.
7. Zhou L, Shi YL, Li K, Hamzavi I, Gao TW, Huggins RH, et al. Increased circulating Th17 cells and elevated serum levels of TGF- beta and IL-21 are correlated with human non-segmental vitiligo development. Pigment Cell Melanoma Res. 2015; 28:324-29.
8. Tu CX, Gu JS, Lin XR. Increased interleukin-6 and granulocyte–macrophage clony stimulating factor levels in the sera of patients with non-segmental vitiligo. J Dermatol sci. 2000; 31(1):73-78.
9. Singh S, Singh U, Pandey SS. Serum concentration of IL-6, IL-2, TNF-α, and IFNγ in Vitiligo patients. Indian J Dermatol. 2012; 57(1):12-14.
10. Sushama S, Dixit N, Gautam RK, Arora P, Khurana A, Anubhuti A. Cytokine profile (IL-2, IL-6, IL-17, IL-22, and TNF-α) in vitiligo-New insight into pathogenesis of disease. J Cosmet Dermatol. 2019; 18(1):337-41.
11. Zaki AM, Abd El Aziz HM, Mohamed MA, Abd El Hamed AA. Evaluation of Serum Interleukin-6 Level in Vitiligo Patients before and after Phototherapy. The EJHM. 2019; 77:5276-78.
12. Abdallah M, El-Mofty M, Anbar T, Rasheed H, Esmat S, A et al. CXCL-10 and Interleukin-6 are reliable serum markers for vitiligo activity: A multicenter cross-sectional study. Pigment Cell Melanoma Res. 2018; 31:330-36.
13. Attwa E, Gamil H, Assaf M, Ghonemy S. Over-expression of tumor necrosis factor-α in vitiligo lesions after narrow-band UVB therapy: an immunohistochemical study. Arch Dermatol Res. 2012; 304:823-30.
14. Webb KC, Tung R, Winterfield LS, Gottlieb AB, Eby JM, Henning SW et al. Tumour necrosis factor-α inhibition can stabilize disease in progressive vitiligo. British J Dermatol. 2015; 173(3):641-50.
15. Randhawa M, Huff T, Valencia JC, et al. Evidence for the ectopic synthesis of melanin in human adipose tissue. The FASEB Jl. 2009; 23:835-43.
16. Page S, Chandhoke V, Baranova A. Melanin and melanogenesis in adipose tissue: possible mechanisms for abating oxidative stress and inflammation?. Obes Rev. 2011; 12:e21-31.
17. Baodong Qin, Ning Ma, Qingqin Tang, Tingting Wei, Min Yang, Haitao Fu et al. Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) were useful markers in assessment of inflammatory response and disease activity in SLE patients. Mod Rheumatol. 2016; 26(3):372-76.